XXVI -Annual Meeting of the Brazilian Society of Protozoology

# XXVI Annual Meeting of the Brazilian Society of Protozoology

# XXXVII Annual Meeting on Basic Research in Chagas' Disease

Control of Gene Expression

Evolution and Epidemiology

Biochemistry and Chemotherapy

Vaccine Development

Immunomodulation

Structural Biology

Vector Control

Cell Biology

SBPz

**October 25-27, 2010**

**Foz do Iguaçu - PR - Brasil**

# IMAGEM DA CAPA

*Ocupar um vazio.*
*As idéias proliferam afastando as complexidades técnicas e as abstrações indecifráveis.*
*O caminho é buscar as cores e enfeitá-las com o emblema.*

Anita Szochor Colli

Ficha Técnica
Título: SBPz
Acrílica s/ lona plástica
127 x 70 cm
Anita Szochor Colli
2009

***PROCEEDINGS***

XXVI Meeting of the Brazilian Society of Protozoology
XXXVII Annual Meeting on Basic Research in Chagas' Disease

*Hotel Rafain Palace, Foz do Iguaçu, PR, BRASIL- 25-27, October 2010*

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## ACKNOWLEDGMENTS

On behalf of the Organizing Committee I welcome all the participants of the XXXVII Annual Meeting of Basic Research in Chagas´s Disease and the XXVI Meeting of the Brazilian Society of Protozoology. We hope you enjoy the new location of the traditional "Caxambu Meeting", Foz do Iguaçu, and above of all, we hope you like the Program we organized for the 2010 year. In this regard, we would like to express our gratitude to all the advisors who helped to assemble the Program and all the invited speakers for making the meeting possible, and hopefully, successful.

The Scientific Program contains abstracts that highlight the recent advances achieved in Protozoology research, including biochemistry, cell biology, chemotherapy, control of gene expression, evolution, epidemiology, immunology, structural biology, vaccine development and vector biology and control. I In addition, besides Conferences and Round-Tables, as established in previous years, we organized sessions of oral presentations, which were selected from the submitted abstracts, giving the opportunity and highlighting the work conducted by the youngsters. The Organizing Committee wishes to express gratitude to all the participants for their support to these annual meetings.We are particularly indebted to the secretaries Ana Paula Lopes Vidal and Vilma de Araújo Andrade for the excellence of their work and dedication during the organization of these meetings. We would also like to acknowledge all those colleagues who have contributed to raising funds and selecting the abstracts.

Finally, we would also to thank the sponsors: CAPES, CNPq, FAPEMIG, FAPERJ, FAPESP, e Ministério da Saúde.

The Organizing Committee wishes to all participants a fruitful Meeting in Foz do Iguaçu.

Foz do Iguaçu, October 25th to 27th, 2010
Angela Kaysel Cruz – President of SBPz

## AGRADECIMENTOS

Em nome da Comissão Organizadora, eu dou as boas vindas a todos os participantes da XXXVII Reunião Anual de Pesquisa Básica em Doença de Chagas e a XXVI Reunião da Sociedade Brasileira de Protozoologia. Este ano, A tradicional "Reunião de Caxambu" acontece em Foz do Iguaçu, e esperamos que o local escolhido seja do agrado da maioria. Gostaríamos de expressar nossa gratidão a todos os colegas que nos ajudaram a compor o programacientífico e a todos os convidados que aceitaram participar e que são a essência do sucesso da Reunião.

O Programa Científico contém resumos que mostram os mais recentes avanços na pesquisa em Protozoologia, incluindo bioquímica, biologia celular, quimioterapia, controle da expressão gênica, evolução, epidemiologia, imunologia, biologia estrutural, desenvolvimento de vacina e biologia e controle de vetores. Repetindo o que foi estabelecido em anos anteriores, e em reconhecimento a contribuição científica dos estudantes de graduação, pós graduação e pós doutores no desenvolvimento destas áreas, além das Conferências e Mesas Redondas, selecionamos a partir do resumo oferecido, 52 apresentações orais que ocorrerão em 8 sessões nos dois dias da Reunião.

A Comissão Organizadora expressa sua gratidão a todos os colegas que, como participantes, apoiam e abrilhantam estas Reuniões Anuais. Em particular, agradece a constante assistência, o excelente trabalho e a grande dedicação das secretárias Ana Paula Vidal e Vilma Andrade esseciais para o sucesso da Reunião. Agradecemos a todos os colegas que contribuíram para obtenção do suporte financeiro das diferentes agências e aos que incansavelmente contribuem anualmente com a avaliação dos resumos submetidos.

Finalmente, nós gostaríamos de agradecer o apoio das agências de fomento: CAPES, CNPq, FAPEMIG, FAPERJ, FAPESP e Ministério da saúde.

A Comissão Organizadora deseja a todos os participantes uma agradável e proveitosa reunião em Foz do Iguaçu.

Foz do Iguaçu, 25 a 27 de outubro de 2010
Angela Kaysel Cruz – Presidente da SBPz

# Meeting Program (Programa Científico)
# October 24, 2010

**14h00 - 18h00 - Expo Center IV - Room C - PM-01 - PRE-MEETING COURSES**
OFICINA DE REDAÇÃO CIENTÍFICA

# October 25, 2010

**08h00 - 12h00 - Expo Center IV - Room C - PM-02 - PRE-MEETING COURSES**
OFICINA DE REDAÇÃO CIENTÍFICA

**14h00 - 19h00 - Expo Center III - REGISTRATION**

**17h00 - 17h20 - Expo Center II - Room A - MEETING OPENING**
Welcome & Announcements

**17h20 - 18h20 - Expo Center II - Room A - SPC - SAMUEL PESSOA CONFERENCE**

**RESEARCH IN BRAZIL AS AN OPTION: 40 YEARS OF EXPERIENCE AND DEDICATION**
*Antoniana Ursine Krettli (FIOCRUZ Brasil)*
**Chair:** Alvaro Jose Romanha (FIOCRUZ Brasil)

**19h00 - 20h30 - Swimming Pool - COCKTAIL**

**20h30 - 22h30 - Expo Center III - PS - 01 - POSTER SESSION**
ODD Numbers / Números ÍMPARES

# October 26, 2010

**09h00 - 11h00 - Expo Center II - Room A – OP.01 - ORAL PRESENTATIONS**
**Molecular Biology**
**Chair:** Renata Torres de Souza and Cristina Henriques

BM007 - GENOMIC AND TRANSCRIPTOMIC ANALYSIS OF Leishmania (Viannia) braziliensis RESISTANT TO POTASSIUM ANTIMONY TARTRATE
*Daniel Barbosa Liarte (Centro de Pesquisas René Rachou-FIOCRUZ Brazil)*

BM023 - FUNCTIONAL CHARACTERIZATION OF THREE LEISHMANIA PABP HOMOLOGUES WITH DISTINCT BINDING PROPERTIES TO RNA AND PROTEIN PARTNERS
*Tamara De Carli da Costa Lima (Centro de Pesquisas Aggeu Magalhães Brazil)*

BM050 - A PLATFORM FOR TRYPANOSOMA CRUZI REVERSE GENETICS: A STEP CLOSER FOR HIGH-THROUGHPUT GENE CHARACTERIZATION
*Fernanda Grande Kugeratski (Instituto Carlos Chagas Brazil)*

BM069 - EXPRESSION AND IMMUNOCYTOLOCALIZATION OF THE COHESIN SUBUNIT SCC1 IN *TRYPANOSOMA CRUZI*
*Renata Cristina Grangeiro Ferreira (Universidade de Brasília Brazil)*

BM079 - CHARACTERIZATION OF TRNA-DERIVED SMALL RNAS IN THE METACYCLIC *TRYPANOSOMA CRUZI*
*Saloe Bispo Poubel (Instituto carlos chagas Brazil)*

BM086 - CTLA-4 +49 and PD-1.3 POLYMORPHISMS CAN CONTRIBUTE FOR DIGESTIVE FORM IN CHAGASIC PATIENTS.
*Fabrício César Dias (Faculdade de Medicina de Ribeirão Preto/USP Brazil)*

BM106 - THE FOUR TRYPANOSOMATID eIF4E HOMOLOGUES FALL INTO TWO DISTINCT GROUPS, WITH FUNCTIONS IN TRANSLATION AND OTHER CRITICAL PROCESSES
*Rodrigo Pontes de Lima (Centro de Pesquisas Aggeu Magalhães Brazil)*

**09h00 - 11h00 - Expo Center IV - Room B – OP.02 - ORAL PRESENTATIONS**
**Immunology**
**Chair:** Tiago W. P. Mineo and Fabio T. M. Costa

IM07 - APOPTOSIS IN THE SPLEEN AND PERIPHERAL BLOOD IN DOGS NATURALLY INFECTED BY LEISHMANIA (L.) CHAGASI
*Valéria Marçal Felix de Lima (Universidade Estadual Paulista Brazil)*

IM17 - RESTRICTION OF LEISHMANIA AMAZONENSIS INFECTION BY THE INFLAMMASOMES
*Djalma de Souza Lima Júnior (Faculdade de Medicina de Ribeirão/USP Brazil)*

IM21 - STUDY OF THE INTERACTION BETWEEN CANINE NEUTROPHILS AND *LEISHMANIA CHAGASI* POSTER
*Amanda Brito Wardini (Universidade Federal do Rio de Janeiro Brazil)*

IM25 - RELATIONSHIP BETWEEN DENDRITIC AND CD4+/CD8+T CELLS IN THE SKIN OF BALB/C MICE INFECTED WITH L. (L.) AMAZONENSIS AND L. (V.) BRAZILIENSIS
*Ana Kely de Carvalho (Faculdade de Medicina Universidade de São Paulo Brazil)*

IM32 - VACCINATION WITH THE LEISHMUNE®S NUCLEOSIDE HYDROLASE MAPS THE C-TERMINAL DOMAIN AS THE TARGET OF THE PROTECTIVE IMMUNE RESPONSE
*Dirlei Nico (Universidade Federal do Rio de Janeiro Brazil)*

IM45 - ACTIVATION OF PLATELETS BY *LEISHMANIA MAJOR* PARASITES TO ATTRACT A POPULATION OF KILLER MONOCYTES TO THE SITE OF INFECTION
*Ricardo Gonçalves (Universidade Federal de Ouro Preto Brazil)*

IM53 - STUDY OF REGULATORY T CELLS (TREGS) FUNCTION IN HUMAN CUTANEOUS LEISHMANIASIS DUE TO *LEISHMANIA BRAZILIENSIS* INFECTION
*Diego Luís Costa (Faculdade de Medicina de Ribeirão Preto - Universi Brazil)*

Meeting Program

# October 26, 2010

**09h00 - 11h00 - Expo Center IV - Room C – OP.03 - ORAL PRESENTATIONS**
**Biochemistry and Immunology**
**Chair:** Luiz Carlos Crocco Afonso and Renata R. Tonelli

BQ10 - QUERCETIN INDUCES DEATH IN *LEISHMANIA AMAZONENSIS* BY MITOCHONDRIAL DYSFUNCTION AND REACTIVE OXYGEN SPECIES PRODUCTION
*Fernanda da Fonseca e Silva (Instituto Oswaldo Cruz / FIOCRUZ Brazil)*

BQ14 - CHARACTERIZATION OF A SIR2-RELATED PROTEIN FROM LEISHMANIA (L.) AMAZONENSIS (LaSir2) AS A CYTOPLASMIIC, GLYCOSYLATED NAD+-DEPENDENT DEACETYLASE.
*Melissa Regina Fessel (Unicamp Brazil)*

BQ36 - INVASION OF HOST CELLS BY *TRYPANOSOMA CRUZI:* A NEW PUTATIVE RECEPTOR
*Ketna Guilhermino Khusal (Universidade de São Paulo Brazil)*

BQ38 - MECHANISM OF TUNICAMYCIN RESISTANCE AND EVALUATION OF N-GLYCOSYLATION INHIBITION OVER VIRULENCE IN LEISHMANIA PARASITES
*Juliano Simões de Toledo (Universidade de São Paulo Brazil)*

IM16 - MODULATION OF DENDRITIC CELL RESPONSE BY DIFFERENT SPECIES OF Leishmania AND THE PARTICIPATION OF EXTRACELLULAR-ATP AND ADENOSINE ON THIS PROCESS
*Amanda Braga de Figueiredo (Universidade Federal de Ouro Preto Brazil)*

IM51 - VITAMIN A-DEFICIENCY IMPAIRS INTESTINAL CD4+ FOXP3+ REGULATORY T CELL EXPANSION AND THE EFFICACY OF THE ORAL LAAG VACCINE AGAINST *LEISHMANIA AMAZONENSIS* INFECTION
*Izabella Pereira da Silva Bezerra (Universidade Federal do Rio de Janeiro Brazil)*

**09h00 - 11h00 - Expo Center IV - Room D – OP.04 - ORAL PRESENTATIONS**
**Epidemiology**
**Chair:** Gerhard Wunderlich and Ana Lucia Falavigna-Guilherme

EP02 - GENES INFLUENCE SEROPOSITIVITY FOR *T. CRUZI* INFECTION AND EKG MEASURES OF CHAGAS DISEASE PROGRESSION
*Sarah Williams-Blangero (Southwest Foundation for Biomedical Research United States)*

EP09 - THE EPIDEMIOLOGY OF THE ACUTE CHAGAS DISEASE IN THE STATE OF PARÁ, BRAZIL
*Adriana de Jesus Benevides Almeida (Universidade de Brasilia Brazil)*

EP21 - EVALUATION OF PARASITE RECOMBINANT PROTEIN AS ANTIGENS FOR CUTANEOUS LEISHMANIASIS SERODIAGNOSIS.
*Ana Paula Almeida de Souza (Fundação Oswaldo Cruz Brazil)*

EP23 - A PROTEOMIC APPROACH TO THE RECOGNOME OF THE *PLASMODIUM FALCIPARUM* INFECTED RED BLOOD CELL SURFACE
*Gerhard Wunderlich (Instituto de Ciencias Biomedicas Brazil)*

**11h00 - 11h25 - Foyer - COFFEE BREAK**

# October 26, 2010

**11h30 - 12h00 - Expo Center II - Room A – MC.01 - MINI-CONFERENCE**

**THE DNA REPAIR AND OXIDATIVE STRESS IN *TRYPANOSOMA CRUZI***
*Carlos Renato Machado (Universidade Federal de Minas Gerais Brasil)*
**Chair:** Bruno Dallagiovanna Muñiz (ICC - FIOCRUZ Brasil)

**11h30 - 12h00 - Expo Center IV - Room B – MC.02 - MINI-CONFERENCE**

**LEISHMANIA PARASITES INDUCE AND ARE KILLED BY NETTING NEUTROPHILS**
*Elvira Maria Saraiva (UFRJ, Inst. de Microbiologia Prof Paulo de Góes Brasil)*
**Chair:** Camila Indiani de Oliveira (FIOCRUZ Brasil)

**12h00 - 12h30 - Expo Center II - Room A – MC.03 - MINI-CONFERENCE**

**MITOCHONDRIA AND CELL DEATH IN *T. CRUZI***
*Anibal Eugenio Vercesi (Univ. Est. de Campinas, Fac. de Ciências Médicas Brasil)*
**Chair:** Fernanda Ramos Gadelha (UNICAMP Brasil)

**12h00 - 12h30 - Expo Center IV - Room B – MC.04 - MINI-CONFERENCE**

**THE CELL BIOLOGY OF *LEISHMANIA* - SAND FLY INTERACTIONS**
*David Sacks (National Inst. of Allergy and Infectious Diseases USA)*
**Chair:** Camila Indiani de Oliveira (FIOCRUZ Brasil)

**14h30 - 16h00 - Expo Center II - Room A – RT.01 - ROUND TABLE**
**HOST-PARASITE INTERACTION DURING LEISHMANIA INFECTION**
**Chair:** Camila Indiani de Oliveira (CPqGM-FIOCRUZ Brasil)

**IMMUNOREGULATION IN CUTANEOUS LEISHMANIASIS: A ROLE FOR NOTCH SIGNALING**
*Lucas Pedreira de Carvalho (UFBA, Brasil)*

**ROLE OF NEUTROPHILS IN RESISTANCE AGAINST EXPERIMENTAL *L. BRAZILIENSIS* INFECTION**
*Camila I. de Oliveira (CPqGM-FIOCRUZ Brasil)*

**THE IMPORTANCE OF MICRORNAS IN MACROPHAGE POLARIZATION AND IN SURVIVAL OF LEISHMANIA**
*Joel kraff (University of Iowa USA)*

**14h30 - 16h00 - Expo Center IV - Room B – RT.02 - ROUND TABLE**
**NEGLECTED DISEASES: CHALLENGES AND OPPORTUNITIES**
**Chair:** Adriano Defini Andricopulo (IFSC-USP Brasil)

**CHARACTERIZATION OF ARTEMISININ RESISTANT *Plasmodium falciparum***
*Dennis E. Kyle (University of South Florida USA)*

**STRUCTURE- AND LIGAND-BASED DRUG DESIGN APPROACHES FOR NEGLECTED TROPICAL DISEASES**
*Adriano Defini Andricopulo (IFSC-USP Brasil)*

**PHENOTYPIC HIGH-THROUGHPUT SCREENING FOR NEGLECTED DISEASES**
*Lucio Holanda Gondim de Freitas Junior (Institut Pasteur Korea Korea)*

**14h30 - 16h00 - Expo Center IV - Room C – RT.03 - ROUND TABLE**
**VECTOR-PARASITE INTERACTION**
**Chair:** Yara Maria Traub-Cseko (FIOCRUZ Brasil)

**CHARACTERIZATION OF THE *ANOPHELES AQUASALIS* IMMUNE RESPONSE TO *Plasmodium vivax***
*Yara Maria Traub-Cseko (Fundação Oswaldo Cruz, Instituto Oswaldo Cruz Brasil)*

**PROTEOPHOSPHOGLYCAN CONFERS RESISTANCE OF LEISHMANIA MAJOR TO MIDGUT DIGESTIVE ENZYMES INDUCED BY BLOOD FEEDING IN VECTOR SAND FLIES**
*Nagila Francinete Costa Secundino (CPqRR, Fiocruz Brasil)*

**INFLUENCES OF THE MICROBIOME ON PATHOGEN TRANSMISSION: LESSONS LEARNED FROM THE TSETSE FLY**
*Serap Aksoy (Yale University School of Public Health USA)*

**14h30 - 16h00 - Expo Center IV - Room D – RT.04 - ROUND TABLE**
**NUCLEAR DNA REPLICATION IN PROTOZOA**
**Chair:** Maria Carolina Elias (Instituto Butantan Brasil)

**THE BIOLOGY OF NUCLEAR DNA REPLICATION IN TRIPANOSOME**
*Maria Carolina Elias (Instituto Butantan Brasil)*

***Plasmodium falciparum* ORIGIN RECOGNITION COMPLEX (ORC): ROLE IN THE REGULATION OF PARASITE DNA REPLICATION AND VIRULENCE GENE EXPRESSION**
*Suman Kumar DHAR (Jawaharlal Nehru University India)*

**ANALYSIS OF THE ARCHITECTURE AND FUNCTION OF THE NUCLEAR DNA REPLICATION APPARATUS IN *TRYPANOSOMA BRUCEI*.**
*Richard McCulloch (University of Glasgow UK)*

**16h00 - 16h25 - Foyer - COFFEE BREAK**

**16h30 - 17h10 - Expo Center II - Room A – CO.01 – CONFERENCE**

**INSIGHTS INTO THE PATHOGENESIS OF VIVAX MALARIA**
*Manoel Barral Netto (FIOCRUZ, Centro de Pesquisas Gonçalo Muniz Brasil)*
**Chair:** João Santana da Silva (FMRP - USP Brasil)

**17h10 - 17h50 - Expo Center II - Room A – CO.02 – CONFERENCE**

**DARING TO BE DIFFERENT: UNUSUAL ASPECTS OF GENE TRANSCRIPTION IN *TRYPANOSOMA BRUCEI***
*George A. M. Cross (The Rockefeller University USA)*
**Chair:** Yara Maria Traub-Cseko(IOC-FIOCRUZ Brasil)

**20h30 - 22h30 - Expo Center III - PS-02 - POSTER SESSION**
EVEN Numbers / Números PARES

# October 27, 2010

**09h00 - 11h00 - Expo Center II - Room A – OP.05 - ORAL PRESENTATIONS**
**Immunology**
**Chair:** Leda Quercia and Dario Zamboni

IM65 - NEUROLOGICAL MANIFESTATION IN EXPERIMENTAL TRYPANOSOMA CRUZI INFECTION: DEPRESSION AND MEMORY
*Glaucia Vilar Pereira (Universidade Federal Fluminense Brazil)*
IM66 - CD8 + T CELL FUNCTION IN THE PATHOGENESIS OF CHRONIC CHAGASIC CARDIOMYOPATHY
*Jaline Coutinho Silverio (Instituto Oswaldo Cruz Brazil)*
IM69 - REGULATORY EFFECTS OF IL-18 ON CYTOKINE PROFILES AND DEVELOPMENT OF MYOCARDITIS DURING TRYPANOSOMA CRUZI INFECTION
*Lísia Maria Esper (Universidade Federal de Minas Gerais Brazil)*
IM71 - INFLAMMATORY ANGIOGENESIS INDUCED BY TRYPANOSOMA CRUZI ANTIGENS IN MURINE SPONGE MODEL
*Francisca Hildemagna Guedes da Silva (Universidade Federal de Ouro Preto Brazil)*
IM73 - ESSENCIAL ROLE OF CASPASE-1 FOR THE HOST INNATE IMMUNE RESPONSE AGAINST PARASITE TRYPANOSOMA CRUZI
*Grace Kelly da Silva (Faculdade de medicina de Ribeirão Preto-USP Brazil)*
IM77 - ANTIBODIES PRODUCTION AND CIRCULATING LEUCOCYTES IN BALB/c MICE REINFECTED WITH RECOMBINANT TOXOPLASMA GONDII STRAINS AFTER IMMUNOSUPPRESSION WITH CYCLOPHOSPHAMIDE
*Letícia de Azevedo Silva (Universidade Federal de Minas Gerais Brazil)*
IM79 - Detection of Th17 and T regulatory cells during the course of experimental toxoplasmosis in susceptible and resistant mice
*Denise Morais da Fonseca (Faculdade de Medicina de Ribeirão Preto Brazil)*

**09h00 - 11h00 - Expo Center IV - Room B – OP.06 - ORAL PRESENTATIONS**
**Cell Biology and Molecular Biology**
**Chair:** Maria Isabel N. Cano and Silvia Reni Bortolin Uliana

BC013 - LIMP-1 IS IMPORTANT FOR *TRYPANOSOMA CRUZI* EXTRACELLULAR AMASTIGOTE INTERNALIZATION INTO MEF CELLS
*Viviane Martinelli Gonçalves (Universidade Federal de São Paulo Brazil)*
BC028 - THE DIFFERENTIAL ROLE OF LYSOSOMAL PROTEINS LAMP-1 AND LAMP-2 IN HOST CELL INVASION BY T. CRUZI TISSUE CULTURE TRYPOMASTIGOTES
*Ludmila Fernandes Kelles (Universidade Federal de Minas Gerais Brazil)*
BC037 - TOR-like 1 KINASE IS INVOLVED IN THE CONTROL OF OSMOTIC STRESS RESPONSE IN *TRYPANOSOMA BRUCEI*
*Teresa Cristina Leandro de Jesus (Universidade Federal de São Paulo Brazil)*
BC060 - MORPHOLOGICAL ASPECTS OF TOXOPLASMA GONDII-FELINE ENTEROCYTES INTERACTIONIN VITRO
*Renata Morley de Muno (FIOCRUZ Brazil)*
BC065 - ANALYSIS OF THE INDUCTION OF NEUTROPHIL EXTRACELLULAR TRAPS (NETS) BY *Toxoplasma gondii*
*Tatiana Christina Paredes Santos (Universidade Federal do Rio de Janeiro Brazil)*
BC071 - GENE EXPRESSION PROFILES OF HUMAN MACROPHAGES INFECTED WITH *LEISHMANIA BRAZILIENSIS* IN VITRO
*Luana Guimaraes de Sousa (UFBA Brazil)*
BC072 - NEW ASPECTS ABOUT INTERACTION BETWEEN *GIARDIA LAMBLIA* AND INTESTINAL CELLS
*Claudia Maia Brigagão (Universidade Federal do Rio de Janeiro Brazil)*
BM011 - UV IRRADIATION INDUCES ALTERATIONS IN THE EXPRESSION OF LEISHMANIA SPP. TELOMERIC PROTEIN RBP38 (LaRBP38)
*Arina Marina Perez (Universidade Estadual Paulista Julio de Mesquita F Brazil)*
BM068 - The GTPase RJL IS INVOLVED IN METACYCLOGENESIS OF *TRYPANOSOMA CRUZI*
*Guilherme Rodrigo R Monteiro dos Santos (Universidade Federal do Rio de Janeiro Brazil)*

Meeting Program

# October 27, 2010

**09h00 - 11h00 - Expo Center IV - Room C – OP.07 - ORAL PRESENTATIONS**
**Chemotherapy**
**Chair:** Renata Tonhosolo and Lisvane Paes

QT13 - ALTERATION OF Trypanosoma cruzi CELL MEMBRANE INTEGRITY INDUCED BY ELATOL
*Vânia Cristina Desoti (Universidade Estadual de Maringá Brazil)*

QT37 - EVALUATION OF MECHANISM OF ACTION, IN VITRO AND IN VIVO ACTIVITIES OF LQB118, A NEW ANTILEISHMANIAL PROTOTYPE
*Grazielle Alves Ribeiro (Fundação Oswaldo Cruz Brazil)*

QT47 - Immunochemotherapy in BALB / c mice infected with Leishmania (L.) amazonensis
*Carolina de Siqueira Paladi (Universidade Federal de São Paulo Brazil)*

QT61 - EFFICACY OF CHALCONE-CONTAINING ELASTIC LIPOSOMES FOR TOPICAL TREATMENT OF CUTANEOUS LEISHMANIASIS
*Camila Alves Bandeira Falcão (Universidade Federal do Rio de Janeiro Brazil)*

QT62 - EFFICACY OF MILTEFOSINE IN THE TREATMENT OF MURINE MODEL OF CUTANEOUS LEISHMANIASIS BY *LEISHMANIA AMAZONENSIS*
*Joseane Lima Prado Godinho (Universidade Federal do Rio de Janeiro Brazil)*

QT73 - ANTIMALARIAL ACTIVITY OF BIOPRODUCTS FROM Aspidosperma sp PLANTS TESTED IN BLOOD CULTURES OF Plasmodium falciparum
*Isabela Oliveira de Freitas (Centro de Pesquisas René Rachou Brazil)*

**09h00 - 11h00 - Expo Center IV - Room D – OP.08 - ORAL PRESENTATIONS**
**Vector**
**Chair:** Adriana Coelho Soares and Luiz Claudio Miletti

VE02 - IN VIVO ANALYSIS OF *TRYPANOSOMA CRUZI* AND *TRYPANOSOMA RANGELI* DISTRIBUTION OVER TIME IN THE MIDGUT OF *RHODNIUS PROLIXUS*
*Alessandra Aparecida Guarneri (Centro de Pesquisas René Rachou Brazil)*

VE06 - THE INFECTION BY *TRYPANOSOMA RANGELI* CHANGES THE SHELTER USE BEHAVIOUR OF *RHODNIUS PROLIXUS*
*Newmar Pinto Marliére (Centro de Pesquisas René Rachou Brazil)*

VE07 - TRIATOMA INFESTANS SALIVA AS AN ENHANCER OF T. CRUZI INFECTION.
*Michele Souza Lima (Universidade Federla do Rio de Janeiro Brazil)*

VE14 - INSIGHT INTO THE SALIVARY TRANSCRIPTOME AND PROTEOME OF DIPETALOGASTER MAXIMA
*Jaime Martins de Santana (Universidade de Brasília Brazil)*

VE17 - CASPAR AND TGF-BETA ARE POTENTIALLY INVOLVED IN *LUTZOMYIA LONGIPALPIS*-PATHOGEN INTERACTION
*Erich Loza Telleria (Instituto Oswaldo Cruz - Fiocruz Brazil)*

**11h00 - 11h25 - Foyer - COFFEE BREAK**

**11h30 - 12h00 - Expo Center II - Room A – MC.05 - MINI-CONFERENCE**

**ON CYTOADHESION OF *PLASMODIUM VIVAX*-INFECTED ERYTHROCYTES**
*Fabio Trindade Maranhão Costa (UNICAMP, Instituto de Biologia Brasil)*
**Chair:** Gerhard Wunderlich (ICB-USP Brasil)

**11h30 - 12h00 - Expo Center IV - Room B – MC.06 - MINI-CONFERENCE**

**FROM PROLINE TO GLUTAMATE: SEVERAL ROLES FOR A BIOCHEMICAL PATHWAY**
*Ariel Mariano Silber (USP, Instituto de Ciências Biomédicas Brasil)*
**Chair:** Maria Julia Manso Alves (IQ-USP Brasil)

# October 27, 2010

**12h00 - 12h30 - Expo Center II - Room A – MC.07 - MINI-CONFERENCE**
**ADPTATIVE STRATEGIES IN THE INTERACTION LEISHMANIA WITH THE MACROPHAGE: MODULATION OF THE NF-KB ACTIVATION AND PKR-SIGNALING**
*Ulisses Gazos Lopes (Instituto de Biofisica Carlos Chagas Filho - UFRJ Brasil)*
**Chair:** Gerhard Wunderlich (ICB-USP Brasil)

**12h00 - 12h30 - Expo Center IV - Room B – MC.08 - MINI-CONFERENCE**

**STRESS-INDUCED ACTIVATION OF THE *L. MAJOR* MAP KINASE LMAMPK7 INCREASES PARASITE RESISTANCE AGAINST THE LEISHMANICIDAL DRUG MILTEFOSINE**
*Gerald Spaeth (Institut Pasteur France)*
**Chair:** Maria Julia Manso Alves (IQ-USP Brasil)

**14h30 - 16h00 - Expo Center II - Room A – RT.05 - ROUND TABLE**
**ACTIVATION OF INNATE IMMUNITY AND EVASION STRATEGIES BY PROTOZOAN PARASITES**
**Chair:** Tiago Wilson Patriarca Mineo (UFMG Brasil)

**INNATE IMMUNE RESPONSES TO *Neospora caninum***
*Tiago Wilson Patriarca Mineo (UFMG Brasil)*

**TOXOPLASMA CYCLING INTO THE HOST CELL**
*Vernon B. Carruthers (University of Michigan Medical School USA)*

**RECOGNITION OF INTRACELLULAR PARASITE INFECTION BY NOD-LIKE RECEPTORS**
*Dario S. Zamboni (FMRP-USP Brasil)*

**14h30 - 16h00 - Expo Center IV - Room B – RT.06 - ROUND TABLE**
**THE TELOMERES OF TRYPANOSOMATIDS**
**Chair:** Luiz Ricardo Orsini Tosi (FMRP-USP Brasil)

**TELOMERE DYNAMICS AND ANTIGENIC VARIATION IN TRYPANOSOMA BRUCEI**
*George A. M. Cross (The Rockefeller University USA)*

**UNVEILING PROTEIN:PROTEIN INTERACTIONS AT *LEISHMANIA* SPP. TELOMERES**
*Maria Isabel Nogueira Cano (IBB, UNESP-Botucatu Brasil)*

**DNA REPAIR AND TELOMERE EXPRESSION IN *LEISHMANIA MAJOR*.**
*Luiz Ricardo Orsini Tosi (FMRP-USP Brasil)*

**14h30 - 16h00 - Expo Center IV - Room C – RT.07 - ROUND TABLE**
**HOST-PARSAITE INTERACTIONS AT THE SUBCELLULAR LEVEL**
**Chair:** Renato Mortara (UNIFESP Brasil)

**MULTIDIMENSIONAL IMAGING OF MACROPHAGES DOUBLY INFECTED BY *LEISHMANIA AMAZONENSIS* AND *LEISHMANIA MAJOR***
*Fernando Roberto Oliveira Real, (UNIFESP Brazil)*

**HOST CELL MEMBRANE-LYSOSOMES INTERPLAY DURING *TRYPANOSOMA CRUZI* INVASION**
*Luciana de Oliveira Andrade (UFMG Brazil)*

***Toxoplasma gondii* INTERACTION VARIES ACCORDING TO HOST CELL MICRODOMAINS**
*Marcia Attias (IBCCF-UFRJ Brazil)*

Meeting Program

# October 27, 2010

**14h30 - 16h00 - Expo Center IV - Room D – RT.08 - ROUND TABLE**
**DIFFERENT APPROACHES USED IN THE SEARCH FOR A MORE SPECIFIC CHAGAS DISEASE THERAPY**
**Chair:** Fernanda Ramos Gadelha (IB-Unicamp Brazil)

***Trypanosoma rangeli*: A BANE OR A BLESSING FOR CHAGAS DISEASE?**
*Edmundo Carlos Grisard (UFSC Brazil)*

**ANALYSIS OF PARASITE METABOLISM IN VIVO USING METABOLOMIC APPROACHES**
*Malcolm McConville (University of Melbourne Australia)*

**UNDERSTANDING TRYPANOSOMATIDS ANTIOXIDANT MECHANISMS: HOW FAR ARE WE?**
*Fernanda Ramos Gadelha (IB-Unicamp Brazil)*

**16h00 - 16h25 - Foyer - COFFEE BREAK**

**16h30 - 17h10 - Expo Center II - Room A – CO.03 – CONFERENCE**

**RNA-BINDING PROTEINS AND EXORIBONUCLEASES INVOLVED IN MRNA DEGRADATION IN *TRYPANOSOMA BRUCEI***
*Christine Clayton (University of Heidelberg Germany)*
**Chair:** Bruno Dallagiovanna Muñiz (ICC - FIOCRUZ Brasil)

**17h10 - 17h50 - Expo Center II - Room A – CO.04 – CONFERENCE**

**TOXOPLASMA PERFORIN-LIKE PROTEIN 1 DICTATES PARASITE EGRESS AND ACUTE VIRULENCE**
*Vernon B. Carruthers (University of Michigan School of Medicine USA)*
**Chair:** Clara Lúcia Barbiéri Mestriner (UNIFESP Brasil)

**18h00 - 19h30 - BS - BRAINSTORM**
The programming will be published in the POSTER session

**20h30 – 21h30 - Expo Center II - Room A - CC - CLOSURE CONFERENCE**

**TALES OF EVOLUTIONARY DIVERSITY: HOW BLOOD-SUCKING INSECTS LEARNED TO USE OUR BLOOD**
*Pedro Lagerblad de Oliveira (Universidade Federal do Rio de Janeiro Brasil)*
**Chair:** Hatisaburo Masuda (UFRJ - IBqM Brasil)

**21h30 - 22h30 - Expo Center II - Room A - ZB - ZIGMAN BRENER AWARDS**
Closure remarks and Announcement of the Zigman Brener Awards Best Posters

# *Scientific Program*

## SPC-RESEARCH IN BRAZIL AS AN OPTION: 40 YEARS OF EXPERIENCE AND DEDICATION

Antoniana U. **Krettli**

Laboratory of Malaria, Centro de Pesquisas René Rachou / FIOCRUZ &
Universidade Federal de Minas Gerais, Belo Horizonte, MG
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Research entered my life by chance, as a student of Zigman Brener. Chloroquine treatment was no longer efficient against *P. falciparum* human malaria, and we tested drugs against murine malaria. Among hundreds of molecules, sulfadiazines (Hoffman la Roche) were active against a chloroquine resistant strain selected by drug pressure. My master thesis was on *P. juxtanuclearem* a chicken malaria parasite discovered in Belo Horizonte, MG. Later, I went to New York, working with Ruth Nussenzweig on malaria anti-sporozoite vaccine and on immunopathology. These 30 months at NYU were intense and exciting, especially learning about neutralizing antibodies after vaccination. Back home, decided to study this antibody mediated protection and not finding vectors to raise sporozoites - a research drawback up to now - I began with *Trypanosoma cruzi* antibody mediated immunity using mice chronically infected. Soon had a paper published (J. Immunol. 1976) and for two decades, in collaboration with several groups, in and outside Brazil, I focus on antibodies against live trypomastigotes describing the “lytic antibodies”.

My students worked with malaria models, less dangerous, rather amusing and important. We infected *Aedes fluviatilis* mosquitoes with *Plasmodium gallinaceum*, successfully, and produced sporozoites used to: vaccinate chickens; characterize antibodies during infections; infect macrophages; and raise monoclonal antibodies to the circum-sporozoite protein (CS). Our work in human malaria, driven to the anti-CS immune response, used recombinant *P. falciparum* (Pf) and *P. vivax* CS proteins and sera from individuals living in malaria endemic areas, or those briefly exposed to transmission. All sera had similar levels of antibodies. Later, as a visiting researcher at NIH (1990), I worked with *P. gallinaceum* (Pg) and aspects of sporozoite and vector interactions. We cloned and sequenced the PgCS protein, a data which confirmed previous hypothesis of Pg and *Pf* being closely related. Back home, we studied possible reactivity of Pg sporozoites with human malaria. Sera from subjects exposed to malaria in hyper-endemic areas of intense Pf transmission strongly reacted with PgCS, not sera from *P. vivax* infections. If Pg sporozoites protect humans against Pf infections is yet to be tested.

At present, our research aims the discovery and development of new antimalarials exploring the Brazilian biodiversity and ethnopharmacology. With phytochemists groups we select and test substances against *P. falciparum* blood stage (comparing traditional microscopy, hypoxanthine incorporation, enzymatic tests, green fluorescent parasites). Those selectively active (low toxicity) are tested in rodent malaria. Among many synthesized molecules, a hybrid between mefloquine and artesunate, more active and less toxic than the antimalarial combination, should undergo clinical trials. The financial support for this multidisciplinary project has allowed our groups to work with students and technicians, train specialists at various levels, from undergraduate to postdoctoral fellows, work on chemistry and malaria, important for the country development. Learning, teaching and doing science has been great fun, and hope to transmit my enthusiasm to future generations. To show them how pleasant research can be, in spite of the intense labor it requires, might be my best contribution to science. Supported by CNPQ, FIOCRUZ-MS.

## CO.01 – INSIGHTS INTO THE PATHOGENESIS OF VIVAX MALARIA

Barral-Netto, Manoel

Centro de Pesquisas Gonçalo Moniz (FIOCRUZ), Bahia, Brazil, Faculdade de Medicina da Bahia, Universidade Federal da Bahia (UFBA), Brazil, Instituto de Investigação em Imunologia - Instituto Nacional de Ciência e Tecnologia (iii-INCT), São Paulo, Brazil

Malaria is considered one of the most important infectious diseases that ever threaten the world. This disease is caused mainly by the infection with *Plasmodium falciparum* or *Plasmodium vivax* transmitted by *Anopheles* mosquitoes. Despite governmental and private efforts for the development of key strategies for the disease control, the actual panorama of the *Plasmodium* infection is getting worse due to the emergence of drug resistant parasite strains. The lethal cases are reported mostly in Africa and are caused by *Plasmodium falciparum*. Albeit being less lethal, *Plasmodium vivax* infections are more widely distributed and can cause high morbidity and eventually death. In most endemic areas, studies have indentified a number of factors related to clinical immunity or susceptibility to the parasites. Thus, at least regarding the falciparum malaria, age, genetic polymorphisms and repeated exposure to *Plasmodium* are considered most important determinants of the disease outcome. Unfortunately, little has been made in the screening of reliable predicting factors that could be ultimately used for clinical evaluations. This landscape is even worse for vivax malaria, probably because many researches consider it as a benign disease. Moreover, as most of the current knowledge about the malaria pathogenesis did not truly help to relieve the disease burden, new insights are necessary to overcame unfavorable scenario. The lecture will present data that aim to identify potential determinants of the vivax malaria severity linked to the immunopathogenesis in an endemic area from the western Brazilian Amazon. First, a precise and effective method for malaria diagnosis was screening by comparing multiple tests, including a software based of artificial neural networks. The molecular assay showed to be the most efficient for the diagnosis of symptomatic and asymptomatic malaria. In addition, the rational use of a rapid test for the diagnosis of malaria may be promising in areas where there is difficulty in continued training of technical human resources. The artificial neural network indicated that the cytokine balance is a strong determinant of the clinical presentation. In another study, the use of serology for measuring IgG antibodies against the sonicate salivary gland of the *Anopheles darlingi* vector showed to be a powerful tool for the estimation of exposure to *Plasmodium vivax* and also to predict clinical immunity. Intriguingly, the natural exposure to the hepatitis B virus appeared as an important factor associated with reduced clinical severity for both vivax and falciparum malaria. Concerning solely the vivax malaria, severe cases have an intense and unregulated inflammatory response. In these patients, the antioxidant enzyme superoxide dismutase-1 has emerged as an excellent marker of severity and was involved in the pathogenesis of the severe disease in which there is a release of large amounts of free heme. Together, these data add important information in understanding the mechanisms that determine the severity of vivax malaria.

## CO.02 – TELOMERE DYNAMICS AND ANTIGENIC VARIATION IN *Trypanosoma brucei*

CROSS, G. A. M.
The Rockefeller University, 1230 York Avenue, New York, NY 10065, USA

Telomeres are specialized DNA-protein complexes that stabilize chromosome ends, protecting them from nucleolytic degradation and illegitimate recombination. Telomeres form a heterochromatic structure that can suppress the transcription of adjacent genes. Telomeres may have additional roles in *Trypanosoma brucei,* whose variant surface glycoproteins, the mediators of antigenic variation and the primary cause of persistent host infections, are expressed from one subtelomeric locus at any given time. Many silent VSG genes are stored at subtelomeric loci, both at the 44 ends of the diploid chromosomes and in minichromosomes, whose abundance is unique to *T. brucei*. Telomere-induced silencing is probably partially but not entirely responsible for VSG silencing. I will summarize our principal findings about telomere structure and dynamics in *T. brucei*. Based on observations of the structure and dynamics of telomeres in the absence of telomerase and after its restoration, we have proposed that growth and breakage of telomeric repeats plays an important role in regulating the rate of antigenic switching, for which I will present new evidence.

## CO.03 – RNA-BINDING PROTEINS AND EXORIBONUCLEASES INVOLVED IN mRNA DEGRADATION IN *Trypanosoma brucei*

Clayton, CE

Zentrum für Molekularbiologie der Universität Heidelberg

Kinetoplastid protists control their gene expression primarily at the levels of mRNA processing, degradation and translation. We have shown that in *Trypanosoma brucei,* the 5'-3' exoribonuclease XRNA is important in the rapid degradation of developmentally regulated mRNAs, whereas the deadenylase CAF1 is required for general, constitutive mRNA degradation.
The rates of mRNA degradation and translation can be influenced by association with specific RNA-binding proteins. We have discovered that a pumilio domain protein, PUF9, specifically stabilises a few mRNAs during S-phase, while a zinc-finger-domain protein is involved in the stress response. Examples of this control will be described.
To investigate the importance of mRNA degradation in more detail, we have used deep RNA sequencing to analyze the transcriptomes of trypanosomes that have reduced expression of XRNA, and to correlate the half-lives of mRNAs with their abundances. Some preliminary results of these experiments will be presented.

## CO.04 – TOXOPLASMA PERFORIN-LIKE PROTEIN 1 DICTATES PARASITE EGRESS AND ACUTE VIRULENCE

Kafsack, B.F.C.[1], Commodaro, A.G.[2], Nepomuceno De Oliveira, N.[2], Hugunin, K.[3], Schultz, T.[1], Roiko, M[1], Eaton, K.[3], Pena, J.[4], Rizzo, L.V.[5], Carruthers, V.B.[1]

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Pore-forming proteins are important virulence factors for many pathogens across the tree of life. With the exception of *Cryptosporidium spp.*, all of the sequenced apicomplexan genomes encode for multiple members of the Membrane Attack Complex/Perforin family of pore-forming proteins. Recent work has illustrated the importance of these so-called Perforin-Like Proteins (PLPs) in the lifecycles of both *Plasmodium spp*. and *Toxoplasma gondii*. In *Toxoplasma* PLP1 plays an important role in egress from host-cells by facilitating the breakdown of the parasitophorous vacuolar membrane. Virulence in Swiss Webster and C57BL/6 mice was attenuated for a PLP1-deficient strain with an $LD_{50}$ of greater than 100,000 parasites, compared to less than 10 parasites for the wild-type RH strain. Using parasites expressing firefly luciferase, we monitored the infection of mice over-time by bioluminescence imaging. PLP1-defficient parasites replicated similar to WT at high doses of intra-peritoneal inoculation (100,000 and 10,000 tachyzoites) but lagged by approximately 2 days at a lower dose (1,000 tachyzoites). At a dose of 10,000 tachyzoites there was no apparent difference in time of dissemination from the peritoneal cavity to other organs. We also showed that the host mounts a milder cellular and inflammatory cytokine response against PLP1-deficient parasites compared to WT. Finally, we demonstrated that MyD88, IL12, and IFNgamma are not necessary for controlling infection with PLP1-deficient parasites, further underscoring the markedly attenuated virulence of this strain. Collectively, our findings suggest that the egress defect in PLP-deficient parasites leads to a moderately slower expansion of infection in mice, which survive by avoiding the severe immune pathology that normally accompanies infection with WT parasites.

## CC – TALES OF EVOLUTIONARY DIVERSITY: HOW BLOOD-SUCKING INSECTS LEARNED TO USE OUR BLOOD.

Pedro L Oliveira

Instituto de Bioquímica Médica, Universidade Federal do Rio de Janeiro, RJ, Brazil and
Instituto Nacional de Ciência e Tecnologia em Entomologia Molecular – INCT-EM

Infectious diseases that are prevalent in developing countries are either transmitted by blood feeding-arthropod vectors as in malaria, Chagas' disease and dengue, or have a blood-feeding organism as the etiologic agent, as in schistosomiasis and malaria. Half of the protein content of vertebrate blood is hemoglobin, and huge amounts of free heme (hemoglobin prosthetic group) are released during blood digestion. Heme is potentially cytotoxic, promoting oxidative damage to lipids, proteins and DNA. Hence, the avoidance of deleterious effects of free heme should be mandatory for blood-feeding organisms and to built protective mechanisms is a major evolutionary trend in the adaptation of these animals to hematophagy. This hypothesis is supported by the description of several protective mechanisms against heme toxicity in different species of hemoglobin-eating animals such as the kissing-bug (*R prolixus*), ticks (*R microplus*), mosquitoes (*A aegypti*) and the blood fluke (*S mansoni)*. During the course of evolution, these phylogenetically distinct groups of blood-feeding animals developed protective mechanisms that may be classified in two main groups: a) mechanisms that specifically deal with the heme molecule; such as heme aggregation, heme degradation by heme oxygenases and heme-binding proteins; b) strategies to control the redox balance, including both reactive oxygen species (ROS) depleting devices such as antioxidant enzymes and radical scavengers, together with regulation of radical production by cell metabolism. Besides the toxic effects of reactive oxygen species (ROS), it has been shown that heme have important roles in a wide range of physiological processes, from signal transduction, modulation of cellular response to stress and infection and microbial killing. This led us to study the regulatory effects of heme on redox metabolism of the midgut of blood feeding organisms. We have found that the production of ROS in the midgut of several blood sucking animals is markedly lowered after a blood meal, through heme-mediated cell signaling, affecting also the interaction with the midgut microbiota. We suggest that down-regulating production of ROS is a novel antioxidant mechanism to compensate for the ingestion of heme. Taken together, these data shows that the heme is a major component of the midgut scenario, profoundly affecting both the insect physiology and the midgut microbial ecology.
Supported by CNPq, FAPERJ and HHMI.

## MC.01 – THE DNA REPAIR AND OXIDATIVE STRESS IN *Trypanosoma cruzi*

Carlos Renato Machado
*Universidade Federal de Minas Gerais, Depto de Bioquímica e Imunologia, Belo Horizonte, MG, Brasil*

*Trypanosoma cruzi* is the causative agent of Chagas disease, a malady of great economic importance in the Americas. As an obligatory intracellular organism with a digenetic life cycle, this protozoan comes across several sources of oxidative stress within its different host environments. It has not only to deal with oxygen species produced by its own or its host metabolism, as well as to survive ROS produced as part of the host immune response. Hence the reports that these parasites have limited ability to deal with reactive oxygen species, based on their lack of a full set of enzymes involved in handling oxidative stress, seem somewhat paradoxical. 8-hydroxy-2'-deoxyguanosine (8-OxoG) is one of the main mutagenic modifications induced in DNA by oxidative stress. To counteract the mutagenic effects of 8-oxoG, organisms have developed a multi-defense mechanism, the so-called GO system, composed by the products of *fpg, mutY* and *mutT* genes in *Escherichia coli* or by the functional homologues *OGG1, MYH* and *MTH1* in eukaryotes. We have been studding the GO system from the *T. cruzi* to better understand the mechanisms involved in the oxidative DNA damage response in this parasite. We are investigating the activity of *T. cruzi* by *in vivo* studies performed with parasites, bacterial and yeast cells overexpressing these genes. The data that we have been obtaining indicate the importance of these genes in the response to oxidative stress, treatment with benznidazole and in the infection of *T. cruzi.*
Financial Support: CNPq, FAPEMIG, HHMI

## MC.02 – LEISHMANIA PARASITES INDUCE AND ARE KILLED BY NETTING NEUTROPHILS

Anderson B. Guimarães-Costa[1], Michelle T. C. Nascimento[1], Giselle S. Froment[1], Rodrigo P. P. Soares[2], Fernanda N. Morgado[3], Fátima Conceição-Silva[3], Elvira M. Saraiva[1]

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Neutrophils are short-lived leukocytes that die by apoptosis, necrosis and by a recently described mechanism named NETosis. Upon death by NETosis, neutrophils release fibrous traps of DNA, histones and granule proteins named neutrophil extracellular traps (NETs), which can kill bacteria and fungi. Inoculation of the protozoan *Leishmania* into the mammalian skin causes local inflammation with neutrophil recruitment. Here, we are describing the release of NETs by human neutrophils upon their interaction with *Leishmania* parasites, as well as NETs' ability to kill this protozoan. The NET constituents DNA, elastase and histones were detected in traps associated to promastigotes by immunofluorescence. Electron microscopy revealed that *Leishmania* was ensnared by NETs released by neutrophils, and promastigotes trapped by NETs presented a thin, flat body with protrusions, indicative of cell damage. Moreover, *Leishmania* and its surface lipophosphoglycan induced NET release by neutrophils in a parasite number- and dose-dependent manner. Disruption of NETs by DNAse treatment during *Leishmania*-neutrophil interaction increased parasite survival, evidencing NETs' leishmanicidal effect. *Leishmania* killing was also elicited by NET-rich supernatants from PMA-activated neutrophils. Immunoneutralization of histone during *Leishmania*-neutrophil interaction partially reverted *Leishmania* killing, and purified histone killed the parasites. Meshes composed of DNA and elastase were evidenced in biopsies of human cutaneous leishmaniasis and nests of amastigotes were seen associated with NETs in these lesions. NET is an innate response that might contribute to contain the infection preventing systemic spreading and diminish parasite burden in the *Leishmania* inoculation site.

## MC.03 – MITOCHONDRIA AND CELL DEATH IN *T. CRUZI*

ANIBAL E. VERCESI

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The coupling between respiration and oxidative phosphorylation is mediated by a proton electrochemical potential generated by the pumping of protons across the inner mitochondrial membrane when electrons flow through the respiratory chain. This provides the energy for ATP synthesis by the ATP-synthase but can also be consumed by electrophoretic influx of cations to the mitochondrial matrix or by the uncoupling proteins (UCPs) that promote protons return to the matrix, thus uncoupling respiration from ATP synthesis and dissipating the energy of the proton gradient as heat. The current understanding on these molecular mechanisms has provided new insights into processes involved in the pathophysiology of mitochondria-dependent programmed or accidental cell death. The same redox reactions that generate the proton electrochemical potential lead to the physiological generation of reactive oxygen species (ROS). In addition, multiple biochemical stimuli such as mitochondrial $Ca^{2+}$ overload leads to overproduction of ROS that may cause mitochondrial dysfunction via opening of pores in the inner (permeability transition pore, PTP) and outer membranes (mitochondrial outer membrane permeabilization, MOMP). These pores are components of the cell death machinery that render mitochondria as attractive targets for chemotherapy. For a long time it was assumed that programmed cell death arose along with multicellular organisms, however, apoptosis-like cell alterations have also been reported in a variety of unicellular eukaryotes including several members of Kinetoplastida. In this presentation will be reported data on the $Ca^{2+}$-dependent mechanismsunderlying T. cruzi epimastigotes apoptosis or necrosis mediated by mitochondrion dysfunction induced by fresh human serum (Irigoin F et al., Biochem J 418: 595-604, 2009) or the lectin Cramoll 1,4 (Fernandes MP et al., J Bioenerg Biomembr 42: 69-78, 2010), respectively.

## MC.04 – THE CELL BIOLOGY OF LEISHMANIA - SAND FLY INTERACTIONS

SACKS, DL

NATIONAL INSTITUTE OF ALLERGY AND INFECTIOUS DISEASES, NIH

The spectrum of clinical outcomes associated with Leishmania infections, ranging from localized cutaneous to disseminated visceral disease, is accounted for by the remarkable diversity of Leishmania species, which are themselves transmitted by an even greater species diversity of sand fly vectors. The identification of parasite- and sand fly-derived molecules that play a role in the development of transmissible infections in the fly has been facilitated by the fact that vector competent sand fly species are in some cases only permissive to the complete development of the species of Leishmania that they transmit in nature. Thus it has been the identification of species- or strain-specific parasite molecules, and the generation of Leishmania mutants specifically deficient in these molecules, that have led to most of the advances in our understanding of the molecular basis of vector competence. Cell surface and secreted phosphoglycans protect the parasite from the proteolytic activities of the blood-fed midgut. The surface lipophosphoglycan (LPG) mediates attachment to the gut wall so as to maintain infection during excretion of the digested bloodmeal. Developmentally regulated modifications in LPG explain the ability of infectious stage, metacyclic promastigotes to position themselves for efficient transmission by bite. LPG also displays inter- and intra-species polymorphisms in their phosphoglycan domains that in most cases can account for species- and strain-specific vector competence. The polygalactose eptiopes expressed by the LPG of most *L. major* strains are required for midgut attachment and promastigote persistence within their natural vector, *P. papatasi*. High-throughput sequencing of a midgut cDNA library of *P. papatasi* identified a novel galectin gene homologue termed *PpGalec* that is used by *L. major* as a receptor for mediating species-restricted binding to the midgut. The possibility that Leishmania diversity is generated, at least in part, by genetic exchange is supported by the first formal demonstration of a sexual cycle during parasite development in the sand fly vector. Co-infection of sand flies with parental lines bearing distinct drug resistance markers yielded hybrid parasites that were resistant to both drugs, and that inherited a full set of chromosomes from each parent. The frequency, timing, and anatomical location of genetic excahnge within the sand fly midgut, and the existence of a haploid, gamete stage are currently being explored.

## MC.05 – ON CYTOADHESION OF *Plasmodium vivax*-INFECTED ERYTHROCYTES

Carvalho, B.O.[1,2]; Lopes, S.C.P.[1]; Nogueira, P.A.[2]; Orlandi, P.P.[2]; Bargieri, D.Y.[3,4]; Blanco, Y.C.[1]; Mamoni, R.[1]; Leite, J.A.[1]; Rodrigues, M.R.[3,4]; Soares, I.S.[5]; Oliveira, T.R.[5]; Wunderlich, G.[6]; Lacerda, M.V.G.[7,8]; Del Portillo, H.A.[9], Russell, B.[10]; Suwanarusk, R.[10]; Snounou, G.[10]; Rénia, L.[10]; Costa, F.T.M. [1]

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*Plasmodium falciparum* and *P. vivax* are responsible for most of the global malaria burden. While accentuated pathogenicity of *P. falciparum* occurs due to sequestration of the mature erythrocytic forms in microvasculature, this phenomenon was not yet noted in *P. vivax*. The rising number of severe manifestations in *P. vivax* infections, similar to those observed for severe falciparum malaria, suggests that key pathogenic mechanisms (e.g. cytoadherence) might be shared by the two parasites.Mature *P. vivax*-infected erythrocytes (Pv-iE) were isolated from blood samples collected from 34 infected patients. Pv-iE enriched on Percoll gradients were used in cytoadhesion assays to human lung endothelial cells (HLEC), to *Saimiri* brain endothelial cells (SBEC), and to placental cryosections.Pv-iE were able to cytoadhere under static and flow conditions to cells expressing endothelial receptors known to mediate cytoadhesion of *P. falciparum*. Although Pv-iE cytoadhesion levels were 10-fold lower than those observed for Pf-iE, the strength of the interaction was similar. Cytoadhesion of Pv-iE was in part mediated by VIR proteins, since specific antisera inhibited the iE-endothelial cell interaction. These observations prompt a modification of the current paradigms of malaria pathogenesis, and open the way to investigate the pathophysiology of *P. vivax* infections. Financial support: CAPES, CNPq, FAPESP, The National Institute for Vaccine Development and Technology (CNPq-INCTV) and Rede Malaria (CNPq).

## MC.06 – FROM PROLINE TO GLUTAMATE: SEVERAL ROLES FOR A BIOCHEMICAL PATHWAY

Silber AM*
Departamento de Parasitologia – Instituto de Ciências Biomédicas – USP. *email: asilber@usp.br

The flagellated parasite *Trypanosoma cruzi* uses proline and glutamate, among other amino acids, as energy source. It was established that proline is involved in the metacyclogenesis, the process occurring in the insect vector, in which replicative non-infective epimastigotes differentiate into infective, non-replicative metacyclic trypomastigotes. It was also demonstrated the participation of proline in the differentiation between the intracellular epimastigote-like and the trypomastigote stages. More recent studies describing a proline racemase presenting a mitogenic activity contributed also to highlight the possible roles of proline in the biology of *T. cruzi.* Together, these data led us to deepen the study of the metabolism of this amino acid. Our work evidenced that *T. cruzi* is equipped with genes coding for actives proline dehydrogenase (PRODH) (EC 1.5.1.2) and a pyrroline-5-carboxylate dehydrogenase (P5CDH) (EC 1.5.1.12) which are able to oxidize L-proline into glutamate. TcPRODH and TcP5CDH genes complemented null mutants of *Saccharomyces cerevisiae* for the genes PUT1 or PUT2, demonstrating the activity of their products. Yeasts complemented with the TcPRODH gene showed lower free intracellular proline levels which correlated with enhanced sensitivity to oxidative stress. The ratio GSS/GSH confirmed the relationship between PDH activity, free intracellular proline levels and sensitivity to oxidative stress. The role of free proline in the resistance to oxidative stress was also confirmed in epimastigote forms. Since proline metabolism seems to be a central pathway for *T. cruzi*, we approached its study by looking for inhibitors among several structural analogues of proline and glutamate. Due to their performance, L,4 thiazolidine carboxylic acid (T4C) was choose for further *in vivo* studies. T4C behaved as a specific competitive inhibitor of the proline transport systems in *T. cruzi*. The treatment of epimastigotes with this analogue reduced their intracellular free proline content, making them more sensitive to oxidative stress. Besides, treated parasites were more sensitive to metabolic and thermal stress. All these data point the proline - glutamate pathway as part of a resistance mechanism against stresses that challenge the parasite along its natural life cycle, and prompt its component as interesting targets for drugs against *T. cruzi* infection.
Supported by: FAPESP and CNPq

## MC.07 - ADAPTTIVE ESTRATEGIES IN THE INTERACTION OF *LEISHMANIA* WITH THE CELL HOST: MODULATION OF NF-KB DIMERIZATION AND PKR ACTIVATION

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*Leishmania* species are obligate intracellular parasites that reside and multiply within mammalian host macrophages. Macrophage infection typically leads to the induction of numerous cellular genes, several of which encode cytokines capable of stimulating both pathogen resistance and an inflammatory response. Adaptive strategies have been evolved and ultimately tailored sophisticated mechanisms leading to the evasion of microbicide macrophage functions. In this work we present our results on the role of the transcription factor NF-kB and the double-stranded RNA-activated protein kinase (PKR) in the infection by *L.amazonensis.* The transcription factor NF-kB is activated by a number of pathogens, including *Leishmania* species, and regulate the expression of key immunological mediators. We have investigated the KF-kB activation and the NF-kB-dependent regulation of the Inductible Nitric Oxide Synthase (**iNOS**) expression by *L.amazonensis* in human and murine macrophages. We demonstrated that *L.amazonensis* infection determined a peculiar and specific activation of the NF-kB homodimer p50/50, which lacks the transactivator transcription domain. NF-kB expression luciferase-reporter assays with macrophages infected with *L.amazonensis* and treated with the LPS (a classical NF-kB inducer) revealed the repression of NF-kB-dependent expression induced by LPS. These results corroborate the notion that *L.amazonensis* activated- NF-kB -homodimer is a transcription repressor. We aimed to investigate the activity of the iNOS promoter which is regulated by NF-kB. Luciferase reporter assays confirmed that *L.amazonensis* infection repressed the activity of the iNOS promoter, containing nk-sites, induced by a mixture of LPS, TNF-$\alpha$ and IFN-$\gamma$. In addition, we also observed that during the infection there is an increase of nuclear histone deacetylase-1 levels (HDAC1) in macrophage nuclei accompanied by an augment of HDAC-activity. The double-stranded RNA-activated protein kinase (PKR) is activated during viral-infections and mediates the antivirus response leading to the expression of Type I interferon (IFN1) and other cytokines such as IL-10 and TNF. In addition, PKR may also be activated through TLR2 and TLR4 and plays a role in the innate immune response. To investigate the role of PKR during the infection by *L.amazonensis,* we infected murine and human macrophages with this parasite and treated with the synthetic RNA molecule Poly I:C. Strikingly, Poly I:C treatment enhanced the infection and this effect was abrogated by the PKR inhibitor 2-AP. Poly I:C treatment did not lead any significant effect in the infection of macrophages transfected with a dominant-negative PKR construction (DN-PKR). Strikingly, *L.amazonenis*, in the absence of exogenous PKR-inducer, was able to activate and increase PKR levels. In fact, PKR signaling was proven to be important for the success of the infection, since the infection index of macrophages of either PKR-KO mice or RAW 264.7 DN-PKR was dramatically reduced compared to wt cells. Interestingly, activation of PKR through the TLR4 ligand LPS also favored the infection in a PKR-dependent fashion. Moreover, we could demonstrate that IL-10 expression was one of the PKR determinant effectors in the infection. Current studies are in progress to investigate the role of TLR receptors in the PKR activation by *L.amazonensis*. We are also addressing the role of IFN1 in the infection and activity of the PKR promoter in this context.

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## MC.08 – STRESS-INDUCED ACTIVATION OF THE *L. MAJOR* MAP KINASE LMAMPK7 INCREASES PARASITE RESISTANCE AGAINST THE LEISHMANICIDAL DRUG MILTEFOSINE

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During the infectious cycle, *Leishmania* is exposed to various environmental stress signals in insect and mammalian hosts, which trigger differentiation of the virulent metacyclic and pathogenic amastigote life cycle stages. Despite the importance of stress sensing and response in transmission and intracellular parasite infection, our understanding on mechanisms underlying these processes remain very poor. We previously used a transgenic strategy to gain insight into the functions of the mitogen-activated *Leishmania major* protein kinase LmaMPK7 in parasite virulence. Establishment of *L. major* and *Leishmania donovani* lines expressing episomal green fluorescent protein (GFP)-LmaMPK7 fusion proteins revealed environmentally-induced activity of this kinase in response to low pH and high temperature, and linked this kinase to amastigote growth regulation and translational control. Here we show that LmaMPK7 confers increased resistance to nutritional stress at stationary growth phase, and treatment with the anti-leishmanial drug miltefosine. Drug resistance was specific to LmaMPK7 as transgenic expression of related LmaMPK4 and LmaMPK10 had no effect. We showed by *in vitro* kinase assay that LmaMPK7 activity itself is induced during miltefosine treatment and thus identified for the first time a regulatory interaction between an anti-leishmanial drug and a parasite signaling pathway that results in enhanced *Leishmania* drug resistance. Transgenic parasites expressing an LmaMPK7 kinase dead mutant were susceptible to miltefosine, demonstrating a direct role of LmaMPK7 phosphotransferase activity and its downstream substrates in parasite drug resistance. Quantitative phosphoproteomics analysis of LmaMPK7 transgenic parasites correlated increased LmaMPK7 activity with enhanced phosphorylation of LmjF29.1240, a hypothetical protein with homology to TPR domain-containing chaperones implicated in stress response. Based on these results we hypothesize that LmaMPK7 regulates *Leishmania* stress resistance through chaperone phosphorylation, which may alter parasite susceptibility to anti-microbial drugs. Our data thus unravel a novel mechanism of drug resistance regulated at the post-translational level, and propose the *Leishmania* stress response in general and LmaMPK7 in particular as attractive targets for anti-leishmanial intervention.

**RT.01-A – Immunoregulation in cutaneous leishmaniasis: A role for Notch signaling**

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*Leishmania braziliensis* infection results in a variety of immunological and clinical spectrum of disease, including the cutaneous (CL) and mucosal (ML) inflammatory forms of leishmaniasis. Upon stimulation with soluble *Leishmania* antigen, CD4+ T cells from CL and ML patients secrete high levels of IFN-gamma and TNF-alpha, cytokines known to participate in the pathogenesis of CL and ML, and low levels of IL-5 and IL-10. The use of recombinant IL-10 and antibodies against IL-12, failed to downmodulate *Leishmania* antigen-induced inflammatory responses by mononuclear cells from CL and ML individuals, suggesting other mechanism as responsible for the sustained IFN-gamma and TNF-alpha production in these patients. Recently, attention has been given to a role for Notch signaling in Th1 differentiation, T cell proliferation and mononuclear phagocyte activation. Notch signaling takes place upon the cleavage of Notch receptor after binding of the ligands Delta or Jagged. Here, we found that *Leishmania* antigen-induced IFN-gamma and TNF-alpha production is blunted by a gamma secretase inhibitor that prevents Notch signaling by interfering with the cleavage of Notch at the cell membrane. Moreover, we found that *Leishmania*-infected human monocytes become activated in a gamma secretase activity-dependent manner. These discoveries show a role for Notch signaling in controlling inflammatory responses in human leishmaniasis, opening perspectives for investigations of new forms of treatment for inflammatory diseases. Supported by NIH, USA.

**RT.01-B - ROLE OF NEUTROPHILS IN RESISTANCE AGAINST EXPERIMENTAL *L. BRAZILIENSIS* INFECTION**

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Experimenal cutaneous leishmaniasis caused by *L. braziliensis* is characterized by a necrotic lesion in the skin that heals spontaneously after ten weeks. In parallel to lesion development and cicatrization, an intense influx of polimorfonuclears neutrophils (PMNs) is observed at the infection site. It has been shown that neutrophil depletion leads to enhanced *Leishmania* infection and that that neutrophils cooperate with macrophages in eliminating *Leishmania*. Moreover, recent studies have also demonstrated the involvement of PMNs in the induction of the adaptive immune response. Data will be shown on the role of neutrophils in promoting resistance against *L. braziliensis* infection, considering the different scenarios described above.

## RT.01-C- THE IMPORTANCE OF microRNAs IN MACROPHAGE POLARIZATION AND INTRACELLULAR SURVIVAL OF LEISHMANIA

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The *Leishmania* spp. protozoa reside intracellularly in their mammalian hosts, and are most often observed in host macrophages. Intracellular Leishmania cause dramatic changes in macrophage gene expression, suppressing microbicidal activities and IFN-gamma responses. Indeed, microarrays from several labs including our own show that infection with *Leishmania* promastigotes induces the expression of a set of genes characteristic of non-classical macrophage activation, similar but not identical to alternative (M2a) activation. This pattern is distinct from the set of classical activation (M1) genes induced by bacterial infection or LPS. MicroRNAs are 18-30 nt noncoding RNAs that globally regulate gene expression. We hypothesized that microRNAs could provide molecular triggers that underlie or modify the global changes in macrophage gene expression induced by leishmania infection or other polarizing stimuli. Therefore, we studied microRNA expression in human monocyte-derived macrophages (MDMs) treated with polarizing agents. Treatments were designed to induce MDMs to differentiate toward each of four distinct phenotypes: M1 (20 ng IFN-gamma/mL;10 ng LPS/mL), M2a (20 ng IL-4/mL ), M2b (IgG complexes; 100 ng LPS/mL), and M2c (0.5 ng TGF-b1/mL). Polarization was verified by increased expression of the predicted chemokines or cytokines. Using multiplex TaqMan qPCR arrays (ABI), we identified microRNAs whose expression in MDMs was altered after incubation in polarizing conditions. Seven microRNAs were significantly altered in the different polarized states compared to control untreated MDMs. One of these microRNAs (miR-155) has already been reported to respond to LPS in the literature. The functions of significantly altered microRNAs were tested using either HEK-293 or the THP-1 monocytic cell line transfected with pre-miRs. First, studies of HEK-293 cells showed that several minor microRNA strands complementary to the strand loaded in the RISC complex were transiently altered by polarization. However, only the corresponding major microRNA strand,i.e. the strand loaded into RISC, was functional in suppressing expression of reporter luciferase activity. Second, studies of transfected THP-1 cells showed that IL-6, TNF-alpha, CXCL9, and IL-10 expression was modified both in unstimulated cells and in response to cytokines and/or LPS by several of the introduced pre-microRNAs. We conclude that microRNAs influence the type of macrophage polarization that occurs in response to external stimuli. Delineation of these microRNAs, and their effects on the intracellular survival of *Leishmania,* may provide novel therapeutic targets for *Leishmania* and other intracellular pathogens.

## RT.02-A – CHARACTERIZATION OF ARTEMISININ RESISTANT *Plasmodium falciparum*

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The emergence of artemisinin resistant falciparum malaria represents a major threat to malaria elimination and is among the most important issues that could undermine global malaria control efforts. Recent studies suggest that prolonged parasite clearance times in patients treated with an artemisinin drug is a harbinger of reduced cure rates in the near future. The problems associated with understanding artemisinin resistance range from a lack of understanding of the mechanism of resistance, the absence of validated molecular markers, and poor correlation of the resistance phenotype in current in vitro antimalarial drug susceptibility tests. In response to these pressing needs we have characterized the response of *P. falciparum* to various artemisinin derivatives in vitro and have identified artemisinin-induced dormancy in ring stages as a probable mechanism of recrudescence. Furthermore, we have developed several independent lines of *P. falciparum* that tolerate > 3 μM artemisinin. Characterization of these resistant lines has identified amplification of pfmdr1 as an accessory to developing resistance. Putative molecular markers of resistance have emerged from analysis of the unique transcriptional profile of resistant parasites as well as full length sequencing the genomes of parent and resistant lines. These data provide insight into the possible mechanisms of resistance and can be rapidly translated into studies with clinically resistant isolates in the field.

## RT.02-B – STRUCTURE- AND LIGAND-BASED DRUG DESIGN APPROACHES FOR NEGLECTED TROPICAL DISEASES

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The modern drug discovery process is increasingly becoming more information driven. Recent years have seen a tremendous increase in new technologies and methods for the design of new chemical entities (NCEs). A broad variety of medicinal chemistry approaches can be used for the identification of promising hits, generation of leads, as well as to accelerate the development of high quality drug candidates. Structure- (SBDD) and ligand-based drug design (LBDD) methods are becoming increasingly powerful, versatile and more widely employed. The use of these drug design strategies has increased enormously because of the availability of databases with millions of commercially available compounds, as well as 3D structures of several target proteins. This presentation will provide a perspective of the utility of SBDD and LBDD approaches, and the integration of experimental and computational methods in medicinal chemistry, highlighting recent examples of successful applications in the area of neglected tropical diseases (NTDs).

## RT.02-C – PHENOTYPIC HIGH-THROUGHPUT SCREENING FOR NEGLECTED DISEASES

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The Center for Neglected Diseases Drug Discovery (CND3) in Institut Pasteur Korea (IPK) develops high-content/high-throughput screening assays (HCS/HTS) for finding new drugs for the treatment of Leishmaniasis, Chagas disease and malaria. Taking advantage of automated confocal microscopy, we developed a whole cell-based approach for screening 200,000 compounds for their ability to inhibit *Leishmania donovani* amastigotes growth in the human macrophage cell line THP-1. Among the 200,000 compounds, the 179 most active compounds, able to inhibit parasite growth without harming macrophages host cells were selected for activity confirmation by dose response curves. We have also developed a whole-cell model assay for discovery of compounds active against *Trypanosoma cruzi*, the causative agent of Chagas disease. Two different *T. cruzi* strains, Y and Dm28c, representing two of *T. cruzi* six groups, were used in the screening of 4,000 compounds of know drug properties. Our data shows, for the first time to our knowledge, that Y and Dm28c strains differ both on their infectivity profile and also differ partly but significantly on their sensitivity to , 4,000 compounds. Currently both leishmaniasis and Chagas assays are being used for screening an additional library of 150,000 compounds in collaboration with Drugs for Neglected Diseases *initiative* (DNDi) and Pfizer, thus further increasing the number of potential drug candidates in the pipeline of Chagas and leishmaniasis drug discovery. In parallel to amastigote-host cell screening system, we developed a secondary high-content assay for kinetoplast-directed drug discovery for *Leishmania* and *T. cruzi* parasites. The kinetoplast is a single mitochondrion, exclusive to order Kinetoplastida (*Leishmania* and *Trypanosoma cruzi*, among other parasites) and contains a number of excellent chemotherapeutic targets that are very unlikely to be found in the human host. Among the kinetoplast targets, the kinetoplast DNA (kDNA) replication proteins are one of the best-known parasite specific machinery. The hits found in the amastigoste-host cell assays for *T. cruzi* and *Leishmania* will be further tested in the kinetoplast assay for assessment of their potential kinetoplast-targeting mechanism of action. This screening consists on incubating *Leishmania* promastigote forms with selected anti-leishmanial compounds in the presence of EdU, a thimidine analog that is incorporated into the replicating kDNA. Parasites are then fixed and stained for EdU-containing DNA. Desired hits will be able to inhibit kDNA, but not nuclear DNA, replication. Our approach to malaria consists on developing HCS/HTS that target the invasion of human red blood cells (RBCs) by *Plasmodium falciparum*, the deadliest of all malaria parasites. We have validated, by means of 4,000-compound screening, a quantitative enzymatic approach to assay for *P. falciparum* viability. This assay was used to screen 80,000 compounds, and the hits emerging from this assay will be tested on a secondary image based high-content assay for their ability to interfere with the red blood cell invasion pathway.

## RT.03-A – CHARACTERIZATION OF THE *ANOPHELES AQUASALIS* IMMUNE RESPONSE TO *Plasmodium vivax*

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Malaria affects millions of people worldwide annually, and 450.000 only in Brazil. The interaction of malaria vectors and parasites has been extensively studied, but very little is known about the pair *Anopheles aquasalis-Plasmodium vivax*, of great importance in the malaria scenario in Brazil. One of the reasons for this lack of information relies on the virtual impossibility of *P. vivax* cultivation, and the recently challenged belief that disease caused by this parasite is not serious. We are characterizing the immune response of *A. aquasalis* to *P. vivax*. We constructed subtraction libraries, comparing infected and non-infected insects. Surprisingly, few immunity genes were identified 2 and 24 hours after infection (hAI). Among these were a serine proteinase with diminished expression, and a carboxipeptidase with increased expression. We also identified a GATA transcription factor, more expressed in males than females and induced (almost 15 times) 36 hAI. Infection increased 63% after GATA knock-down, confirming its importance in the immune response of *A. aquasalis* against *P. vivax.* In parallel, specific genes were amplified using degenerate primers and characterized. The immune response genes STAT, PIAS and NOS were induced by infection, demonstrating the importance of the JAK/STAT pathway in response against the parasite. Silencing of STAT caused an increase in oocysts count. In relation to the detoxification enzymes, we observed an increased expression of SOD and catalase 36 hAI and a decreased activity at 24 hAI. Fluorescence microscopy using a redox state probe showed a reduction of free radicals in both blood fed and infected insects when compared to sugar fed insects.

## RT.03-B – PROTEOPHOSPHOGLYCAN CONFERS RESISTANCE OF LEISHMANIA MAJOR TO MIDGUT DIGESTIVE ENZYMES INDUCED BY BLOOD FEEDING IN VECTOR SAND FLIES

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Leishmania synthesize abundant phosphoglycancontaining molecules made up of [Gal-Man-PO4] repeating units, including the surface lipophosphoglycan (LPG), and the surface and secreted proteophosphoglycan (PPG). The vector competence of *Phlebotomus duboscqi* and *Lutzomyia longipalpis* sand flies was tested using *L. major* knockout mutants deficient in either total phosphoglycans (lpg2- or lpg5A-/5B-) or LPG alone (lpg1-) along with their respective gene add-back controls. Our results confirm that LPG, the major cell surface molecule of Leishmania promastigotes known to mediate attachment to the vector midgut, is necessary to prevent the loss of infection during excretion of the blood meal remnants from a natural vector, *P. duboscqi* , but not an unnatural vector, *L. longipalpis*. Midgut digestive enzymes induced by blood feeding pose another potential barrier to parasite survival. Our results show that 36–72 h after the infective feed, all parasites developed well except the lpg2- and lpg5A-/5B- mutants, which showed significantly reduced survival and growth. Protease inhibitors promoted the early survival and growth of lpg2- in the blood meal. PPG was shown to be the key molecule conferring resistance to midgut digestive enzymes, as it prevented killing of lpg2- promastigotes exposed to midgut lysates prepared from blood-fed flies. The protection was not associated with inhibition of enzyme activities, but with cell surface acquisition of the PPG, which appears to function similar to mammalian mucins to protect the surface of developing promastigotes against proteolytic damage.

## RT.03-C – INFLUENCES OF THE MICROBIOME ON PATHOGEN TRANSMISSION: LESSONS LEARNED FROM THE TSETSE FLY

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The role of microbial organisms in eukaryotic functions is increasingly being recognized. These interactions range from transient environmental associations to coevolved symbiotic interactions. in particular insect are rich in such symbiotic interactions, which may have enabled them to exist in specialized niches. Similarly, tsetse flies, sole vectors of African trypanosomes, have coevolved with multiple symbionts: mutualistic endosymbiont *Wigglesworthia,* commensal *Sodalis* and parasitic *Wolbachia.* Recent results show that the functions of symbiotic bacteria in tsetse range from host nutrient provisioning to host immune competence and reproductive fitness outcomes. It has been possible to maintain tsetse lines through dietary supplementation that either lack *Wigglesworthia* or all three endosymbionts. Absence of *Wigglesworthia* alone compromises tsetse's fertility and also immune resistance to pathogenic trypanosomes. Comparison of select immune gene expression between *Wigglesworthia* cured flies and their age-matched normal counterparts indicate that the absence of *Wigglesworthia* during host immature development particularly affects the maturation of cellular immune responses in the adults.

## RT.04-A – THE BIOLOGY OF NUCLEAR DNA REPLICATION IN TRIPANOSOME

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DNA replication is a complex multistep process that is comprised of initiation, elongation, and DNA damage repair. Chromosomal replication initiates with the assembly of the prereplication complex (pre-RC) at DNA sites along the chro- mosomes that are called origins of replication. In eukaryotes, the pre-RC is composed of an origin recognition complex (ORC) containing six proteins, Orc1 to Orc6, two proteins named Cdc6 and Cdt1, and the minichromosome maintenance (MCM) complex, which is composed of Mcm2 to Mcm7 proteins. Different from eukaryotes and similar to Archaea, tripanosomes contain a single protein, homologous to Orc1 and Cdc6, named Orc1/Cdc6 that presents a functional role at pre-replication machinery. This protein is expressed during the entire cell cycle in *Trypanosoma cruzi* and *Trypanosoma bruce* and it remains bound to DNA in any stage of the cell cycle. This result suggests that Orc1/Cdc6 is not involved in the control of DNA replication in these organisms. We analyzed the localization of Orc1/Cdc6 and PCNA, the replication machinery component, during the *T. cruzi* cell cycle. Therefore the organization of DNA replication in the nuclear space of *T. cruzi* will also be discussed.
Supported by FAPESP.

## RT.04-B – *Plasmodium falciparum* ORIGIN RECOGNITION COMPLEX (ORC): ROLE IN THE REGULATION OF PARASITE DNA REPLICATION AND VIRULENCE GENE EXPRESSION

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DNA replication, a fundamental process central to the parasite proliferation and pathogenesis is poorly understood in human malaria parasite *Plasmodium falciparum*. A six-protein initiator complex known as origin recognition complex, is essential for DNA replication initiation in eukaryotes. *Plasmodium* ORC contains the largest subunit of ORC, ORC1 and putative ORC5 and ORC2 subunits only. The C-terminal PfORC1 shows the replication-specific function whereas N-terminus may have different function (1). We find that ORC5 forms distinct nuclear foci co-localized with replication foci marker proliferating cell nuclear antigen (PCNA) with the onset of DNA replication in the parasites. However, PCNA and ORC5 foci separate from each other at the end of DNA replication suggesting the presence of replication factory model in the parasites. Surprisingly, PfORC1 co-localizes with both PCNA and PfORC5 at the beginning of DNA replication but gets degraded at the end of the erythrocytic cycle possibly mediated through phosphorylation in a CDK-dependent manner ensuring the regulation of DNA replication in the parasites (2). *Plasmodium falciparum* sirtuin, PfSir2, contains histone deacetylase (HDAC) activity that may regulate virulence gene expression in the parasites (3). PfORC may co-operate with PfSir2 to regulate the telomeric gene silencing. The possible role of ORC in gene silencing apart from DNA replication will also be discussed.

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## RT.04-C – ANALYSIS OF THE ARCHITECTURE AND FUNCTION OF THE NUCLEAR DNA REPLICATION APPARATUS IN *TRYPANOSOMA BRUCEI*.

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DNA replication is central to the propagation of life and initiates by the designation of genome sequences as origins, where synthesis of a copy of the genetic material begins once per cell division. Despite considerable progress in understanding mitochondrial (kinetoplast) DNA replication in kinetoplastid parasites, little is known about nuclear DNA replication. The mechanism and machinery of DNA replication initiation is well-conserved amongst characterised eukaryotes. The six-protein origin recognition complex (ORC; Orc1-Orc6), Cdc6 and Cdt1 are recruited sequentially to DNA and, once bound, load the replicative helicase complex (Mcm2-7) to form a pre-replicative complex at origins. Orc1 and Cdc6 are related in sequence, indicative of derivation from a common ancestor. Such an ancestral molecule is found in present-day archaea, prokaryotes in which an Orc1/Cdc6 protein appears to provide all ORC functions, since orthologues of Orcs2-6 are absent. Comparative genome analysis of *Trypanosoma brucei* and related trypanosomatids revealed, remarkably, only a single ORC protein, equally related to eukaryotic Orc1 and Cdc6 (named TbORC1/CDC6 in *T. brucei*). In addition, no clear homologue of Cdt1 was found. These observations have been interpreted as suggesting that origin designation in trypanosomatids, although eukaryotic, may be archaeal-like, raising numerous mechanistic and evolutionary questions. To test this hypothesis, and to dissect the process of nuclear DNA replication in *T. brucei*, we have taken a number of approaches. First, we have used RNAi to examine TbORC1/CDC6 function, and our findings are in agreement with work from other labs in suggesting an essential nuclear replication function in the parasite. Second, we have used chromatin immunoprecipitation of TbORC1/CDC6, coupled with microarray hybridisation, to map genome-wide binding sites of the protein. These data suggest sparse binding in the conserved core of the *T. brucei* chromosomes as well as high-density binding in the more variant subtelomeres, perhaps consistent with origin and non-origin functions. Consistent with this, analysis of gene expression following RNAi suggests that TbORC1/CDC6 plays an important role in putative epigenetic silencing of some *T. brucei* genes. Finally, we have searched for factors that interact with TbORC1/CDC6 and the replicative helicase. This has revealed a number of diverged proteins with roles in DNA replication, which shed new light on the architecture and potential functioning of the origin designation machinery in these parasites.

## RT.05-A – INNATE IMMUNE RESPONSES TO *Neospora caninum*

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*Neospora caninum*, the causative agent of neosporosis, is an obligate intracellular parasite considered to be a major cause of abortion in cattle throughout the world. Most studies concerning *N. caninum* have focused on life cycle, seroepidemiology, pathology and vaccination, while data on host–parasite interaction, such as host cell innate recognition, cell migration, mechanisms of evasion and dissemination of this parasite during the early phase of infection are still poorly understood. Recently, we have focused our research in order to unravel the initial interactions between *N. caninum* and innate immune cells and, along with comparative experiments with closely related *T. gondii*, the results yielded have clarified some key features of the parasite's biology and the generated knowledge may soon be applied in therapeutical or prophylactic protocols towards the disease.

## RT.05-B – TOXOPLASMA CYCLING INTO THE HOST CELL

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Cell-to-cell transmission, also termed intercellular transmission, is a critical step in the life cycle of any intracellular pathogen. To successfully make this treacherous transition, the pathogen must escape from one host cell, avoid a battery of immune insults, and hastily infect a new host cell. During acute infection, *Toxoplasma* replicates particularly well in lymphoid organs, which are laden with immune effector cells. Thus the parasite has presumably evolved maximally efficiency mechanisms for intercellular transmission. Here we show that *Toxoplasma* preferentially egresses in the early part of G1 when it has maximal invasion proficiency. Using cell cycle synchronized and non-synchronized populations, we demonstrate that G1 parasites are substantially more efficient at egress and invasion than parasites in S phase or mitosis/cytokinesis (M/C) phase. Although extracellular parasites display the cellular features of G1, they don't show a normal G1 transcription profile. Instead, they express a subset of genes from the "invasion transcriptome" normally seen in late S and M/C when invasion organelles are being made. This altered expression profile may be due to "spill over" of S/M/G transcripts into G1 parasites or it could represent a unique expression profile optimized for intercellular transmission. Upon invasion, the parasite quickly initiates progression through G1 with a "basal transcriptome" expression profile indicative of metabolic escalation and DNA replication. Interestingly, when the time of invasion is restricted, the parasite population is initially cell cycle synchronized by the invasion event through at least the first cell division. By obviating the need to chemically synchronize populations, this observation should greatly aid analysis of organelle biogenesis during daughter cell formation.

## RT.05-C – RECOGNITION OF INTRACELLULAR PARASITE INFECTION BY NOD-LIKE RECEPTORS

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Immune responses against infectious agents are usually initiated upon recognition of pathogen-associated molecular patterns (PAMPs) by the pattern recognition receptors (PRRs) present in the host cells. Among PRR are the Toll-like receptors (TLRs), which have being extensively characterized and are well known to recognize intracellular and extracellular parasites. However, the recognition of parasites by non-TLR PRR remains largely obscure. A family of cytosolic proteins containing a nucleotide-binding oligomerization domain (NOD)-like receptors (NLRs) has being recently described as important sensors of microbial infection. Among the well characterized NLRs are proteins (such as Nod1, Nod2, Naip5, Nlrc4, Nlrp1 and Nalp3), which contributes for detection of different PAMPs. In contrast to TLRs, which detect extracellular and vacuole-containing PAMPs, the NLRs detect PAMPs contained in the host cell cytoplasm. This information has led to the speculation that NLRs are able to discriminate avirulent from the pathogenic microbes (because only the latter can survive intracellular and release PAMPs in the host cell cytoplasm). In fact, it has being recently demonstrated that in contrast to TLRs, NLRs are able to recognize virulent bacteria that either lyses the vacuolar membranes or that secrete proteins into the host cell cytoplasm by using specialized secretion system. Although important pathogenic parasites (such as *Leishmania, Trypanosoma, Toxoplasma, Plasmodium*) inhabits the host cell cytoplasm, there is virtually no information regarding the recognition of these pathogens by NLRs. Data to be presented will highlight the important role of NLRs for recognition of pathogenic parasites and the mechanisms by which NLRs triggers host resistance against infection.
Supported by FAPESP, CNPq, PEW and WHO/TDR.

## RT.06-A – DARING TO BE DIFFERENT: UNUSUAL ASPECTS OF GENE TRANSCRIPTION IN *Trypanosoma brucei*

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I will present some contemporary observations on the regulation of gene transcription in *Trypanosoma brucei* — the agent of African Sleeping Sickness. Trypanosomes have three RNA polymerases, but that seems to be where the similarity to more intensively studied organisms ends. Evolving differently, trypanosomes have largely eschewed introns and —with a few exceptions — do not appear to regulate the initiation of RNA transcription. Differential mRNA trans-splicing and stability appear to be major determinants of mRNA abundance. Histone modifications are central to the regulation of gene transcription in all organisms, and we expected chromatin structure to play some role in the regulation of transcription in *T. brucei*, especially for the allelic exclusion involved in regulating variant surface glycoprotein transcription, where previous work has demonstrated differences in chromatin structure. Although histone sequences are generally highly conserved, the amino acid sequences of trypanosome histones are unusually divergent. This necessitated identifying histone modifications ab initio and raising specific antibodies to them, as no commercial antibodies were suitable. Using mass spectrometry and Edman degradation chemistry, we identified more than 30 modifications but have studied only a few of them in detail. Each of the four core histones are encoded by arrays of identical genes, preventing genetic approaches to identifying the role of specific amino-acid modifications until recently, when we have replaced both tandem alleles of H3 and H4 with single genes. For each core histone, there is a single-gene-encoded variant, allowing them to be studied by genetic manipulation. The variants of H3 and H4 are not essential, at least separately, but the variants of H2A and H2B are. To investigate the genome-wide distribution of various histone posttranslational modifications and all four histone variants in *T. brucei*, we used chromatin immunoprecipitation followed by high-throughput DNA sequencing (ChIP-seq). We have identified striking profiles at regions that are probably associated with the initiation and termination of polycistronic transcription by RNA Polymerase II. Identification of the precise points at which transcription starts is an ongoing challenge. The current status and proposed future directions of this work will be discussed.

## RT.06-B – UNVEILING PROTEIN:PROTEIN INTERACTIONS AT *LEISHMANIA* SPP. TELOMERES

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In most eukaryotes, telomere binding proteins such as POT1 and TRF2 play crucial roles in telomere biology by interacting with several other telomere regulators to ensure proper telomere maintenance and to form high order complexes known as telosome or shelterin. *Leishmania* spp. telomeres are composed by the conserved TTAGGG repeats which are maintained by telomerase. The basic *Leishmania* telomeric protein complex is formed by the proteins LaRPA-1 and LaRbp38, which bind *in vitro* and *in vivo*, with high affinity, to the G-rich single-stranded DNA, and by proteins that interact with the double-stranded region of telomeres such as the recently described TRF homologue. The *Leishmania* spp. genome, like other trypanosomatid, lacks many of the conserved single-stranded telomeric proteins found in other eukaryotes, such as the CDC13 and POT1 protein homologues. Thus, we speculate that the *Leishmania* RPA-1 homologue may play the same roles as POT1/CDC13 at parasite telomeres, although it can also bind to other single-stranded DNA with high affinity and in a sequence-independent manner. LaRPA-1 together with the multifunctional LaRbp38 protein, which also interacts with a wide range of GT-rich sequences, including telomeres, seems to form part of a parasite telomeric complex that resembles the recently described CST complex. The CST complex is being considered a second telomere capping mode occurring in a broad variety of species, except budding yeast, and is mainly formed by RPA-like proteins. In this report we used different approaches to show that LaRPA-1 interacts with both LaRbp38 and with telomerase. We are currently investigating if these protein:protein interactions occur in a cell-cycle dependent manner. In addition, LaRPA-1 partially co-localizes with both proteins, probably reflecting its functions in DNA metabolism. We speculate whether these protein interactions reflect the entire telomeric complex or the presence of functionally distinct subcomplexes at parasite telomeres.

## RT.06-C – DNA REPAIR AND TELOMERE EXPRESSION IN *LEISHMANIA MAJOR*.

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The *Leishmania* genome presents hallmarks of genome instability such as gene amplification and chromosome polymorphisms that are compatible with the presence of a robust mechanism to deal with such high plasticity. Genome stability is constantly challenged by DNA damage that is caused by the metabolism of the cell or inflicted by exogenous factors. Several types of DNA damage result in the formation of single stranded DNA which is recognized by the single strand binding protein RPA. The RPA-coated DNA serves as the substrate for the recruitment of checkpoint protein complexes. One of the first players recruited to the DNA lesion sites in eukaryote genomes is a PCNA-related trimeric complex formed by the proteins Rad9, Rad1 and Hus1. Loading of the 911 complex onto the chromatin initiates the signaling events necessary to halt cell cycle progression and to allow DNA repair to take place. In mammals, the 911 complex also associates with telomeres and plays a role in chromosome stability. Despite its importance in genome maintenance, the 911 complex has not been studied in trypanosomatids. We have investigated the existence and functioning of the 911 complex in the protozoan *Leishmania major*. We identified the parasite gene that is a homolog of Hus1 from other organisms. The predicted secondary structure of the parasite Hus1 revealed the presence of putative beta-sheets and alpha-helices in conserved positions indicating the presence of the PCNA-like domains that may enable this protein to participate in DNA metabolism. Using specific antibodies we found that the parasite Hus1 protein localizes to the nucleus. Upon exposure to DNA damaging drugs Hus1 not only relocates to the nuclear periphery, but also exhibit an increased co-localization with RPA1, which has been shown to be a telomeric protein in *L. amazonensis*. In fact, Hus1 also associates to telomeres and seems to affect the expression of a selectable marker integrated at a subtelomere. Transfectant cell lines with an increased expression of Hus1 are clearly resistant to hydroxyurea and methyl methanesulfonate. However, resistance to phleomycin is not observed upon Hus1 overexpression. Interestingly, exposure to phleomycin leads to a significant increase in telomeric repeat-containing transcripts. Transcription of telomere repeats in protozoa have been investigated and regarded as result from read-through downstream of telomeric genes. However, it has been recently demonstrated that the transcription of mammalian and yeast telomeres is finely regulated resulting in telomeric repeat-containing RNA (TERRA) that associates with chromosomal ends. *Leishmania* TERRA transcription proceeds exclusively toward the telomeres irrespective of the direction of the polycistron suggesting that it is not a mere transcription by-product. The data gathered so far support our hypothesis that LmTERRA is a functional RNA class that might be explored by the cell to regulate its genome expression and maintenance.
Supported by FAPESP and CNPq

## RT.07-A – MULTIDIMENSIONAL IMAGING OF MACROPHAGES DOUBLY INFECTED BY *LEISHMANIA AMAZONENSIS* AND *LEISHMANIA MAJOR*

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Protozoan parasites of the genus *Leishmania* alternate between flagellated, elongated extracellular promastigotes found in insect vectors, and round-shaped amastigotes enclosed in phagolysosome-like Parasitophorous Vacuoles (PVs) of infected mammalian host cells. *Leishmania amazonensis* amastigotes occupy large PVs which may contain many parasites; in contrast, single amastigotes of *Leishmania major* lodge in small, tight PVs, which undergo fission as parasites divide. To determine if PVs of these *Leishmania* species can fuse with each other, mouse macrophages in culture were infected with non-fluorescent *L. amazonensis* amastigotes and, 48h later, superinfected with fluorescent *L. major* amastigotes or promastigotes. Fusion was directly investigated by time lapse image acquisition of living cells and inferred as well from the presence of parasites of the two species in the same PVs. Survival, multiplication and differentiation of parasites that did or not share the same vacuoles were also investigated. Fusion of PVs containing *L. amazonensis* and *L. major* amastigotes was not found. However, PVs containing *L. major* promastigotes did fuse with pre-established *L. amazonensis* PVs. In these chimeric vacuoles, *L. major* promastigotes remained motile and multiplied, but did not differentiate into amastigotes. In contrast, within their own, unfused PVs in doubly infected cells, metacyclic-enriched *L. major* promastigotes, but not log phase promastigotes - which were destroyed - differentiated in proliferating amastigotes. The results indicate that PVs presumably customized by *L. major* amastigotes or promastigotes, differ in their ability to fuse with *L. amazonensis* PVs. Additionally, a species-specific PV was required for *L. major* destruction or differentiation – a requirement which mechanisms remain unknown. This descriptive experimental work should be useful in further studies of the permissiveness of PVs to different species of *Leishmania* parasites, and of the mechanisms involved in the recognition and fusion of PVs.

## RT.07-B – HOST CELL MEMBRANE-LYSOSOMES INTERPLAY DURING *TRYPANOSOMA CRUZI* INVASION

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*Trypanosoma cruzi*, the etiological agent of Chagas´Disease, can invade a wide variety of cell types, varying from phagocytic to non-pahgocytic cells. The parasitic invasion of the latter occurs through two convergent paths: host membrane invagination with subsequent lysosomal fusion or direct lysosomal fusion at the parasite attachment site. The interaction between parasite and host cell membrane is then one of the critical steps to the establishment of a well-succeeded invasion. Two relevant steps during this interaction are: 1. the interaction of *T. cruzi* with host cell plasma membrane, which will direct signaling events that will culminate with host cell lysosome recruitment for the formation of the parasitophorous vacuole; 2. *T. cruzi* interaction with its parasitophorous vacuole membrane which will help its invasion in host cell and will influence parasite vacuole escape during parasite intracellular development. Concerning parasite interaction with host plasma membrane, recently, it has been shown that cholesterol and cell microdomains might influence parasite-host cell invasion in fibroblasts. Our work demonstrated that these host cell microdomains, also known as membrane rafts, are also important during parasite invasion in cardiomyocytes, important cells during *T. cruzi* infection in humans. These host cell membrane domains seem to be hot spots of parasite interaction and entrance. We also investigated the participation of the membrane rafts in lysosome fusion during *T. cruzi* entry. Cardiomyocytes treatment with MβCD, a drug which can deplete cellular cholesterol content, led to decreased *T. cruzi* invasion in a dose dependent manner without interfering with cell viability, showing that cholesterol is important for efficient *T. cruzi* entry. However upon MβCD treatment lysosomal fusion during parasite entry became altered, suggesting that these regions might be involved with directing lysosome fusion to the site of parasite attachment. After lysosome recruitment and fusion, *T. cruzi* interaction with lysosomal membrane is imperative for *T. cruzi* retention in host cells. Studies of parasite infection in cells lacking LAMP 1 and 2 (LAMP-KO cells), two integral lysosome membrane proteins, show that this two proteins interfere with parasite entry and later with its intracellular development in the host. Absence of these two proteins lead to decreased cell infection, however it facilitates parasite intracellular development, suggesting that parasite interaction with its vacuolar membrane might determine the success of host infection.

## RT.07-C – *Toxoplasma gondii* INTERACTION VARIES ACCORDING TO HOST CELL MICRODOMAINS

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Lipid rafts are dynamic nanoscale assemblies enriched in sterol-sphingolipid. The exploitation of rafts by intracellular pathogens may facilitate invasion. *Toxoplasma gondii* is a pathogen that actively invades host cells. Most host cell proteins do not pass beyond the moving junction and are excluded from the parasitophorous vacuole (PVM). However, many components of PVM derive from host cell membrane, including microdomains. We evaluated the participation of cholesterol enriched microdomains in invasion of *T. gondii* into LLC-MK2 and murine macrophages through transient depletion of host cells cholesterol with either methyl-beta-cyclodextrin (MβCD) [final concentrations of 5, 10 and 20 mM for 30 min before interaction]; or Filipin [final concentrations of 1, 3 and 6 nM for 30 min before interaction]. Reversibility for MβCD was also tested. The interaction of the parasite with the host cells after treatment with cholera toxin B subunit (CTB) that binds to GM1 ganglioside was also tested. After interaction (10 min, 50 parasites per cell), adhesion and internalization indexes were determined by light microscopy. These were significantly diminished in cells treated with MβCD compared to controls. With 20 mM of MβCD, inhibition of internalization in LLC-MK2 reached 80% and almost 100% in macrophages. Pretreatment with MβCD followed by cholesterol reposition before interaction completely reverted inhibition in macrophages, but not in LLC-MK2. Filipin did not interfere in interactions with LLC-MK2 cells, but in macrophages, inhibition reached 76%. CTB did not interfere in adhesion to LLC-MK2 cells, but internalization was inhibited in 70%. Furthermore, macrophages treated with CTB inhibited the adhesion and internalization of the parasite in almost 80%. These results indicate that host cell membrane cholesterol enriched domains participate in the process of adhesion and active invasion of Toxoplasma and the differences observed between macrophages and LLC-MK2 cells may be due to different levels of this molecule in its membranes. Support: CNPq, CAPES, FAPERJ.

## RT.08-A – *Trypanosoma rangeli*: A BANE OR A BLESSING FOR CHAGAS DISEASE?

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*Trypanosoma rangeli* is non-pathogenic, but is frequently mistaken for the related Chagas disease agent, *T. cruzi*, with which it shares vectors, hosts, significant antigenicity and a sympatric distribution over a wide geographical area. Trypanosomes infective to humans have been extensively studied at a cell and molecular level, but study of *T. rangeli*, remains in relative infancy. Since human infection by *T. rangeli* can induce cross-reactivity with *T. cruzi* confounding serological detection of Chagas disease, reliable discrimination of *T. rangeli* from *T. cruzi* remains of utmost importance.

Aiming to increment the knowledge on the *T. rangeli* genome and to perform comparative analyzes, assessment of the parasite transcriptome through the generation of EST and ORESTES tags from both epimastigote and trypomastigote forms increased 26-fold the number of parasite sequences on the GenBank. The results allowed an estimate of ~8,500 genes for *T. rangeli* with an average G+C content of 55%. Biological functions were assigned to around 70% of the generated sequences, allowing the establishment of an annotated *T. rangeli* gene expression database.

Comparative analyses of *T. rangeli* sequences were carried out with *T. cruzi* and a wide range of pathogenic and non-pathogenic protozoan sequences now available, resulting in a relatively low number of unmatched sequences. Most *T. rangeli* sequences showed similarity to their homologs on the TriTryps genomes (3,128), revealing 625 exclusive hits with *T. cruzi*, which is consistent with former reports on the close phylogenetic relationship of these two taxa. Almost 23% of the sequences failed to yield blast hits with the TriTryps genomes. The presence of these *novel T. rangeli* specific sequences may provide new biological insights and/or diagnostic targets. Excluding the 289 *T. rangeli* protein coding sequences described in this study which were similar to those already in the GenBank database, a total of 2,228 sequences are described for this taxon for the first time.

Genes associated with virulence in pathogenic kinetoplastids, including GP63, TS, Cruzipain, Oligopeptidase B and Cysteine proteases, were found to be expressed in *T. rangeli*, but no transcripts related to mucin-associated proteins (MASPs) were among the generated ESTs. Groups of cDNA related to transcription and translation (RNA editing, RNA genes, RNA binding proteins and ribosome proteins), division (cyclins and protein kinases) and metabolism pathways like sterol synthesis were observed. Also, several retrotransposon hot spot proteins, like RHS1, RHS2c and RHS4 (a, e, f and g), are now described for this taxon. Based on their similarity to proteins of unknown function in related species a total of 1,076 hypothetical and hypothetical conserved proteins were observed for *T. rangeli*, representing 36.6% of all annotated sequences described.

These results represent the first large-scale analysis of the parasite genome, describing a draft of the gene expression profile of epimastigote and trypomastigote forms from polar strains. Furthermore, comparative analysis of these sequences with kinetoplastid genomes suggests that the gene repertoire of *T. rangeli* is closely related with pathogenic *T. cruzi*, reinforcing the importance of *T. rangeli* as a model to address studies involving mechanisms of virulence and pathogenicity. Currently, the Brazilian Genome Consortia is sequencing the *T. rangeli* genome, which will dramatically increase the knowledge of this neglected parasite, allowing comparative and functional genomics studies.

## RT.08-B – ANALYSIS OF PARASITE METABOLISM IN VIVO USING METABOLOMIC APPROACHES

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The genomes of many parasitic protozoa have now been sequenced providing a detailed overview of the metabolic capacity of these pathogens. However, it is likely that many metabolic and regulatory processes remain to be discovered as a large number of genes within the sequenced parasite genomes (typically >50%) have no assigned function. Moreover, almost nothing is known about the relationship between gene/protein expression and metabolic fluxes in these organisms. This is particularly striking in some trypanosomatid parasites that constitutively express the majority of genes in all life-cycle stages. Metabolomics is complementary to other profiling approaches (transcriptomics, proteomics) and is increasingly being used to identify stage-specific changes in parasite metabolism and physiology and parasite responses to various environmental and chemical stimuli (drugs etc). Here we provide an overview of analytical methods that are being used in metabolomic studies. We also highlight the unique insights that can be obtained using $^{13}C$ stable isotope labeling methods to measure metabolic dynamics and develop quantitative models of metabolism. We have applied these approaches to assess the metabolic state of the major developmental stages of *Leishmania*. Our results demonstrate that each stage enters a unique physiological state that are not predicted from other profiling approaches.

## RT.08-C – UNDERSTANDING TRYPANOSOMATIDS ANTIOXIDANT MECHANISMS: HOW FAR ARE WE?

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*Trypanosoma brucei* and *Trypanosoma cruzi* are responsible for two major tropical diseases: human African trypanosomiasis and Chagas disease, respectively. With no immediate prospect of vaccines, and no satisfactory treatment, the search for targets to develop a more specific therapy is a priority. One potential Achilles? heel is the systems the parasite employs to detoxify reactive oxygen species (ROS). Since the pioneering studies which identified trypanothione ($N^1$,$N^8$-bis-glutathionylspermidine), a protein unique to the members of the Kinetoplastida order and ascribed nowadays to an important role in ROS detoxification, numerous work have been undertaken trying to elucidate the molecular mechanisms that underlie the parasite's complex network of antioxidant devices. A review of the advances made throughout the years and the biological relevance of the proteins involved in this system will be presented.

## BC.01 - *Trypanosoma cruzi* eIF2α IS PHOSPHORYLATED DURING NUTRITIONAL STRESS REQUIRED FOR DIFFERENTIATION INTO THE INFECTIVE FORMS.

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Unlike other eukaryotes, in trypanosomatids regulation of gene expression is essentially post-transcriptional. Nevertheless, mechanisms of regulation of translation initiation have not been described in these parasites, although they express typical initiation factors and also three eIF2$\alpha$ kinases. The transformation of *Trypanosoma cruzi* epimastigotes into infective metacyclic trypomastigotes (metacyclogenesis), occurs naturally in the last portion of the digestive tract of triatomine insect vector, when nutrients are scarce. Metacyclogenesis can be reproduced *in vitro* when epimastigotes are submitted to a nutrient poor medium that mimics the composition of triatomine urine (TAU) followed by incubation in TAU supplemented with amino acids and glucose (TAU3AAG). Here we investigated whether the phosphorylation of the alpha subunit of eIF2, which causes translational arrest in response to amino acid starvation in other eukaryotes, is involved in this differentiation process. Trypanosomatids' eIF2$\alpha$ diverge from other eukaryotes in having a threonine residue ($T^{169}$) in place of the typical serine 51 that is phosphorylated in all other eukaryotes. We generated antibodies that recognize the phosphorylated form of *T. cruzi* eIF2α specifically at $Thr^{169}$. The specificity of this antibody was determined by showing that recognition was abolished after treatment with active λ-phosphatase and the antibodies did not react with the eIF2$\alpha$ protein of trypanosomes containing a $Thr^{169}$Ala mutation. When exponentially growing epimastigotes were incubated in TAU medium, an increase in Tc-eIF2α phosphorylation was observed along with a simultaneous decrease in polysomes and in total protein synthesis. Upon transfer to TAU3AAG medium, there was a decrease in eIF2$\alpha$ phosphorylation concomitant with partial recovery of translation. These results indicate that nutritional stress in trypanosomatids result in phosphorylation of Tc-eIF2$\alpha$ and translational arrest. Furthermore, our analysis also indicated that translational inhibition mediated by eIF2$\alpha$ phosphorylation is involved in the generation of infective forms of *T. cruzi*. Supported by FAPESP and CNPq

## BC.02 - DIFFERENTIAL EXPRESSION AND CELLULAR ADDRESSING OF GP82 AND GP90 SURFACE PROTEINS DURING *TRYPANOSOMA CRUZI* METACYCLOGENESIS

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*Trypanosoma cruzi* GP82 and GP90 are GPI anchored glycoproteins, members of the *trans*-sialidase-like family, expressed on the surface of metacyclic trypomastigote. GP82 is an adhesin involved in mammalian cell invasion while GP90 seems to act in a negative manner. Previous studies demonstrated that GP82 and GP90 mRNAs are stabilized and associated to polyssomes at the metacyclic trypomastigote stage. Metacyclogenesis involves the differentiation of replicating non-infective epimastigotes into non-replicating metacyclic trypomastigotes. This process is characterized by the presence of intermediate forms accompanied by several morphological changes and structural alterations. In this work we analyzed the expression of GP82 and GP90 genes during *in vitro* metacyclogenesis, giving special attention to intermediate forms. Epimastigotes were incubated in TAU medium and the following time points were analyzed: stationary epimastigotes before and after 2 h stress, epimastigotes and intermediate forms attached to culture flask 24 h and 48 h after inoculum in TAU3AAG. We observed increased levels of GP82 and GP90 mRNAs by real-time PCR in epimastigotes after 2 h of stress and also in epimastigotes and intermediate forms attached to culture flask. Protein levels were determined by western blot. While GP90 seems to increase continuously after 2 h stress, GP82 peaks in attached cells at 48 h. Immunofluorescence analysis of intermediate forms showed that GP90 localizes in the flagellar pocket (FP) and plasma membrane (PM), indicating that it is being produced and exported from Golgi to FP and then to PM. In contrast, GP82 localizes in reservosomes and PM, being easily detected at 48 h in those organelles. This indicates that GP82 proceed in a different way passing through reservosomes before reaching the PM. It is possible that GP82 undergoes some processing step in reservosomes before going to the PM. Further characterization of GP82 and GP90 processing during metacyclogenesis is underway in our laboratory. Supported by FAPESP and CNPq.

## BC.03 - HeLa CELL INVASION BY AMASTIGOTES: REMARKABLE DIFFERENCES BETWEEN *Trypanosoma cruzi* AND *Leishmania L. amazonensis*

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*Trypanosoma cruzi* amastigotes of the G strain promptly invade mammalian cells in culture. During the invasion of HeLa cells, actin-rich surface protrusions originally named 'cups' are formed. *Leishmania (L.) amazonensis* axenic amastigotes also invade HeLa cells, but with a much lower efficiency. The aim of this study was to examine the membrane protrusions formed during the invasion of HeLa cells by amastigotes of the two parasites. HeLa cells were infected for 30 min-1h with *T. cruzi* or for 2-4h with *L. L. amazonensis* amastigotes and: 1: imaged live on a spinning disk system with HeLa cells transfected with GPI-YFP and LAMP1-RFP or; 2: processed for field emission scanning electron microscopy. Alternatively, samples on coverslips were lysed with 1% Triton X-100 in the presence of 10 μg/ml of taxol and phalloidin to stabilize microtubules and actin microfilaments. Samples were fixed in aldehydes and post-fixed with $OsO_4$ and tannic acid. Contrast was also applied using rotary shadowing with Pt. *T. cruzi* amastigotes promptly invade HeLa cells and by live microscopy, cups are detectable around 10 min. after parasites were added to the cells. By contrast, *Leishmania* parasites do not cause significant alterations on the markers even after 1h. By SEM *T. cruzi* EA readily attach to and deform the tips of surface microvilli whereas *Leishmania* amastigotesare rarely seen in contact with the surface projections. TX-100 extracted material reveal parasites surrounded by meshwork of filaments at the early stages of *T. cruzi* invasion whereas *Leishmania* parasites are only detectable inside the cells at 2 and 24 h post infection. These results reveal that amastigotes of the two species engage distinct mechanisms to colonize HeLa cells. Supported by FAPESP, CNPq and CAPES.

## BC.04 - THE PROTEIN KINASE D IS RECRUITED TO INVASION SITES OF *TRYPANOSOMA CRUZI* EXTRACELLULAR AMASTIGOTES (EA)

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*Trypanosoma cruzi* life cycle comprises distinct developmental stages. Extracellular Amastigotes (EA) are generated by the extracellular differentiation of trypomastigotes and are dependent on host actin filaments polymerization to invade cultured cells. Signaling events surrounding these processes are poorly understood. The protein kinase D (PKD) family comprises three different but closely related serine-threonine kinases, PKD1, PKD2, and PKD3, all of which have a highly conserved N-terminal regulatory domain containing two cysteine-rich diacylglycerol (DAG) binding domains and an autoinhibitory pleckstrin homology (PH) domain. PKD not only is a direct DAG target but also lies downstream of PKCs in a novel signal transduction pathway implicated in the regulation of multiple fundamental biological processes such as cell shape, adhesion, and migration. At the leading edge of migrating cells active PKD co-localizes with F-actin, Arp3 and cortactin. Cortactin has emerged as a key signaling protein in cellular processes such as endocytosis and tumor invasion. The ability of cortactin to interact with and alter the cortical actin network is central to its role in regulating these processes. We attempted to evaluate the structural requirements of PKD in the EA uptake of HeLa cells. HeLa cells were transfected with cortactin and PKD GFP-vectors, infected with EA and examined for the acquisition of these markers. Wild type PKD1 and 2, but not PKD3 are recruited to sites of actin remodeling and EA invasion, which also contain cortactin. Both PKD1 pleckstrin homology-deleted and PKD1-kinase-dead were not recruited to EA invasion sites. PKD1 lacking N-terminal domain was recruited to and colocalized with actin and cortactin. These results together with previous literature data suggest that EA internalization could be regulated by different processes from those which regulate other actin related events like lamellipodia formation. Support: FAPESP, CAPES

## BC.05 - INDUCTION OF PROINFLAMMATORY CYTOKINES AND NITRIC OXIDE BY *TRYPANOSOMA CRUZI* IN RENAL CELLS

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Chagas disease, caused by the protozoan parasite *Trypanosoma cruzi*, is tipically associated with cardiac involvement. In the murine model of *T. cruzi* infection, myocarditis may develop during the acute phase, but prior to the cardiac alteration the infected animals present renal inflammatory infiltration causing acute kidney injury (AKY) due to an ischemia/reperfusion lesion. In an attempt to understand the genesis of AKI in *T. cruzi* infection, the various aspects of parasite-renal cell interactions were examined in this study by using three cell lines (HMC, MDCK and LLC-PK1). The susceptibility to infection of these cells was low, even after 72 h interaction with trypomastigotes. HMC cells were the most resistant and LLC-PK1 cells the most susceptible to *T. cruzi*, about 13% of the latter being infected and supporting robust intracellular parasite multiplication. Upon interaction with *T. cruzi*, some cell line-dependent alterations in the cellular integrity and in the levels of inflammatory mediators, such as nitric oxide (NO), tumor factor necrosis-alpha (TNF-α) and interferon-gamma (INF-σ), were observed. Mesangial HMC cells, but not the other cell types, had their viability diminished and NO release augmented upon 72h contact with the parasites. NO production was induced in HMC and MDCK cells upon 3 h or 72 h incubation with *T. cruzi* secreted factors, and this NO-inducing property was abolished by heating. The recombinant *T. cruzi* trans-sialidase, an enzyme that is secreted during the acute infection in mice and humans, displayed a temperature-sensitive NO-inducing capacity toward HMC and MDCK cells. Increased levels of TNF-α and INF-σ were detected in HMC cell cultures at 72 h post-infection. Our results suggest that ischemia/reperfusion lesions in acute *T. cruzi* infection do not result from cellular parasitism; they are apparently associated with the alterations in renal cells and generation of inflammatory mediators. Supported by FAPERJ; CNPq and FIOCRUZ/RJ

## BC.06 – DOES PHOSPHATIDYLSERINE MODULATE *Trypanosoma cruzi* ENTRY'S PROCESS INTO PERITONEAL MACROPHAGES?

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*Trypanosoma cruzi*, the etiological agent of Chagas disease, is an intracellular parasite that as others intracellular pathogens developed evasion mechanisms, enabling the establishment of infection. It has been shown that virus and parasitic protozoa, including *Leishmania amazonensis* and *Toxoplasma gondii*, are capable of mimicking mammalian apoptotic cell death by the exposure of phosphatidylserine (PS). This process, called "apoptotic mimicry", justify the occurrence of apoptotic features in a unicellular pathogen. Seabra *et al* (2006) demonstrated that the exposure of PS by a population of *T.Cruzi* trypomastigotes, but not epimastigotes or intracellular amastigotes, leads to the deactivation of macrophages through a TGF-β signaling pathway. Here, we investigated if the presence of PS modulates trypomastigotes' entry process into macrophages. In order to quantify the percentage of $PS^+$ trypomastigotes, we used flow cytometry and a low percentage was found. Then, we separated $PS^-$ and $PS^+$ trypomastigotes, using an Annexin V microbead kit, which allowed us to interact separately these subpopulations with macrophages for 1, 24 and 48 hours, always comparing with total population ($PS^-$ and $PS^+$). The subpopulations' separation through this Kit showed a consonance with the percentage of $PS^+$ trypomastigotes found through flow cytometry, validating this method. We could observe that the $PS^+$ parasite's internalization was drastic diminished (higher than 90%) within 24 and 48 hours of interaction. The *T. cruzi*'s adhesion index was not altered. Besides that, $PS^-$ parasites showed an infection's progression of 60% higher when compared with total population within 48 hours. By field emission scanning electron microscopy, we observed that $PS^-$ parasites enter in macrophages preferentially by anterior region, but further quantifications will be done to confirm this data. Together, our findings suggest the participation of PS in trypomastigotes' entry process into peritoneal macrophages, indicating cooperation between $PS^-$ and $PS^+$ parasites in the establishment of the infection. Supported by CNPq, CAPES and Faperj.

## BC.07 - MURINE INFECTION WITH *TRYPANOSOMA CRUZI* LIKE ISOLATED FROM BATS: THE FIRST FIVE DAYS

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Bats are important reservoirs of trypanosomes of the subgenus *Schizotrypanum* including *Trypanosoma cruzi* and other trypanosomatids. This study aims to evaluate the kinetics of infection within the first 120 hours in mice inoculated with trypanosomes isolated from bat species *Artibeus planirostris* (EM437), *Phyllostomus hastatus* (EM239 and EM245) and *Phyllostomus discolor* (EM465). The isolates were genetically characterized by multiplex-PCR with primers D72/D75/RG3 and PCR-RFLP analysis of the mitochondrial cytochrome oxidase subunit II gene (COII-RFLP). To study the kinetics of infection, groups of 13 non-isogenic mice per isolate were inoculated intradermally in the psoas muscle with 2 x 10ϖ culture trypomastigotes/mL. Mice were euthanized at 2, 4, 8, 12, 24, 36, 48, 60, 72, 84, 96, 108 and 120 hours after infection and their paws were removed for histopathological studies by HE stain. In multiplex-PCR and COII-RFLP all isolates showed amplification products of 250 bp and 263 bp, respectively, which are compatible with *T. cruzi* I. In all animals inoculated with four isolates, the histopathological study of the psoas muscle showed the presence of intense inflammatory infiltrates characterized by polymorphonuclear, neutrophils and eosinophils in the first 48 hours. After 60 hours the infiltrate was composed primarily of the eosinophils. Unlike what is observed in infections with *T. cruzi* isolated from human infections, where there are high numbers of amastigotes within macrophages, infection by these isolates found only one to six amastigotes forms within scarce macrophages from 36 hours to 120 hours. This can tell us: 1) that these isolates are not virulent and pathogenic, 2) that the forms of amastigotes observed early in infection did not correspond to *T. cruzi* but other trypanosomatids the subgenus *Schizotrypanum*. Supported by CAPES. CNPq, FAPEMIG and FUNEPU.

## BC.08 - BIOLOGICAL BEHAVIOR OF A STRAIN OF Trypanosoma cruzi TcV GROUP ISOLATED FROM A HUMAN CONGENITAL TRANSMISSION

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Data about biological behavior of *Trypanosoma cruzi* TcV group is scarce in the literature, however, suggest its association with congenital transmission in some endemic regions for Chagas disease. In this study, we analyzed the in vitro and in vivo behavior of TcV strain 3048, isolated from a Brazilian child infected by congenital transmission. Balb/C females mice were infected with 1 x $10^5$ trypomastigotes by the intraperitoneal route. The parasitemia was evaluated during 40 days using the microhematocrit and blood fresh examination. After 4 months of infection (chronic phase), the animals were killed and 16 tissue/organs were collected to histopathological analysis. The metacyclogenesis was performed by cultivation in TAU (Artificial Urine Triatomine) medium and the infectivity for cells was assessed by exposition of MK2 cells to trypomastigotes. During the acute phase the parasitemia was subpatent and was detectable in 100% of animals only by the microhematocrit. No mortality was observed among animals in both acute and chronic phases. No intracellular amastigotes nests were detected by hematoxylin-eosin staining, althought, different degrees of inflammatory foci (IF) with mononuclear cells and macrophages were seen: moderate/intense IF in spleen (66,6%), smooth muscle (55.6% gastroesophageal and 44.5% gastroduodenal junctions), lungs (44.4%) and uterus (22.2%); mild/moderate IF in liver (88,9%), skeletal muscle and diaphragm (55.5%); mild IF in heart (88.9%). Metacyclogenesis rate after 7 days in TAU medium was 9% (90% broad forms).The rate of infected MK2 cells was 74.3% and 74.6% after 3 and 72 hours respectively, the number of amastigotes/cell (72 h exposure) was 1.6. The strain 3048 (TcV), despite the high in vitro infectivity for Mk2 cells, presented a low blood and tissue parasitism in Balb/C mice, with greater impairment of smooth muscle and organs of the mononuclear phagocytic system as liver and spleen, but in a few cases in uterus.
Supported by Capes, CNPq Universal 2008; FUNEPU and FAPEMIG.

## BC.09 - CONGENITAL TRANSMISSION OF EXPERIMENTAL CHAGAS DISEASE INDUCED BY *Trypanosoma cruzi* STRAIN GROUP TcV

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The factors involved in congenital transmission of Chagas disease are not completely understood and include multifactorial mechanisms related to the host and the *Trypanosoma cruzi* strain. In this study, we evaluate the congenital transmission in mice infected with strain 3048 (TcV), isolated from a child infected via the same pathway. A total of twenty female BALB/c mice were intraperitonially infected with 1 x $10^5$ trypomastigotes. Ten of these females were mated during the acute phase of the infection and another ten in the chronic phase. The microhematocrit technique was performed to evaluate the parasitemia during pregnancy. Congenital infection was diagnosed either by hemoculture in LIT (Liver Infusion Tryptose) and by PCR (polymerase chain reaction) using 121 and 122 primers. A half of the offspring from each litter was submitted to euthanasia after birth when heart, liver and spleen were obtained focusing the PCR tests. The other half of the offspring provided sources of blood for hemoculture and to perform PCR at 30 and 60 days of life. In the acute phase, the maternal mortality rate was 40% (4/10) and no female reached pregnancy. In the chronic phase, this rate was reduced to 10% (1/10), in which all females reached pregnancy, the mean litter size per offspring was 8.67 and the mortality rate was 6.41% (5/78). Parasitemia levels presented a subpatent profile and were detectable only by the microhematocrit method during pregnancy. Hemoculture tests were negative for all offspring. The congenital transmission rate detected by PCR were 43.84% (32/73), in which 45.45% (15/33) corresponded to tissue samples obtained from newborn offspring and 42.50% (17/40) to blood samples obtained after 30 days of life. The hemoculture test showed itself ineffective in the diagnosis of congenital transmission. Strain 3048 (TcV) was able to vertically transmit Chagas disease, even in the presence of low maternal parasitaemia levels.
Supported by Capes, CNPq Universal 2008; FUNEPU and FAPEMIG

## BC.10 - DIFFERENT CHANGES IN THE NUMBER OF CIRCULATING LEUKOCYTES IN PERIPHERAL BLOOD OF MICE INFECTED WITH BLOOD OR METACYCLIC TRYPOMASTIGOTES OF *TRYPANOSOMA CRUZI*

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Although it was shown that metacyclic and blood trypomastigotes are completely functional in relation to parasite-host interaction and invasion of the target cell, they differ in the molecules present on the surface. Based on this, the aim of this study was to investigate changes in peripheral blood leukocytes before and after infection with metacyclic (MT) or blood (BT) forms of Be-78 *T. cruzi* strain. Animals of the MT group showed an increase in total leukocyte at 28 days after infection, and this increase was maintained until 42 days after infection. However animals of the BT group showed an increase of these cells only in 42 days after infection. Moreover, animals in the MT group presented an increase in the number of eosinophils throughout infection, this increase was not observed in BT group. Regarding monocytes, animals in the BT group showed an early increase of these cells on the seventh day after infection, but this increase was not maintained throughout the infection, returning to baseline already on the fourteenth day after infection. The values of lymphocytes were found elevated after the twenty-first day after infection in group MT, but in group BT there was an early increase on the seventh day, declining after this day. Increased again only in the forty-second day after infection. Taking together, these results demonstrate that the source of inoculum can influence the course of Chagas' disease, because infection with metacyclic forms it takes change early in a greater number of cells which could explain the lower parasitemia of this group compared to the animals infected by blood forms.
Supported by FAPEMIG (PPM, Redes Toxifar e Bioterismo), CNPq and UFOP.

## BC.11 - B1 CELLS ARE TARGETS OF INFECTION BY *TRYPANOSOMA CRUZI* IN VITRO AND IN VIVO

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*T. cruzi* has the ability to infect different mammalian cell types in vitro and in vivo, but infectivity may vary considerably depending on the parasite strain and the host cell type. Trypomastigotes of CL strain used in this study efficiently enter cultured epithelial cells, as well fibroblasts, and replicate intracellularly as amastigotes. Mouse peritoneal macrophages are also invaded but the number of intracellular parasites decreases with time. Here we examined the ability of CL strain trypomastigotes to invade and develop in C57BL/6 mouse B1 cells, which predominate in the peritoneal cavity and can be distinguished from conventional B cells. Of all cell types examined to date, B1 cells (CD23+CD11b+CD19+), which transform in a novel type of mononuclear phagocytes in culture while maintaining lymphoid characteristics, were the most susceptible to *T. cruzi* infection. Incubation of B1 cells with trypomastigotes for 1 h, at 1:1 parasite:cell ratio, resulted in about 60% infected cells, whereas under the same condition the percentage of infected HeLa cells was <1%. B1 cells also supported intense parasite multiplication and trypomastigotes were released into medium by 96 h. To determine whether B1 cells were targets for *T. cruzi* in vivo, C57BL/6 mice were separated in two groups. The control group received PBS intraperitoneally and the other group received heat-inactivated *Propionibacterium acnes*, which induces an increase in macrophages, immature dendritic cells and B1 cells. Twenty four hours later, the animals were challenged with *T. cruzi* trypomastigotes labeled with fluorescent dye CFSE. Cells were collected from the peritoneal cavity of control and experimental mice 2 hours after challenge and analyzed by flow cytometry. The number of B1 cells infected with *T. cruzi*, i.e., cells containing labeled parasites was about 6 fold higher in cells from mice inoculated with *P. acnes* than in cells derived from control mice. Work supported by FAPESP and CNPq.

## BC.12 - FAK SIGNALING PATHWAY IS INVOLVED IN *T.CRUZI*-CARDIOMYOCYTE INTERACTION

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Trypanosoma cruzi invasion is mediated by receptor-ligand recognition between the surface of both parasite and target cell. *Different signaling pathways may be activated during mammalian cells invasion, including protein tyrosine kinases activation that can be important in the regulation of parasite entry.* In this work, we demonstrate the participation of protein tyrosine kinases, especially focal adhesion kinase (FAK) and SRC, during T. cruzi invasion in cardiomyocytes. Treatment of cardiomyocytes with genistein, a protein tyrosine kinase inhibitor, PP1, a potent SRC-family protein inhibitor and PF573228, a potent FAK inhibitor, reduced in a dose-dependent manner the invasion of T. cruzi. Furthermore, T. cruzi entry was accompanied by changes in c-SRC expression and FAK phosphorylation levels. Enhancement of FAK activation takes place during initial stage of T. cruzi-cardiomyocyte interaction (30 and 60 min) with concomitant 2-fold increase in the level of c-SRC expression. Dephosphorylation of FAK also coincided with lower level of c-SRC expression after 2h of interaction, suggesting that FAK/c-SRC promotes an integrated signaling that coordinates parasite entry. These data provide novel insight into signaling pathway involved in T. cruzi uptake in cardiomyocytes. Another approach of this study was to evaluate the effect of T. cruzi infection on focal adhesion proteins, since structural changes, including breakdown of myofibrillar (Pereira et al., 1993), besides downregulation of α-cardiac-actin mRNA, were evidenced in cardiomyocytes infected by T. cruzi (Pereira et al., 2000). Our results demonstrated that the localization of focal adhesion proteins remained unaltered in 24h-infected cardiomyocytes. In contrast, biochemical assays demonstrated a decline in focal adhesion proteins expression after 72h of infection. Our data demonstrate that T. cruzi infection disturbs the structural integrity in cardiomyocytes in vitro, which can result in loss of cardiac tension, suggesting that structural changes can contribute to the cardiac dysfunction evidenced in Chagas' disease. Supported by FIOCRUZ, CAPES and FAPERJ.

## BC.13 - LIMP-1 IS IMPORTANT FOR *TRYPANOSOMA CRUZI* EXTRACELLULAR AMASTIGOTE INTERNALIZATION INTO MEF CELLS

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The parasitophorous vacuole membrane formed during cell infection by *Trypanosoma cruzi*, the intracellular protozoan that causes Chagas' disease in humans, may display endocytic pathway markers such as Associated and Integrated lysosomal membrane glycoproteins, LAMPs (LAMP-1 and LAMP-2) and LIMPs (LIMP-1 and LIMP-2), respectively. Previous studies have suggested that extracellular amastigotes (EA) cell invasion, but not the multiplication of parasites within cells, could be differentially modulated in LAMP knockout mammalian cells suggesting that LAMPs might play a role in internalization processes, although the exact function has not yet been elucidated. Our study aimed to evaluate the role of LAMP and LIMP proteins during the invasion by EA of *T. cruzi* (G strain) using mouse embryonic fibroblast cell lines (MEFs) derived from LAMP-1, LAMP-2, LIMP-1 and LIMP-2 single knockout and LAMP-1 and 2 double knockout mice. Our results indicate that the lack of LIMP-1 decreases cell invasion by the parasite, while in LAMP-2 or LIMP-2 knockout cells we observed an increased level of parasite internalization. We propose that lysosomal glycoproteins can modulate events related to cell internalization processes.
Supported by CNPq and FAPESP.

## BC.14 - HOST-PATHOGEN INTERPLAY: HOW INHIBITORS CAN AFFECT BOTH PARTNERS AND MODULATE INFECTIVITY

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*Trypanosoma cruzi* amastigotes which are generated by the extracellular differentiation of trypomastigotes are referred to as Extracellular Amastigotes (EAs) and are able to invade mammalian cells. EAs of the G strain promptly aggregate actin filaments by attaching to dorsal microvilli of HeLa cells and, as a result, cup-like structures are formed underneath the parasites. EAs is therefore dependent on host actin filament polymerization to invade cells. However, detailed signaling events surrounding these processes are still obscure. In the present study, we aimed to evaluate the effect of signaling pathways' inhibitors on both parasite and host cells. Interacting partners were pre-treated with PP2 and DASATINIB (Src kinase pathway inhibitors); wortmannin, 3-methyladenine and LY294002 (conventional phosphatidylinositol kinase inhibitors); rapamycin and ATM/ATR inhibitor (phosphatidylinositol-related kinase inhibitors) and a protein kinase D inhibitor. Control cells and parasites were left untreated. HeLa or Vero cells were then infected with EAs. The treatment of host cells with Src inhibitors decreased EA entry, thus suggesting that EA may need Fyn-Src kinase pathway to invade cells. By contrast, after treatment with conventional phosphatidylinositol kinase inhibitors, parasites acquired LAMP-1 (Lysosomal Associated Membrane Protein-1) markers faster than the controls and remained longer in LAMP-1 positive vacuoles. The inhibition of the phosphatidylinositol-related kinase pathways in host cells led to the enhancement of parasite entry. Here, we propose a signaling pathway model of EA entry into host cells different from that already described for trypomastigotes (TCTs).
Supported by: FAPESP.

## BC.15 – CHOLESTEROL DEPLETION IN PRIMARY MURINE CARDIOMYOCYTES DIMINISHES *TRYPANOSOMA CRUZI* ENTRY AND ALTERS LYSOSOMAL FUSION FOR THE FORMATION OF THE PARASITOPHOROUS VACUOLE

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*Trypanosoma cruzi,* the etiological agent of Chagas´disease, can invade several types of non-professional phagocytic cells including myocytes and others. Invasion occurs when parasites attach to and stimulate host cells, producing intracellular calcium signaling events that culminate with lysosome recruitment and fusion with the host cell plasma membrane for the formation of the parasitophorous vacuole. Several factors influence *T. cruzi* entry in host cells: *membrane rafts*, sphingolipid and cholesterol enriched plasma membrane domains, interferes in parasite invasion of fibroblasts and macrophages. Recently we have also shown that both membrane rafts and cholesterol are important for parasite invasion in murine cardiomyocytes. However this mechanism of *T. cruzi* host cell invasion through rafts is still under investigation. Since lysosomes are crucial for parasite stable host cell infection, we decided to study the effect of cholesterol depletion in the recruitment of these organelles during *T. cruzi* entry. We verified that lysosomal fusion during parasite invasion diminished when cholesterol was depleted by incubation with MβCD. Treatment with HγCD, a cyclodextrin with less affinity for cholesterol, did not show any difference in relation to control cells. We also studied the effect of host PI-3 kinase inhibition in cholesterol depleted cells, since this enzyme apparently regulates lysosomal fusion during parasite invasion. No significant differences have been observed. We then investigated if cholesterol depletion deregulates lysosomal exocytosis. Our results show that cholesterol depletion alone leads to lysosomal exocytosis, suggesting that these membrane domains might regulate lysosomal fusion events. We have also tested the effects of cholesterol depletion in host cell actin distribution and observed a more organized cortical cytoskeleton pattern in cells with less cholesterol. Altogether, our results suggest that cholesterol depletion alters host cell lysosomal fusion with the plasma membrane interfering with the process of *T. cruzi* host cell invasion. Supported by Capes/INCT, CNPq and FAPEMIG

## BC-16 - ANALYSIS OF BIOLOGICAL BEHAVIOR AND CONGENITAL TRANSMISSION IN EXPERIMENTAL CHAGAS' DISEASE INDUCED BY *Trypanosoma cruzi* STRAIN GROUP TcI

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Nowadays congenital Chagas' disease has become a public health problem in several countries because of migration of infected people from endemic countries. We evaluate the biological behavior and congenital transmission in mice with strain AQ1-7 (TcI), isolated from *Triatoma sordida*. We used 20 female Balb/C, half for analyzing the biological behavior and the remainder for mating in the chronic phase. The inoculum used was $1x10^5$ trypomastigotes by the intraperitoneal route. Parasitemia was monitored by microhematocrit and blood fresh examination. The rate of infectivity was analyzed in MK2 cells. The histological analysis of 16 organs, obtained in the chronic phase, was performed with hematoxylin-eosin. The females were mated after 30 days of infection and congenital transmission was diagnosed by hemoculture in LIT medium and PCR (primers 121 and 122). Half of the offspring was killed at birth and heart, liver and spleen were obtained to perform PCR. The other half were killed 60 days after birth and blood was collected for hemoculture and PCR. Parasitemia presented subpatent and experimental infection was confirmed only by hemoculture. The histological analysis revealed inflammation of mild to moderate and the parasite has been found in the skeletal muscle in only one animal. The infectivity in MK2 cells was 69.7% after 3 hours of infection, reaching 100% after 72 hours. During mating occurred the death of a female (1/10) and one was not pregnant (1/9). The average number of offspring per female was 5.33 (43 offspring in total). Hemoculture resulted negative for all offsprings. However, the blood PCR was positive in 33.33% (8/24) and PCR tissue in 89.47% (17/19), representing an overall rate of congenital transmission of 58.14% (25/43). The strain AQ1-7 (TcI) showed a high rate of congenital transmission, suggesting the importance of this genotype be better studied in the context of vertical transmission. Supported by Capes, CNPq Universal 2008; FUNEPU and FAPEMIG.

## BC.17 - AMASTIN AS A VIRULENCE FACTOR OF *TRYPANOSOMA CRUZI*

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*Trypanosoma cruzi* extracellular amastigote (EA) from G strain (*T. cruzi* I) shows high infectivity towards host cells *in vitro* when compared to the traditionally more infectious CL strain (*T. cruzi* IV). We observed in a microarray data that amastin, an amastigote stage-specific surface protein, was 21 times more expressed in EA from CL strain. In order to study the putative involvement of amastin in cell cycle, we cloned, expressed and purified the less hydrophobic region of amastin in fusion with GST to developed polyclonal antibodies in rabbits. Immunolocalization of amastin in *T. cruzi* amastigotes confirmed its location on the parasite surface. HeLa cells pre-incubated with 5 μg/ml of the recombinant protein showed a decreased in cell invasion by EA. Parasites from G strain were transfected with pTREX-Amastin-GFP and pTREX-GFP: a significant decrease in cell invasion of EA that over-expressed amastin was observed when compared to the controls. However, the number of trypomastigotes released into the supernatant of infected HeLa cells 96h and 120h after the invasion was higher in cells infected with the parasites that over-expressed amastin. In susceptible mouse strains (A/J) EA overexpressing amastin were precociously observed in liver and spleen nests whereas parasitemia was never detected. These results show that amastin plays a key role in the course of intracellular *T. cruzi* infection both *in vitro* as well as *in vivo.*

Financial support: CNPq, CAPES and FAPESP.

## BC.18 - CELL SURFACE MODULATION PROTECTS Y STRAIN TRYPOMASTIGOTES OF *TRYPANOSOMA CRUZI* FROM ANTI-α-GAL ANTIBODY LYSIS

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*Trypanosoma cruzi* trypomastigotes present a negative charge mainly attributed to the presence of sialic acids that is incorporated mostly into mucin-like molecules and that prevent the parasite killing by complement and Chagasic anti-α-Gal antibodies (anti-α-Gal). We previously showed that tissue culture trypomastigotes from Y strain and CL-Brener clone have distinct capacities to modulate the composition of their surfaces in the presence of cationized ferritin (CF) and Concanavalin A (Con A). In the present study we analyzed whether those cell surface modifications could inhibit the anti-α-Gal mediated lysis. Trypomastigotes were incubated for 10 min at 37°C in the presence of CF and Con A (both at 10μg/ml concentration). Then 8μl of anti-α-Gal (20μg/ml) was added, and the mixture was further incubated for 30 minutes at 37℃. As the anti-α-Gal induces both agglutination and lysis, we measure the number of motile and free (non-agglutinated) trypomastigotes in a hemocytometer. Control Y strain trypomastigotes were more susceptible to lysis by anti-α-Gal compared to CL-Brener parasites. Incubation with CF and Con A, protected Y parasites from lysis by anti-α-Gal while did not cause significant changes in CL-Brener trypomastigotes. Flow cytometry analysis of the intensity of fluorescence of Y and CL-Brener parasites after anti-α-Gal antibody labeling showed that Y parasites presented two different cell populations and that after CF and Con A incubation, the pattern of fluorescence of Y parasites changed and the most fluorescent population is lost. No changes were observed in CL-Brener parasites submitted to the same procedures. The data suggested that some anti-α-Gal binding sites in Y parasites were shed under the influence of CF and Con A and that the decrease in the amount and/or redistribution of those binding sites protect the Y parasites from the anti-α-Gal induced lysis.

Supported by: CNPq, CAPES, FAPERJ, Pronex

## BC.19 - DELETION OF ALPHA-2,3-SIALILTRASFERASE IV DECREASES PARASITEMIA AND MORTALITY OF MICE INFECTED WITH *TRYPANOSOMA CRUZI*

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Chagas' disease is the leading cause of death from heart problems in endemic areas in Latin America. The most severe manifestation of the disease caused by the protozoan *Trypanosoma cruzi* is a chronic cardiomyopathy, which is mainly caused by an intense inflammatory response triggered by the parasite's persistence in heart tissue. Interaction between selectins and cell surface sialyl Lewis$^x$ ligands promote tethering and rolling of leukocyte on vascular endothelium, an important step for lymphocyte homing to secondary lymphoid organs and for leukocytes recruitment to injured non-lymphoid tissues. The aim of this study was to investigate the impact of deletion of the enzyme alpha-2,3- sialiltrasferase IV (ST3Gal IV), witch catalyses the final glycosylation step in sialyl Lewis$^x$ synthesis, in the infection by *T. cruzi* (Y strain). ST3Gal-IV deficient (ST3Gal IV KO) or wild type mice (WT) were infected i.p. with trypanomastigotes ($1x10^4$). Our results demonstrate that, despite the decrease of pro-inflammatory cytokines, such as IL-6, TNF-alpha and INF-gamma in intra-peritoneal lavage, 6 h after infection, ST3Gal-IV knockout mice infected with *T. cruzi* had a significant reduction of parasitemia and mortality, when compared with wild type mice. Consistent with these observations, $CD8^+$ T cells isolated from infected ST3Gal-IV KO mice showed increased cytotoxic activity. These results open a new perspective for the role of ST3Gal-IV in the development of adaptive immunity during infection by *T. cruzi*.

## BC.20 - RHO-ACTIN SIGNALING *PATHWAY* IS LINKED WITH CELLULAR EVENTS SUCH AS ADHESION AND ENDOCYTOSIS IN *TRYPANOSOMA CRUZI*

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Rho family GTPases play critical roles in motility, phagocytosis, intracellular transport, adhesion and morphology, performing these functions via the actin cytoskeleton. The etiologic agent of Chagas disease, *Trypanosoma cruzi*, has orthologs for Rho, actin, and several actin-binding proteins. Knowing how the Rho orthologue acts on the actin cytoskeleton may provide a better understanding of cellular physiology of the parasite, providing new therapeutic targets. Previous experiments performed by our group showed that evolutionarily conserved Rho retains its function on cell-substrate adhesion. Previous assays showed that constitutively active Rho potentiated the event, while the inactive Rho mutant limited adhesion. In the current work, we carry out assays to link the Rho phenotypes with actin cytoskeleton. Parasites overexpressing actin showed high levels of adhesion on substrate under *in vitro* conditions. Similar results were also observed in an *ex vivo* model, incubating epimastigotes on the epithelial tissue of insects *Rhodnius prolixus* dissected. In summary, these results allow us to suggest that Rho activation and actin levels themselves leads to an enhancement of adhesion in parasites. Moreover, having prior knowledge of the damages caused by the expression of inactive Rho mutant on proliferation and receptor-mediated endocytosis, we carry out electron microscopy analysis to investigate the morphology of these parasites, was identified a raised accumulation of reservossomes in epimastigotes expressing inactive Rho mutant. These morphological changes in endocytic organelles may explain the effects already observed in endocytosis and proliferation after inactive Rho mutant expression. A better understanding of how environmental stimuli act on the regulatory proteins of GTPases, their effectors, and how they transmit stimuli to the actin cytoskeleton modulating the phenotypes observed depends on the identification of protein-protein interactions of the *pathway*. Currently, a strategy of yeast two-hybrid system is underway to identify the molecular partners of Rho-actin signaling *pathway in T. cruzi.* This work was supported by FAPERJ, CNPQ, and IFRJ-PROCIÊNCIA

## BC.21 - TARGETING THE KDNA: PROMISSORY CHEMOTHERAPIES APPROACHES AGAINST TRYPANOSOMATIDS

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The mitochondrial DNA of trypanosomatids protozoa, termed kinetoplast DNA (kDNA), is a complex network, composed of topologically interlocked DNA circles, which are of two types: maxicircles and minicircles. The maxicircles, which encode ribosomal RNAs and several mitochondrial proteins, are similar in structure and genetic function to the mitochondrial DNA of other eukaryotes. A most remarkable feature of maxicircles is that most of their transcripts undergo RNA editing to form a functional mRNA, a process involving the insertion or deletion of uridine residues. The minicircles encode small guide RNA´s that control the specificity of editing. Aiming to arrest crucial steps of RNA editing in trypanosomatids that could represent an efficient chemotherapeutic approach to combat the diseases caused by these parasites, based on literature and bioinformatics analyses, presently we are searching for potential conserved sequences in the mitochondrial genome of trypanosomatids involved in the RNA anchors coding, which could finally target maxicircle sequences that should be edited. These selected sequences were found to be conserved in *Trypanosoma cruzi*, *T. brucei* and *Leishmania spp*. Next, the sequences were synthesized and assayed, through biophysical interaction studies, on eight different aromatic compounds (aromatic diamidines (AD) – including DB75 and DB569 - and arylimidamides (AIA) - including DB766, DB709, DB613, DB1831 and DB1852. Although AD and AIA present high activity and selectivity against a wide range of organisms, including *T. brucei and T. cruzi*, their mechanism of action is still poorly known. Also, literature data show morphological and biophysical evidences that many ADs are strong DNA binders triggering striking alterations on the mitochondria and KDNA of these parasite, but still very little is known regarding AIA effects. Thus, using Circular Dichroism (CD) and Tm studies we evaluated the mode of interaction and level of affinity of these aromatic compounds upon the RNAg anchor sequence. Our data show that all compounds display a typical CD spectrum of minor groove binders, with some of them leading to a considerable perturbation on the DNA molecules. Interestingly, although some of them, like DB1831 and DB766, do not bind strongly to the DNA sequence, they do present a considerable trypanocidal activity against *T. cruzi* suggesting that only their direct interaction and/or perturbation of the KDNA topology and sequence *per se* may not be the only cause of the parasite death and that other secondary effects could participate in the parasite killing processes. Supported by: Fiocruz/PAPES V, FAPERJ, CNPq, CPDD, NIH and the Gates Foundation

## BC.22 - ENDOTHELIN AND BRADYKININ RECEPTORS ACTING INTERDEPENDENTLY, INCREASE VASCULAR PERMEABILITY AND DRIVE THE UPTAKE OF *TRYPANOSOMA CRUZI* BY CARDIOVASCULAR CELLS

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Infection-associated vasculopathy in Chagas' disease was ascribed to upregulated expression of endothelin-1 in parasitized myocardial tissues. Another evidence linking *T. cruzi* function to microvascular derangement came from research on pro-inflammatory proteolytic responses evoked by trypomastigotes. Using the Dm28 strain as a model, we have previously shown that tissue culture trypomastigotes (TCT) evoke neutrophil-dependent plasma leakage through mechanisms involving cooperation between TLR2, CXCR2 and bradykinin B2 receptors (BK2R). Here we examined the possibility that the endothelin pathway integrates the above mentioned activation axis. Using the hamster cheek pouch as a model, control experiments showed that topically applied TCT induced the accumulation (15-60 min) of rhodamine-labeled leukocytes in microvascular beds. These effects were markedly reduced either by BQ-788 (ETBR antagonist) or BQ-123 (ETAR antagonist), however, these ETR antagonists did not interfere with the parasite competence to evoke plasma leakage. Notwithstanding this, measurements of footpad swelling made 3 h p.i. Balb/c mice indicated that the inflammatory edema was drastically reduced by the ETR antagonists (BQ-123 or BQ-788) or by HOE-140 (BK2R antagonist). We then asked if TCT were able to invade mammalian cells *in vitro* through the signaling of ETRs. First, we found that BQ-123 or BQ-788, respectively inhibited extent of infection of CHO cells overexpressing ETARs or ETBRs. Invasion assays with mouse cardiomyocytes or human smooth muscle cells revealed that parasite-uptake was partially, albeit significantly inhibited by BQ-123, BQ-788 or HOE-140, whereas HUVECs were protected by BQ-788 or HOE-140, but not by BQ-123. Interestingly, host cell invasion was not further reduced by the combined addition of ETR antagonists and HOE-140. These results indicate that kinin-releasing strains of *T. cruzi* elicit the activation of BK2R and ETAR/ETBR in interdependent manner. Further studies may clarify if the ETR/BK2R pathway jointly contributes to infection-associated vasculopathy in Chagas' disease. Supported by CNPq and FAPERJ

## BC.23 – TGF-BETA EXERTS DIFFERENTIAL EFFECT ON EXTRACELLULAR MATRIX REMODELING OF TRYPANOSOMA CRUZI- INFECTED CULTURES.

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Extracellular matrix (ECM) components are involved in the pathogenesis of the chronic phase of Chagas' disease due to its progressive accumulation. TGF-beta, a cytokine related to ECM stimulation, is implicated with chagasic fibrosis. The fact that cardiac cells present low responsiveness to TGF-beta stimulus *in vitro* opened the question whether TGF-beta treatment alters ECM remodeling after infection of different cell types with *T. cruzi*. Confocal laser scanning microscopy revealed enhancement of FN signal in cardiomyocytes (CMs) stimulated with doses higher than 10ng/ml of TGF-beta as previously demonstrated. Curiously, addition of only 1ng/ml of TGF-beta resulted in augmented FN fibril formation in skeletal myoblasts (L6E9 cells) and cardiac fibroblasts. In contrast, *T. cruzi* infection (Y strain) reduced fibrillar FN matrix in CM and L6E9, while uninfected cells in *T. cruzi*-infected culture presented FN staining similar to control. Remarkably, FN disorganization in highly infected cells was visualized even after addition of 10-15 ng/ml doses of TGF-beta. In contrast, *T cruzi* infection seems not to alter FN distribution in cardiac fibroblasts. Western blot assay revealed a raise of 2.39 and 3.35 folds in FN levels in L6E9 stimulated with 1 ng/ml and 10 ng/ml of TGF-beta, respectively. In CMs, only a 15ng/ml dose of TGF-beta induced a raise of 2 fold in FN expression, suggesting that CM are less responsive to TGF-beta treatment than skeletal mioblasts and cardiac fibroblasts concerning FN expression. This differential response can be due to distinct intracellular signaling mechanisms, since skeletal myoblasts presents phosphorylated Smad 2 levels 3 folds higher than cardiomyocytes. This way, our data raises new perspectives to evaluate alterations in TGF-beta signaling and the mechanisms that result in ECM reduction in *T. cruzi* infected cells, including cytoskeleton alterations and receptors modulation. Supported by CNPq, FAPERJ, FIOCRUZ, PAPES V.

## BC.24 - *TRYPANOSOMA CRUZI*: *IN VITRO* AND *IN VIVO* EFFECT OF CARRAGEENAN

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Carrangeenans (CAR) are heterogeneous mixtures of sulfated polygalactans obtained from the cell wall of *Chondrus crispus*, a red alga found on rocky areas of the Atlantic coast of Europe and North America. CAR inhibit the replication of viruses (dengue, herpes simplex and hepatitis A), bacteria such as *Helicobacter pylor*i (Girond et al. 1991; Utt 1997; Carlucci et al. 1999; Adams et al., 2005; Buck et al, 2008) and also the binding of growth factors, such as transforming growth factor-1 (TGF-1), fibroblast growth factor-2 (FGF-2) and platelet-derived growth factor (PDGF) to cells, modulating cell invasion and proliferation. However, only a few studies were done investigating CAR effect on others pathogenic protozoa, including *Trypanosoma cruzi*. At present, the only accepted drugs for treatment of Chagas disease are nifurtimox and benznidazole. This controversy is primarily due to the undesirable side effects that frequently force the abandonment of treatment and poor indices of apparent cure from the disease (Janin and Villa 2007; Soeiro and De Castro 2009). In the present work the objective was verify the effects of CAR on *T. cruzi* evaluating both *in vitro* and *in vivo* systems. In conclusion we observed that CAR administration in Swiss mice before parasite inoculation provoked inhibition of the multiplication of circulating parasites, suggesting an antiparasite response produced by CAR.
Supported by FIOCRUZ and FAPERJ

## BC.25 - COMPARATIVE ANALYSIS OF *TRYPANOSOMA* OF SUBGENUS *SCHIZOTRYPANUM* FROM BATS AND HUMAN

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Within the genus *Trypanosoma*, species of the subgenus *Schizotrypanum* can invade and develop in mammalian cells. Here we analysed *T. dionisii* from bats, which does not infect humans, *T. cruzi* strain Y from Chagasic patient and an isolate of a new lineage of *T. cruzi* associated with bats (TcBat). Metacyclic trypomastigotes of Y strain and TcBat efficiently infected human epithelial cells in medium containing serum whereas infection by *T. dionisii* was effective in PBS containing $Ca^{2+}$/$Mg^{2+}$, in absence of glucose or aminoacids. The ability of Y strain and TcBat metacyclic forms to enter host cells was mediated by the surface molecule gp82 whereas *T. dionisii* apparently relied on mucin-like molecules. Expression of members of gp82 family was detected in Y strain and TcBat but not in *T. dionisii* by monoclonal antibody (mAb) 3F6 and anti-gp82 polyclonal antibodies. Analysis of genomic organization of gp82 gene family, and the chromosomal mapping of gp82 genes, showed distinct profiles in these parasites. Profiles of surface mucin-like molecules were also different in Y strain, TcBat and *T. dionisii.* Unlike the double bands of 35 and 50 kDa detected by mAb 2B10 in immunoblots in different *T. cruzi* strains examined to date, TcBat metacylic trypomastigotes exhibited bands of approximately 90, 55 and 30 KDa. When administered orally into mice, Y strain metacyclid forms produced high parasitemias, compatible with the gastric mucin- binding and epithelial cell invasion properties of gp82. Although expressing gp82 at high levels, TcBat metacyclic forms were poorly infective in mice by the oral route, what may be due to their relative susceptibility to complement-mediated lysis. In contrast to Y strain, TcBat metacyclic forms were lysed upon incubation with undiluted normal human serum as source of complement. *T. dionisii* metacyclic forms, which are highly susceptible to complement-mediated lyis, failed to infect mice. Supported by FAPESP and CNPq.

## BC.27 - IDENTIFICATION AND CLASSIFICATION OF NC-RNAS IN *TRYPANOSOMA CRUZI*: A MULTISTEP APPROACH

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Non-coding RNAs (ncRNAs) prediction has become a vast field of research and several classes of ncRNAs with different regulatory, catalytic and structural functions have been discovered. Few years ago, some kinetoplastid genomes have been finalized, and a recent study to predict ncRNAs in *Leishmania braziliensis* and *Trypanosoma brucei* has been published. Similarly, we propose to predict and classify ncRNAs for the complete genome of *Trypanosoma cruzi*. For this purpose, we used eQRNA, an algorithm for comparative analysis of biological sequences that performs probabilistic inference on genomic alignments. The entire genomes of *T. brucei* and *T. cruzi* were used to generate the initial alignments submitted to eQRNA, and 4195 ncRNA candidate sequences equal to or longer than 30 nucleotides were found. The candidate sequences were used for blastx search (e-value = 10e-05) against *T. cruzi* annotated proteins. 2816 candidates matched protein-coding sequences and the remaining 1382 candidates were submitted to a pipeline that included search against 25 different ncRNA databases, ab initio RNA tools and structural analysis. 1301 candidates had no evidence to be classified as ncRNAs and 49 candidates are tRNAs or rRNAs. Twenty-nine candidates presented similarity with ncRNAs from several databases. Our next goal is to identify putative regulatory ncRNAs that may be directed to UTR elements by matching the 29 ncRNAs to a catalog of 5' and 3' UTR sequences of *T. cruzi* transcripts retrieved from dbEST. *In silico* approaches concerning energy parameters will be employed to test the validity of these findings.
Supported by CAPES, Faperj, Fapemig

## BC.28 - THE DIFFERENTIAL ROLE OF LYSOSOMAL PROTEINS LAMP-1 AND LAMP-2 IN HOST CELL INVASION BY *T. CRUZI* TISSUE CULTURE TRYPOMASTIGOTES

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Many pathogens have developed mechanisms to prevent fusion with lysosomes during cell invasion. For *Trypanosoma cruzi* (etiologic agent of Chagas disease), however, lysosomes are essential in this process, participating in the formation of the parasitophorus vacuole and in the anchoring of parasites inside host cells. Lysosomal anchorage of *T. cruzi* inside host cells is probably a consequence of the interaction of parasite surface with lysosomal integral membrane proteins. Among the lysosomal integral membrane proteins, the main ones are LAMP-1 and 2 (Lysosomal Associated Membrane Protein-1 and 2), belonging to groups lgpA and lgpB, respectively. Recently we have demonstrated that lack of this two proteins have a profound effect on host cell infection by *T. cruzi*. Invasion assays studies of *T. cruzi* trypomastigotes in fibroblasts derived from mice knocked out for these two proteins (LAMP-1/2 KO cells) showed a reduction of 50% in parasite cell entry when compared to wild type (WT) fibroblasts. On the other hand, they also revealed that parasite intracellular development is higher in LAMP-1/ 2 KO when compared to WT cells. Whether the phenotypes observed are a consequence of the absence of both isoforms concomitantly is still uncertain. Despite being both highly sialilated proteins and presenting similar structure and biochemical properties, LAMP-1 and 2 are distinct proteins that have diverged during evolution. Intraspecies comparison between the two groups, A and B, show that despite their high similarity they are still less related to each other than are, for example, mouse and mammalian proteins from the same group. Therefore, more studies are needed to reveal the unique role of each isoform of LAMP in the interaction of the parasite with the host cell. In order to investigate this issue we decided to study whether these proteins have functional differences in the interaction process of the cell with the parasite. We then performed the same invasion assays described above, now using LAMP-1 or 2 single Knock out cells (LAMP-1 KO or LAMP-2 KO). Preliminary results indicate a differential role for each LAMP isoform. Cells lacking LAMP1 (LAMP-1 KO cells), at first, seem to better reproduce the results obtained with LAMP-1/2 KO cells, showing a reduction in *T. cruzi* host cell entry as compared to WT cells. On the other hand, cells lacking LAMP-2 (LAMP-2 KO cells) showed parasite invasion rates more similar to WT cells. Thus, these results suggest the existence of a differential role of these proteins in the invasion of this parasite in the host cell. We are now investigating the effect of the absence of each LAMP isoform in the intracellular development of *T. cruzi*, aiming to determine whether the isoforms also have different roles in the intracellular multiplication of the parasite. Supported by CNPq and FAPEMIG

## BC.29 – DISORGANIZATION OF TGF-BETA RECEPTOR TYPE II COSTAMERIC DISTRIBUTION IN CARDIOMYOCYTES AFFECTS TGF-BETA RESPONSE: ROLE OF *TRYPANOSOMA CRUZI* INFECTION

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Transforming growth factor beta (TGF-beta) family cytokines have been found to regulate growth, differentiation, immune response and fibrosis. TGF-beta is involved in Chagas disease establishment and progression, participating in *T. cruzi* host cell invasion, intracellular parasite cycle, regulation of immune response and heart remodeling. However, TGF-beta receptors in the host cell have been poorly studied. Since previous reports demonstrated that *T. cruzi* can modulate host cell receptors, we were interested to analyze the TGF-beta receptor type II (TbetaRII) expression and distribution during *T. cruzi* – cardiomyocyte interaction. TbetaRII staining by indirect immunofluorescence revealed an unexpected striated organization of in cardiomyocytes, which was enhanced (38%) after TGF-beta treatment. Double labeling with anti-vinculin and anti-TbetaRII antibodies showed a co-localization of TbetaRII with costameres of vinculin by confocal microscopy. The association of TbetaRII with the cytoskeleton was also demonstrated by cytochalasin D treatment, which resulted in a decrease of 45.3% in the ratio of cardiomyocytes presenting TbetaRII striations. This association of TbetaRII with the cytoskeleton may be involved in triggering TGF-beta signaling, since western blot analysis showed that cytochalasin D significantly inhibited Smad 2 phosphorylation and fibronectin stimulation after TGF-beta treatment in cardiomyocytes. *T. cruzi* infection elicited a decrease of 79.8% in the frequency of cardiomyocytes presenting TbetaRII striations. The treatment of *T. cruzi*-infected cultures with TGF-beta did not provoke any significant alteration in the frequency of TbetaRII striations, still showing low TbetaRII striation percentage, in contrast with the raise observed in control cultures. Together, these results suggest that the co-localization of TbetaRII with costameres are important to activate TGF-beta signaling cascade, and *T. cruzi* derived cytoskeleton disorganization could result in altered or low TGF-beta response in infected cardiomyocytes. **Supported by:** FIOCRUZ, FAPERJ, PAPES V and CNPq

## BC.30 - INFLUENCE OF ASF1 AND THE ACETYLATION OF HISTONE H4 IN THE DNA DOUBLE STRAND BREAK IN *TRYPANOSOMA*

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Histone post-translational modifications are involved in replication, transcription, chromatin assembly and DNA repair. We have previously identified acetylations at the lysine residues 4, 10 and 14 of the histone H4 of *Trypanosoma cruzi.* K10 and K14 were also shown to increase after gamma irradiation (Nardelli et al, 2009, Chromosoma, 118:487). To understand how these modifications are associated with the DNA repair process, we overexpressed the histone H4 with the lysine 4, 10 and 14 replaced by arginine. Parasites containing mutated K10 and K14 were more sensitive to irradiation. Resistance to irradiation can be increased by overexpressing TcRad51, a protein that is required for DNA repair by homologous recombination (HR). In this overexpressor, K10 and K14, but not K4 modifications are already increased in non-irradiated cells. Upon irradiation, immunofluorescence labeling with specific antibodies for K10 and K14 modifications were found to colocalize with TcRAD51 in the nucleus. We also investigated the effect of overexpression of the histone chaperone Anti-silencing factor 1 (Asf1) in *Trypanosoma brucei.* Asf1 stabilizes the heterodimer H3/H4 and forms a complex with the histone acetyltransferase 1 (Hat1) that acetylates histone H4 at position K5 and K12 just after the synthesis. These modifications seem to correspond to K4 and K10 of *Trypanosoma* histone H4. Overexpressors of Asf1 are more sensible to DNA damage induced by gamma irradiation. Taken together, these results suggest that histone H4 acetylations at K10 and K14 residues participate in repair mechanism of double strand breaks in *Trypanosoma.* Supported by FAPESP and CNPq

## BC.31 - EXPRESSION AND CHARACTERIZATION OF CLATHRIN HEAVY CHAIN IN *TRYPANOSOMA CRUZI*

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Clathrin-coated vesicles mediate cellular endocytosis of nutrients and molecules that are involved in a variety of biological processes. Basic components of the vesicle coat are clathrin heavy chain (Chc) and clathrin light chain molecules. Previous ultrastructural studies in *Trypanosoma cruzi* epimastigote and trypomastigote forms have indicated the presence of coated pits and vesicles at the flagellar pocket region, which were morphologically similar to clathrin-coated vesicles. Thus, in the present study we have identified, cloned and expressed the gene corresponding to the clathrin heavy chain of *T. cruzi*. Thereafter, we have produced a mouse polyclonal antibody and expression of the protein in epimastigote and trypomastigote forms was confirmed by Western blot. Cellular localization of clathrin in epimastigote forms was performed by immunofluorescence using a Leica SP5 confocal laser microscope, showing a location close to the flagellar pocket region. Further studies are underway to demonstrate the subcellular localization of clathrin heavy chain in epimastigotes and trypomastigotes by immunocytochemistry (transmission electron microscopy). Supported by CNPq and Fiocruz.

## BC.32 - EXPRESSION AND CHARACTERIZATION OF CLATHRIN LIGHT CHAIN IN *TRYPANOSOMA CRUZI*

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The molecules involved in transport of nutrients from the cell plasma membrane into the cytoplasm (endocytosis) are poorly known in *Trypanosoma cruzi*. Coated vesicles have been already shown in the cytoplasm of *T. cruzi* epimastigotes and trypomastigotes, suggesting that this parasite is able to perform clathrin-mediated endocytosis. The clathrin protein is formed by the assembly of three heavy chains and three light chains. A previous study has indicated the expression of clathrin heavy chain in *T. cruzi*, but nothing is known about the light chain. Therefore, aim of this work is to express and characterize the subcellular localization of the clathrin light chain in *T. cruzi*. For this, we have identified the gene in the genome of this parasite, which was amplified and cloned into bacterial vectors for the expression of recombinant proteins. Specific antibodies were produced in mice and these antibodies were used to evaluate the gene expression, using immunoassays, immunofluorescence and immunocytochemistry. Our data indicate the expression of the clathrin light chain in *T. cruzi*, as demonstrated by Western blot, cellular localization by immunofluorescence using confocal laser microscopy, and subcellular localization by transmission electron microscopy by using immunocytochemistry. Characterization of the expression of clathrin light chain in *T. cruzi* helps to shed some light on the processes of endocytosis performed by this parasite, which are essential for obtaining nutrients and survival of the parasite inside the hosts.Supported by CNPq.

## BC.33 - TRANSFERRIN RECYCLING IN EPIMASTIGOTES FROM *TRYPANOSOMA CRUZI*

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Eukaryotic cells internalize a wide range of molecules from the extracellular medium. One of the most extensively studied endocytic system has been the process of transferrin uptake. In mammal cells, transferring is recycled back to the cell surface coupled to its receptor. *Trypanosoma cruzi* has a characteristic organelle called reservosome, considered the final organelle of the endocytic pathway. Reservosomes are acidic compartments where endocytic cargo is stored and eventually degraded. It also concentrates proteolytic enzymes, such as cruzipain and serine carboxypeptidase, and has been considered as a late endosome or a lysosome like organelle. In 2000, Mendonça *et al* a localized TcRab11, a homologue of the molecular marker of recycling endosomes in mammal cells, at the reservosomes. In order to investigate the reservosome role as a recycling compartment, we used FITC-transferrin or gold-labeled transferrin as tracers. After 30 min of incubation with the tracer, when it is concentrated inside reservosomes, parasites was washed and incubated in fresh medium. We collected the supernatants after different times and quantified the tracer in a microplate reader. The amount of FITC-transferrin in the supernatant increased with time, decreasing in cells concomitantly. We did not detect the presence of gold labeled transferrin in the supernatants. It is possible that gold coupling impairs transferrin from going on the recycling route. Considering that we are assaying recycling from reservosomes, it is possible that transferrin arrives in the supernatant total or partially degraded. To investigate this possibility, we are performing the whole assays in the presence or absence of the inhibitors of the main reservosome proteases. Intact FITC-transferrin or its fragments were detected in the supernatant by western blot using anti-FITC antibodies. We are using the same strategy in electron microscopy assays to follow the exocytic pathway of transferrin and/or transferrin fragments from reservosomes towards cell exterior. Supported by: CAPES, FAPERJ, CNPq

## BC.34 - VARIATIONS IN THE FORMS OF RNA POL II IN THE PARASITE *TRYPANOSOMA CRUZI*

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RNA polymerase II is a large, multisubunit enzyme which catalyzes the transcription of protein coding genes in eukaryotic cells. The largest subunit of RNAP II (Rpb1) contains a highly conserved heptapeptide repeat at its C-terminus (CTD) which is absent in trypanosomes. The CTD is modified by phosphorylation. The enzyme switches between the phosphorylated state, referred to as RNAP IIo and the unphosphorylated state, referred to as RNAP IIa. These variations are control switching from transcription initiation to transcription elongation, transcription termination, mRNA capping, RNA splicing and RNA polyadenylation. It has been shown recently that in *T. brucei* CTD is modified by phosphorylation, despite the lack of the heptapeptide repeats. In the present work, we subjected *T. cruzi* parasite cultures to a series of different stimulus in order to analyze the variations of the phosphorylated state of Rpb1. Our results showed that during exponential growth there was a predominance of the Rpb1 IIo form in strains Y, DM28c and CL Brener. Stationary phase was marked by an increase of the levels of Rpb1 IIa form in the same strains. Addition of proflavine (a transcription inhibitor) led to a slightly decrease in the Rpb1 IIo form and a marked increase in the Rpb1 IIa form in DM28c and CL Brener strains. When these same strains were subjected to heat shock, soon after the first 30 minutes at 42ºC the IIo form of Rpb1 decreased markedly while IIa form increased substantially. During metacyclogenesis using DM28c strain, both Rpb1 IIo and IIa forms remained equal throughout the process. Our results lead us to conclude that RNA pol II state in *T. cruzi* varies switching between two forms, one possibly phosphorylated and other unphosphorylated.
Supported by Fapesp and CNPq.

## BC.35 - BIODEMES, ISOENZYMES AND MOLECULAR GENOTYPING OF *TRYPANOSOMA CRUZI* STRAINS FROM SANTA CATARINA STATE (2005) ACCORDING WITH NEW CONSENSUSFOR MOLECULAR NOMECLATURE (2009).

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In 2005 an outbreak of acute Chagas disease occurred in Santa Catarina State-Br. Twenty four persons became infected by the ingestion of sugar cane juice accidentally contaminated with this parasite in the locality of Navegantes, with three fatal cases. We received 09 strains isolated from the human cases, identified as SC94, SC95, SC96, SC97, SC98, SC99, SC100, SC101, SC102, and 2 cultures isolated in the same area from one marsupial (*Didelphis aurita*) SC90, and from a Triatomine naturally infected *(T. tibiamaculata)* SC93. *Biodemes* characterization was performed after 2 to 5 passages in mice to establish the patterns of parasitemia, mortality rates, histopathological alterations, virulence and pathogenicity. The tissue lesions in the different groups, revealed early lesions in the myocardium and skeletal muscles characteristics of the *Type II Biodeme* . In six cases the presence of late alterations together with increasing of parasitemic levels suggested double infection with *Types II / III Biodemes* corresponding to Taxas *T.cruzi II* and *T. cruzi I (1999).* For isoenzymic characterization the enzymes ASAT, ALAT, PGM and GPI were used. With the Biological methods, the strains were identified as from the *Biodeme Type II* but the isoenzymic patterns were variable, suggesting the mixture of two types of zymodemes Z1 and Z2. The findings of the present investigation are in accordance with the molecular characterization established in the 2009 Consensus, which was afterwards performed by analyzing three polymorphic genes (Cytochrome Oxidase –COII, spliced leader intergenic region - SL-IR, and 24Sα rRNA genes and six microsatellite loci (SCLE10, SCLE11, MCLF10, TcAAAT6, TcTAC15 and TcTAT20). The molecular characterization confirms that the majority of the isolates were classified into T. cruzi II lineage, but three isolates were composed by populations mixture : Tc I + Tc II or TcII + Tc VI. Supported by: FAPESB, FAPEMIG, CNPq and CAPES

## BC.36 - A ROLE OF TRANSLATION INITIATION FACTOR 2 PHOSPHORYLATION IN THE DIFFERENTIATION OF *TRYPANOSOMA CRUZI*

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Control of translation initiation has an important role in the adaptation to nutrient stress in many eukaryotes. One of the key regulatory steps in protein synthesis occurs through the phosphorylation of the alpha subunit of the translation initiation factor 2 (eIF2α) by a family of proteins kinases activated under stress conditions. The phosphorylation of the serine 51 in mammalian eIF2α inhibits the GDP/GTP exchange in eiF2 preventing the formation of new t-RNA/GTP/eiF2 complexes, which are required to the start the scanning of mRNAs. In trypanosomatids eIF2α has an extra N-terminus domain and alignments with several eukaryote eIF2α revealed that Ser 51 corresponds to the Thr 169 and that an additional serine at position 43 could also be phosphorylated. Here we show that specific antibodies against a peptide containing phosphorylated Thr 169 and antibodies to mammalian Ser 51 recognizes in western blot a protein of 50 kDa corresponding to eIF2α of *Trypanosoma cruzi* epimastigotes. The reactivity of these antibodies increases upon several nutritional stress, which decrease protein synthesis and are able to induce differentiation into metacyclic forms of the parasites. *T. cruzi* expressing eIF2a mutants (T169A and S43A) were generated and used to confirm the lack of reactivity in these cases. We are currently investigating whether parasites over expressing these mutants are able to differentiate after nutritional stress.
Supported by Fapesp and CNPq.

## BC.37 - TOR-like 1 kinase is involved in the control of osmotic stress response in *Trypanosoma brucei*

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Target of rapamycin (TOR) kinases are highly conserved protein kinases that integrate signals from nutrients and growth factors to coordinate cell growth and cell cycle progression. Two TOR kinases were described with the function of controlling cell growth in the protozoan parasite *Trypanosoma brucei,* the causative agent of African trypanosomiasis. However, two additional sequences were described as encoding putative kinases with high domain similarity to TOR kinases in *T. brucei*: TbTOR-like 1 and TbTOR-like 2. Here we studied the TOR-like 1 protein, which contains an unusual PDZ domain, not found in any other TOR kinase. PDZ domains are though to be involved in protein-protein interactions, mediating binding of a class of submembranous proteins to membrane receptors and ion channels. We previously found that ablation of TbTOR-like 1 by RNAi causes a progressive inhibition of cell proliferation with parasites accumulating in S/G2 phase of the cell cycle and showing increased cell size. Moreover, RNAi cells presented an increase in the acidocalcisomes size and higher levels of polyphosphate and pyrophosphate content. Now we show that TbTOR-like 1 localizes to unique cytosolic granules. After hyperosmotic stress, the localization of the protein shifts to the cell periphery, differently from other organelle markers. We also found that cultivating *T. brucei* under hyperosmotic conditions reduced parasite growth and increased the levels of polyphosphate within acidocalcisomes. Importantly, TbTOR-like 1 ablation resulted in an increment of polyphosphate early after induction and before growth arrest. These results suggest that TbTOR-like 1 kinase participates in the control of osmotic stress response as a consequence of changes in the acidocalcisomes and polyphosphate content in *T. brucei*. The data establishes a link between the control of cell growth by TOR kinases and the polyphosphate metabolism.
Supported by: FAPESP, CNPq and Ellison Medical Foundation.

## BC.38 - The subcellular localization of phosphatidylinositol-related kinase TOR1 and TOR 2 (Target Of Rapamycin 1 and 2) in *Trypanosoma cruzi* is distinct from that in *Trypanosoma brucei*

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Target of rapamycin (TOR) is a serine/threonine (phosphatidylinositol-related) kinase that couples nutrient availability to activation of processes including metabolism, transcription and translation that lead to cell growth. TOR participates in the maintenance of energy and amino acid homeostasis. Upon nutrient starvation or energy depletion, TOR activity is inhibited, triggering processes such as autophagy aimed to maintain cellular viability and overcome unfavorable conditions. TOR acts as two functionally and structurally distinct complexes, TORC1 and TORC2. This dual function of TOR confers the capability of governing spatial and temporal cell growth separately. TORC1 controls temporal aspects of cell growth through processes such as ribosome biogenesis, transcription, translation and repression of autophagy, while TORC2 controls spatial aspects of cell growth by actin cytoskeleton remodeling. The overall TOR functions may be conserved in early-branching eukaryote. However, several new findings have emerged that differ from those described for other eukaryotes. Localization of signaling molecules is key in regulating their function and specificity. *Trypanosoma brucei* TOR, TbTOR1, has been shown to be predominantly nuclear, and TbTOR2 associated with the endoplasmic reticulum (ER) and mitochondria. Surprisingly, the subcellular localization of *Trypanosoma cruzi* TOR1 and TOR2 (TcTOR1 and TcTOR2) is completely distinct from that previously observed in *Trypanosoma brucei*. ***Rabbit and mouse serum raised against specific TcTOR1 and TcTOR2 peptides coupled to KLH were IgG-purified*** by using ***biochemical affinity column.*** **T. cruzi** ***was fixed and imaged by*** three-dimensional (3D) microscopy. Unlike TbTOR1, TcTOR1 is excluded from nucleus, concentrated at the posterior portion in punctuated compartments which coincide with reservosomes. TcTOR2, unlike TbTOR2, is excluded from ER and mitochondria and is dispersed in the cytoplasm, concentrating around TcTOR1 location. This unusual localization of TOR proteins may shed new lights to TOR function in metabolism, differentiation and invasion process of *Trypanosoma cruzi*, which are the current challenges of our group.Supported by: FAPESP

## BC.39 - IDENTIFICATION AND ALIGNMENT OF THE GENES CORRESPONDING TO THE FOUR SUBUNITS OF THE ADAPTOR COMPLEX 1 IN *TRYPANOSOMA CRUZI*

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Adaptor complexes are formed by heterotetrameric subunits and play a role in both recruiting clathrin and binding to membrane receptors to make endocytic vesicles. In eukaryotic cells the AP1 complex is involved in the traffic of vesicles that carry acid hydrolases between the Trans Golgi Network and the lysosomes. The AP1 adaptor complex is formed by two large (γ and β1), a medium (μ1) and a small (σ1) subunits. Genes encoding the AP1 proteins have been already described as part of the genome of *Trypanosoma cruzi*. Aim of this work is to further characterize and align the genes corresponding to the four subunits of the AP1 adaptor complex in *Trypanosoma cruzi* and other pathogenic trypanosomatids. Furthermore, the genes identified in T. cruzi were compared with corresponding genes found in human (*H. sapiens*), fruit fly (*D. melanogaster*), rooster (*G. gallus*) and fish (*D. rerio*), in order to identify the similarity and conservation rates. A genomic data bank search allowed the identification of all genes encoding the four subunits of the AP1 adapter complex in *T. cruzi*. A comparison between these genes and those of other higher eukaryotic organisms showed a low identity (between 30 to 50%), as expected for the evolutive divergence of this protozoan parasite. On the other hand, a higher identity (between 50 and 70%) was observed when *T. cruzi* genes were compared with those of other trypanosomatids (*L. brasiliensis, L. major, L. infantum and T. brucei*), with the highest identity between *T. cruzi* and *T. brucei*. These results indicate that due to its important role in the cell metabolism the AP1 complex adaptor has been conserved in the eukaryonts. Studies are underway to obtain antibodies against the *T. cruzi* AP1 subunits in order to perform subcellular localization of the proteins.
This work has been supported by CNPq and Fiocruz.

## BC.40 - SEARCHING FOR THE CONTROL OF NUCLEAR DNA REPLICATION IN TRYPANOSOMES

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Chromosomal replication initiates with the assembly of the prereplication complex (pre-RC) at replication origins. In eukaryotes, the pre-RC is composed of ORC complex containing six proteins, Orc1-Orc6, two proteins named Cdc6 and Cdt1, and the minichromosome mainenance (MCM) complex, which is composed of Mcm2 to Mcm7 proteins and presents helicase activity, essential for DNA replication. As long as the pre-RC is organized on the chromatin, origins become licensed to replicate. Since ORC, Cdc6, and Cdt1 are required for loading MCM onto DNA, but are not required for the continued MCM-DNA interaction, the downregulation of their expression and/or activity at the end of G1 represents, in eukaryotes, an effective way to block DNA replication. Trypanosomes do not contain the complex ORC, Cdc6 or Cdt1. Instead, they contain a protein homologous to Orc1 and Cdc6, named Orc1/Cdc6 that are a component of pre-RC. Orc1/Cdc6, however, does not seem to be involved at DNA replication control, since it is bound to DNA during the entire cell cycle. Therefore, we asked if Mcm proteins could be involved in this control in trypanosomes. In this work, we searched for sequences in trypanosomes databases and we found 10 genes for *T. cruzi* and 8 genes for *T. brucei* annotated as Mcms. By alignment analysis we identified a putathive Mcm7 gene. *T. cruzi* Mcm7 was then cloned and expressed by a prokaryote. The recombinat protein rTcMcm7 was used to immunize mouses. The obtained antibody was able to recognize the *T. cruzi* recombinant Mcm7 protein as well as the putative *T. brucei* recombinant Mcm7, expressed by Sf9 cells using the bacullovirus system. The anti-rTcMcm7 serum will be used in western blotting and immunofluorescence assays in order to analyze the expression and localization of Mcm7 during the cell cycle of trypanosomes.
Supported by FAPESP

## BC.41 - DETERMINATION AND EVALUATION OF TRACE ELEMENTES OF DOGS NATURALLY INFECTED WITH *Leishmania (Leishmania) infantum*

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Canine Visceral Leishmaniosis is a chronic and systematic disease involving many organs. The trace elements cupper (Cu), iron (Fe) are related to the immunological effectiveness. Cu alterations are associated to anemia, abnormal collagen synthesis and Fe is related of parasite-host interactions, Nitric oxide (NO) synthesis and fibrogenesis. Several works have related Cu and Fe with diseases, but this isn´t well defined in dogs, and neither is a possible correlation with CVL. Graphite Furnace Atomic Absorption Spectrometry was used for trace elements detection. Morfometric studies were done for Fe (Prussia Blue) and Cu (Timm`s) in tissue samples. NO detection was done by Griess technique. The results obtained for variables pyrolysis and atomization temperatures were: 1460 - 2090 $^{o}$C for Cu and 1530 - 2500 $^{o}$C for Fe. We found matrix effect for Fe but not for Cu. Permanent modifiers using Ruthenium for Cu and without modifier for Fe. Canine sera were collected and distribute: 9 from uninfected dogs, 9 from symptomatic (SD) and 4 from asymptomatic (ASD). Statistical difference shown higher levels of Cu and Fe serumin ASD than SD and controls group. In contrast, morfometrical analysis showed higher deposition of Fe in all tissues samples of SD than AS and control groups. In parallel, we observed higher levels of NO in serum of AS than SD and control groups. However, no correlation was found between Fe serum and NO serum levels. Chronic infection is correlated to Fe deposition in tissues, NO disturb and worse prognostic. In this study we have found this Fe deposition mainly in SD dogs in parallel to lower levels of Fe serum. However, Fe and Cu serum levels were not concluded yet and even with NO serum levels correlations. Thus, weare improving our results to make correlations to the progression of the CVL. Supported: FAPEMIG nº14138

## BC.42 - THE ROLE OF ADENOSINE ON *Leishmania (Leishmania) amazonensis* METACYCLOGENESIS

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Metacyclogenesis is an important stage in the life cycle of parasitic trypanosomatids in which infective forms of the parasite are generated. However, little is known about the conditions in which these forms are generated. In this work, we investigated the effects of adenosine in the in vitro development of infective metacyclic promastigotes of *L. amazonensis*. Parasites were cultured in the presence of CGS15943, a potent adenosine receptor antagonist. CGS15943 treated cultures showed a significant increase in the number of metacyclic promastigotes (10X) if compared to the control culture, as assessed by morphology, motility and isolation in density gradient. This increased metacyclogenesis also correlated with a significant increase in survival (15X) to complement-mediated lysis and a low adherence to a sand fly gut by CGS15943 treated parasites. To investigate possible changes on parasite infectivity, peritoneal macrophages were inoculated with promastigotes obtained from control or CGS15943 treated cultures and the amount of infected cells was highly increased in the group inoculated with treated parasites. Thereafter, C57BL6 mice were inoculated on the ear with a low dose ($1x10^3$) of control or treated promastigotes. Both lesion size and tissue parasitism were significant increased in the group inoculated with parasites obtained from the treated culture. Interestingly, metacyclogenesis induction was completely reversed in *L. amazonensis* cultured in the presence of CGS15943 plus adenosine. Corroborating these results, we found increased levels of metacyclogenesis in parasites incubated with dipyridamole, a specific adenosine transport inhibitor. Moreover, ours results indicate that the effects observed for *L. amazonensis* was conserved for other species of trypanosomatids like *L. brasiliensis*, *L. major*, *L. chagasi* and *T. cruzi*. In conclusion, our data suggest that acquisition and/or metabolism of adenosine is important to control the differentiation of *L. amazonensis* promastigotes into infective forms. Supported by CNPq, CAPES, FAPEMIG and Rede Mineira de Bioterismo.

## BC.43 - THE POSSIBLE RELATIONSHIP BETWEEN ECTO-ENZYMES AND DRUG TRANSPORT IN RESISTANT *LEISHMANIA*

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Ecto-nucleotidases are surface membrane-bound enzymes able to hydrolyze extracellular nucleotides. Some functions are suggested for these proteins: cell adhesion, purine acquisition, protection against cytotoxic effects of extracellular ATP and, recently, MDR phenomenon. P-glycoprotein (P-gp) is involved in the removal of drugs, most of them positively charged, from the cytoplasm. P-gp is also associated with movement of ATP, an anion, from the cytoplasm to the extracellular space. The central question of this study is to establish the relationship between ecto-enzyme activities and a possible mechanism of drug transport in *Leishmania amazonensis. L. amazonensis* promastigotes were selected by gradual increasing concentrations of the vinblastine from 10 µM to 100µM, and the cells were maintained continuously under drug pressure. The cells were used to determine $Mg^{+2}$ dependent ecto-ATPase activity by measuring $^{32}Pi$ release from the substrate $\gamma^{32}Pi$ ATP. In addition, ecto-phosphatase, ecto-5'- and ecto-3'-nucleotidases were assessed by colorimetric method by the complex formation of Pi (Fiske and Subbarow, 1925). Our results show that ecto-ATPase activity from resistant *Leishmania* presented a higher activity rate compared with wild-type *Leishmania* (8,15 ± 0,74 and 19,94 ± 0,6 nmolPi x h x $10^7$cels respectively). This increase is progressive with increasing drug concentrations. In addition, other ecto-enzyme activities did not presented this increment with drug pressure. We compared the ecto-ATPase activity in cells resistant to another drug. In cells resistant to chalcone the ecto-ATPasic activity is about three times higher than in with type cells. This initial result suggests a possible relationship between resistance and ecto-ATPase activity and this can contribute to elucidate the mechanism of drug transport. Supported by: CNPq, FAPERJ, IOC/FIOCRUZ.

## BC.44 - HISTOPATHOLOGICAL AND PARASITOLOGICAL STUDY OF THE GASTROINTESTINAL TRACT OF DOGS NATURALLY INFECTED WITH *LEISHMANIA (LEISHMANIA) INFANTUM*

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The aim of this study was to provide a systematic pathological and parasitological overview of the gastrointestinal tract (GIT) of dogs including the stomach, duodenum, jejunum, ileum, cecum and colon, naturally infected by *Leishmania*. An epidemiological survey of 20 mongrel dogs with visceral leishmaniasis was carried out by the municipality of Belo Horizonte, MG, Brazil. The infected animals had an increased number of macrophages, plasma cells and lymphocytes. Parasite distribution in the GIT was evident in all intestinal segments and layers of the intestinal wall (mucosal, muscular and submucosal) irrespective of the clinical status of the animals. However, the parasite load was statistically higher in the cecum and colon than in other segments of the GIT. The high parasite burden evident throughout the GIT mucosa without marked pathological alterations led us to consider whether *Leishmania* gains an advantage from the intestinal immunoregulatory response (immunological tolerance). Sponsors (FAPEMIG Processo 15548/2009) ; CNPq (Processo 473601/2009-5), UFMG.

## BC.45 - CYTOPHATIC EFFECTS OF *TRITRICHOMONAS FOETUS* ON BOVINE OVIDUCT CELLS-COMPARISON WITH *TRICHOMONAS VAGINALIS*

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*Tritrichomonas foetus* is an extracellular parasite of the reproductive tract in cattle. In order to investigate the cytophatic effects of *T. foetus* in deeper parts of the reproductive tract, a bovine primary oviduct epithelial cell system (BOECs) was developed. Reproductive tracts were obtained from cows and the effect of co-incubation of *T. foetus* and *T. vaginalis* with BOECs was analyzed by scanning, transmission and fluorescence microscopy. Viability tests were performed using colorimetric methods, *TUNEL* (*Terminal deoxynucleotidyltransferase-mediated dUTP nick-end labeling*), JC-1 and annexin-V. The results reported here demonstrate that: (1) the *in vitro* oviduct epithelium has been shown to be useful in interaction experiments with *T. foetus* and *T. vaginalis*; (2) *T. foetus* adheres to the BOECs as single separate cells, and posteriorly the cells aggregate in large clusters; (3) *T. foetus* provoked a severe damage to BOECs leaving imprints in the epithelial cells, wide intercellular spaces, and provoking large lesions in the epithelium; (4) no species-specific host-parasite interactions was observed; (5) fresh *T. vaginalis* is more aggressive to BOECs than *T. foetus*, but long-term *T. vaginalis* has similar effects to *T. foetus*. Here we show that both *T. vaginalis* and *T. foetus* are able to adhere and damage bovine oviduct cells and lead to cell death showing no species- specificity. Thus, both parasites are likely to be important in mediating infertility, since oviduct is the natural passage of early stage of embryo development. Our findings raise the possibility that trichomonads are able to attack the oviduct and thus could contribute to infertility in cows. Further studies *in vivo* are in course to elucidate this proposal.
Supported by AUSU, CNPq, FAPERJ, and PRONEX.

## BC.46 - ROLE OF THE CALCIUM INDEPENDENT PHOSPHOLIPASE A2 IN DISTRIBUTION INTRA / EXTRACELLULAR ACID PHOSPHATASE ACTIVITY IN *LEISHMANIA AMAZONENSIS*

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Compartments of the endocytic and exocytic pathways in protozoa of the Trypanosomatidae family present structural and functional characteristics distinct from those described for the same compartments in mammalian cells. The dynamics of these compartments is of fundamental importance in processes such as secretion, removal and exposure of molecules on the surface of the parasite thus modulating process like adhesion, virulence, and defense against the host immune system. Although the structure and composition of many compartments of the endo/exocytic pathway in *Leishmania* have been elucidated, very little is known about mechanisms controlling their recognition and fusion. Several proteins, such as phospholipase A2 (PLA2), are involved in the process of fusion of membrane bound compartments in different cells. In this study, we investigated the effect of Bromoenol lactone (BEL), a specific and irreversible inhibitor of a calcium independent phospholipase A2 (iPLA2), on the intra and extracellular activity of different acid phosphatases in *Leishmania amazonensis*. Parasites were grown for 72h in Schneider medium, incubated for 1 h with 2.5 μM of BEL and analyzed by ultrastrucutral cytochemistry and biochemical assays. The enzymatic activity detected in the cell culture medium was reduced by 43% after BEL treatment with an accumulation in the cell as demonstrated by the total extract analysis of the parasite. Cytochemical analysis of BEL treated parasites showed a significant reduction in the electrondense labeling of the flagellar pocket membrane with an accumulation in the multivesicullar tubules, in the Golgi and in different vesicular and tubular compartments near the flagellar pocket region. Another important finding on effect of BEL was the significant reduction of the number of membrane bound vesicles within the flagellar pocket. The results suggest that iPLA2 is involved in the control fusion of different compartments of the exocytic pathway in *Leishmania*. Supported by CNPq, CAPES, FAPERJ

## BC.47 - CELL DAMAGE BY *LEISHMANIA AMAZONENSIS* LEISHPORIN: AN ATOMIC FORCE MICROSCOPY STUDY

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Leishporin is a pore-forming cytolysin produced by species of the Genus *Leishmania*. Because it is optimally active at acidic pH (5,5) and at 37 ℃, we have postulated that it may act in the mammalian host, being involved in phagolysosome and plasma membrane rupture, crucial steps for parasite survival and infection recrudescence. In previous works, we showed that leishporin does not need proteins or carbohydrates as receptors to lyse cells. We found that lipids from target membranes are sufficient for cytolysin binding and membrane rupture. In the present work we studied the damage caused on target membranes by leishporin using the Atomic Force Microscopy tapping-mode technique. As membrane models, we used erythrocytes and DPPC-liposomes both highly susceptible to leishporin activity. After hemolytic or lipsomes-lysis assays we analyzed the damage caused by parasite extracts in both membrane surfaces. The images obtained showed pore-like forms in both models. The circular structures measured about 25-200 nm of diameter and presented 4-8 nm of depth, the latter being sufficient to cross lipid bilayer. All observed structures are certainly enough to permeate the used membranes models and to lead to lysis. This work provided for the first time a visual evidence of leishporin activity. Support: OMS – CNPq – FAPEMIG – CAPES.

## BC.48 - ROLE OF HEME-OXYGENASE 1 (HO-1) IN RESPONSE TO *LEISHMANIA CHAGASI* INFECTION WITHIN MURINE MACROPHAGES

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Visceral leishmaniasis (VL) is commonly associated with hematologic manifestations. In this scenario, mechanisms related to hemolysis, released of heme and activity of the enzyme heme oxygenase 1 (HO-1) may be involved in the immunopathogenesis of VL. However whether HO-1 influences *Leishmania (L.) chagasi* infection, etiologic agent of VL in Brazil, is poorly comprehended. Here, we evaluate the role of HO-1 in *L. chagasi* infection of murine peritoneal macrophages from C57BL/6 mice stimulated with thioglycolate and infected in presence of CoProtoporphyrinIX (CoPPIX) the HO-1 inducer, besides one of the products of HO-1 activity (Biliverdin). *L. chagasi* infection induced HO-1 production compared to uninfected macrophages. The percentage of infected macrophages and quantity of amastigotes by 100 macrophages was increased with CoPPIX treatment to infected macrophage. Beyond this, we observed that bone marrow derived macrophages knockout to HO-1 gene have a significant low parasite load when infected by *L. chagasi* than their wild type counterparts. Furthermore, upregulation of HO-1 by CoPP resulted in inhibition of TNF- α, IL-1β, IL-6, MCP-1, PGE-2 and Nitrite levels upon LPS stimulation and simultaneously induced a higher IL-10/TNF-α ratio in peritoneal macrophages contributing to the anti inflammatory pathway that favors *L. chagasi* replication. Our findings are the first evidence that HO-1 is induced during *L. chagasi* infection and favors an increased parasite load, suggesting an anti inflammatory mechanism of HO-1 in VL, an important hemolytic parasite disease. Supported by: CNPq

## BC.49 - CANINE VISCERAL LEISHMANIASIS LIKE A FIBROTIC DISEASE MODEL

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The aim of this work was study the extracellular matrix alterations in liver, spleen, cervical lymph nodes, lungs and kidneys in symptomatic dogs naturally infected with *Leishmania (Leishmania) chagasi* correlating with clinical and anatomical pathological aspects. This study was carried out with 30 dogs, divided at two groups: six not infected animals (group control) and twenty four symptomatic infected animals. All them was mongrel dogs with undefined age, obtained from the municipality of Santa Luzia/MG and Ribeirão das Neves/MG municipalities. The group denominated symptomatic was composed by animals with classical clinical signals of the disease as skin lesions (alopecia, eczemas and ulcers), loss weight and lymphopathy. Paraffined sections of the tissues were stained by Hematoxylin-Eosin (HE); Gomori's ammoniacal silver staining for reticular fibers and strepto-avidin peroxidase Immunohistochemical method for tissue *Leishmania* amastigotes detection. The tissue images were transferred to a computer video screen by means of the software KS300 and relayed to a computer-assisted image analysis system (Kontron Elektronic/Carl Zeiss, Germany) for morphometrical analysis. A significant increasing of collagen deposition in all organs was found when compared to the controls. Positive correlation between the parasite load and collagen deposition was found in all organs expect the lungs of infected animals. The organs that showed higher colagenogenesis were livers, kidneys and lungs. Our results have indicated that canine visceral leishmaniasis is a fibrotic disease model.
Supported by CNPq.

## BC.50 - IDENTIFICATION OF *TRITRICHOMONAS FOETUS* PSEUDOCYSTS IN FRESH PREPUTIAL SECRETION SAMPLES FROM BULLS

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*Tritrichomonas foetus* is a serious veterinary pathogen that causes bovine trichomonosis, a sexually transmitted disease that eventually leads to abortion and infertility. *T. foetus* has a simple life cycle that consists of only a trophozoitic form. During unfavorable environmental conditions, the trophozoites, which are polar and flagellated, can adopt a spherical shape and internalize their flagella. These rounded organisms are known as pseudocysts. Although it is currently assumed that *T. foetus* pseudocyst formation is reversible and that it represents a response to stressful conditions, there are no reports showing the presence of this form *in vivo*. For this reason, the aim of this study was to verify whether *T. foetus* pseudocysts are encountered in naturally infected bulls. Towards this goal, fresh preputial samples obtained from seven mature bulls that were naturally infected with *T. foetus* were analyzed using complementary techniques, such as video microscopy, fluorescence microscopy, scanning and transmission electron microscopy. The analyses revealed that approximately 55% of the parasites were in pseudocyst form in each preputial sample, whereas approximately 25% of *T. foetus* displayed pear-shaped bodies. Previous research demonstrated that *in vitro T. foetus* pseudocysts are able to divide by a budding process. Here, this division mode was observed in approximately 20% of fresh *T. foetus* obtained from preputial bovine samples. Thus, this study shows that in infected bulls, pseudocysts are present and occur more frequently than the pear-shaped parasites. Because *T. foetus* pseudocysts are capable of generating multinucleated organisms that release single organisms when environmental conditions become favorable, we hypothesize that *T. foetus* might be more likely transferred to a new host as a pseudocyst than as a piriform cell, which could therefore contribute to a more efficient infection of the new host. Supported by CNPq, FAPERJ, PRONEX, AUSU.

## BC.51 - ULTRASTRUCTURAL ALTERATIONS INDUCED BY $\Delta^{24(25)}$-STEROL METHYLTRANSFERASE INHIBITORS ON *TRICHOMONAS VAGINALIS*

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*Trichomonas vaginalis* is an important human parasite that causes trichomoniasis, a cosmopolitan sexually transmitted disease. Currently, the treatment of choice for *T. vaginalis* infections is metronidazole. However, the increase in metronidazole-resistant trichomoniasis and the common and undesirable side effects of this drug make the search for alternative chemotherapeutic approaches a top priority for the management of this disease. In the present work, the antiproliferative and ultrastructural effects of sterol biosynthesis inhibitors against *T. vaginalis* were investigated. It was found that 22,26-azasterol and 24(*R,S)*,25-epiminolanosterol, known inhibitors of $\Delta^{24(25)}$-sterol methyltransferase, exhibited antiproliferative effects on *T. vaginalis* trophozoites cultured *in vitro*. Morphological analyses showed that azasterols induced changes in the ultrastructure of *T. vaginalis*. The most significant alterations were (1) membrane blebbing and disruption, (2) cell wrinkling and (3) the formation of cell clusters. In addition, autophagic vacuoles, Golgi duplication arrest, an abnormal Golgi enlargement and damaged hydrogenosomes were also observed. Nonspecific cytotoxicity assays using the cultured mammalian cell lines MDCK showed no effect of the azasterols on the viability and proliferation of these cells at a concentration that significantly inhibited the proliferation of *T. vaginalis*, indicating a selective antiparasitic action. Taken together, these results suggest that azasterols could be important compounds in the development of novel chemotherapeutic approaches against *T. vaginalis*.
Supported by CNPq, PRONEX, FAPERJ, AUSU.

## BC.52 - Identification of Lipid Rafts-like in *Tritrichomonas foetus*

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*Tritrichomonas foetus* is a parasite that causes bovine trichomoniasis, a sexually transmitted disease that eventually leads to abortion and infertility. *T. foetus* is an extracellular parasite that adheres to epithelial cells, however, the cellular mechanism by which colonizes mucosal surfaces is not well defined. The involvement of lipid rafts has been described on parasite adhesion to host cells. However, these membrane domains have not been reported in *Trichomonas* yet. Thus, the purpose of this study was to identify rafts-like domains in *T. foetus*. For this, *T. foetus* was cultured in serum-free medium. In some trials, rafts were disrupted by Methyl-β-cyclodextrin (MBCD) or filipin for 30min. To determine whether *T. foetus* cells possess rafts-like microdomains and to distinguish them from more-fluid-phase membrane regions, cells were stained with either the order-preferring lipid analog DiIC16 or the non-order-preferring lipid analog FAST-DiI with or without chemical previous disruption of rafts . The cells were then fixed and observed in the fluorescence microscope. In addition, cells were stained with cholera toxin B subunit FITC conjugated (FITC-CTX-B), another marker of membrane rafts. Both lipid analogs and FITC-CTX-B were found in the plasma membrane and in some intracellular organelles of *T. foetus*. Treatment with MBCD or filipin abolished staining in the plasma membrane by $DiIC_{16}$. In addition, disrupted treatment resulted in alteration of the $DiIC_{16}$ staining pattern. Similar results were observed after FITC-CTX-B labeling. As expected, the raft-disrupting agents did not alter the FAST-DiI staining. Taken together, these data authenticate the colocalization of DiIC16 and FITC-CTX-B with cholesterol-rich membrane regions and suggest the existence of rafts-like domains in *T. foetus*. We verified that these raft-like microdomains had no effect on adhesion of the parasites to MDCK monolayer. However, the lipids rafts might be necessary for other events concerning cytotoxicity exerted by *T. foetus*.
Supported by CNPq, FAPERJ, PRONEX, AUSU

## BC.53 - CYTOTOXIC EFFECTS EXERTED BY *TRITRICHOMONAS FOETUS* PSEUDOCYSTS

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*Tritrichomonas foetus* is a parasite of cattle and other animals that has a simple life cycle consisting only of a trophozoitic form. Under unfavorable environmental conditions, the trophozoites, which are polar and flagellated, can adopt a spherical shape and internalize their flagella. These rounded organisms are known as pseudocysts. It is currently believed that this form is reversible and that its formation represents a defense mechanism against stress conditions. However, there are still several open questions about pseudocyst biology, such as if it is cytotoxic. Consequently, the aim of the present study is to assess whether pseudocysts exert cytotoxic effects in interaction with epithelial cells and compare their behavior to the behavior of the pear-shaped parasites. To clarify these questions, a long-term grown and a fresh *T. foetus* isolates were used and interaction trials of both parasite forms with MDCK (an epithelial kidney canine cell) were carried out. These interactions were analyzed using complementary techniques, such as light and electron microscopy. Cytotoxicity assays were performed using the MTT (Methylthiazolyldiphenyl-tetrazolium bromide) viability staining method. This work demonstrates that both *T. foetus* isolates were able to exert cytotoxic effects in host cells. However, the fresh isolate provoked a higher damage in MDCK cells when compared with the long-term *T. foetus* strain. In both isolates, pseudocysts were more cytotoxic when in contact with host cells as compared to the flagellated pear-shaped parasites. Our results suggest that the *T. foetus* pseudocyst might be a more aggressive and infective form.
Supported by AUSU, CNPq, FAPERJ, and PRONEX.

## BC.54 - MORPHOLOGICAL CHANGES IN THE CYST WALL OF THE PARASITE *GIARDIA LAMBLIA* DURING EXCYSTATION PROCESS

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Differentiation from one life cycle stage into another is an elegant adaptation by which many parasites ensure their transmission and survival. The protozoan *Giardia lamblia* is a major cause of water-borne diarrheal disease. This parasite exhibits two forms in its life cycle that includes trophozoite and cyst. The cyst presents a lower metabolic rate than trophozoites and it can survive in water for weeks. Encystation and excystation are crucial processes for establishment and maintenance of *Giardia* infection. The cyst wall of the parasite is known to be composed by carbohydrates and proteins and it provides the resistance of the cyst. Few studies were performed using the excystation as a model. Therefore, the aim of this study is to analyze the ultrastructural modifications of the cyst wall during the excystation process. Trophozoites of *G. lamblia* were grown in TYI medium and induced to encystation and excystation *in vitro*. The encystation was verified with immunofluorescence assay using the monoclonal antibody anti-CWP. The excystation process was analyzed using complementary techniques, such as scanning and transmission electron microscopy. A field emission scanning electron microscopy (FESEM) was used to compare the mature cysts and the beginning of excystation process. The *in vitro* encystation was performed successfully, because a positive staining was observed using the anti-CWP antibody. The following alterations in cell morphology during the excystation were seen: (1) a change in the cyst shape and (2) the presence of new electron-dense vesicles close to the cyst wall. In addition, the fibrillar composition of the cyst wall was better analyzed with the use of FESEM. In mature cysts, the microfibrils of the cyst wall were compacted although during of the excystation process this tight arrangement was lost. Our results suggest that the modifications of cyst wall fibrils are necessary for the development of excystation process.
Supported by AUSU, CNPq, FAPERJ, and PRONEX.

## BC.55 – THE BACTERIUM ENDOSYMBIONT OF *CRITHIDIA DEANEI* UNDERGOES COORDINATED DIVISION WITH THE HOST CELL STRUCTURES

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In trypanosomatids, cell division involves morphological changes and requires coordinated replication and segregation of single copy organelles as the nucleus, kinetoplast, flagellum and basal body. Some trypanosomatids also present a symbiotic bacterium in their cytoplasm that co-evolves through a mutualistic relationship with the host protozoan. Previous studies have reported that this symbiont divides in synchrony with the host structures in such way that each daughter cell carries only one bacterium. Thus, symbiont-bearing trypanosomatids constitutes an interesting model to understand the relationship between cell cycle and organelle division processes. In the present work, we used light and electron microscopy techniques to describe the morphological events that occur during *Crithidia deanei* cell cycle, in particular the chronological division of the symbiont relative to other trypanosomatid structures. Immunofluorescence assays showed that the symbiont can present different shapes and positions during the protozoan cell cycle. Furthermore, we obtained clear evidences that the endosymbiont divides before the basal body and kinetoplast segregation and that the nucleus is the last organelle to divide, just before cytokinesis. The basal body position was not seen in coincidence with that of the symbiont during all *C. deanei* cell cycle, indicating that this microtubule-organizing structure is not directly involved in the bacterium division and segregation.These results are based on counts of a thousand cells, where structures of interest were labeled with specific antibodies and DNA-binding compounds, as DAPI. Preliminarily assays using inhibitors to DNA polymerase and to eukaryotic protein synthesis blocked the nucleus and kinetoplast segregation, as well as the symbiont duplication, suggesting that bacterium division is coordinated with other trypanosomatid structures. Supported by CNPq, CAPES and FAPERJ.

## BC.56 - EFFECT OF THE MAIN CONSTITUENTS OF ESSENTIAL OILS FROM *SYZYGIUM AROMATICUM* L., *THYMUS VULGARIS* L. AND *CYMBOPOGON CITRATUS* (DC) STAPF., ALONE OR COMBINED, ON THE TRYPANOSOMATID PROTOZOAN *CRITHIDIA FASCICULATA*

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Essential oil is a complex mixture of secondary metabolites produced by plants. Those oils, or their compounds, have a broad spectrum of pharmacological effects including effects against some parasites that cause human parasitoses. For example, it has been demonstrated that some essential oil have activity against *Trypanosoma cruzi*, *Leishmania amazonensis* and *L.chagasi*. In this work we analyzed the combination of eugenol, thymol and citral, which are the major compounds of the essential oil of *Syzygium aromaticum* L. (clove), *Thymus vulgaris* L. (thyme) and *Cympobogon citratus* (DC) Stapf (lemon grass),respectively, on the growth of *C. fasciculata*. Initially the components were added separately at different concentrations to cultures of *C. fasciculata* in the exponential growth phase and then used to calculate the $IC_{50}$ (dose that inhibited the growth of the culture at 50%) and $IC_{90}$ for each component after 24 hours of treatment. Thymol gave the best results when given alone, with $IC_{50}$ = 32.5 µg/ml and $IC_{90}$ = 62.5 µg/ml, followed by citral ($IC_{50}$ = 76.28 µg/ml, $IC_{90}$ = 146.05 µg/ml) and eugenol ($IC_{50}$ = 93.75 µg/ml, $IC_{90}$ = 300 µg/ml). When we applied the combination of the constituents the best result was obtained with the combination of the three components, resulting in the decrease of $IC_{50}$ dose to 16.66 µg/ml of thymol, 39.12 µg/ml of citral and 48.08 µg/ml of eugenol. Treatment with benznidazole at concentrations ranging 20-500 µg/ml did not affect culture growth. Analysis by SEM demonstrated that treatment with constituents has led to a rounding of the protozoa body. Our data showed that the combination of constituents of essential oils led to an increase in inhibitory activity on growth of *C. fasciculata*, suggesting that this procedure can be effective when applied against pathogenic trypanosomatids. Supported by CNPq.

## BC.57 - LIPID BODY INDUCTION IN MACROPHAGES BY *CRITHIDIA DEANEI* AND *TOXOPLASMA GONDII*

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Lipid bodies (LB) are organelles rich in lipid present in many cell types modulated by specific signals such as interaction with parasites and apoptotic cells. Apoptotic cells and some parasites expose phosphatidylserine (PS) at their plasma membrane. This phospholipid might be involved in the induction of LB. To test this hypothesis LB in macrophages were analyzed after the interaction with *Crithidia deanei* and *Toxoplasma gondii* (RH and ME-49, virulent and less virulent strains respectively). *C. deanei* presented an exponential growth until day 4, followed by a sharp decline at day 6, and a gradual decrease up to day 21; no morphological change during the growth curve was detected. *C. deanei* cultured for 3 and 8 days and tachyzoites of both *T. gondii* strains interacted with macrophages for 1h and the LB induction was assayed by Nile red staining after 24h. Macrophages cultured with fetal bovine serum (FBS) were used as negative control due to the low induction of LB. *C. deanei* cultured for 8 days induced more LB than the ones cultured for 3 days. The number of necrotic cells in axenic culture increases along culture time and may be inducing LB in macrophages during the interactions with *C. deanei*. However, purified necrotic *C. deanei* was unable to induce LB in macrophages. The exposure of PS in *C. deanei* revealed a 4 fold increase of the PS positive population in cells from 8 days culture compared to those from 3 days. Both *T. gondii* strains were able to induce LB in macrophages similarly. These results indicate that both protozoa induce LB in macrophages. The hypothesis that PS exposure by these protozoa may be responsible for the induction of LB in macrophages is being further investigated.
Supported by CAPES, CNPq, FAPERJ and UENF

## BC.58 - CELL CYCLE STUDY OF *BLASTOCRITHIDIA CULICIS*, AN ENDOSYMBIONT-HARBORING TRYPANOSOMATID

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As other member of the Trypanosomatidae family, *Blastocrithidia culicis* presents single-copy structures, as the nucleus, the kinetoplast and the flagellum. This species also contains a symbiotic bacterium, which establishes an obligatory relationship with the host protozoan. Investigation of the cell cycle in symbiont-harboring trypanosomatids suggests that the bacterium divides in coordination with other host cell structures, as the nucleus and kinetoplast. In this work we used optical and electron microscopy methods to study the *B. culicis* cell cycle. The immunofluorescence approach showed that the symbiont presents different shapes, sizes and positions in the host as the cell cycle proceeds. Thus, at the beginning of the cell cycle, the bacterium is located in the posterior region of the protozoan cell body and presents an elongated rod-shape form. Later on, the symbiont lies down over the protozoan nucleus and duplicates. Thereupon both symbionts acquire a constricted shape, remaining in the posterior end of the host cell body. During cytokinesis the cleavage furrow position ensures that each daughter cell will inherit a single endosymbiont. The rod shape bacterium measures about 1.5µm, while the constricted or dividing form may vary in size from 2.5µm to 4µm. Both shapes present approximately 0.5µm in diameter. The symbiont format was also studied by transmission electron microscopy and analysis by scanning electron microscopy was useful to characterize the protozoan cytokinesis. Preliminary tests using aphidicolin, a DNA polymerase inhibitor promoted cell proliferation arrest in *Blastocrithidia culicis*. Thus, our next step is to check if the bacterium division proceeds, even if nuclear DNA duplication is blocked.
Supported by CNPq e FAPERJ

## BC.59 - THE ENERGETIC METABOLISM IN *CRITHIDIA DEANEI*, AN ENDOSYMBIONT-HARBORING TRYPANOSOMATID

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In trypanosomatids two organelles are involved in ATP production: the mitochondrion and glycosomes, an special type of peroxisomes Some monoxenous trypanosomatids harbor an intracellular bacterium which co-evolves with the host protozoan through a mutualist relationship, thus constituting an excellent model to study organelle origin and cellular evolution. The presence of the endosymbiont is associated to morphological alterations in the host protozoan and an intense metabolic exchange occurs between both partners. Conversely, the symbiont is capable of obtaining part of the required energetic molecules from the host glycosomes. It is well established that endosymbiont-bearing strains presents a lower generation time and a higher metabolic capacity than the aposymbiotic cells. Our previous results showed that the symbiont-containing strain of *Crithidia deanei* presents a higher $O_2$ consumption when compared with endosymbiont-free cells. In this work, 3-D reconstruction revealed that glycosomes are usually around the endosymbiont. This proximity suggests the occurrence of metabolic exchanges between this organelle and the symbiotic bacterium. The mitochondrial metabolism of the symbiont-bearing strain was investigated by using inhibitors of the respiratory chain. The obtained results showed: no effect by oligomycin (0.5 – 16 µg/mL), an inhibitor of FoF1ATP synthase; stimulus of the $O_2$ uptake up to 30% after using 2.5 µM carbonyl cyanide p-trifluoromethoxyphenylhydrazone (FCCP), a proton ionophore that uncouples $O_2$ consumption from ATP synthesis; and 71.5 % decrease of $O_2$ comsuption after treatment with 1.4 mM cyanide, a complex IV inhibitor. After cell fractioning, isolated mitochondria presented higher rates of $O_2$ consumption when compared to symbionts. Our data showed that the $O_2$ uptake rates are similar when assays are performed in Warren's medium or in Krebs-Ringer solution. Our next goal is to investigate the $O_2$ consumption by the aposymbiotic strain and to study the endosymbiont influence on the host respiration. Supported by: FAPERJ

## BC.60 - MORPHOLOGICAL ASPECTS OF *TOXOPLASMA GONDII*-FELINE ENTEROCYTES INTERACTION *IN VITRO*

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Primary cultures of the feline intestinal epithelial cell (FIEC) have significant importance for the study of the normal development and differentiation of intestinal epithelium. Many mechanisms involved in the pathogenesis of chronic enteropathies or host-pathogen interactions in feline intestine have not been elucidated so far. This cellular model potentially applies to the investigation of the infection process provoked by enteropathogenous, in particular to the protozoan, *Toxoplasma gondii*, a coccidian which has the feline as its definitive host, and which maintains its sexual life cycle in the feline gut. Primary cultures of FIECs obtained of fetus small gut of feline collected surgically were cultivated and infected after seven days with bradyzoites forms isolated of intracerebral cysts collected of C57BL6 mice. The analysis of interaction *T. gondii*-enterocytes showed that the parasite-host cell ratio could be pointed out as a decisive factor which determines the intracellular fate of bradyzoites forms. The development of the syncytial forms of *T. gondii* was observed using the 1:20 bradyzoites-host cell ratio resulting in similar forms described in *in vivo* systems. After 6 days of parasite-host cell interaction, it was possible to show by ultrastructural analysis vacuoles containing parasites inside a vacuolar matrix full of the well developed tubulovesicular membrane network like schizont of the sexual cycle of *T. gondii* as previously described in the *in vivo* system. This alternative study potentially opens up the field for investigation of the molecular aspects of this interaction that can contribute to the developing of new strategies for intervention in one of the main routes by which toxoplasmosis spreads. Supported by FAPERJ, IOC/Fiocruz and CNPq

## BC.61 - *TOXOPLASMA GONDII* DECREASES THE EXPRESSION OF CADHERIN IN SKELETAL MUSCLE CELL

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Mouse primary culture of skeletal muscle cells (SkMC) was employed as a model for experimental toxoplasmosis studies. In the present study, we examined: (i) the influence of *T. gondii* infection on the myogenesis process; (ii) the parasite's role on M-cadherin expression by infected SkMC and, (iii) its correlation with the myogenesis process. The M-cadherins (M for muscle) are involved in the initial cell-cell recognition, allowing initiation of myoblasts fusion to form multinucleated myotubes. We observed that even with a relation of only 1:1 (parasite-cell host) after 24 h of interaction, the infection was of only 43% leading to inhibition of 75% on the myogenesis process. The modulation of cadherin expression during *T.gondii*-SkMC interaction was investigated. Initialy, we demonstrated the cadherin localized at the contact areas between myoblasts and myotubes during the myogenesis process by confocal microscopy. SkMC infected with *T. gondii* analyzed by immunofluorescent and immunoblotting assays after 24 h of interaction showed that a reduction of 54% in expression of cadherin protein, leading the inhibition of the cell membrane fusion process. By PCR assays we analyzed the regulation of M-cadherin (M for muscle) by mRNA levels in SkMC in the presence and absence of infection by *T. gondii.* Our data demonstrated reduction the M-cadherin mRNA expression after 3h of interaction being higher after 24 h. These data corrobore the suggestion of that the *T. gondii* is able negatively to modulate the cadherin expression, interfering molecularly with the surface of host cell, inhibiting the membranes fusion and consequently affect the myogenesis process.
Suportted by: FIOCRUZ/IOC, FAPERJ and CNPq

## BC.62 - KOJIC ACID, A SECONDARY METABOLITE PRODUCED BY THE FUNGUS *Aspergillus sp*., WAS NOT ABLE TO CONTROL THE *in vitro* DEVELOPMENT OF *Toxoplasma gondii* IN MACROPHAGES.

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Toxoplasmosis is a highly prevalent infection disease caused by *Toxoplasma gondii*, which is an obligate intracellular protozoan parasite. The disease is an important issue for public health due the existence of just a few drugs that are used to treat patients. Kojic acid, a secondary metabolite from *Aspergillus sp.*, increases the microbicidal capacity of macrophages against *Leishmania amazonensis*. For this work, we have tested if this metabolite may control *T. gondii* infection *in vitro*. Thus, the progress of *T. gondii* infection in culture of macrophages treated or not with this metabolite was assayed. To this end, the J774.A1 macrophage cell line was seeded in 24-well plates on coverslips, treated with the metabolite, activated or not with lipopolysaccharide and interferon-gamma, and infected with *T. gondii*. Coverslips and supernatants were collected after 2, 24 and 48 hours of infection, to evaluate the entrance and the development of the parasite and NO production. The metabolite treatment was not able to control *in vitro* infection of *T. gondii* in resident and activated macrophages. NO production was not altered after the metabolite treatment of non-infected macrophages. Furthermore, treatment with the metabolite was not able to revert the inhibitory capacity on NO production caused by *T. gondii* infection. *T. gondii* development was similar between treated and control group during 2, 24 and 48h of interaction. Therefore, this metabolite was not able to inhibit *T. gondii* growth.
Supported by CAPES, CNPq, FAPERJ, UENF.

## BC.63 - OSTRICHES MONOCYTE-DERIVED MACROPHAGES CONTROL *TOXOPLASMA GONDII* GROWTH AFTER ACTIVATION WITH LIPOPOLYSACHARIDE

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Ostriches have gain attention as a relevant economic poultry. *Toxoplasma gondii* serum positive ostriches have been reported, thus, this bird is a potential intermediate host for human toxoplasmosis. However, little is known about its immunology, including macrophage biology, and *T. gondii* cellular infection. The aim of the present study was to adapt a methodology to obtain ostriches macrophages derived from blood monocytes and to study their behavior when infected with tachyzoites of the RH strain of *T. gondii*. Blood was collected, leukocytes separated by centrifugation, and cultured over glass coverslips in 24-well plates with Dulbecco's Modified Eagle's Medium containing 10% fetal bovine serum for 6 days at 37℃ in a 5% $CO_2$ atmosphere. One day before experiments, half of the cells were activated with 0.1 µg/mL of lipopolysaccharide. Cells were infected with *T. gondii*, washed, cultured for 24h, fixed and the culture supernatant collected. The NO production was evaluated indirectly by measuring nitrite in macrophage culture supernatants by the Griess reagent. The percentage of infected macrophages and the mean number of *T. gondii* in macrophages were scored under a light microscope. Lipopolysaccharide did not induce NO production. However, this treatment turned macrophages more microbicidal as seen by a reduction of the percentage of infected macrophages and of the mean number of *T. gondii* in macrophages after 24h of culture. In non-activated macrophages parasites grew as expected. These results indicate that macrophage of ostrich were activated by LPS *in vitro* becoming more microbicidal against *T. gondii*. However, the microbicidal mechanism was not related to NO production. More studies are in progress to better understand the relationship between ostrich macrophage and *T. gondii*. Supported by CAPES, CNPq, FAPERJ and UENF

## BC.64 - KINETICS OF NITRIC OXIDE INHIBITION AND PERSISTANCE OF TWO *TOXOPLASMA GONDII* STRAINS WITH DIFFERENT VIRULENCE

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Toxoplasmosis is a worldwide disease caused by *Toxoplasma gondii*. Like others obligate intracellular parasites, *T. gondii* can inhibit the microbicidal action of the host. One of the mechanisms in infected macrophages may be related to the exposure of phosphatidylserine (PS) by tachyzoites that causes the disappearance of the enzyme iNOS, which catalyzes the production of the microbicidal agent nitric oxide (NO). To better understand this mechanism, mouse peritoneal macrophages were activated with interferon-gamma and lipopolysaccharide and infected with tachyzoites of the RH (virulent) and ME-49 (less virulent) strains. Phosphatidylserine exposure was assayed by flow cytometry. Macrophages were infected with parasites for 2h and cultured up to 96h. Infectivity and development of *T. gondii* in macrophages were analyzed by Giemsa staining. NO production was measured by Griess reagent and iNOS localized by immunofluorescence. Tachyzoites recovered from infected macrophages by mechanical disruption were inoculated in mice to determine its infectivity. Both strains exposed PS, infected macrophage and caused disappearance of iNOS after 2h of interaction in a similar way. NO production after 24 and 48h was effectively inhibited by RH that persisted in iNOS negative macrophages. ME-49 strain infected macrophages produced intermediate levels of NO after 24h and showed similar production to non-infected macrophages after 48h, no parasites were detected inside macrophages at this period. Macrophages infected with ME-49 strain for 24 and 48h expressed iNOS as non-infected cells, confirming NO production results. Recovered RH tachyzoites from infected macrophages at all time points killed infected mice, but no cysts were found in the brains of mice inoculated with recovered ME-49. Collectively, these results indicate that both strains have the ability to initially inhibit NO production by the disappearance of iNOS probably because of PS exposure; however, ME-49 is killed probably by another microbicidal mechanism and cannot persist in activated macrophages. Supported by CAPES, CNPq, FAPERJ and UENF

## BC.65 - ANALYSIS OF THE INDUCTION OF NEUTROPHIL EXTRACELLULAR TRAPS (NETS) BY *Toxoplasma gondii*

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*Toxoplasma gondii* is an obligate intracellular parasite that can invade any nucleated cell from warm blood animals. *T. gondii* is the etiologic agent of toxoplasmosis, a disease with worldwide distribution. Toxoplasmosis can be a serious and deadly disease for unborn children and immunocompromised patients. Neutrophils are essential for innate immune response, fundamental to control the infection, being the first cells to be recruited at the inflammation sites. They kill pathogens either by phagocytosis or by releasing cytoplasmic granules. Recently, a new mechanism of neutrophil cell death was described: NETosis. It involves the release of neutrophil extracellular traps (NETs) rich in DNA and proteins, such as histones and elastase. These NETs can immobilize and kill bacteria, fungi and parasites, also providing a high concentration of antimicrobial molecules. We evaluated if *T. gondii* is able to induce NETs release. Human neutrophils were incubated with *T. gondii* of the RH strain harvested from 48h in vitro cell cultures at several ratios, for 30 minutes. The samples were observed by immunofluorescence (IFA) and scanning electron microscopy (SEM). The amount of DNA released was measured from the culture supernatant by the picogreen method. In IFA assays DNA was stained with DAPI to localize the NETs, the parasites were labeled with SAG-1. Many parasites were seen trapped in NET filaments, maintaining their normal shape. These results were corroborated by SEM where we visualized the ultrastructural aspects of these extracellular traps in close contact with the parasite. Analyzing the quantification of DNA, it is possible to conclude that *Toxoplasma* is able to induce NET release. We will evaluate NET toxicity to the parasites to determine whether they are dead or just immobilized by these webs. Supported by CNPq and FAPERJ

## BC.66 - BRADYKININ B2 RECEPTORS ARE NOT INVOLVED IN THE INVASION PROCESS OF *TOXOPLASMA GONDII* INTO MICE MACROPHAGES

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Bradykinin B2 receptors (BK2R) are implicated in *Trypanosoma cruzi* invasion of cardiovascular cells and *Leishmania chagasi* infection of macrophages. In both systems parasite proteases mediates the release of kinins from kininogens essential for invasion. Considering that *Toxoplasma gondii* relies on serine proteases activity to invade mammalian cells, we evaluated if BK2R may be involved in invasion and development of this parasite in macrophages as well as in the modulation of nitric oxide production. To this end, mice peritoneal macrophages and two cell lines (J774-A1 and alveolar) were seeded on coverslips in 24-well plate, activated with lipopolysaccharide and interferon-gamma and cultured with Dulbecco's modified Eagle's medium supplemented with 10% fetal bovine serum at 37℃, 5% $CO_2$ atmosphere. Macrophages were incubated for 1 hour with Hoe-140 and infected with *T. gondii* of the RH strain. Coverslips were collected at 2 and 24 hours after infection, stained with Giemsa and parasite invasion and development evaluated by direct count; nitric oxide was evaluated by the Griess reagent. HOE-140 decreased the entry of *T. gondii* in activated and resident macrophages; however, the values were not statistically significant. Moreover, the development of *T. gondii* and nitric oxide production of macrophages treated with HOE-140 was similar to control cells. These data suggest that the BK2R is not essential to the entry of *T. gondii* in the different lineages of macrophages.
Supported by CAPES, CNPq, FAPERJ and UENF

## BC.67 - *TOXOPLASMA GONDII* EVADES NITRIC OXIDE DEPENDENT IMMUNITY OF INTESTINAL EPITHELIAL CELLS (IEC-6).

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*Toxoplasma gondii*, the agent of toxoplasmosis, is an obligate intracellular protozoan able to infect a wide range of vertebrate cells including nonprofessional phagocytes. The natural route of infection of *T. gondii* is oral. Thus, the intestinal barrier composed by a monolayer of polarized enterocytes must be crossed by *T. gondii* for dissemination into deep tissues. This transmigration required viable and actively motile parasite, but the integrity of the host cell barrier is not altered during parasite crossing as evidenced by *ex vivo* and *in vitro* experiment. Furthermore, in *T. gondii*-induced ileitis enterocytes are one of the most resistant cells of this tissue. Thus, it seems that this cell type has microbicidal mechanisms against this parasite. It has been shown that *T. gondii* partially inhibits nitric oxide (NO) production of activated macrophages, promoting its persistence in the host cell. We analyzed the ability of an intestinal epithelial cell line (IEC-6) to express iNOS and produce NO after the infection by tachyzoites of *T. gondii*. IEC-6 was cultured at 37ºC in a 5% $CO_2$ atmosphere over coverslips and activated with recombinant interferon-gamma for 24h. Activated IEC-6 was infected with *T. gondii* (RH and ME-49 strains) for 2h and further cultured. After 24h the cells were fixed, and iNOS and *T. gondii* immunolocalized. NO production was evaluated at the culture supernatant by the Griess reagent. Infection by *T. gondii* of both strains at 24h was able to inhibit NO production and expression of iNOS of IEC-6. However, after 48h only the RH strains maintained NO inhibition; IEC-6 infected with the ME-49 strain by this time produced similar NO levels as non-infected IEC-6. We conclude that *T. gondii* was able to inhibit NO production and the expression of iNOS also in IEC-6 similarly as described for macrophages.
Supported by CAPES, CNPq, FAPERJ, UENF.

## BC.68 - A LIGHT AND ELECTRON MICROSCOPY STUDY OF TRYPANOSOMES ISOLATED FROM *LEPTODACTYLUS OCELLATUS* FROGS

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Purpose of this study was to analyze the morphology of trypanosomes isolated from frogs by light and electron microscopy. Trypanosomes were isolated from naturally infected *Leptodactylus ocellatus* frogs collected in Seropédica City, RJ, Brazil ($22^0$ 44' 08"S and $43^0$ 42' 27"W). Blood samples were collected by heart puncture and macerations of heart, liver, spleen and kidney and then inoculated into culture tubes containing blood-agar medium. After incubation for four days at $22^oC$, parasites from the supernatant of positive cultures were collected by centrifugation, fixed and then processed for light microscopy (Giemsa staining), scanning electron microscopy (SEM) and transmission electron microscopy (TEM). In culture, most bloodstream trypomastigotes differentiated into epimastigotes (elongated and stump forms) and amastigotes, although a few esphaeromastigotes and trypomastigotes were also observed. Coil-shaped epimastigotes were observed by scanning electron microscopy and were characterized by TEM by the presence of an oval nucleus, a rod-shaped kinetoplast and a well-developed cytostome-cytopharynx complex. Large electron-dense vesicles similar to lipid inclusions and spherical electron-dense vesicles similar to acidocacisomes could be observed randomly distributed in the cytoplasm. Some parasites presented rod-shaped bacterium-like organisms (BLO) apparently free in the cytoplasm. The BLO were surrounded by two unit membranes that were separated by an electron-dense space, and presented ribosome-like particles and electron-lucent areas distributed throughout the cytoplasm. Further studies are underway for complementary characterization of the ultrastructure of these trypanosomes and bacterium-like organisms.
Supported by CNPq and FAPERJ

## BC.69 - COMPARATIVE MORPHOLOGICAL STUDIES OF TWO CLOSE TRYPANOSOMATIDAE SPECIES: *TRYPANOSOMA CRUZI* AND *TRYPANOSOMA DIONISII*

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*Trypanosoma cruzi* is the protozoan that causes Chagas disease. It divides into the insect vector gut or into cytosol of an infected mammalian cell. *Trypanosoma dionisii* was isolated from a bat and is phylogenetically close to *T. cruzi.* Both present similar morphological stages and are able to infect mammalian cells in culture. Here we compared their cellular 3D ultrastructure obtained from serial sections of epimastigote forms. Ultrathin serial sections were obtained from Epon embedded parasites and were photographed under a transmission electron microscope. 3D models were generated using Reconstruct and Blender modelling softwares. Both species have an interconnected dense chromatin in the nucleus leaving an internal space for a centrally located nucleolus. They also present the kinetoplast accommodated within a separated branch from the tubular and single mitochondrion. Their cytostoma progresses from the parasite surface towards the posterior end contouring the kinetoplast and the nucleus. As the cell cycle progresses from G1 to G2 phase their cytostoma retract. As major differences we found that *T. dionissi* presents larger multivesicular structures in the posterior region that could be related to *T. cruzi* reservosomes. Also,*T. dionisii* mitochondrion is smaller and the flagellar pocket is larger than *T. cruzi* related structures. We propose that the similarities reflect the conserved features of cell division and life cycle, while the differences would be a consequence of differences in the parasite metabolism.
Supported by CNPq and FAPESP

## BC.70 - PURIFICATION OF ANTI-NTPDASE 1 RECOMBINANT ANTIBODIES AND IMMUNOLOCALIZATION IN *TRYPANOSOMA CRUZI*

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*T. cruzi* is the etiological agent of Chagas disease an important tropical neglected disease that affects people mainly in South America. Parasites NTPDases are enzymes that can hydrolyze ecto localized tri and di-phosphate nucleotides controlling the purinergic signaling in hosts. Classical NTPDase activity was previously demonstrated in *T. cruzi* surface and a gene coding an NTPDase was isolated, cloned and expressed in bacterial system. This NTPDase-1 protein has been demonstrated to be a new virulence *T. cruzi* molecule. The main goal of this work was to purify specific anti-NTPDase-1 antibodies and use this purified antibodies to investigate the localization of NTPDase in live parasites. To achieve these goals the recombinant purified T. cruzi NTPDase-1 was immobilized on Sepharose 4 Fast Flow (GE). This resin was used to purify specific anti-NTPDase-1 antibodies. The purified antibodies, polyclonal antiserum and commercial anti-CD39 policlonal anti-serum were used in western blot analysis with epimastigotes and trypomastigotes showing higher specificity to the purified antibodies. The purified antibodies were used to analyze the immunolocalization of NTPDase1 by confocal technique. Our results showed specific points of fluorescence at surface e of all morphological T. cruzi forms. Electronic microscopy using purified antibodies will be the next step in this work in order to elucidate the real localization of this protein in the parasite.
Supported by FAPEMIG.

## BC.71 - GENE EXPRESSION PROFILES OF HUMAN MACROPHAGES INFECTED WITH *LEISHMANIA BRAZILIENSIS* IN VITRO.

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The protozoan parasite *Leishmania braziliensis* has a high degree of intra-species genetic and phenotypic polymorphism, which is accompanied by a spectrum of clinical presentations in the infected human host, including: localized cutaneous leishmaniasis (CL), mucosal leishmaniasis (ML) and the more recently described disseminated leishmaniasis (DL). Our hypotheses are (1) that these parasites interfere with the gene expression of infected cells in a manner that is beneficial to their infectivity, and (2) that strains of *L. braziliensis* drawn from patients with either CL, ML or DL lead to different gene expression profiles in the infected macrophages. Employing DNA micro-array we compared the global gene expression profiles in human monocyte derived macrophages (MDM), obtained from healthy donors and infected in parallel with one *L. braziliensis* isolated from a CL, one from a ML and one from a DL case of the same endemic region in Northeastern Brazil. We also assessed how infected MDM compared with non-infected cells. Overall, *L. brazilienis* caused the repression of the majority of the genes that presented significant changes of their expression levels in infected MDM as compared to non-infected cells. Immune and non-immune response genes were affected. Among the three isolates tested, the two drawn from metastatic disease cases (i.e. ML and DL) induced more similar gene expression patterns in the MDM. These suggest that these parasites may increase their chance of survival by down regulating host cell genes during the infection process, and that strains associated with different forms of disease elicit somewhat diverse behaviors in host cells, which may be related to the different clinical outcomes of the disease.
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## BC.72 - NEW ASPECTS ABOUT INTERACTION BETWEEN *GIARDIA LAMBLIA* AND INTESTINAL CELLS

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Our group previously showed studies about interaction between intestinal cells and *Giardia lamblia*. This is a parasitic protozoa that causes diarrheal and other intestinal diseases, being the mechanisms of the pathogenesis poorly understood. We presented that adhesion of parasites caused reduction in transepithelial electrical resistance (TER), concomitant with ultrastructural changes in intestinal cells. Our current results show that both samples (control and interaction) remain viable after 24 hours, although the TER has been reduced in approximately 40%. These data confirm the previous suggestion that ions probably pass through the paracellular region (junctional area). Here we also demonstrate that there was no change in expression of junctional proteins (tight and adherens junction proteins); nevertheless, the cellular distribution of these proteins in Caco-2 was significantly altered, as observed under laser scanning confocal microscope. 3D reconstructions allowed a fine analysis of the rearrangement of junctional proteins in epithelial monolayers after parasite adhesion. This study provides a deep review of the data published so far in the literature regarding the interaction *in vitro* between intestinal cells and *Giardia*.

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## BC.73 - *Plasmodium chabaudi* EXPOSE PHOSPHATIDYLSERINE AS ESCAPE MECHANISM OF IMUNE SYSTEM

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Has been shown that exposure of phosphatidylserine in outside of the plasma membrane in parasitic protozoa such as *Leishmania amazonensis*, *Toxoplasma gondii* and *Trypanosoma cruzi*, is important in the escape mechanism used by these protozoan parasites. In this paper we analyze the existence this inhibition mechanism of immune activity in *Plasmodium chabaudi*, a parasite that causes malaria in rodents. For this, the *P. chabaudi* was maintained by intraperitoneal passages in mice. During 24 hours, the blood of mice was collected and made himself a count of the forms of the parasite contained in temporal kinetics, making it possible to choose the way you would use for the study. After analysis by flow cytometry of parasites, it was observed that 90 % of parasite population exposed phosphatidylserine on the outside of membrane. Images obtained by scanning electron microscopy of the interaction of parasites with peritoneal macrophages of mice revealed that the form studied is able to penetrate the macrophages and modulate the microbicidal action of same. With these results, we conclude that exposure of phosphatidylserine may be a common escape mechanism between protozoan parasites that interact during their biological cycles with effector cells (macrophages).

Supported by FAPERJ

## BC.74 - ANALYSIS OF THE INTERACTION OF MURINE MACROPHAGES WITH SUBPOPULATION OF TACHYZOITES OF *Toxoplasma gondii* THAT EXPOSE OR NOT PHOSPHATIDYLSERINE

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Exposure of phosphatidylserine (PS) by cells indicates apoptosis. This exposure is essential for signaling the release of transforming growth factor-beta1 (TGF-b1) by macrophages, which induces an anti-inflammatory response during phagocytosis of apoptotic cells. Toxoplasmosis is a worldwide disease caused by *Toxoplasma gondii*. The active invasion of *T. gondii* inhibits nitric oxide (NO) production, allowing the persistence of the parasite in macrophages. Our group showed that the mechanism used by *T. gondii* to inhibit the production of NO in activated macrophages is similar to *Leishmania* and involves the exposure of PS. In this work the *T. gondii* tachyzoite population was separated into PS positive and PS negative subsets (by annexin V conjugated to magnetic beads) and *in vitro* interactions with murine macrophages were performed for the analysis of the penetration mechanism and survival of the parasite. Analysis by flow cytometry confirmed the efficiency of the isolation procedure of *T. gondii* PS subpopulations. Nitrite measurements in culture medium after the interaction showed a significant decrease in NO production after interaction of macrophages with PS positive subset of *T. gondii* compared to the PS negative. Scanning electron microscopy showed that the PS positive subpopulation invaded macrophages by active penetration, but the PS negative subset entered these cells by macropinocytosis. Treatment of cells with dynasore (inhibitor of macropinocytosis) showed that the invasion of the PS negative *T. gondii* subset in macrophages was inhibited with increasing concentration of this compound confirming the result. *In vivo* experiments shows that survival of mice infected with subsets of T. gondii were lower when compared to mice infected with both parasite total population simultaneously. These results suggest that the PS positive and PS negative population of *T. gondii* invade macrophages by different mechanisms and the growth of the parasite depends on both populations.
Supported by: FAPERJ, CNPq, CAPES.

## BM.001 - NEW RECOMBINANT ANTIGENS FOR VISCERAL LEISHMANIASIS DIAGNOSIS

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Visceral Leishmaniasis (VL) in Brazil is caused by *Leishmania chagasi* and is a major public health problem. In the last two years we have developed a proteomic study of *L. chagasi* for identification of new antigens with potential use in diagnosis and vaccine. These tests allowed us to identify over 40 promising protein. In this study the goal was to select the proteins by bioinformatics the most promising for use as recombinant antigen in the diagnosis of VL. Therefore, initially, we made the prediction for B cell epitopes using the programs ABCPRED, BCPREDS and BEPIPRED. It was observed that the HIP400 protein had 12, 6, 6 epitopes predicted by the respective programs, with an overlap of 93 amino acids (approximately 47% protein) amongst at least two programs simultaneously and HIP1000 protein showed 27, 12 and 12 predicted epitopes, with 214 amino acids (approximately 60% protein) overlapped at least two programs simultaneously. Moreover, they are proteins with a size smaller than 1kDa without signal peptide which may indicate hydrophobicity. For the production of recombinant proteins, we used the pGEM cloning vector and *E. coli* - XL1-Blue as host cell. The host cell was grown in liquid environment and then plated on agar containing ampicillin. The positive colonies confirmed by PCR, were lysed and digested for obtaining the fragment. This was inserted into the expression vector (pET-Tev) and incorporated by another host (*E. coli* - BL-21). The host cell was grown and then plated on agar containing kanamycin. The positive colonies confirmed by PCR, were incubated with an inducing factor expression (IPTG). After the process of expression, cells were lysed to obtain a purified protein that was bound to the column on the Akta Histrap. The purified proteins are going to be used in standardized testing and validation of VL diagnosis by ELISA.
Supported by: FAPEMIG and CNPq

## BM.002 - SEARCH FOR PHOSPHATIDYLSERINE FLOPPASES IN *LEISHMANIA (LEISHMANIA) AMAZONENSIS*

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In its life cycle the protozoan *Leishmania* alternates the two forms between the insect and the mammalian hosts. However some aspects of the infection of the mammalian macrophage remain to be elucidated. It has been suggested that lipid organisation of the plasmatic membrane of the parasite can play a role in the phagocytic process as well as in the ability to survive in mammal host. Phosphatidylserine (PS) exposure at the exoplasmic leaflet of the plasma membrane could be one of the signals delivered by amastigotes promotes the phagocytosis but inhibits the macrophages activation (Balanco et al., 2001). Floppase is a translocase that is responsible for the transport of PS from the cytoplasmic to the exoplasmic leaflet. In the present comunication we described a strategy to isolate genes responsible for the externalisation of PS in *L. (L.) amazonensis*. Cells, originated from a transfection with a genomic library of *L. (L.) amazonensis* (Uliana et. al., 1999), were selected with Annexin-V coupled to magnetic microbeads. The cosmids from the selected cells were recovered and DNA was cloned into *E. coli*. *Bam*HI restriction profiles of those cosmids were analyzed showing the existence of two distinct cosmids that were then used on a new transfection of wild type *L. (L.) amazonensis*. The produced cells were labeled with Annexin-V-FITC and their fluorescence was assessed by flow citometry (FACS). The culture produced by the transfection with one of the cosmid showed a differential fluorescence, suggesting a change in the fenotipe, probably induced by the expression of cosmid information. We intend to analyze the genes present in the cosmid to isolate the one responsible for this differential fluorescence. Supported by FAPESP and CNPq.

## BM.003 - MOLECULAR AND FUNCTIONAL CHARACTERIZATION OF A GENE ENCODING A LEUCINE RICH PROTEIN IN *LEISHMANIA*

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Leucine-rich repeats (LRRs) are versatile binding motifs found in a variety of proteins involved in protein-protein interactions. During the process of characterizing the *Leishmania amazonensis META1* genomic region, we identified a 2.03 Kb ORF encoding a protein with 6 LRRs in its central region and presenting similarity with the human NOD3 protein. The *LaLRR17* protein is found in increased abundance in amastigotes and is also secreted to the cytoplasm of *L. amazonensis*-infected macrophages. where it might interact with macrophage proteins and modulate the cell's response to infection. In *L. braziliensis* we identified an orthologue to *LaLRR17* gene in chromosome 17 (*Lbr*M17_V2.0920, http://www.genedb.org). A more detailed study of this genomic region identified a second, truncated, copy of this gene, encoding the first 84 amino acids of the protein. However, hybridization analysis and attempts to characterise this second gene copy indicated that there is an assembly error in this region of the genome. To study the function of the LRR17 protein in *L. braziliensis* and *L. amazonensis* we developed specific policlonal antibodies against peptides derived from amino and carboxyl regions conserved between *L. braziliensis, L. major, L. amazonensis* and *L infantum* LRR17 proteins. We found that *LbLRR17* gene expression is differentially regulated during the life cycle of *L. braziliensis* and the protein is found with increased abundance in promastigotes. We also obtained mutant lines of *L. amazonensis* overexpressing the *LaLRR17* gene and of *L. braziliensis* overexpressing the *LbLRR17* gene. These mutant strains were more infective to macrophages *in vitro* when compared with the wild type strains, indicating that the LRR17 protein may interact with macrophage molecules through their LRRs, modulating the cellular response to increase parasite survival. The phenotype of these mutants will be analyzed both *in vitro,* and *in vivo* to test for increased virulence in experimental models of infection. Support: FAPESP, CNPq, CAPES.

## BM.004 - FUNCTIONAL CHARACTERIZATION OF A LEUCINE RICH PROTEIN OF *LEISHMANIA MAJOR*

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Proteins containing leucine rich repeats (LRR) are known to be involved in macromolecular interactions in many processes such as bacterial colonization of host cells, immune response in plants and inhibition of RNA binding. A previous study in our laboratory identified a *L. amazonensis* gene encoding a protein containing 6 LRRs (*LaLRR17*). *LaLRR17* is a stage-regulated gene expressed with increased abundance in the amastigote stage. Additionally, the LaLRR17 protein has been shown to be secreted to the macrophage's cytoplasm. Highly conserved homologues of LaLRR17 were found in all *Leishmania* species analyzed. Therefore, the aim of this study was to characterize the homologous protein of *L. major* (LmLRR17). Antibodies raised against peptide sequences common to LaLRR17 and LmLRR17 allowed the study of the steady-state protein abundance. Interestingly, LmLRR17 protein was found to be up-regulated in procyclic promastigotes, instead of amastigotes. To investigate the function of this protein in *L. major*, mutants over expressing a myc-tagged version of LmLRR17 or of LaLRR17 protein were obtained by stable transfection. Chimeric proteins in mutant strains were expressed following the same pattern of expression observed in the wild type parasites, in spite of the increase in gene copy number. The phenotype of these mutants was assessed *in vitro* through macrophage infections. Overexpression of LmLRR17 protein in *L. major* resulted in a decrease in the percentage of infected cells as well as in the number of intracellular amastigotes. On the other hand, overexpression of LaLRR17 in *L. major* induced an increase in virulence with a higher number of infected cells and intracellular parasites. These results indicate that the expression of LmLRR17 protein in *L. major* is tightly regulated and an increase in LmLRR17 protein levels seems to be detrimental to the parasite, while the expression of the heterologous LaLRR17 protein increased infectivity *in vitro*. Support: FAPESP, CNPq.

## BM.005 - INVESTIGATION OF AN EXTRA-RIBOSOMAL FUNCTION FOR THE RIBOSOMAL PROTEIN L19 OF *LEISHMANIA*.

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Several ribosomal proteins are reported to play diverse extra-ribosomal functions. However, little is known about the ribosomal proteins in trypanosomatids. Thus, we decided to investigate a possible extra-ribosomal role in gene expression for RPL19, a protein whose transcript is present at variable levels during the parasite life cycle. We generated a transfectant of *L. major* (LV39) that overexpresses the transcript and L19 protein. Promastigotes of independent clones showed an impaired growth. To verify if the phenotype was due to the L19 overexpression, we cured clones and observed that they rescue the wild type growth curves patterns. We have previously observed that the endogenous and overexpressed L19 are dispersed throughout the cytoplasm in a granular appearance. We are further investigating whether the L19 excess leads to an increment of ribosomes in the cell or if the overexpressed L19 remains free in the cytosol. We investigated proteome profile differences between the *L. major* transfectants with parental line. The differentially expressed proteins detected were identified by mass spectrometry. Analysis of identified genes did not reveal any common or complementary function for them, neither allowed to rescue conserved motifs (from the untranslated regions) potentially involved in control of gene expression. Regarding a possible protein or RNA interaction with L19 immunoprecipitation assays with anti-L19 have been unsuccessful. Pull-down experiments are in course to investigate L19 interaction molecules.
Supported by FAPESP.

## BM.006 - MOLECULAR AND FUNCTIONALCHARACTERIZATION OF *Leishmania amazonensis* CERAMIDE SYNTHASE

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Sphingolipids are involved in fundamental aspects of celullar biology, including growth, differentiation, apoptosis and oncogenesis and are essential membrane components of eukariotes, including Kinetoplastidae protozoans such as *Leishmania*. Ceramide synthase (CerS) catalyzes the acetylation of sphinganine and sphingosine to form the N-acyl derivates dihydroceramide and ceramide, respectively, which constitute the sphingolipid backbone. Due to the fundamental role of this enzyme we decided to identify and characterize the CerS of *L. amazonensis*. The identification of consensus elements in the alignment of CerS sequences of several organisms allowed the search for a *Leishmania* homologue in genome databanks. The nucleotide sequence of a putative *L. major* CerS gene (LmjF31.1780) identified in this search was used to design oligonucleotides that were then applied to identify the *L. amazonensis* corresponding sequence. The *L. amazonensis* putative CerS gene encodes a 460 aminoacid protein with six potential transmembrane domains. The translated sequence contains a TRAM_LAG1_CLN8 motif (PF03798), found in all CerS previously characterized. Yeast complementation tests were used to determine gene function. The *L. amazonensis* putative CerS gene was able to rescue *Saccharomyces cerevisiae* YPK9 strains with a deletion in the endogenous CerS gene. Since CerS activity is essential in this yeast strain, functional complementation showed that the *L. amazonensis* encoded protein possesses CerS activity. In conclusion, we have identified and characterized the CerS gene of *L. amazonensis*. The characterization of sphingolipid biosynthesis in *Leishmania* may prove important to identify new targets for the chemotherapy of leishmaniasis.
Funding: FAPESPand CNPq.

## BM.007 - GENOMIC AND TRANSCRIPTOMIC ANALYSIS OF *Leishmania (Viannia) braziliensis* RESISTANT TO POTASSIUM ANTIMONY TARTRATE

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Mechanisms of drug resistance in New World *Leishmania* species are poorly understood. Recently, we selected *in vitro* populations of *Leishmania braziliensis,* which are 20-fold more resistant to potassium antimony tartrate Sb III (Lb SbR) than its susceptible counterpart (Lb WTS) (Liarte & Murta, 2010). In this study we analyzed gene amplification and deletion and identified transcripts differentially expressed in Lb WTS and Lb SbR populations using DNA microarray methodology. The final array consisted of 13,321 *Leishmania* oligonucleotides: 13,011 from *L. major*, 112 from *L. infantum*, 66 control oligos. The oligonucleotide sequences were clustered by similarity in 12,146 contigs. The genomic analysis (GCH – comparative Genomic hybridization) allowed the identification of 124 sequences amplified 2 to 21-fold (1.78%) and 128 deleted 2 to 9-fold (1.71%) in the Lb SbR population compared to Lb WTS. The amplification of the H region and regions from chromosomes 17, 20 and 31 was observed in the SbIII-resistant population from *L. braziliensis*. The transcriptomic analysis showed 560 up-regulated transcripts and 397 down-regulated transcripts in SbIII-resistant *L. braziliensis* population. Functional annotation data suggest an increased expression of transcripts related to DNA replication and transcription and a decreased expression of transcripts associated with metabolism of lipids and carbohydrates and transport of proteins. Taking into account only the genes indentified by two techniques simultaneously, we observed that 46 genes are up-regulated/amplified in Lb SbR and 15 genes are down-regulated/deleted in this population. Sequence analysis showed that 60% of those 61 genes encode hypothetical proteins. The other genes encode proteins involved with metabolism, replication, DNA repair, transcription, glycoconjugate biosynthesis and others. The identification of genes that are differentially expressed in SbIII-resistant and -susceptible *L. braziliensis* populations may help our understanding of the molecular basis of drug resistance in this parasite and also provide information on drug targets for chemotherapy.Financial Support: CNPq, FAPEMIG, CPqRR and UNICEF/UNDP/World Bank/WHO/TDR.

## BM.008 - PROTEOMIC ANALYSIS OF MILTEFOSINE RESISTANCE IN *LEISHMANIA (VIANNIA) PANAMENSIS*

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Miltefosine is the first orally effective drug for the treatment of visceral and cutaneous leishmaniasis. However, despite its recent approval (2002) failures in miltefosine treatment have been reported, which indicates that there is probability of finding drug-resistant field isolates of *Leishmania*. In Colombia, miltefosine is used as therapeutic alternative in the treatment of cutaneous leishmaniasis, which is mainly caused by *L. (V.) panamensis*. In this work we conducted a comparative proteomics study using two-dimensional electrophoresis and mass spectrometry to identify proteins differentially expressed between wild-type and miltefosine-resistant *L. (V.) panamensis* promastigotes. Five gels from five different sample preparations for both sensitive and resistant lines were processed, and were detected 572 ± 60 y 526 ± 48 proteins, respectively, in two-dimensional proteome maps. Comparative analysis of the two proteomes allowed the identification of seven differentially expressed protein spots between wild-type and miltefosine-resistant *L. (V.) panamensis* promastigotes, among of proteins we found heat shock proteins (Hsp60, Hsp70), ribosomal proteins (P2, S12), and proteins involved in cellular metabolic pathways (glutamine synthetase, peroxidoxin, p-nitrophenylphosphatase). This is the first study of miltefosine resistance in *Leishmania* parasites belonging to the *Viannia* subgenus using a proteomic approach, in which was achieved the identification of proteins that had not been previously implicated in miltefosine resistance. This comparative proteomic analysis highlights the variety of cellular responses required for the acquisition and/or maintenance of miltefosine resistance in *Leishmania*, which besides being mediated by membrane transporters, also can be modulated directly or indirectly by cytoplasmic and mitochondrial proteins.

## BM.009 - ASF1 (*Anti Silencing Factor 1*) OF *LEISHMANIA MAJOR*

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The mechanisms and genes involved in chromatin remodeling in trypanosomatid have been the subject of intense investigation in recent years. Studied in different organisms, ASF1 (Anti-Silencing Factor 1) was identified as a histone chaperone that contributes to histones deposition during nucleosome assembly in newly replicated DNA. This protein is also involved in cellular response to DNA damage and transcriptional silencing. We are investigating a putative ASF1 from *L. major*, which seems to be involved in chromatin packaging and preservation of DNA integrity. In an attempt to evaluate the involvement of ASF1 in the control of gene expression in *L. major*, we analyzed the proteome of a *L. major* transfectant that overexpresses ASF1 (*Lm[pXNeo-ASF1]*) by comparison with the one of a control line (*Lm[pXNeo]*). We analyzed the protein profile differences by two-dimensional gel electrophoresis and we have detected 19 differentially expressed proteins that were identified by mass spectrometry. To specifically investigate a possible role of LmASF1 to control levels of expression of genes present at the telomeric ends, as it is the case of the *Saccharomyces cerevisiae* ASF1, we compared transcript levels of 42 genes in *Lm[pXNeo-ASF1]* with *Lm[pXNeo]*, by Real time PCR. Our results suggest that LmASF1 does not modify expression of these genes. To evaluate the role of LmASF1 in DNA repair, we submitted *Lm[pXNeo-ASF1]* and *Lm[pXNeo]* to different genotoxic agents that trigger distinct DNA repair pathways. Our results suggest that overexpression of ASF1 contributes significantly to the resistance of the cells to oxidative stress since *Lm[pXNeo-ASF1]* is more resistant to damage caused by the hydrogen peroxide ($H_2O_2$). In addition, after gamma irradiation transfectants overexpressing LmASF1 recovers more slowly than the control line, which may suggest that LmASF1 is involved in cell cycle control. This set of complementary approaches allows understanding ASF1 roles in *Leishmania* parasites. Supported by FAPESP.

## BM.010 - EVALUATION OF ASSOCIATIONS BETWEEN POLYMORPHISMS IN THE NRAMP1 GENE (natural resistance-associated macrophage protein 1) AND DOGS SUSCEPTIBILITY TO LEISHMANIASIS

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Studies have shown that polymorphisms in some genes are involved in genetic predisposition and/or susceptibility to infections by *Leishmania* spp in dogs. The aim of this work is to analyze the relationship between polymorphisms of a microsatellite in intron 1 of the nramp1 gene and susceptibility of dogs from canine visceral leishmaniasis endemic areas to infection by *Leishmania* (*Leishmania*) *chagasi*. Previously. 191 dogs with serology (IFAT and ELISA) and PCR positive for *L.* (*L.*) *chagasi* (infected group - IG) and 61 dogs with serology and PCR negative (uninfected group – UG) were examined. All animals participating in this study were crossbreeds. We identified seven alleles that differ to each other by four base pairs (bp) and ranged from 133-157 bp in length. The allele 145 was the most frequent in IG (0.35 vs. 0.28 in UG) and allele 141 was the most frequent in UG (0.33 versus 0.28 in IG). Allele 157 appeared only in IG (0.005). The homozygous genotype 149/149 was more frequent in IG group (0.33) and 145/145 in the UG group (0.35). Differences in allelic and genotypic frequencies between case and control groups were not statistically significant ($p > 0.05$) but the presence of alleles such as 157 can suggest a protective effect. Altet et al. (2002), in a study conducted in Spain using breed dogs, observed a significantly higher frequency of the allele 145 in IG ($p < 0.025$), which appeared exclusively in homozygosis. Although preliminary, our data suggest a positive association between allelic frequencies and dog susceptibility or resistance to infection by *L.* (*L.*) *chagasi*.

## BM.011 - UV IRRADIATION INDUCES ALTERATIONS IN THE EXPRESSION OF LEISHMANIA SPP. TELOMERIC PROTEIN RBP38 (LARBP38)

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Recent results demonstrated that Rbp38 is a protein exclusively expressed in trypanosomatid parasites, including *Leishmania* spp. Rbp38 is probably a multifunctional protein since it seems to exert different functions in the kinetoplast and in the nucleus of the parasites. The *L. amazonensis* Rbp38 (LaRbp38) protein was co-purified with LaRPA-1 from nuclear extracts positive for telomerase activity. LaRbp38 can also bind in vitro and in vivo with telomeric DNA and with kDNA, although its biological function is yet unknown. We recently demonstrated that LaRbp38 forms part of a minimum telomeric complex at *Leishmania* telomeres where we can also find LaRPA-1 and telomerase. Using different protein extracts obtained from total and subcellular fractions of promastigote forms, we were able to show by Western Blot, that under normal conditions, LaRBP38 can be found in both nuclear and mitochondrial fractions. By indirect immunofluorescence using anti-LaRbp38 serum, the protein was mainly localized in the kinetoplast, showing an antipodal distribution, but it was also present to a lesser extent, in the nucleus and in some cells it was possible to visualize the protein in both compartments. We further exposed *L. major* promastigotes to 1200J/$m^2$ of UV radiation for 2-10 min and let parasites recover in culture in the dark, for 1h-24h. Protein extracts were obtained from irradiated and control parasites at all time points and then submitted to Western blot analysis. The results showed a gradual increase in LaRbp38 expression in irradiated parasites and during recovery in culture. No changes were seen in alfa-tubulin expression, used as the loading control. When we analysed the expression of LaRBP38 in a nuclear fraction from irradiated parasites, we observed a slight increase in protein expression after 8-10 min of UV exposition and that after 6h of recovery no protein was detected in the extracts. The expression of LaRbp38 in the nucleus did not return to the normal levels even after 24h, when compared to non-irradiated parasites. Curiously, the expression of LaRBP38 was unaltered in the mitochondrial fraction even after UV irradiation and recovery. We are currently checking if alterations in LaRBP38 expression are associated with the recruitment of DNA repair proteins and other telomeric proteins as well.

## BM.012 - CLONING OF GENES FOR PUTATIVES PROLINE DEHYDROGENASE AND $\Delta^1$-PYRROLINE-5-CARBOXYLATE DEHYDROGENASE FROM *LEISHMANIA* (*LEISHMANIA*) *AMAZONENSIS* AND FUNCTIONAL CHARACTERIZATION OF THEIR PRODUCTS

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It is known that trypanosomatids use amino acids, particularly proline, as a carbon and energy sources. In addition, it was involved in other physiological processes such as metacyclogenesis, osmorregulation or resistance to different stress conditions. The proline-glutamate redox pathway is being studied in *Leishmania (Leishmania) amazonensis* by our group. Two enzymes are involved in this route: 1. proline dehydrogenase (PRODH) which converts L-proline into $\Delta^1$-pyrroline-5-carboxylate (P5C) and 2. $\Delta^1$-pyrroline-5-carboxylate dehydrogenase (P5CDh) which converts P5C into L-glutamate. Although, the presence of this pathway in some species of the *Leishmania* genus is strongly supported by their genome project data, no biochemical evidences of their activities were reported up to now. In this work we focus on some aspects of the proline-glutamate pathway in *L. (L.) amazonensis.* The genome sequence of this specie has not been completed yet. Using the protein sequences available on databases from *L.* (*L.*) *major, L.* (*V.*) *braziliensis, L.* (*L.*) *infantun* and *L.* (*L.*) *mexicana*, we have obtained a consensus sequence for both genes. Degenerated oligonucleotides from conserved domains were designed and used to amplify by PCR, clone and sequence the corresponding DNA fragments for *La*PRODH and *La*P5CDh. The ORFs sizes were 1,7 Kb and 1,68 Kb for *La*PRODH and *La*P5CDh, respectively. For protein analysis, the recombinant enzymes were cloned in the pRSET-C vector and expressed fused to an N-terminus his-tag. The purified recombinant proteins showed an apparent molecular weights of 62 and 66 kDa, as expected. The functionality of these genes is being analyzed by using yeast strains lacking the orthologues genes (Δput1 and Δput2). The Δput2 mutant transformed with *La*P5CDh regained the ability to grow in selective medium with proline as a sole nitrogen source. Our data suggest the presence and functionality of this pathway in *L. (L.) amazonensis*. Supported by FAPESP, USP and CNPq.

## BM.013 - THE BEHAVIOUR OF TELOMERIC AND REPAIR PROTEINS UPON DNA DAMAGE IN *LEISHMANIA*

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Replication protein A is a complex of single-stranded DNA-binding proteins implicated in DNA metabolism, including DNA repair and telomere maintenance. In *Leishmania* the subunit RPA-1 (LaRPA-1) binds and co-localizes *in vivo* with telomeres. In yeast and human telomeres RPA also works as a telomerase recruiter and as a DNA damage sensor. Its involvement with the DNA damage response triggers its hyperphosphorylation and the recruitment of proteins from the RAD51 group. We intend to determine if in *Leishmania*, RPA is also required to protect chromosome ends from being detected by the DNA damage machinery. Our first results showed that even high doses (~500 ug/ml) of the DNA damaging agent phleomycin was unable to kill *L. amazonensis* promastigotes. In contrast, low doses of the drug (~20-40 ug/ml) induced G1/S cell cycle arrest. Then, we verified if phleomycin treatment triggered changes in expression of telomeric (RPA, RBP38 and telomerase) and repair proteins (RAD51, MRE11 and H2Ax). The results indicated that the expression of LaRPA-1 and other telomeric proteins slightly diminished in parasites treated with phleomycin whereas a gradual increase in the expression of RAD51 occurred probably in response to DNA damage. Moreover, it was possible to see by chromatin immunoprecipitation that more LaRPA-1 was immediately recruited to the G-rich telomere strand upon phleomycin-induced damage, compared with the C-rich telomeric strand, suggesting that the presence of LaRPA-1 may prevent loss of single-stranded telomeric DNA or elicit activation of a local DNA damage response. We are currently checking if double strand breaks on DNA alters the known protein:protein interactions at parasite telomeres.
Supported by CNPq.

## BM.014 - *Leishmania infantum chagasi* NTPDase as New Antigen in Canine Leishmaniasis Diagnosis

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Leishmaniasis are tropical neglected diseases caused by parasites from the genus *Leishmania*. *Leishmania infantum chagasi* is aetiological agent of American Visceral Leishmaniasis and canine Leishmaniasis. Actually dogs had been pointed as the main epidemiological reservoir for Leishmania and fast and effective epidemiological vigilance is crucial to the control of this antropozoonosis. The main goal of this work is the development of a strategy to do rapid canine leishmaniasis diagnosis using the new recombinant *L. infantum chagasi* (NTPDase) as antigen (LicNTPDase) an lateral flow immunocromatography. NTPDase is a leishmania ecto-localized virulence molecule. The soluble portion of NTPDase gene was cloned in pET21b vector. This construction pET21b-NTPDase was used to transform *Escherichia coli* BL21-DE3 strain. Transformed bacteria were isolated and used to express the recombinant *L. infantum chagasi* NTPDase. The LicNTPDase was expressed after 1 hour of IPTG induction, recovered and purified from inclusion bodies using Ni-NTA agarose affinity chromatography. The recombinant protein was tested as antigen in a direct ELISA assay using standard dog sera and presented good differentiation of negative and positive sera. These results showed the potential use of LicNTPDase as new antigen to be used in canine Leishmaniasis immune diagnosis. Our perspectives include the development of a new rapid immunochromatography kit to be used in epidemiological inquiries helping in the control of this important tropical disease.
Supported: FAPEMIG, UFV.

## BM.015 - CELL CYCLE DETERMINATION AND CHARACTERIZATION OF THE PROMASTIGOTE STAGE OF *LEISHMANIA (L.) AMAZONENSIS*.

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The eukaryotic cell cycle is a precisely controlled phenomena involving the replication and segregation of organelles culminating in the production of two identical daughter cells. In kinetoplastid protozoa, the cell cycle is characterized by the duplication of the kinetoplast (mitochondria), the nucleus and growth of an extra flagellum prior to cytokinesis. We present in this study the morphological events and the timing of these events during the cell cycle of *Leishmania amazonensis* promastigotes. *L. amazonensis* is the protozoan parasite that causes tegumentar leishmaniasis, an important neglected disease in Brazil. Cell cycle characterization was done using culture synchronization with hrydroxyurea treatment followed by flow cytometry analysis, DAPI DNA staining, flagellum labeling, bromodeoxyuridine incorporation, and indirect immunofluorescence analysis. Results showed that DNA replication takes around 6-7 hours with the nucleus generally completing division shortly after the kinetoplast. The new flagellum is formed during late S phase and G2, reaching its final size during the final stages of the cycle (G2/M). Complete separation of daughter cells may take up to 2 hours after the cycle is completed. During S phase, replication protein A subunit 1 colocalized with bromodeoxyuridine incorporation confirming that this protein participates in the DNA replication machinery in the nucleus. Successful synchronization using hydroxyurea treatment allowed us to determine for the first time the timing and order of the various events that compose the cell cycle in *L. amazonensis*. The determination of telomere and kinetoplast DNA replication in synchronized parasites is currently under way.
Supported by FAPESP, CNPQ and PROPe (UNESP).

## BM.016 - UNVEILING PROTEIN: PROTEIN INTERACTIONS AT *LEISHMANIA* SPP. TELOMERES

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In most eukaryotes, telomere binding proteins such as POT1 and TRF2 play crucial roles in telomere biology by interacting with several other telomere regulators to ensure proper telomere maintenance and to form high order complexes known as telosome or shelterin. *Leishmania* spp. telomeres are composed by the conserved TTAGGG repeats which are maintained by telomerase. The basic *Leishmania* telomeric protein complex is formed by the proteins LaRPA-1 and LaRbp38, which bind *in vitro* and *in vivo*, with high affinity, to the G-rich single-stranded DNA, and by proteins that interact with the double-stranded region of telomeres such as the recently described TRF homologue. The *Leishmania* spp. genome, like other trypanosomatid, lacks many of the conserved single-stranded telomeric proteins found in other eukaryotes, such as the CDC13 and POT1 protein homologues. Thus, we speculate that the *Leishmania* RPA-1 homologue may play the same roles as POT1/CDC13 at parasite telomeres, although it can also bind to other single-stranded DNA with high affinity and in a sequence-independent manner. LaRPA-1 together with the multifunctional LaRbp38 protein, which also interacts with a wide range of GT-rich sequences, including telomeres, seems to form part of a parasite telomeric complex that resembles the recently described CST complex. The CST complex is being considered a second telomere capping mode occurring in a broad variety of species, except budding yeast, and is mainly formed by RPA-like proteins. In this report we used different approaches to show that LaRPA-1 interacts with both LaRbp38 and with telomerase, and that these protein:protein interactions seem to occur in a cell-cycle independent manner. In addition, LaRPA-1 partially co-localizes with both proteins, probably reflecting its functions in DNA metabolism. We speculate whether these protein interactions reflect the entire telomeric complex or the presence of functionally distinct subcomplexes at parasite telomeres. Agência Financiadora: FAPESP

## BM.017 - SELECTION OF *L. (L.) AMAZONENSIS* AND *L. (L.) MAJOR* MUTANTS RESISTANT TO PURINE ANALOGUE TUBERCIDIN

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Species of the protozoan Leishmania are the causative agents of a wide range of pathologies called leishmaniasis. Gene amplification is a common phenomenon observed in Leishmania cell lines subjected to drug pressure. Tubercidin (TUB), a toxic purine analogue has already been described as a potent antiparasite action. It is incorporated in nucleic acids of microorganism and mammalian cells, inhibiting DNA synthesis and repair. In this study, we obtained two resistant lines, *L. (L.) amazonensis* (La) and *L. (L.) major* (Lm) resistant to TUB ($TUB^r$). First, we selected $LaTUB^r$ by increasing drug pressure from the wild type $IC_{50}$ value concentration (0.23μM) to 5μM of TUB, conferring to the $LaTUB^r$ 5 a resistance level greater than 43 fold when compared to La wild type cells ($IC_{50}$ value of approximately 10μM). As expected, after 2 months without TUB pressure, $LaTUB^r$ 5 (-2months) cells showed a declined resistance ratio (from 43 to 5 fold TUB resistance; an $IC_{50}$ value of approximately 1.0μM). Curiously, when the same experiment was carried out for Lm cells, during the same period, we just reached 0.5μM of TUB, a concentration 10 fold smaller than the TUB concentration reached for La cells (5μM). To understand why such a marked difference in the TUB sensibility occurs among these two leishmania species, we analyzed the presence of circular DNAs in both resistant lines, by a plasmid alkaline lysis preparation and analysis in agarose gel. We also analyzed the presence of some restriction fragment amplified with digestion of total DNA with *BamH*I and *Hind*III restriction enzyme and no fragment was observed. Cross resistance with allopurinol, glucantime and pentamidine was done, and again no cross-resistance was observed. Further analysis within these mutants must be performed to clarify these different characteristics among these leishmania species in order to find new markers for the inter-specific differentiation. Supported by CNPq, CAPES, FAPESP and LIM48-FMUSP.

## BM.018 - IDENTIFICATION OF ONE LOCUS OF LEISHMANIA (LEISHMANIA) MAJOR RELATED TO TUBERCIDIN RESISTANCE

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A rational strategy for therapeutic exploitation of parasitic diseases can be based on identification of fundamental differences between the parasite and its mammalian host. An important pathway is the purine metabolism. Leishmania and other protozoans are unable to synthesize purine nucleotides by *de novo* and must *salvage* them from the host. Tubercidin (TUB), a purine toxic compound can be considered a potential antiparasite agent by inhibition of purine transport for the Leishmania. Gene identification involving drug resistance has contributed to a better understanding of the action mechanisms of antiparasite compounds. Using a transfection and overexpression selection strategy, we isolated a 31kb *locus* of *L. (L.) major* (cosTUB2) capable to confer resistance to TUB, with resistance ratio 4 times greater than the wild type. After a couple of deletion sets we obtained a 3kb fragment containing a gene probably involved with TUB resistance, according to GenBank related as a hypothetical protein. A realtime PCR demonstrated that the hypothetical protein mRNA was about 30 times more expressed than cosTUB2. However, functional analysis showed that overexpression of hypothetical protein alone presented a 2 fold smaller TUB resistance value than cosTUB2. These results suggested that the upstream region can be involved as a regulation factor in this hypothetical protein. A new construction with the hypothetical protein containing the upstream region was done and it is being submitted to amplification copy number. Cross resistance analysis with the allopurinol showed that both, cosTUB2 and wild type cells presented comparables $IC_{50}$ values of approximately 10μg/mL. This indicated that the mechanism used by cosTUB2 cells is selectively of purine pathway and not for pirimidines. Thus, this study can explain better the purine metabolism in Leishmania and can suggest this important pathway as a new target for antileishmania agents.
Supported by: CAPES, CNPq, FAPESP and LIM48-FMUSP.

## BM.019 - IFN-1 SIGNALING AND TLR2 ARE REQUIRED IN *LEISHMANIA AMAZONENSIS* – INDUCED PKR GENE EXPRESSION.

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*Leishmania* parasites are able to modulate the suppressor and microbicidal macrophage functions. Many macrophage functions and signaling pathways can be regulated by PKR (dsRNA-activated protein kinase R), a host anti-viral protein that also favors *Leishmania* infection. PKR is also involved in the modulation of cytokine signaling and transcriptional. We demonstrated in a *previous study that Leishmania amazonensis induced the PKR protein levels* and can modulated the expression of the *pkr* gene. The TATA-less promoter of *pkr* gene drives the basal and inducible expression through the KCS and ISRE elements of the *pkr* promoter. Toll-Like Receptor 2 (TLR2) also contributes to the innate immunity in *Leishmania* infection, mainly through the binding of LPG (lipophosphoglycan), which trigger several signaling pathways. To investigate the possible role of *Leishmania amazonensis* in the modulation of *pkr* gene expression in infected macrophages, and the putative role of IFN-type I and TLR2, we used a pkr-promoter luciferase gene reporter and RT-PCR/Real-time assays in HEK 293T cell and RAW 264.7 macrophages. Moreover, we also assayed wild-type and knockout TLR2 and IFN-I receptors macrophages. We demonstrated that *L. amazonensis* and $LPG_{amaz}$ modulated the expression of *pkr* gene in wild-type cells, but failed in TLR2 and IFN-I-R-Ko macrophages. Luciferase reporter assays with HEK 293T cells co-transfected with the pkr promoter construction and TLR2 demonstrated that *L. amazonensis* and $LPG_{amaz}$ induced the expression of *pkr* gene. In addition, the Luc activity is dependent on the presence of the TLR2. RT-PCR assays revealed that *L. amazonensis* or $LPG_{amaz}$ induced the expression of *pkr* gene in wild-type cells but not in IFN-I-R knockout cells. Taken together, these data indicate that *L. amazonensis* infection or LPG induces the expression of the *pkr* gene through TLR2 and IFN-type I pathways. Financial support: CNPq, FAPERJ and INCT-Amazônia.

## BM.20 - THE ROLE OF TOLL LIKE RECEPTORS AND THE PROTEIN PACT IN PKR ACTIVATION DURING *LEISHMANIA AMAZONENSIS* INFECTION

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The mammalian protein kinase PKR (ds-RNA dependent protein kinase) is a known as a critical component of the innate immune response against viral infection and modulates the expression of immunomodulatory mediators. To be enzymatically active, latent PKR needs to be induced by binding to one of its activators, dsRNA or the PACT protein. We have previously shown that *Leishmania amazonensis* is able to activate and increase the PKR levels in infected macrophages. PKR activation leads to intracellular proliferation in a IL-10-dependent fashion. The signaling pathways triggered by toll like receptors induce the production of innate immune mediators and may rely on PKR activation. In proliferation assays, we report that peritoneal macrophages of TLR2 or TLR4-KO B6 mice, similar to the observed to PKR-KO macrophages, showed a decreased *L. amazonensis* intracellular proliferation compared with wt macrophages. Our data suggest that toll receptors pathways are important for *L. amazonensis* infection. Additionally, western blotting assays showed that infected macrophages from TLR2-KO and TLR4-KO mice showed weaker PKR activation than wild-type macrophages, suggesting that PKR phosphorylation seems to be partially dependent on TLR. We also showed that the supressor cytokine IL-10, is reduced in macrophages from TLR2 and TLR4-KO mice compared with wt macrophages. Finally, we also observed that *L. amazonensis* is able to induce PACT mRNA, suggesting that PACT may activate PKR in presence of *L. amazonensis* infection. Taken together, these data indicate that PKR-dependent intracellular proliferation of *L. amazonensis* may depend on TLR2 and 4 activation. Additional experiments are in progress to determine the signalling pathways connecting TLRs and PKR, as well as the parasite molecules involved with this activation. Supported by FAPERJ, CNPq and INCT – Amazônia.

## BM.021 - HETEROLOGOUE EXPRESSION OF RECOMBINANT SCAVENGER RECEPTOR MARCO BY CHO CELLS INCREASE THE BINDING OF *Leishmania major*

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CBA mice are resistant to *Leishmania major* yet permissive to *L. amazonensis* infection. CBA macrophages control infection by *L. major,* but not *L. amazonensis, in vitro*. Previous studies have demonstrated that the MARCO scavenger receptor gene is upregulated in CBA macrophages infected with *L. major,* but not with *L. amazonensis*. MARCO is blocked by Mab ED31, reducing macrophage infection by *L. major* by 30%, which suggests that MARCO plays a role in *L. major* recognition by host macrophages. This study aimed to identify the proteins associated with MARCO on the surfaces of macrophages during *L. major* recognition. The MARCO receptor gene was cloned into a pTAPC2 vector containing the TAPTAG sequence, which allowed for the use of a dual-tagging purification method. CHO cells were transfected with pTAPC2-MARCO, the cells were lysed, MARCO expression was identified by western blot and the parasite binding ability of recombinant MARCO-TAPTAG was evaluated. *L. major* promastigotes were allowed to bind to transfected CHO cells at 4℃ for 30 minutes and then submitted to immunofluorescence. CHO cells that express MARCO-TAPTAG bind to *L. major* to a greater extent than control cells, indicating that MARCO-TAPTAG is functional and appears to directly interact with this parasite. Furthermore, MARCO-TAPTAG-expressing CHO cells presented lamellipodia-like protrusions, as has been previously demonstrated. Protein complexes from the pTAPC2-MARCO stably transfected-CHO cells that bound to *L. major* were purified using the tandem affinity purification method and identified by western blot. Similar studies will be performed using pTAPC2-MARCO stably transfected RAW cells to identify the protein complexes involved in *L. major* recognition using mass spectrometry. The identification of proteins involved in *L. major* parasite binding and phagocytosis will contribute to the understanding of the mechanisms that influence infection outcome.
Supported by FIOCRUZ and CNPq - 306672/2008-1

## BM.022 - USING PROTEOMIC ANALYSIS TO IDENTIFY MOLECULAR EXPRESSION IN MACROPHAGES INFECTED WITH *Leishmania amazonensis* OR *Leishmania major*

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CBA mice are resistant to *Leishmania major* yet susceptible to *Leishmania amazonensis*. In addition, CBA macrophages control infection by *L. major* and are permissive to *L. amazonensis*, which suggests that macrophages play an important role in the outcome of *Leishmania* infection. In order to evaluate the global macrophage response to *Leishmania* infection, proteomic studies were carried out. Protein expression was identified six and 24 hours after infection with *L. major* or *L. amazonensis*. Protein extracts were obtained from infected cells to identify peptides using LC-MS/MS with a MudPIT approach. The results from six independent experiments were analyzed and 382 proteins were found to be expressed differently, in accordance with infection by *L. amazonensis* or *L. major*. These proteins are involved in a variety of cell functions, including cell death, post-translational modification, lipid metabolism, molecular transport, amino acid metabolism, small molecule biochemistry, cell signaling, cell cycle and cell-mediated immune response. Using IPA software, ten protein networks were constructed. The proteins related to lipid metabolism and small molecule biochemistry were grouped into one network and exhibited higher expression levels in *L. amazonensis*-infected cells compared to *L. major*. Proteins related to cell signaling and cellular assembly, organization and movement formed another network exhibiting higher expression levels in *L. major*-infected cells compared to *L. amazonensis*. These results clearly demonstrate that *L. amazonensis* and *L. major* modulate macrophage functions in different ways. In conclusion, macrophage response to *L. amazonensis*, did not establish a definite activation profile. However, *L. major* activates cell-signaling networks, with respect to cell activation. Taken together, the data indicate that parasites play important roles affecting cell activation related to infection outcome. Currently, western-blot analyses are being performed to corroborate differences in protein expression levels detected by high throughput proteomic analysis. Supported by CNPq (306672/2008-1).

## BM.023 - FUNCTIONAL CHARACTERIZATION OF THREE *LEISHMANIA* PABP HOMOLOGUES WITH DISTINCT BINDING PROPERTIES TO RNA AND PROTEIN PARTNERS

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The Poly(A) binding protein (PABP) is a conserved eukaryotic protein involved in many aspects of mRNA metabolism such as mRNA biogenesis, processing, transport and degradation. It also performs major roles during translation initiation, through an interaction with the translation initiation factor eIF4G, and also possibly during termination. Here we describe the study of the three *Leishmania major* PABP homologues (*Lm*PABP1 through 3) whose genes were found within available genomic sequences. The three proteins are abundantly expressed and cytoplasmic but only *Lm*PABP1 is represented as multiple isoforms. Upon transcription inhibition with actinomicyn D a major shift in protein localization was observed, with both *Lm*PABP2 and 3 migrating to the nucleus, whilst *Lm*PABP1 remained predominantly cytoplasmic. This differential localization of *Lm*PABP2 and 3 is not induced upon inhibition of mRNA processing or translation using the inhibitors sinefungin and cycloheximide, respectively. Through pull-down assays we observed that all three proteins bind to a *Leishmania* eIF4G homologue (*Lm*EIF4G3), with the binding of *Lm*PABP1 being the most efficient. Immunoprecipitation assays (IP) confirmed the binding between *Lm*EIF4G3 and *Lm*PABP1 although we were not able to detect any interaction between *Lm*EIF4G3 and *Lm*PABP2 and 3 or between a second eIF4G homologue, *Lm*EIF4G4, and any of the three PABPs. IP assays also showed an interaction between *Lm*PABP2 and *Lm*PABP3 homologues, in a RNA-independent manner. Through RNA binding assays *Lm*PABP1 was found to bind specifically to A-rich sequences whilst *Lm*PABP2, despite overall strong affinity to RNA, lacked specificity and *Lm*PABP3 displayed an intermediate phenotype. Complementary RNAi analyses of *T. brucei* prociclic forms with the *Lm*PABP1 and 2 orthologues indicate that both proteins are required for cellular viability. Our results imply that the *Lm*PABP1 homologue is the major candidate to play a role in translation and that the *Lm*PABP2 and 3 may participate in novel functions.
Supported by FACEPE, FIOCRUZ, CNPq

## BM.024 - DIAGNOSIS OF AMERICAN TEGUMENTARY LEISHMANIASIS AND *LEISHMANIA* SUBGENUS IDENTIFICATION USING PCR-RFLP IN THE WEST-CENTRAL REGION OF BRAZIL

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American Tegumentary Leishmaniasis (ATL) is a human disease with a broad spectrum of clinical manifestations depending on parasite species and host immune responses. The aim of this study was to evaluate the contribution of PCR-RFLP to the diagnosis and parasite subgenus identification in biopsy fragments from cutaneous and mucosal lesions. Patients clinically suspected of having cutaneous (CL, n = 31) or mucosal (ML, n = 6) leishmaniasis were assisted at the Tropical Disease Hospital, in the West-Central Region of Brazil. Montenegro skin test (MST), indirect immunofluorescence (IFI), and histopathological exam followed by immunohistochemistry analysis to confirm parasite presence were performed. Genomic DNA was extracted from fragment biopsies and a PCR to amplify conserved region of the kDNA minicircles was done. PCR products were further submitted to PCR-restriction fragment length polymorphism (PCR-RFLP) analysis, using Hae III or AVA I enzymes. Among 37 patients, only 33 were confirmed ATL (CL, 29; ML, 4). Confirmed cases of ATL were detected with similar sensitivity by histopathological analysis (60.7%), MST (73.1%), and PCR (72.7%). However, IFI presented low sensitivity (36.6%). Although high level of agreement between results of histopathological and PCR tests to diagnosis of ATL, for ML all histopathological analysis did not detect parasites on lesions while PCR was positive in two samples. PCR-RFLP analysis identified 91.7% of parasites as *L. (Viannia)* (CL, 20; ML. 2) and 8.3% as *L. (L.) amazonensis* parasites (CL, 2). In conclusion, our data support the value of PCR-RFLP as a reliable method for the diagnosis of ATL. Our data from ML suggest that PCR-RFLP can be more sensitive than other methods for diagnosis when very low numbers of parasites are present in lesions. Parasite genus/species identification is helpful to improve treatment schedule, to define the epidemiology of the leishmaniasis in Brazil, and to understand the pathogeny of ATL. Supported by CNPq and FAPEG.

## BM.025 - SEARCHING FOR SIGNALS IN THE TRITRYPS GENOMES

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The protozoans *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania major* (Tritryps), are evolutionarily ancient eukaryotes which cause worldwide human parasitosis. Probably due to the early branching in eukaryotic evolution, they present unique biological features. Particularly, RNA polymerases (RNAP) are distinctive. For RNAPII transcribed genes, the search for transcription initiation sites has been elusive so far. Polycistrons strand switch regions have been implicated in this process and evidence favoring this hypothesis has been accumulating lately. Besides, *Trypanosoma brucei* ARNPI is capable of synthesizing the pre-mRNAm coding for VSG and procyclin proteins. In eukaryotes, RNAPI promoters are characterized by the conservation of conformational elements but no sequence conservation.We aim to determine the structural characteristics of transcription initiation sites in Tritryps to better describe putative binding sites. In this regard, we have collected the sequences corresponding to the RNAPI transcription start sites of about 25 eukaryotes (including the ones for Tritryps) and constructed databases of their conformational characteristics. We found interesting conformational similarities among RNAPI promoter sites from Tritryps and other eukaryotes. We also performed a genome wide analysis for the Tritryps of intrinsic curvature and constructed chromosome specific files that allow visualization of curvature in a global scale using the Artemis genome browser. Apart from this, we plan to carry out an experimental approach including the characterization of each polymerase transcriptome. We are carrying out *run-on* assays in the presence of $\alpha$-amanitin to establish the transcriptome of RNAPI. We conducted these experiments with *T. brucei* procyclic forms. qRT-PCR analysis of the sub-population of RNAs obtained established that the methodology is able to separate RNAs synthesized *de novo* but the conditions for RNAPI inhibition should be adjusted.

## BM.026 - INVESTIGATION OF MECHANISMS AND ELEMENTS INVOLVED IN REGULATION OF GENE EXPRESSION IN *LEISHMANIA*

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Untranslated regions (UTR) of genes may contain elements that interact with protein machineries that will modify mRNA steady state levels or initiation of translation activity of that given gene. This mode of postranscriptional regulation of gene expression in regulatory effects of gene expression in trypanosomatids such as mRNA maturation, mRNA abundance and translational efficiency. We developed an in silico pipeline to search for conserved sequences motifs within the flanking regions of all annotated CDSs of the *Leishmania braziliensis*, *L. major* and *L. infantum* genomes. These conserved sequences (CICEs) were grouped using *L. major* Gene Ontology Family and we selected four conserved elements that flank genes LmjF21.0725, LmjF22.1060, LmjF22.1630 and LmjF33.3190. The elements are oligonucleotides varying in size from 34 to 41 nucleotides. Preliminary results of Electrophoretic Mobility Shift Assays (EMSA) suggest that these RNA sequences interact with proteins. To test the potential role of these elements in modifying transcript stability or level of translation of the controlled gene we generated versions of a construct in which the neomycin phosphotransferase (*NEO*) gene is flanked by the the Dihydrofolate Reductase Thymidilate Synthase (*DHFR-TS*) sequences and each CICE was inserted within the *DHFRTS* 3'UTR . Targeting fragments of these constructs have been transfected in *Leishmania major* to replace one DHFRTS allele. After selection of positive clones, no drug of selection is used and *NEO* works as a reporter gene. Mutants and control cells were compared regarding growth behavior, reporter gene expression levels and transcript stability, results will be presented.
Supported by FAPESP.

## BM.027 - COMPARATIVE ANALYSIS OF THE EXPRESSED GENOME OF *LEISHMANIA BRAZILIENSIS* ISOLATED FROM TWO DISTINCT CLINICAL MANIFESTATIONS

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*Leishmania braziliensis* is a major ethiological agent of cutaneous leishmaniasis in Brazil. The mucosal form of the disease affects about 5% of patients which may present progressive destruction of cartilage or bones of the face, pharynx and larynx. To investigate possible phenotypic modifications between parasites from mucosal and cutaneous sites we evaluated the proteome profile differences of paired mucosal and cutaneous isolates of *L. braziliensis*. Parasites recovered from patients with concomitant mucosal (LBM) and cutaneous lesions (LBC) were investigated. We have previously shown that the molecular karyotypes of all isolates were indiscernible. The proteome comparative analysis revealed among other genes, the differential pattern of expression of LbrM31_V2.2410 (prostaglandin f2-alpha synthase) which was uniquely expressed in $LBC^1$ (patient 1) and was 1.7 times more expressed in $LBC^2$ (patient 2) when compared with $LBM^1$ and $LBM^2$, respectively. To further investigate this gene and the biological significance of the finding we conducted Northern Blotting experiments and we constructed a plasmid bearing LbrM31_V2.2410 to be expressed in *Escherichia coli*. The overexpressed soluble protein has been injected in rabbit and chicken for the production of polyclonal antibodies. Additionally, we have observed in one of the *in vivo* infection experiments a mucosal lesion in one hamster infected with LBM[1]. We could not rescue parasites from the lesion. Next, we conducted *in vivo* infections to reproduce such mucosal lesions, but they were never observed. Histological sections from mucosal and cutaneous tissues of 16 hamsters infected with $LBM^1$, $LBM^2$, $LBC^1$ and $LBC^2$ were examined in search for tissue infection signs, or inflammatory infiltrate. Parasites and inflammation were only detected in the cutaneous lesions of all animals evaluated.
Supported by: FAPESP and CNPq.

## BM.028 - *IN SILICO* WORKFLOW TO MAP AND TO CHARACTERIZE CONSERVED INTERCODING SEQUENCES OF *LEISHMANIA* SPP. GENOMES

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After obtaining the complete genomes of different *Leishmania* species, *in silico* analyses of generated data are now widely used to improve the understanding of these pathogens' biology. *Leishmania spp.,* such as other trypanosomatids, have their protein-coding genes grouped in long polycistronic units of functionally unrelated genes and accumulating evidence indicates that control of gene expression happens by diverse mechanisms at the posttranscriptional level. The high degree of synteny among *Leishmania* species is accompanied by highly conserved coding sequences (CDS) and poorly conserved non-coding sequences (intercoding). Having as our main goal the identification of elements involved in control of gene expression, an *in silico* investigation of conserved sequences in the intercoding regions of *L. major, L. infantum and L. braziliensis* genomes was conducted. A combination of computational tools Linux-Shell[1], PERL[2] and R[3] languages, BLAST[4], MSPcrunch[5] and SSAKE[6] algorithms were used to construct a workflow for: formatting data files (1,2); searching for conservation in the target-regions and creating a redundant FASTA file with millions of conserved intercoding sequences (2, 4, 5), clustering this FASTA file to eliminate redundancy (6), assigning GO biological process and KEGG pathways classification for the generated contigs using *L. major* neighbouring CDS as template (1, 2) and giving statistical support for the gene-enrichment annotation (3). The computational workflow will be presented together with some identified motifs specifically associated (enriched) to GO and/or KEGG terms. These findings may contribute to the prediction of putative regulons in the *Leishmania spp.* genomes. Supported by FAPESP and CNPq

## BM.029 - INVESTIGATION OF THE LEISHMANIA MAJOR HUS1 GENE AND ITS ROLE IN ATM/ATR-MEDIATED RESISTANCE TO REPLICATIVE AND OXIDATIVE STRESS.

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The parasite *Leishmania* has a dynamic and plastic genome in which gene amplification and chromosome translocations are common phenomena. Such plasticity hints at the necessity of dependable genome maintenance pathways. In other eukaryotes, PIK-related kinases ATR and ATM act as principal sensors that govern DNA damage response. Also, the Rad9-Rad1-Hus1 (9-1-1) trimeric complex has a central role in sensing and signaling DNA damage. The binding of 9-1-1 complex at DNA lesion stabilizes ATR allowing an effective activation of effector kinase Chk1. We have investigated the 9-1-1 subunit Hus1 in *Leishmania major.* LmHus1 is a nuclear protein that improves the cell capability to cope with replicative and oxidative stress. Overexpression of LmHus1 mediated resistance to the genotoxic drugs such as hydroxyurea (HU), methyl methanesulfonate (MMS) and Oxigen peroxide ($H_2O_2$). On the other hand, an increase in LmHus1 expression did not confer resistance to phleomycin. Inhibition of ATR and ATM kinases by caffeine abrogates the resistance phenotype associated to LmHus1 overexpression.The LmHus1 involvement in double strand breaks (DSB) repair is being further investigated using gamma-ray assay. Also, a possible homologue of the effector kinase Chk1 has been identified in the parasite genome e is being investigated. Current work is focused not only in the generation of an *LmHUS1* disrupt cell line, but also in the investigation of the LmHus1 expression along parasite life. Preliminary results suggested that the gene is not expressed in the metacyclic form of the parasite. These studies will provide a better understanding of the DNA repair pathways and mechanisms of genome maintenance in the protozoan parasite *Leishmania*.
Financial support: FAPESP and CNPq

## BM.030 - WORKING ON THE CHARACTERIZATION OF THE PSEUDOURIDINE SYNTHASE 7 FROM *TRYPANOSOMATIDS*

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Transfer RNAs (tRNAs) play a central role in protein synthesis, being the translators of the genetic code. As many other molecules, they need to undergo several modifications to become mature and functional. The yeast Pseudouridine Synthase 7 (Pus7) is a multisite and multisubstrate enzyme that is able to modify uridines in several tRNAs, U2 small nuclear RNA and rRNA. In pre-$tRNA^{Tyr}$, it acts on U35, catalyzing the formation of pseudouridine (ψ), a highly phylogenetically conserved modification. We found homologues of the yeast Pus7 enzyme in the genomes of the tritryps (*Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania major*). In order to functionally characterize these enzymes in trypanosomatids we cloned the *T. cruzi pus7*-like gene to produce the recombinant enzyme and test its activity towards different tRNA transcripts as substrates. Furthermore, we have cloned and sequenced an internal region of the *pus7*-like gene from *T. brucei* and generated a RNAi construct to evaluate the importance of this enzyme for the different life stages of the parasite.
Supported by: FAPERJ, OMS, CNPq
Key words: Trypanosoma, tRNA, Pseudouridine, trypanosomatids

### BM.031 - SELENOCYSTEINE INCORPORATION MACHINERY IN *NAEGLERIA GRUBERI*

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Selenium (Se) is an essential trace element *in vivo,* which is mainly found in selenoproteins, that contains the 21st amino acid (Selenocystein – Sec – U). The selenoproteins generally works in the balance of redox state of the cell, playing an important role on cell growth and proliferation. Selenocystein is encoded by a TGA codon in phase in an open reading frame (ORF) when a specific stem-loop structure, designated Sec insertion sequence (SECIS) element, is located in the 3'-untranslated region (UTR) directs the insertion of Sec. The publication of *Naegleria gruberi* (ATCC 30224) genome allowed us to investigate the presence of the Sec-incorporation pathway in a primitive eukaryote. Using a throughly bioinformatics approach, we identified the following genes involved in Sec-incorporation: Phosphoseril tRNA kinase (PSTK), Selenocysteine Synthase (SepSecS), Selenophosphase synthase (SelD or SPS), elongation factor EFSec (SelB) and a SECIS binding protein (SBP). Also, we found two potential tRNASec and one selenoprotein that is homologue to a mitochondrial thioredoxin redutase (TR3). Our predictions were confirmed by total RNA extraction, RT-PCR and sequencing, showing that the selenocysteine incorporation machinery is indeed present in *N. gruberi.* Interestingly, the SelD of *N. gruberi* has two distinct domains. The N-terminal has a predicted methiltransferase activity and the C-terminal is homologue to SPS/SelD. The SelD domain is phylogenetically of prokaryote origin, which could indicate an event of lateral gene transfer to *N. gruberi*. This is also observed in *Spironucleus barkhanus,* a member of the basal eukaryotic infrakingdom Excavata. Such observations suggest the possibility of finding completely new selenoproteins, and it has interesting features that may help to elucidate the evolutionary history of this pathway. For this purpose, a careful search for conserved domains and SECIS using hidden Markov and covariance models is currently underway. Supported by CAPES, CNPq and FAPESP

### BM.032 - CHARACTERIZATION OF KINETOPLAST PROTEINS FROM *Crithidia deanei*

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Trypanosomatids are unicellular organisms that parasitize plants, animals and insects. Many species are monoxenic with single invertebrate host, usually an insect, during life cycle. Among these monoxenic species, *Crithidia deanei, C. oncopleti, C. desouzai, Blastocrithidia culicis* and *Herpetomonas roitmani* harbor a symbiotic bacterium in the cytoplasm. This mutualism is an excellent model to study the origin of organelles and cellular evolution. The mitochondrial DNA in trypanosomatids is localized in the structure named kinetoplast (kDNA). The presence of the endosymbiont causes ultrastructural modification toward a larger unwind kDNA network. Studies of kDNA on protozoans that harbor symbiotic bacteria are unknown. In collaboration with the National Laboratory of the Scientific Computational (LNCC), we have sequenced the genomes of the *C. deanei* and *B. culicis* using the 454 Roche platform. These are the first genomes sequenced from parasite with endosymbiont and they are being annotated in the SABIA system (http://www.sabia.lncc.br). Our objective is to identify kinetoplastid-associated proteins (KAPs) and proteins of the replication mechanism of the kDNA in the genome of *C. deanei*. Approximately 51 million of base pairs were analyzed with 23X genome coverage. The genome has 48% of G+C content and 17,762 ORFs with an average length of 1320 bp. We were able to find sequences coding for proteins involved in the kDNA structure and in the replication mechanism. The universal minicircle sequence binding protein (UMSBP), which has 9 zinc-fingers domains and 70% of the identity with UMSBP of the *T. cruzi*, and the KAPs of C. deanei, with 60% of identity with the *T. cruzi* and *C. fasciculata* orthologs, are examples. The information generated in this project will contribute to a better understanding of the biology of this monoxenic protozoan.
Supported by CNPq, FAPERJ, LNCC.

## BM.033 - COMPARATIVE GENOMICS AND FUNCTIONAL ANALYSIS OF PROTEINS INVOLVED IN MRNA NUCLEOCYTOPLASMATIC EXPORT IN TRYPANOSOMES

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Different RNAs are exported from the nucleus by specialized pathways. It is not known whether RNA export pathways are conserved in deeply diverging eukaryotic lineage, and the question of how complex the RNA nucleocytoplasmic export was in the last eukaryotic common ancestor (LECA) remains open. One of our objectives was to reconstruct the evolutionary history of RNA export pathways across eukaryotes by screening genomes for the presence of homologs in metazoa and fungi, using human and yeast as queries. Our comparisons indicate that several key proteins involved in RNA export pathways are conserved across most eukaryotic lineages, inferring that orthologs were already present in LECA. The mRNA export pathway is the most complex and the least conserved one, suggesting that among deeply diverging eukaryotic lineages is different from what is observed in extant “higher” eukaryotes. To better understand mRNA export in deeply diverging eukaryote lineages, we are investigating the function of well conserved proteins in trypanosomatid protozoa, causative agents of deadly human diseases. The most conserved protein across eukaryotes was Sub2/UAP56, a component of TREX complex that connects transcription with mRNA export. It is a nuclear protein in *L.major*, *T.brucei* and *T.cruzi*. Ultrastructural analysis showed that *T.cruzi* Sub2 localizes in foci, excluding nucleolus, at the interface between dense and non-dense chromatin areas indicating its association to transcription sites. This result was further analyzed by BrUTP incorporation assays and TcSub2 colocalizes with RNA pol. II transcription sites. These evidences strongly suggest that TcSub2 participates in nuclear mRNA metabolism. We are currently investigating the role of Sub2 to elucidate if it’s component of mRNA export in trypanosomatids. Besides, the double knockout of the TcSub2 gene is lethal in *T. cruzi* and TbSub2 iRNA causes a growth defect in *T. brucei*, indicating that it is an essential protein for trypanosomes. Supported by CAPES, CNPq, Fundação Araucária and FIOCRUZ.

## BM.034 - KNOCKOUT OF THE GENE ENCODING THE METACYCLIN II PROTEIN IN *Trypanosoma cruzi*

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The protozoan, *Trypanosoma cruzi* is the causative agent of Chagas disease, the most important parasitic infection in South America. The life-cycle of this parasite involves two intermediate hosts (triatomine insects and mammals) and three well-defined morphological and functional developmental stages: epimastigotes, trypomastigotes and amastigotes. The differentiation of epimastigotes into metacyclic trypomastigotes (metacyclogenesis) involves the transformation of a replicative non-infective form of *T. cruzi* into a non-replicative infective stage. Several major phenotypic changes occur during metacyclogenesis, including the kinetoplast morphology and kinetoplast DNA (kDNA) compaction. The kDNA is an unusual form of mitochondrial DNA consisting of a catenated network of several thousand minicircles and a smaller number of maxicircles. The kDNA is associated with H1 histone-like proteins, known as kinetoplast-associated proteins (KAPs), which condense the kDNA network. Little is known about the role that KAPs play in kDNA remodeling that occurs during the differentiation of *T. cruzi*. Three KAPs (KAP3, KAP4 and KAP6) were identified in all developmental stages of the parasite and present a differential distribution within the kinetoplasts of epimastigote, amastigote and trypomastigote forms (Cavalcanti *et al*., 2009, De Souza *et al*., 2010). Another protein (metacyclin II), which is encoded by the gene *Tcmet2*, was localized mainly at the kinetoplast of the parasite and is up-regulated in metacyclic trypomastigote (Yamada-Ogatta *et al*., 2004). In this work we used gene deletion to investigate the function of TcMET2. *Tcmet2* null mutants are viable and we are investigating possible changes in kinetoplast morphology by light, confocal and electron microscopy. We are also analyzing its fitness during proliferation, differentiation and infectivity. It is still unclear how the compact kinetoplast disk of epimastigotes is converted into a globular structure in the infective trypomastigotes, we believe that TcMET2 could play a role in the kinetoplast morphology and kDNA compaction. Supported by CNPq, CAPES and Fundação Araucária

## BM.035 - *TRYPANOSOMA CRUZI* SUBTELOMERIC GENOMIC CONTEXT AND ITS ROLE IN RECOMBINATION EVENTS

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GP85 gene family, related to cell invasion, display great sequence diversity and is often associated with subtelomeric regions. We previously suggested that the preferred telomeric location of GP85 genes could hold up recombination events further the generation of new variants. The role of subtelomeric regions in DNA recombination has been analyzed with a *T. cruzi* artificial chromosome (pTAC) carrying a GP85 pseudogene. No recombination events involving displacement of pTAC sequences could be detected. Reduced recombination rate has been described in T. brucei laboratory-adapted strains (Boothroyd et al., 2009). Authors demonstrated that the introduction of DNA double strand breaks (DSB) adjacent to the 70-bp repeats upstream of the transcribed VSG increases switching in vitro 250-fold. Our proposal is introduce DSB adjacent to repetitive telomeric sequences near GP85 and verify if there is an increase in rearrangements in T. cruzi genome. To induce DSB, we performed a system established by Taylor MC and Kelly JM, involving plasmids pLEW13/pTcINDEX. T. cruzi (G, CL and Y strains) were transfected with pLEW13 to express T7 RNA polymerase and tetracycline repressor protein constitutively. Afterwards, these clones were transfected with luciferase enzyme cloned in pT7LUC. The system was tested after induction with tetracycline 24h, 48h, 15 and 30 days after transfection. Luciferase activity could be induced up to 75-fold when compared with control, within 48h of tetracycline addition. We selected few clones from each strain and transfected with pTcINDEX-RFP. Preliminary results shown CL clones die in presence of hygromycin, the pTcINDEX resistance gene. Therefore, just clones from G and Y strain were transfected with pTcINDEX- I-SceI. We hope pLEW13/pTcINDEX system can represent a valuable genetic tool for studying recombination process involving telomeric sequences in T. cruzi. Although, recombination events are very rare, artificial DSB in specific points can induce rearrangements in T. cruzi genome. Supported by FAPESP, CNPq and CAPES.

## BM.036 - IDENTIFICATION AND FUNCTIONAL CHARACTERIZATION OF NUCLEOTIDE-SUGAR TRANSPORTERS OF *TRYPANOSOMA CRUZI* BY HETEROLOGOUS EXPRESSION IN YEAST AND MAMMALIAN CELLS

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Glycoconjugates play a fundamental role in many different biological processes such as the development of multicellular organisms and the survival and infectivity of parasites. A variety of human diseases are caused by defects in glycosylation and key processes for parasite infection such as cell invasion and modulation of the host immune system depend on glycoconjugates. The addition of sugars to proteins and lipids occurs in the lumen of the endoplasmic reticulum (ER) and Golgi apparatus and is catalyzed by glycosyltransferases located in these organelles. Nucleotide sugars, the activated sugar donors, are mostly synthesized in the cytosol and therefore must be transported across the ER and Golgi membranes. The intracellular transport of nucleotide sugars by multi-spanning transmembrane proteins known as nucleotide-sugar transporters (NSTs) is essential for glycoconjugate biosynthesis. To functionally characterize nucleotide-sugar transporters from *Trypanosoma cruzi* we initially searched for putative NSTs in the *T. cruzi* genome. By performing Blast searches in the GeneDB and TriTrypDB databases using characterized NSTs of different organisms as queries we have identified a family of 11 putative NSTs. Sequence analyses of the *T. cruzi* genes revealed hydrophobic profiles and characteristic motifs found in other members of the NST family. Heterologous expression of these genes in *Saccharomyces cerevisiae* and *Kluyveromyces lactis* mutants - deficient in GDP-mannose and UDP-N-acetylglucosamine transport, respectively – to rescue the wild-type phenotype were unsuccessful suggesting that trypanosomatid NSTs may not be functional in yeast. Interestingly it has recently been shown that *Leishmania* NSTs are able to rescue glycosylation defects of mammalian mutants deficient in UDP-galactose transport. We are thus using a similar approach to identify and characterize *T. cruzi* NSTs responsible for the uptake of this substrate. Furthermore gene knockout experiments are currently being performed to evaluate the role of glycoconjugates and specific NSTs in the parasite infectious cycle. Supported by Fiocruz and CNPq.

## BM.037 - EXPRESSION AND EPITOPE MAPPING OF SAP (SERINE-, ALANINE-, AND PROLINE-RICH PROTEIN) OF *TRYPANOSOMA CRUZI*

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Some members of SAP (serine-, alanine-, and proline-rich proteins) family are secreted/excreted by metacyclic trypomastigotes in the extracellular medium. The central domain of SAP (SAP-CD, 155 amino acids) binds to mammalian cell in a dose-dependent and saturable manner, inhibiting the cell adhesion and invasion by metacyclic forms. In the present study, to determine the important amino acid sequences for the adhesion, epitope mapping was performed using overlapping recombinant peptides corresponding to the SAP-CD. Monoclonal antibodies (MAb SAP4 and SAP5) and polyclonal monospecific antiserum were generated against the SAP-CD expressed as GST fusion protein. In immunofluorescence assays, MAbs and the monospecific antiserum reacted with cytoplasmic components of epimastigotes and metacyclic trypomastigotes permeabilized with saponin and fixed with formaldehyde. Murine MAbs and polyclonal rabbit antiserum reacted with a 55-kDa SAP shed into the extra-cellular medium by metacyclic forms. The 55-kDa SAP was also detected by the MAbs in the protein extracts of epimastigote and metacyclic forms. To map continous epitopes of SAP, overlapping sequences from the SAP-CD were expressed as GST fusion proteins. All anti-SAP antibodies recognized a peptide of 54 amino acids (aa) located in the middle of SAP-CD. A major B-cell epitope spanning residues 36 to 46 (GSPSPPPPATP) has been predicted in the 54-aa peptide. Recombinant proteins (in fusion with GST) spanning the 54-aa region were generated and tested with MAbs and polyclonal anti-SAP antibodies. Interestingly, a 22-aa peptide (MGAA GSPSPPPPATPGSAGANS) carrying the predicted epitope reacted with the rabbit antiserum but failed to be recognized by the MAbs. This could suggest that the antigenic site recognized by MAbs is defined by a conformation-dependent structure rather than a linear sequence. Recombinant proteins containing additional sequences larger than the 22-aa peptide will be constructed to define the main antigenic site of MAbs.
Supported by CNPq and FAPESP.

## BM.038 - CHARACTERIZATION OF DIFFERENT MEMBERS OF AMASTINS AND THEIR ROLE IN *TRYPANOSOMA CRUZI* HOST CELL INTERACTION

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Amastins are 170-200 amino acids, highly hydrophobic surface glycoproteins expressed not only in *T. cruzi* amastigotes but also in amastigotes from several species of *Leishmania*. The *T. cruzi* genome contains several copies of amastins, which can be subdivided in two groups in this parasite. The $\delta$ amastins are organized in a cluster containing alternated copies of tuzin genes, differently from $\beta$ amastins which are not. Likewise $\delta$ amastins, mRNA from members of the $\beta$ group is more abundant in amastigotes than in the others stages of the parasite. Analyses of amastin genes present in the genome of various strains of *T. cruzi* showed that they encode highly divergent proteins with increased amino acid variability in the protein domain that is likely in contact with the host cell cytoplasm. In order to gain new insights of their function, parasites over expressing amastin were generated and yeast two hybrid (Y2H) experiments were performed to identify human proteins able to interact with amastins. Epimastigotes over expressing a $\delta$ amastin gene in fusion with GFP showed a higher multiplication rate in comparison to wild type parasites. From the Y2H experiments, ten positive clones were identified and IL-15 cDNA was present in two of them. IL-15 is a cytokine which modulates different cell populations and has numerous immune-related functions, as well as antiapoptotic effect in various cell types. In contrast to other cytokines, IL-15 exhibits two isoforms: a secreted and a cytoplasmic one. Moreover, elevated levels of this cytokine were found in heart lesions from people infected with *T. cruzi*. We observed that IL-15 interacts with the surface of intracellular amastigotes and, to confirm a direct interaction of amastins with IL-15, *in vitro* pull-down experiments and co-immunoprecipitation assays are being conducted. Supported by: CNPq, FAPEMIG and HHMI.

## BM.039 - PROTEASOMAL ATPUBIQUITIN-DEPENDENT AND -INDEPENDENT PROTEOLYSIS DURING THE *IN VITRO* METACYCLOGENESIS OF *TRYPANOSOMA CRUZI*

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Proteasomes are large protein complexes, the main function of which is to degrade unneeded or damaged proteins. The inhibition of proteasome activity in *Trypanosoma cruzi* blocks parasite replication and cellular differentiation. We analyzed proteasome-mediated proteolytic degradation during the cellular differentiation of *T. cruzi* from replicative non infectious epimastigotes to non replicative and infectious trypomastigotes (metacyclogenesis). We provide the first demonstration that proteasome-dependent proteolysis occurs during metacyclogenesis. We also present biochemical evidence for the coexistence of proteasomal degradation processes dependent on and independent of ubiquitin. During *in vitro* metacyclogenesis, no increase in protein degradation was observed following the addition of ubiquitin and ATP. No peaks of ubiquitin-mediated degradation were observed and the profile of ubiquitinated conjugates was similar at all stages of differentiation. An analysis of carbonylated proteins showed significant variation in oxidized protein levels at the various stages of differentiation. Proteasome inhibition also increased oxidized protein levels. Proteasomes may therefore be involved in the degradation of oxidized proteins, because oxidized or misfolded proteins are the natural substrates of ubiquitin-independent proteolysis. During metacyclogenesis, several proteasome complexes may act together, and the 20S proteasome may be free or linked to regulatory particles (PA700, PA28/PA26). We hypothesize that the coordinated action of these complexes controls the coexistence or degradation of different proteasomes responsible for degrading ubiquitin-tagged or oxidized proteins.
Supported by Capes

## BM.040 - UBIQUITIN-RELATED PROTEOME OF *Trypanosoma cruzi*

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*Trypanosoma cruzi* metacyclogenesis is a differentiation process driven mainly by post-transcriptional changes in gene expression, that may be controlled at protein level by modulation of the activity, location or amount of stage-specific proteins. This modulation involves a complex combination of signaling systems, in which ubiquitination – modification of target-proteins by ubiquitin (Ub) - plays an important role. Aiming the identification of Ub-related proteins, the present work has generated some important tools for this study, such as the production of antibodies against *T.cruzi* Ub and functional recombinant Ub, besides transfected *T.cruzi* cells that express recombinant Ub fused to different molecular tags. In this way, different ubiquitin-affinity proteins could be identified: an hypothetical protein (4764.t00006) with an ubiquitin-binding domain (UBA); DNA and RNA binding proteins, such as Alba (4859.t00001) and ribosomal subunits; proteins that regulate the translation initiation, such as the initiation factor eIF4a (8728.t00019) and a small GTP-binding protein (8128.t00005) from the endocytic pathway. All above-mentioned mechanisms are regulated by the ubiquitination process in other organisms. We have also identified other proteins with still not described function, such as some hypothetical proteins with no known domains (4718.t00003 and 8445.t00002, among others). Furthermore, we have also identified proteins involved in cellular processes where, up to now, no ubiquitination is known to occur, such as proteins involved in the Krebs cycle (7146.t00001), in the pentoses pathway (8033.t00005), or even the gluconeogenesis (4917.t00002). The present work has initiated investigations on the molecular and cellular mechanisms of ubiquitination in this primitive organism. Identification of ubiquitin-interacting proteins and of targets for this signalization in *T.cruzi* will help to understand the mechanism of regulation of gene expression in this pathogenic protozoan.
Supported by CAPES and CNPq.

## BM.041 - CLONING, EXPRESSION CHARACTERIZATION AND CELLULAR LOCALIZATION OF COMPONENTS OF UBIQUITINATION SYSTEM IN *T. CRUZI* METACYCLOGENESIS

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Ubiquitination is a post-translational mechanism that modifies target-proteins by another protein known as ubiquitin. At least three groups of enzymes are required for ubiquitination: Ub-activating (E1), Ub-conjugating (E2) and Ub-ligase (E3) enzymes. E3 is generally considered to be most important in controlling target specificity, despite E2 also being responsible for target recognition. This mechanism plays an important role in most cellular processes by modulation of the activity, location or amount of many proteins. *Trypanosoma cruzi* metacyclogenesis is a differentiation process driven by environmental stimuli, like population and nutritive stress, and for adaptation to this new environment is necessary a gene expression reprogramming. Control of gene expression in this organism occurs at post-transcriptional level and ubiquitination may be involved tagging stage-specific proteins. In this work, *in vitro* metacyclogenesis was used as an experimental model to investigate the expression of enzymes involved in ubiquitination system. The expressed recombinant proteins from these genes were used to produce polyclonal antibodies, which were subsequently used to characterize the expression (by immunoblot assays) and localization (by immunolocalization using confocal laser microscopy) of these enzymes during metacyclogenesis. The expression analysis disclosed two patterns: decreased (two E2, one E3) and unchanged (one E1, one E3) protein levels during metacyclogenesis. Cellular localization of all enzymes showed a cytoplasmic distribution, some of them with accumulation around the nucleus (one E3), other with a strong localization at flagellar adhesion zone (one E2). These results are the first attempt for the characterization of the ubiquitin system in *T. cruzi*. Supported by CAPES and CNPq.

## BM.042 - GENOME-WIDE ANALYSIS OF TcRBP40 AND ITS ASSOCIATED mRNAs IN *Trypanosoma cruzi*

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Beyond the medical interest on Chagas disease, *Trypanosoma cruzi* is considered a model organism for studying the posttranscriptional mechanisms that regulate gene expression in eukaryotes. These mechanisms are mediated and facilitated by RNA binding proteins (RBPs) and regulatory elements present in the untranslated regions (UTR) of mRNAs. mRNAs associate in functionally related ribonucleoprotein complexes (RNPs) to define regulatory networks known as RNA Regulons. Characterizing the proteins present in the different RNPs and their target mRNAs may contribute to the comprehension of posttranscriptional mechanisms in eukaryotes. We have selected several proteins that are expressed in the epimastigote forms of *T. cruzi* and show some degree of regulation during differentiation, focusing on those containing the RNA Recognition Motif (RRM). We characterized the function of one of these proteins, TcRBP40, using a ribonomic approach. His- and TAP-tagged recombinant TcRBP40 were used in pull-down assays with epimastigote RNA. Recovered RNAs were amplified and analyzed by *T. cruzi* oligonucleotide microarray. 70% of the identified mRNAs correspond to hypothetical proteins, and half had a signal for transmembrane localization. The TcRBP40 recognition element was investigated by EMSA and results point to an A- and G-rich region as the strongest putative element. The expression profile of TcRBP40 targets indicates that they are more abundant in the metacyclic life stage of the trypanosome. On the other hand, TcRBP40 expression was not detected on this same life stage. Overexpression resulted in reduction of the accumulation of its targets. These results suggest an mRNA destabilization function of TcRBP40. The protein was located at the posterior region, in specific cytoplasmic foci. Further analyses will help to fully characterize the mechanisms of target regulation by TcRBP40 and determine its relevance on cell differentiation process.
Supported by FIOCRUZ, CNPq and CAPES.

## BM.043 - INFECTIVITY AND PATHOGENICITY OF TRYPANOSOMATIDS ISOLATED FROM BATS

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The trypanosomes of bats are difficult to be distinguished by morphological examination and have significant genetic diversity. Biological tests, such as inoculation in laboratory animals, assess characteristics of infectivity and pathogenicity of the strains, contributing to the characterization of the species. The purpose of this study was to investigate the parasitemia and tissue parasitism in recent experimental acute infection (5 days) and late (40 days) of mice inoculated with trypanosomes isolated from bat species *Artibeus planirostris* (EM437) and *Phyllostomus discolor* (EM465). Non-isogenic mice were inoculated intradermally in front of their thigh, with 2 x 106 culture trypomastigotes/mL. The investigation of parasitemia and tissue parasitism was made on the 5th and 40th days after infection. Parasitemia was assessed by microhematocrit and blood culture and tissue parasitism by histological analysis by Hematoxilin-Eosin staining and PCR (kDNA) with primers 121 and 122. Mice inoculated with the isolates showed no parasitemia (microhematocrit and blood culture) or tissue parasitism (HE). However, PCR analysis detected the presence of 330bp kDNA band corresponding to *Trypanosoma cruzi* in the following organs and tissues: Fifth day: EM437 - lymphnode, intercostal and psoas muscles; EM465 - lymphnode, heart, ileo-caecal junction and psoas muscle; Forth day: EM437 - lymphnode, diaphragm, intercostal and psoas muscles; EM465 - heart and psoas muscle. It is concluded that assessment of parasitemia and tissue parasitism is variable and insufficient to discriminate and identify trypanosomes of bats. In addition, PCR can indicate the presence of parasite DNA or that the DNA of the parasite was not completely eliminated after infection, it is recommended to carry out additional studies.
Supported by CAPES, CNPq, FAPEMIG and FUNEPU.

## BM.044 - mRNA EXPORT IN *TRYPANOSOMA CRUZI:* CHARACTERIZATION OF THE DEAD-BOX HELICASE TcDbp5

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Transport of mRNA from the nucleus to the cytoplasm is an essential step of gene expression and in trypanossomatids little was described so far. Previous data from our group demonstrated that the mRNA export pathway is the least conserved along eukaryotes lineages. To better understand mRNA export in deeply diverging eukaryote lineages, we start investigating the function of well conserved proteins in *Trypanosoma cruzi*. Dbp5/DDX19 (*Saccharomyces cerevisiae*/Human) is among the most conserved and functions in the final step of mRNA export, releasing the mRNA to translation after crossing the Nuclear Pore Complex (NPC). The ortholog protein in *T. cruzi* is a DEAD-box helicase with 59% of similarity to Dbp5. Molecular modeling analysis showed that TcDbp5 structure is very similar to eIF4AIII and DDX19. Basically, TcDbp5 presents all typical motifs from DEAD-Box family members with major differences in the N-terminal region. Even similar to eIF4AIII, the *T. cruzi* protein is not associated with polysome fractions. Western blot using cytoplasmic and nuclear fractions showed that TcDbp5 is mainly present in the cytoplasm. It was further confirmed by immunofluorescence microscopy, showing that TcDbp5 is disperse in the cytoplasm and more concentrated around the nucleus. To confirm the association of TcDbp5 with nuclear pore proteins, specific antibodies were raised against nucleoporins to be used in immunoprecipitation assays. Besides, the RNA helicase activity, essential for its function, will be tested by in vitro assays. Since Dbp5/DDX19 associates to the mRNA inside the nuclei and shuttles to cytoplasm where it is activated, we intend to evaluate the mobility of the TcDbp5 in *T. cruzi* by blocking the protein transport from nuclei by treatment with Leptomicyn-B and observing if TcDbp5 accumulates in the nucleus. Taking together, our results will provide evidences for the role of TcDbp5 in *T. cruzi* mRNA export. Supported by CNPq, Fundação Araucária, Fiocruz.

## BM.045 - STRUCTURAL AND FUNCTIONAL CHARACTERIZATION OF A NEW FAMILY OF MEMBRANE PROTEINS OF *TRYPANOSOMA CRUZI* THAT SHARES SIMILARITY WITH THE *TRYPANOSOMA BRUCEI* PROCYCLIC FORM SURFACE GLYCOPROTEINS

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We identified a new family of membrane proteins that share 40% identity with the procyclic-form surface glycoproteins from *Trypanosoma brucei.* ***T. brucei*** procyclic-form surface glycoprotein is a stage-specific antigen with features of a typical transmembrane glycoprotein but with unusual cytoplasmic tail composed of proline-rich tandem repeats. The putative *T. cruzi* procyclic-form membrane proteins (Tc-pmp) have conserved 2−3 transmembrane helices. In addition, some of these glycoproteins (i.e., EAN98004, EAN98005, and EAN98006) seem to contain an uncleaved signal anchor sequence, suggesting that they are located on the cell surface. Tc-pmp gene has expanded into a family of duplicated paralogous genes located in the chromosomal band XX of clone CL Brener. Southern hybridization analysis of *T. cruzi* isolates provides evidence for the strikingly conservation of the Tc-pmp gene family, but also for some chromosomal duplication events in isolates from Tc group I. Tc-pmp transcripts and peptides are expressed in epimatigotes and metacyclic trypomastigotes. The subcellular distribution of Tc-pmp will be analyzed using antibodies against the recombinant. Supported by FAPESP, CNPq and CAPES

## BM.046 - EVALUATION OF OXIDATIVE METABOLISM IN NATURAL BENZNIDAZOLE-RESISTANT *Trypanosoma cruzi* STRAINS

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The mechanism of drug resistance of *Trypanosoma cruzi* is poorly understood. Studies have searched potential targets for trypanocidal substances reporting an increase on the production of enzymes involved in the oxidative stress which probably could be responsible for the benznidazole resistance of certain strains. Such enzymes have important roles in the survival and growth of the parasites as: superoxide dismutase (SOD), a metaloenzyme which eliminates superoxide radicals converting them into hydrogen peroxide and molecular oxygen; old yellow enzyme (OYE), an NADPH-flavin oxidoreductase which may be involved in the reduction of some trypanocidal substances and, peroxiredoxin (Prx), which catalyzes the reduction of peroxides. This work aims to evaluate the susceptibility of some different *T. cruzi* strains to benznidazole and also analyse these three enzymes expression. The susceptibility of epimastigotas forms to benznidazole was performed using colorimetric MTT and $IC_{50}$ values of each strain were, respectively: Y = 34.62 µM, Bolivia = 96.06 µM, Santo Inácio (1) = 27.28 µM, Santo Inácio (3) = 105.28 µM, Santo Inácio (8) = 58.40 µM and Quaraí (II) = 63.78 µM. The ORFs (Open Reading Frame) of the three enzymes were cloned and all recombinant proteins were purified. At this moment, the Prx polyclonal antibody was produced in rabbit and probed by Western blot to characterize Prx protein production in benznidazole-treated *T. cruzi* strains. The results showed an increase on Prx-expression in some resistant strains. The anti-Prx antibody also reacted with other isoforms of the enzyme; however, it seems that the higher expression is related to the oxidized form of the enzyme. When the parasites were treated with hydrogen peroxide as a control, the expression of Prx was inhibited. According to other studies, a previous induction may be necessary to improve the resistance of the parasite to oxidative substances.
Supported by FAPESP.

## BM.047 - THE ABC TRANSPORTER SUPERFAMILY IN *TRYPANOSOMA CRUZI*

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The ATP-binding cassette (ABC) protein superfamily is one of the largest evolutionarily conserved families. Most of these proteins are involved in the ATP-dependent transport of molecules across biological membranes, including chemotherapeutic drugs. The goal of this study was to identify and classify ABC transporters in the *T. cruzi* genome. The putative ABC genes were retrieved from the TriTrypDB database with the ABC protein signature motif as query. 56 ORFs coding for putative ABC proteins were identified. This represents 0.22% of the total number of *T. cruzi* genes (~25,000). Of the 56 ORFs, 36 are intrinsic membrane proteins and 20 do not show any transmembrane domain. The two haplotypes of the same ABC gene were identified for most ORFs. However, 21 haplotypes were not encountered, suggesting genic loss or incomplete sequencing/assembly of CL Brener genome. Three pseudogenes were also disclosed. The ABC genes are largely dispersed in the genome and are found on 17 different CL Brener chromosomes. Sequences were assigned as orthologues with *Leishmania* and *Trypanosoma* species if they showed the highest score in BLAST search analyses using OrthoMCL Database. Multiple sequence alignments were performed on the amino acid sequences of the ATP-binding domains by using CLUSTAL W with the default settings. The two Walker A and B nucleotide-binding domains of full-length ABC proteins were treated independently for alignments. The resulting multiple sequence alignments were subjected to analyses using neighbor-joining and bootstrapped maximum parsimony methods. *T. cruzi* ABC proteins were classified into ABCA to ABCH subfamilies, following the HUGO nomenclature adopted for eukaryotes ABC proteins. The present study provides a phylogenic classification of *T. cruzi* ABC proteins and sets the basis for further functional studies on this important class of proteins, some of which are associated to multidrug resistance. Support: FAPESP; CAPES; CNPq.

## BM.048 - GLOBAL MAPPING OF CHROMOSOME-SIZED SCAFFOLDS ON CHROMOSOMAL BANDS OF TRYPANOSOMA CRUZI CLONE CL BRENER

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The genome of *T. cruzi* (clone CL Brener) has been sequenced and compiled as contigs and scaffolds, but specific chromosome location of all scaffolds is unknown. Recently, the genome sequence was assembled in 41 chromosome-sized scaffolds (TcChr) and the sequence deposited in the TriTrypDatabase (Weatherly et al., 2009, BMC Genomics 10:255; http://tritrypdb.org/tritrypdb). To determine which chromosome-sized scaffolds belong to the chromosomal bands, we have selected 123 genetic markers, reaching an average density of one marker every 300 kb. The probes were hybridized to chromosomal bands from CL Brener and G strain separated by PFGE (pulsed field gel electrophoresis). Until now, 31 chromosome-sized scaffolds have been already assigned to the chromosomal bands. Most of chromosome-size scaffolds were ordered and oriented according to in silico assembly. This approach allows the identification of several homologous chromosomes of different sizes, and chromosomal duplication and translocation in *T. cruzi* genome. Several anomalies were addressed and several contigs including subtelomeric sequences were reassigned. For instance, markers at 5´ end of chromosome-sized scaffold 35 (TcChr-35) hybridized with chromosomal bands XI and I, while a third marker distant 442 kb hybridized with band XI and the last marker positioned at 3´ end of TcChr-35 hybridized with chromosomal band XVIII. These misassembled contigs were easily identified by chromoblot hybridization. Our work addresses erroneous computer-based assignment of a few numbers of contigs and emphasizes the need for alternative and confirmatory methods of scaffold construction. Taken together, our results can be useful to the final assembly of *T. cruzi* chromosomes and provide important information about the genomic organization of the parasite.
Supported by FAPESP, CNPq and CAPES.

## BM.049 - PCR AMPLIFICATION, cDNA CLONING AND EXPRESSION OF BRANCHED CHAIN AMINO ACID 2-OXOISOVALERATE DEHYDROGENASE COMPLEX IN *TRYPANOSOMA CRUZI*

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*Trypanosoma cruzi,* is the etiological agent of Chagas´ disease affecting about 10 million individuals in the endemic areas of Americas and with about 40 million people at risk of acquiring the disease. *T. cruzi* has the ability to use carbohydrates and amino acids as carbon and energy sources. Beyond their participation as protein components, amino acids are also involved in osmoregulation, metacyclogenesis and management of nutritional and oxidative stress in *T. cruzi.* 2-oxoisovalerate dehydrogenase complex consists of three polypeptides coded by three genes: 2-oxoisovalerate dehydrogenase E1 component (EC 1.2.4.4), 2-oxoisovalerate dehydrogenase E2 component (EC 2.3.1.168) and dihydro lipoamide dehydrogenase (EC 1.8.1.4), being the whole complex responsible for the catabolism of second step of leucine, isoleucine and valine degradative pathways. In the present work, the three genes were identified in the *T. cruzi* genome and further amplified from *T. cruzi* genomic DNA, rendering amplicons of 1107, 1311 and 1434 bp for E1 component, E2 component and dihydro lipoamide dehydrogenase respectively. Furthermore, the obtained DNA fragments were cloned into vector pGEMT Easy and sub-cloned into expression vector pET28a+. Identities of all genes were confirmed by sequencing. The cDNAs were expressed in different *Escherichia coli* strains, fused to an N-terminal His-tag. 2-oxoisovalerate dehydrogenase E1 component was successfully expressed in BL 21 Codon Plus by the induction of 0.5 mM IPTG for 20 hours at 37˚C, 2-oxoisovalerate dehydrogenase E2 component was successfully expressed in Rosetta pLysS by the induction of 0.1 mM IPTG for 20 hours at 28˚C and the expression of dihydro lipoamide dehydrogenase was achieved by using Rosetta pLysS and induction with 0.1 mM IPTG for 20 hours at 28˚C. In all cases, soluble proteins were obtained, having the predicted sizes (40.18, 47.36 and 50.51 kDA for E1 component, E2 component and dihydro lipoamide dehydrogenase respectively). This research project has financial support from TWAS (The Academy of Sciences for the Developing World), FAPESP and CNPq.

## BM.050 - A PLATFORM FOR *TRYPANOSOMA CRUZI* REVERSE GENETICS: A STEP CLOSER FOR HIGH-THROUGHPUT GENE CHARACTERIZATION

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The sequencing of the *Trypanosoma cruzi* genome revealed the high percentage of genes encoding hypothetical proteins, being necessary a functional characterization of these proteins to better understand the biology of the parasite. Reverse genetics-based tools have been largely employed for many purposes to obtain biological information on genes of unknown function. Actually, there are technical limitations in protein expression in heterologous systems, being of great need the development of a high-throughput reverse genetics platform for *T. cruzi*. We previously constructed plasmid vectors carrying genes for N-terminal fusions, as fluorescent proteins (green, cyan and yellow), and sequences for the *c-myc* epitope, tandem affinity purification (TAP) or poly-histidine tags. These vectors have neomycin or hygromycin as antibiotic resistance marker and to ensure a fast and efficient cloning system the platform is based on Gateway® technology. Here we show some applicabilities of these vectors and modifications made to improve the platform. Using vectors containing N-terminal GFP and CFP tags, we success co-localized two previously characterized proteins (*Tc*Rab7 and PAR2) and using the TAP tag vector we purified two-protein complex (*Tcr*L27-ribosome and *Tc*pr29A-proteasome) validating this platform. We made the exchange of one restriction site, allowing each restriction enzyme to cut only once in the vector. Such modifications become the platform fully flexible allowing the exchange of all elements, such as promoters, fusion tags, intergenic regions or antibiotic resistance markers. In the new version of the vectors, we have add phleomycin as an option for antibiotic resistance marker and created a C-terminal fusion tag vector. The development of this platform is an important step towards improving available methodologies for the characterization of thousands genes whose function remain unknown in *T. cruzi*. Supported by NIH, CNPq and FIOCRUZ.

## BM.051 - MULTIPLEX TAQMAN REAL TIME PCR PROTOCOLFOR IDENTIFICATION OF *TRYPANOSOMA CRUZI* DISCRETE TYPING UNITS

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Diversity of *Trypanosoma cruzi* extensively demonstrated using different biological, biochemical and molecular strategies targeting different genetic markers, allowing the identification of six discrete typing units (DTUs) designated as Tc I – Tc VI (Zingales et al., 2009). In this presentation, we propose a novel algorithm for identifying DTUs, based on two consecutive multiplex real time PCRs using DTUs´specific TaqMan probes. The first PCR strategy involves one common forward primer and four differential reverse primers for amplification of the intergenic region of the spliced leader (SL) genes, and specific LNA Taqman probes for detection of four groups of DTUs: Tc I, Tc III, Tc IV and Tc II/V/VI. This strategy was tested using 27 *T. cruzi* reference strains from Argentina, Chile, Brazil, Colombia, Mexico and USA, belonging to the six DTUs. SL-multiplex PCR was used to characterize *T. cruzi* isolates from faeces of sylvatic triatomines (*Triatoma gerstaeckeri*, *T. protacta*, *T. indictiva*, *T. sanguisuga* and *T. lecticularia*), and from isolates from blood cultures of opossums and raccoons from Southern USA, allowing DTU identification in 31/39 samples, 4 of them being characterized as mixed infections composed of Tc I and Tc IV DTUs. The second PCR further discriminates among Tc II, Tc V and Tc VI DTUs by amplifying 18s rRNA and cytochrome oxidase subunit II (COII) genes using 3 differential TaqMan probes. It was tested using 6 *T. cruzi* reference strains. The protocol herein described, currently under standardization, may constitute a methodological improvement in DTU identification, increasing specificity and reducing costs due to the incorporation of TaqMan probes in multiplex reactions and enabling detection of mixed DTU infections, which have been difficult to be detected by conventional PCR assays.
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## BM.052 - INSIGHTS INTO THE CHROMOSOME ARCHITECTURE AND EVOLUTION IN *TRYPANOSOMA CRUZI*

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We compared the level of synteny between *Trypanosoma cruzi* and *Trypanosoma brucei* by looking at homologous chromosomal segments. We chose genetic markers previously mapped on megabase chromosomal bands XX and XVI of *T. cruzi* (clone CL Brener). Recently, *T. cruzi* genomic sequences were assembled in platforms here named as chromosome-sized scaffolds (TcChr). Taking into consideration that *T. brucei* and *T. cruzi* exhibit a striking conservation of gene order, we compared chromosomes XX and XVI to their homologous regions in *T. brucei.* TcChr37 and TcChr4 were assigned to chromosome XX of CL Brener and they are homologous to *T. brucei* chromosome Tb10. The comparison between the sequences of large chromosomal fragments from each species showed that there is a large segment inversion involving TcChr37. The region located between two chromosome inversion segments in Tb10 corresponds to TcChr4. TcChr39 was assigned to chromosome XVI in CL Brener. It is homologous to *T. brucei* chromosomes Tb9 and Tb11. Trypanosomatid common ancestor seems to present smaller chromosomes and a more fragmented genomic organization. This is in agreement with bioinformatics analysis of TcChr39 where two different fragments have joined to form one single chromosome in *T. cruzi* (TcChr39) while these same fragments have joined to others fragments to form different chromosomes in *T. brucei* (Tb11 and Tb9). Comparative genome analysis showed evidence that there appear to be several cases of chromosome fusions in *T. brucei*. TcChr14, TcChr30 and TcChr35 were assigned to different regions of Tb11 chromosome. We showed that specific markers along Tb11 chromosome hybridized with distinct chromosomal bands in CL Brener. We found overwhelming evidence that many fragments corresponding to entire chromosomes in *T. cruzi* appear to have joined to form a single *T. brucei* chromosome (Tb11). A detailed study of each chromosome must be done to understand chromosome evolution in trypanosomes. Supported by FAPESP, CNPq and CAPES.

## BM.053 - *TRYPANOSOMA CRUZI* TRANSCRIPTOMICS IN RESPONSE TO STEROL BIOSYNTHESIS INHIBITORS

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Similar to fungi, and unlike mammals, *T. cruzi* produces mainly ergosterol. Therefore, its biosynthesis route represents a potential chemotherapy target for Chagas disease treatment. The present work aims to analyze the transcriptome of this parasite in response to two drugs acting at different points of this pathway: Ketoconazole and Lovastatin. First we calculated the drug doses capable of inhibiting the epimastigote culture growth by 50% after 3 days of exposure (IC50), or kill all cells in 24 hours (LD100). Polissomal RNA samples were extracted in short exposure times (30 and 60 minutes) to the LD100 (in triplicate) and in long times (1 to 5 days) to IC50 (in duplicate). The cDNAs generated from purified poly $A^+$ fractions were analyzed by massive parallel sequencing (RNA-Seq) with the SOLiD 3 platform. We generated about 320 million reads of 50 bases for the LD100 experiments, and a preliminary analysis showed more than 1000 genes differentially expressed in response to Lovastatin and about 400 in response to ketoconazole. These genes include heat shock proteins, kinases, cytoskeletal proteins and several RNA binding proteins. Further characterization was performed by transmission electron microscopy and flow cytometry, where high doses of both drugs induced a process similar to autophagy,. This was not corroborated by modulation at the transcriptional level, suggesting a possible role for post-translational regulation. For the IC50 doses, microscopy and flow cytometry experiments showed an increased number of reservosomes, organelles possibly involved in storage and synthesis of sterols. Currently, we are analyzing 220 millions reads produced for the IC50 experiments. The data generated constitute the first global assessment of the transcriptomic regulation of *T. cruzi* to these drugs, leading to a better understanding of the molecular changes in response to the inhibition of the sterol biosynthesis. Keywords: *Trypanosoma cruzi*; transcriptomics; sterol biosynthesis. Financial support: CNPq, CAPES, NIH, Fundação Araucária and FIOCRUZ

## BM.054 - FUNCTIONAL CHARACTERIZATION OF MYOSINS COMMON TO TRYPANOSOMATIDS IN *Trypanosoma cruzi*

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The cytoskeleton of eukaryotic cells consists of three types of polymers that control the shape and mechanics of cells: actin filaments, microtubules and a group of polymers collectively known as intermediate filaments. The cytoskeleton of the Trypanosomatidae family organisms is still very poorly studied. This family includes some organisms that cause human diseases, such as Chagas disease and sleeping sickness, caused by species of the *Trypanosoma* genus, and leishmaniasis, caused by species of *Leishmania* genus. While microtubules are the main filament of the trypanosomatids, it had not been detected yet the presence of the actin filaments, although it has been shown the presence of this protein in its monomeric form by immunoassays. Both microtubules and actin filaments may function as tracks for molecular motors, responsible for the cargo transport. Myosins are motor proteins that move along the actin filaments and are present in almost all eukaryotic organisms; they are classified into classes according to their similarities. Trypanosomatids possess Class I myosin, present in almost all organisms, and a myosin present only in this family. The presence of a myosin exclusive to trypanosomatids is quite intriguing, mainly because little is known about the actin cytoskeleton of these organisms. This work aims the functional characterization of the two myosins of *T. cruzi* common to the trypanosomatids and has as specific purposes: protein expression analysis in different stages of the life cycle and cellular localization using antibodies produced in mice; localization through expression of a GFP-tagged myosin in *T. cruzi*; dominant negative assays and inhibition of protein expression in *T. brucei* by RNA interference. Genes were cloned and proteins were expressed in *Escherichia coli*, antibodies anti-myosin were produced in mice and immunoassays, dominant negative and interference assays are in progress. Supported by CNPq and Fundação Araucária.

## BM.055 - CHARACTERIZATION OF TWO *T.cruzi* PROTEINS: INVOLVEMENT IN REGULATION OF GENE EXPRESSION

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*Trypanosoma cruzi*, the etiological agent of Chagas' disease, is widely studied due to its medical importance and particular features that make it an alternative model for basic biological studies. Repression of messenger RNAs in cytoplasmic granules composed of mRNA-protein (mRNP) complexes are an important pathway of posttranscriptional regulation in eukaryotes, and recently was shown that mRNA granules are present in *T. cruzi*. TIA1/TIAR are nuclear proteins involved in splicing, apoptosis, posttranscriptional regulation and also play an important role in eukaryote´s stress response, since they migrate to the cytoplasm under stress conditions acting in the assembly of stress granules. Two *T. cruzi* proteins similar to the human TIA, named TRRM2 and DRBD9, were chosen for this study. The genes encoding these proteins were cloned using the Gateway® technology, recombinant proteins were obtained and polyclonal antibodies were produced, in order to perform immunoassays to characterize these proteins. The immunofluorescence assay showed that TRRM2 presents nuclear localization and DRBD9 appears in cytoplasmic granules. Further colocalization assays will be performed to analyze the composition of the DRBD9-containing granules. Both proteins are expressed throughout the parasite's life cycle and sucrose gradient assays showed that DRBD9 is associated to non-polysomal heavy complexes, while TRRM2 only appears in the light fractions, suggesting that it is not associated with large protein complexes. The characterization of these proteins will help to elucidate the mechanisms of posttranscriptional regulation in *T. cruzi*.
Supported by FIOCRUZ and CNPq.

## BM.056 - GENE EXPRESSION IN *TRYPANOSOMA CRUZI* PARASITES EXPRESSING A TcBDF2 DOMINANT NEGATIVE MUTANT

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Histones postranslational modifications, their role in gene expression regulation and others nuclear events in trypanosomatids are currently an active field of research. Recently it has been proposed a model for RNA Polymerase II transcription initiation in *Trypanosoma brucei* which involves histone posttranslational modifications that would serve as binding site for a bromodomain factor (BDF). In turn, this BDF would recruit chromatin remodelling proteins involved in incorporating variants of histones into nucleosomes, resulting in less stable nucleosomes, permissive for polymerase binding and transcription initiation. We have reported that *Trypanosma cruzi* Bromodomain Factor2 TcBDF2 is expressed in discrete regions inside the nucleus, between dense and less dense chromatin regions. It binds H4, with preference for K10 and K14 acetylated residues. In order to analyze its function, TcBDF2 fused to c-myc tag and deleted at its C-term (TcBDF2ΔC) were expressed in epimastigotes from a tetracycline regulated promoter. TcBDF2ΔC mutant acts as a dominant negative, inhibiting parasite growth and enhancing their sensitivity to UV irradiation. Global transcriptome from TcBDF2ΔC, TcBDF2-c-myc and control parasites was evaluated 48 h after tetracycline addition by using a 10 K oligonucleotide microarray. Limma software (R) was used to determine mRNA relative abundances (LFC>1.5 and p value =0.01) among the three strains. Just 25 genes were up- and down-regulated between TcBDF2myc and control parasites. However, 100 genes were differentially expressed in TcBDF2ΔC respect to control parasites and this quantity increased to more than 1000 when mRNA relative abundances of TcBDF2ΔC and TcBDF2myc parasites were compared. RT-PCR assays are in progress to corroborate these data. Neither up-regulated nor down-regulated genes showed a pattern of localization in chromosomes respect to strand switch region. Our results support the model proposed and the hypothesis that acetylated histones and BDF2 are taking part in gene expression process throw chromatin remodeling. Supported by ANPCyT and CONICET

## BM.057 - GENE EXPRESSION AND REGULATION OF HSP10 CHAPERONIN GENES OF *TRYPANOSOMA CRUZI*

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The heat shock proteins (HSPs) are a group of chaperone proteins that have their gene expression increased in response to heat shock and other stressing agents. Some HSP families have received more attention because of their important function in the cell. Among these is the chaperonin (HSP60 and HSP10) families. In our study, we are investigating how gene expression of HSP10 is regulated in *Trypanosoma cruzi*, that cause Chagas disease, and also how it is coordinated with HSP60 gene expression. It was shown in earlier studies by our lab that the gene is more similar to *the T. brucei* ortholog, and that HSP10 gene mRNA level remains unchanged after heat shock. In the present study we investigated the mRNA processing sites. A single trans-splicing acceptor site was identified 110 nucleotides upstream of the HSP10 coding region, which apparently is used in each of the three gene copies. We are currently mapping the polyadenylation site(s). After identifying the lengths of both 5' and 3' UTRs, reporter plasmids will be contructed in which both UTRs of the central copy of the HSP10 gene will be placed flanking the reporter gene, chloramphenicol acetyltransferase (CAT). Stable cell lines will be generated by transfection of epimastigotes and used to investigate the presence of heat shock-responsive elements in the CAT mRNA. We are also investigating the stability of the endogenous HSP10 mRNA by measuring its half-life using real-time RT-PCR and northern blots. Finally, with the purpose of obtaining HSP10-specific antibodies, we produced a GST-HSP10 fusion protein after cloning the coding region in an expression vector. The HSP10-specific antibodies will be used for western blot and intracellular localizations analysis. Suported by CNPq and FAPERJ.

## BM.058 - EVALUATION OF PCR ASSAYS SENSITIVITY FOR MOLECULAR MARKERS EMPLOYING ARTIFICIAL MIXTURES OF DIFFERENT *Trypanosoma cruzi* Strains

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Chagas disease is a tropical disease caused by the protozoan *Trypanosoma cruzi* that is endemic in Latin America and affects approximately 10 million individuals. Patients in endemic areas may be infected by multiple contacts with different triatomines, and these, in turn, may feed on different infected individuals. This scenario propitiates the formation of multiclonal populations in hosts and vectors. By using microsatellite analyses it was demonstrated that the percentage of multiclonal populations decrease progressively when we compare strains isolated from the sylvatic cycle with those isolated from man in acute or chronic phase of the infection. However, the identification of mixed populations in a host depends primarily on the sensitivity of the molecular markers used. Thus, the major goal of this work was investigate the sensibility degree of PCR assays for different molecular markers using artificial polyclonal populations. Twenty and seven DNA mixtures from Sílvio X10 cl1 (TcI) and CL Brener (TcVI) in different percentages ranging from 99,9% Silvio/0,1% CL Brener to 0,1% Silvio/99,9% CL Brener, were initially analyzed. These mixtures containing a total of 1 ng of parasite DNA were submitted to PCR assays for 24Sα rDNA and Cytochrome Oxidase II (CO II) genes and seven microsatellite polymorphic loci: SCLE10, SCLE11, MCLF10, TcAAAT6, TcATT14, TcTAT20 and TcCAA10. Preliminary results indicated that PCR assays for 24Sα rDNA and COII genes presented more sensitive in detecting mixture of different parasite populations than those accessed by microsatellite analyses. The detection limits found for 24Sα rDNA and COII were 0,5 and 5% for Silvio and 2 and 8% for CL Brener, respectively. The microsatellite loci more sensitive were TcAAAT6, SCLE10 and SCLE11 with sensitivity of 1, 4 and 10% for Silvio and 2, 3 and 5% for CL Brener, respectively. However, to confirm these results additional experiments will be performed using new combinations of DNA from *T. cruzi* strains belonging to the six different phylogenetic lineages.
Supported by FAPEMIG, CNPq, CAPES.

## BM.059 - GENOMIC MARKERS FOR THE DIFFERENTIAL PCR DIAGNOSIS OF *TRYPANOSOMA CRUZI* AND *TRYPANOSOMA RANGELI* INFECTIONS IN HUMAN BLOOD SAMPLES

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Recent reports of human infections by *Trypanosoma rangeli*, a protozoan parasite non-pathogenic to humans, and of outbreaks caused by *Trypanosoma cruzi* infections point out the necessity of new approaches for the reliable differential diagnosis of the infections caused by these trypanosomatid species. The aim of this work is to develop PCR protocols for detection of *T. rangeli* and *T. cruzi* DNA in culture and in experimentally infected human blood samples. Primers used belonged to three classes: (i) conserved *T. rangeli* and *T. cruzi* telomeric (T189Fw2/Tc189Rv3 and TrF3/TrR8) and Chaperone (Tr3/Tr4) sequences were used as controls; (ii) species-specific amplification of *T. rangeli* DNA (Tr1/Tr2, Tr5/Tr6 and Tr7/Tr8, no predicted function); and (iii) species-specific amplification of *T. cruzi* DNA (Serine Carboxipeptidase e Ubiquitin intergenic region). PCR was conducted in DNA samples obtained from axenic cultures of *T. cruzi* (four strains), *T. rangeli* (four KP1+ strains and two KP1- strains), and *Leishmania sp*. (*L. major*, *L. infantum* and *L. braziliensis*) and in DNA samples obtained from human blood experimentally contaminated with serial dilutions of *T. rangeli* P07 and *T. cruzi* Y parasite strains (0; 0,1; 1; 10; 100 e 1.000 parasites/mL). Control reactions allowed the species confirmation and the assessment of DNA integrity. Expected amplicons were specifically observed for all targets. Primers Tr05/Tr06 allowed the observation of a differential amplification pattern in *T. rangeli* samples, entirely coincident with the parasite genotypes defined by kDNA classification. Considering the experimental conditions used, each reaction permitted the detection of five genomic copies of *T. rangeli* or *T. cruzi*, a detection limit which can be improved with the optimization of PCR protocols. Furthermore, the use of primers Tr05/Tr06 allows the determination of intra-specific polymorphism in *T. rangeli* suggesting the possibility of being used as a marker for diagnosis and testing of new genotyping and phylogenetic relationships.
Supported by FAPEMIG and CNPq.

## BM.060 - *Trypanosoma cruzi* GENES INVOLVED IN THE GPI BIOSYNTHETIC PATHWAY: CELLULAR LOCALIZATION, FUNCTIONAL COMPLEMENTATION AND GENE DELETION

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Glycosylphosphatidylinositol (GPI) is an important anchoring molecule for cell surface proteins involved in many aspects of host-parasite interactions, such as adhesion and invasion of host cells and evasion of the host immune response. Moreover, protozoan-derived GPI anchors exert various immunostimulatory and regulatory activities, including their ability to elicit the synthesis of pro-inflammatory cytokines by host macrophages. Therefore, the biosynthesis of *T.cruzi* GPI anchors is an important aspect of the study of the parasite cell biology and offers potential targets for drug development towards treatment of Chagas disease. Here we present the characterization of nine *T.cruzi* genes encoding homologs of proteins involved in GPI biosynthesis: DPM1, PIG-A, GPI1, PIG-L, PIG-M, PIG-V, GPI10, GAA-1, and GPI8. To verify whether these genes encode protein homologues of *Saccharomyces cerevisiae* enzymes, we transformed yeast mutants defective in each GPI biosynthesis gene and showed that DPM1, PIG-A, GPI1, PIG-L, and GPI10 from *T.cruzi* restore the growth of mutant yeasts in non-permissive condition. Sequences corresponding to PIG-A and PIG-L genes of *T.cruzi*, cloned in fusion with GFP and transfected into epimastigotes, showed a cellular localization compatible with endoplasmic reticulum. Similar localization was observed after transfecting HT1080 human fibrosarcoma cells with *T.cruzi* DPM1, PIG-A, PIG-L, and GPI8 genes in fusion with GFP, which were found to co-localize with a red fluorescent reporter protein containing an endoplasmic reticulum localization signal. To investigate the role of GPI anchored proteins in *T.cruzi*, we disrupt the GPI8 gene, which encodes the catalytic subunit of the GPI:protein transamidase complex. After transfection with the knockout construct and selection for neomycin resistant parasites, characterization of the mutants was realized by PCR, which confirmed the integration of the NEO gene and disruption of one allele of GPI8 gene. Further characterization of the single allele mutant, as well as deletion of the second allele, are currently underway.
Supported by CNPq, FAPEMIG and HHMI.

## BM.061 - PURIFICATION OF ACTIVE RECOMBINANT *Trypanosoma cruzi* APYRASE (NTPDase 1): REFOLDING, STABILITY AND EVALUATION OF OLIGOMERIZATION

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An ecto-NTP diphosphohydrolase activity was previously characterized on the surface of live *T. cruzi* parasites. Additionally, trypomastigotes (infective form) were shown to have a 2:1 ATP/ADP hydrolysis ratio, while epimastigotes (non-infective form) presented a 1:1 ratio, suggesting a possible role for the NTPDase in the parasite's virulence mechanisms. Nowadays, it's known that E-NTPDases act as virulence factor and infectivity facilitators. Therefore, these enzymes appear as good targets for the development of new approaches to chemotherapy, immunotherapy and diagnosis of Chagas disease. Using the soluble portion of *T. cruzi* NTPDase-1 cloned in pET21b (Novagen), we performed its heterologous expression in *E. coli* BL21-DE3 system, purified it from inclusion bodies, renaturated and analyzed the stability and oligomerization state of the recombinant protein. The stability of the purified protein was tested at -22 ℃, 4 ° C and 22 °C for 20 days. The enzyme activity was stable at 4 ℃ for 11 days, with active peak in 48h. The oligomerization state was evaluated by molecular exclusion chromatography in Sephadex G200 column in AKTA-Purifier system (GE). This analysis showed that recombinant NTPDase-1 present more than one protein peak. One of them is the monomeric protein and another fraction of the protein is in an agglomerated or oligomerizated state. Only the monomeric protein remains active after the chromatographic separation. Now, the active protein is been tested with adenosine analogs as possible inhibitors. These studies will open a new range of possibilities, leading us in the search for new drugs, to be applied in Chagas disease chemotherapy.
Supported by: Capes, FAPESP, UFV, FAPEMIG and CNPq

## BM.062 - mRNA CIS-ACTING ELEMENTS INVOLVED IN STAGE-SPECIFIC REGULATION OF GENE EXPRESSION IN *TRYPANOSOMA CRUZI*

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Several studies have shown that transcription in trypanosomatids is policistronic and that sequences present in untranslated regions of *T.cruzi* transcripts are involved in post-transcription regulation of gene expression. Proteins bound to specific sequences in the 3'UTR of mRNAs are known to affect mRNA stability, thus controlling individual mRNA steady levels during the parasite life cycle. In order to evaluate the role of 3'UTRs from stage-specific mRNAs of *T cruzi*, we generate a new vector, named pTcDuaLuc, in which sequences present in the 3 UTR can be inserted downstream from the firefly-luciferase reporter gene. Because the *Renilla*-luciferase gene is also cloned in this vector, it can be used to normalize the values, avoiding the requirement of co-transfection with a control plasmid. In transient transfection assays, using the circular vector, we showed that luciferase activity is higher in epimastigotes transfected with pTcDuaLuc containing the 3'UTR of alpha tubulin, whose mRNA is up-regulated in epimastigotes. After generating stable transfected cell lines from epimastigote cultures transfected with pTcDuaLuc containing 3´UTR from the alpha tubulin gene, we showed that the spliced leader sequence and poly-A tail were inserted in the predicted sites in the firefly luciferase mRNA, thus indicating the correct processing of the transcripts. We also showed that deletion of a U-rich region in the same 3'UTR results in decreased luciferase activity and mRNA levels because it affects poly-A addition. More importantly, we showed that luciferase activity as well mRNA levels changed during the *T. cruzi* life cycle according with the 3'UTR sequences inserted downstream from the luciferase coding region: they are higher in epimastigote when the 3´UTR is derived from the alpha tubulin mRNA, in amastigote when the 3´UTR is derived from the amastin mRNA and in trypomastigotes when the 3´UTR is derived from the trans-sialidase and MASP mRNAs. Financial support:CNPq, FAPEMIG, HHMI.

## BM.063 - A SENSITIVE MULTIPLEX PCR SYSTEM FOR DOUBLE INVESTIGATION IN *Trypanosoma cruzi* POPULATION STUDIES: CLASSIFICATION INTO SIX DISCRETE TAXONOMIC UNITS AND INDIVIDUAL TYPING

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*Trypanosoma cruzi*, aetiologic agent of Chagas disease, presents a great genetic heterogeneity evidenced by several molecular markers. Recently, a second consensus was reached for *T. cruzi* nomenclature: *T. cruzi* strains are now referred to six Discrete Taxonomic Units (DTUs) named *T. cruzi* I to *T. cruzi* VI. However, there is no consensus about how many and which markers should be used to have a suitable classification for *T. cruzi* strains within these six DTUs. In addition, to date there is no methodology able to identify the *T. cruzi* DTUs and simultaneously characterize intraspecific variability among the parasite strains. Herein, we propose a sensitive multiplex PCR system based on a Full Nested strategy composed of seven polymorphic markers: RFLP-Cytochrome Oxidase subunit II, Spliced Leader Intergenic Region, 24Sα rDNA and four microsatellite loci (TcTAc15, TcTAT20, TcATT14 and TcAAAT6), to improve the procedure for molecular characterization of *T. cruzi* strains. Preliminary results revealed that after the second round of PCR assays. All DNA markers presented the expected amplification patterns while was used 1ng of parasite DNA, demonstrating that the multiplex strategy is suitable for typing cultured parasite strains. However, when evaluating the sensitivity of this multiplex system, employing serial parasite DNA dilutions, positive amplifications were obtained only up to 10 picograms, quantity much higher than the 200 fentograms frequently found in biological samples. These findings demonstrated that further experiments will be necessary to improve the sensitivity of this methodology to detect parasite DNA directly in biological samples such as blood and other tissues from chronic chagasic patients. The optimization of PCR sensitivity will open new possibilities in the molecular characterization *T. cruzi* procedures allowing to determine with assurance the *T. cruzi* DTUs as well as individually characterize each parasite strain using only a single sample of DNA.
Supported by FAPEMIG, CAPES, CNPq.

## BM.064 - *TRYPANOSOMA CRUZI* : FUNCTIONAL CHARACTERIZATION OF A POLY-Q RICH PROTEIN ORTHOLOGOUS TO *T. BRUCEI* GAP2

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In trypanosomatids, regulation of gene expression occurs mainly at the post-transcriptional level. The stability of mRNA and the access to polysomes must be tightly regulated, allowing *Trypanosoma cruzi* to adapt to the different environmental conditions during its life cycle. Post-transcriptional regulation requires the association between mRNAs and certain proteins to form mRNP complexes. To characterize protein complexes associated with non-translated or translated mRNAs, mRNPs from epimastigotes and epimastigotes under nutritional stress were isolated using poly-(T) beads and the protein complexes bound to poly-($A^+$) mRNAs were analyzed by mass spectrometry (LC-MS/MS). Among these proteins, a hypothetical poly-Q rich protein was identified in the polysomal fraction of epimastigotes under nutritional stress. The aim of this study is to characterize this protein and to determine if it is involved with regulation of gene expression. The gene encoding this protein was cloned in an expression vector and the recombinant protein was used for the production of polyclonal antiserum. Western blot analysis was performed and showed that this protein is downregulated in metacyclic forms. When epimastigotes and nutritionally stressed epimastigotes lysates were loaded onto a 15-55% sucrose gradient and the fractions were analysed by immunoblot, we observed that the protein was present in polysome-dependent complexes only in the latter. Immunofluorescence analysis showed that the protein is localized mostly in the kinetoplast in both forms. *In situ* labeling of the nicks and gaps in network minicircles with terminal deoxynucleotidyl transferase (TdT) and fluorescent dUTP followed by immunofluorescence showed partial colocalization indicating some involvement with minicircle replication. These data suggest that this protein can be part of mitochondrial mRNPs or can have different roles depending on its localization. Supported by CNPq and CAPES.

## BM.065 - MOLECULAR KARYOTYPE MAINTENANCE IN *TRYPANOSOMA CRUZI* AFTER DNA DOUBLE STRAND BREAKS (DSBs) INDUCED BY IONIZING RADIATION.

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*T. cruzi* displays a pronounced resistance to lethal effects of ionizing radiation and exhibit high DNA repair capabilities. Gamma radiation induces cell growth arrest and chromosomal fragmentation, which can be associated with DNA double-strand breaks (DSBs). However, 48h after irradiation, normal sized chromosomal bands have been detected by pulsed field gel electrophoresis (PFGE) (Silva et al. Mol Biochem Parasitol 149:191, 2009). Although very effective recombination repair mediated by Rad51gene has been proposed as a contributing factor, a detailed understanding of *T. cruzi* irradiation responses has not yet been obtained. Therefore, our study was carried out to understand the chromosome reconstruction after exposition of epimastigotes to gamma radiation. Exponentially growing epimastigotes (clone CL Brener and G strain) were exposed to gamma-radiation doses of 500 to 2000 Gy. Cell survival, growth inhibition, DNA damage and chromosomal recovering were studied at various post-irradiation time intervals. Growth inhibition starting immediately after irradiation and extending up to 96h was observed at dose of 500 Gy, while a large delay (12 days) was observed at 1000 Gy. Parasites irradiated at higher doses (1500 and 2000 Gy) did not survive. Using assays detecting DSBs (TUNEL) and chromosomal fragmentation (PFGE), we evaluated the genotoxic effect of irradiation on epimastigotes. Six days after irradiation, the percentage of TUNEL-positive cells in samples irradiated with 500 Gy is near to that found in non irradiated parasites and 3-fold higher than those cells receiving 1000 to 2000 Gy. We found that TUNEL positivity and chromosomal fragmentation seems to exist in unison. We compared the level of synteny between irradiated and non irradiated parasites by looking at homologous chromosomal segments. Irradiated cells exhibit a striking conservation of gene order when compared to non treated parasites. Factors causing the recovery of chromosomes from radiation-induced damage are presently being investigated.
Support: FAPESP, CNPq and CAPES.

## BM.066 - FURTHER MOLECULAR CHARACTERIZATION OF *TRYPANOSOMA CRUZI* STRAINS ISOLATED IN SANTA CATARINA STATE – BRAZIL, AFTER AN OUTBREAK OF ORAL TRANSMISSION IN 2005.

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In March 2005, several cases of acute Chagas disease occurred in Navegantes, Santa Catarina-BR, related to the ingestion of *Trypanosoma cruzi* in sugar cane juice, which during its preparation was contaminated by the naturally-infected insect vectors. All the infected individuals had ingested the juice in February 13 afternoon, in the same place. A total of 10 strains were isolated from different hosts next to the outbreak area or from the infected patients: SC90 (isolated from *Didelphis aurita*), SC93 (isolated from *Triatomma tibiamaculata)*, SC94, SC95, SC96, SC97, SC98, SC99, SC101 e SC102 (isolated from humans) and were characterized as *T. cruzi* I and *T. cruzi* II populations at that time. Herein, we characterized these populations by analyzing three polymorphic genes (Cytochrome Oxidase –COII, spliced leader intergenic region - SL-IR, and 24Sα rRNA genes and six microsatellite loci (SCLE10, SCLE11, MCLF10, TcAAAT6, TcTAC15 and TcTAT20). The main goal was not only to determine the major lineages of the strains in according to the classification proposed in the Second Satellite Meeting held in Búzios-RJ, but also to characterize the intra-lineage variability identifying eventual polyclonal or mixed populations. As expected, the majority of the isolates were classified into *T. cruzi* II lineage, but 3 isolates were composed by population mixtures (*T. cruzi* I + *T. cruzi* II or *T. cruzi* II + *T. cruzi* VI). In addition to the expected fragments of 110 and 125bp, the 24Sα rDNA analysis revealed anomalous fragments of 117 and 119bp demonstrating the great variability for this marker in these isolates. Furthermore, the presence of identical microsatellites profiles among isolates obtained from different vectors and hosts suggests the presence of characteristic genotypes circulating in this endemic region. Further work is ongoing to analyze single cells derived of these polyclonal populations using FACS Cell Sorter apparatus. Supported by FAPEMIG, CNPq, and CAPES.

**BM.067 - IDENTIFICATION OF DNA TARGETS OF THE NUCLEAR PERIPHERY COILED-COIL PROTEIN TcNUP-1 IN *Trypanosoma cruzi*.**

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The nuclear lamina is a structure that lines the inner nuclear membrane. In metazoans, lamins are the primary structural components of the nuclear lamina and are involved in several processes. Eukaryotes that lack lamins have distinct proteins with homologous functions. Some years ago, a coiled-coil protein in *Trypanosoma brucei*, NUP-1, was identified as the major filamentous component of its nuclear lamina. However, its precise role has not been determined. We characterized a homologous protein in *Trypanosoma cruzi*, TcNUP-1, and identified its *in vivo* DNA binding sites using a chromatin immunoprecipitation assay. We demonstrate for the first time that TcNUP-1 associates with chromosomal regions containing large non-tandem arrays of genes encoding surface proteins. We therefore suggest that TcNUP-1 is a structural protein that plays an essential role in nuclear organization by anchoring *T. cruzi* chromosomes to the nuclear envelope.
Supported by CNPq and Fundação Araucaria.

**BM.068 - THE GTPase RJL IS INVOLVED IN METACYCLOGENESIS OF *Trypanosoma cruzi***

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RJL proteins belong to the Ras superfamily of small GTPases. The cellular function of this new family is still uncertain. We have previously showed the sequence conservation of RJL genes in the genome of trypanosomatids and the expression in the three evolutive stages of *Trypanosoma cruzi*. In this work we aimed to uncover the function of TcRJL in *T. cruzi*. The wild type (TcRJL) and the mutant (TcRJL-S37N), a dominant-negative mutation in the GTPase, were cloned in the trypanosomatid expression vector (pTEX-GFP) and transfected in epimastigotes. After selection we pursued a number of observations in order to reveal a specific phenotype. Our results with fluorescent microscopy showed that RJL is dispersed in the cytoplasm. Growth curve analysis did not reveal any significant differences compared to the control DM28c strain. However, metacyclogenesis assays showed that cells overexpressing RJL did not complete the differentiation process and were arrested in the intermediate stage. Pull down and co-immunoprecipitation assays revealed an associated 20kDa protein which is under characterization. Moreover, TLC experiments showed a poor hydrolysis of GTP by RJL. In conclusion, our results suggest an important involvement of RJL in metacyclogenesis and a poor GTPase activity. Experiments of protein-protein interaction has showed a putative associated protein.
Supported by CAPES,FAPERJ and CNPq

## BM.069 - EXPRESSION AND IMMUNOCYTOLOCALIZATION OF THE COHESIN SUBUNIT SCC1 IN *TRYPANOSOMA CRUZI*

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The segregation of sister chromatids to opposite poles of the cell during division is the most complex and, at the same time, the most important event during the life cycle of a eukaryotic cell. Both in mitosis and meiosis cohesion between sister chromatids is essential for the occurrence of the correct chromosomal segregation. The protein complex responsible for cohesion between chromatids is called Cohesin. The Cohesin complex is well known in yeast and mammals, consisting of two SMC (structural maintenance of chromosomes) proteins, SMC1 and SMC3, and two proteins SCC (sister chromatid cohesion) proteins, the SCC1 and SCC3 (SA1 and SA2 in mammalian cells). The Cohesin keeps sister chromatids together from S phase until the transition between metaphase and anaphase in cell cycle, when sister chromatids separate to the opposite poles of the cell. In trypanosomatids, there are few studies about this complex and the genome project revealed the presence of all Cohesin complex genes in *Trypanosoma cruzi*, *Trypanosoma brucei* and *Leishmania major*. In this work we proposed the analysis of the expression of the Cohesin TcSCC1 subunit and its imunocytolocalization in *T. cruzi* cells. For this, TcSCC1 gene was amplified by PCR, cloned and expressed in *E. coli* cells. The protein TcSCC1 was then used for production of polyclonal anti-TcSCC1 antibody in rabbit. This antibody was used in Western blots and immunofluorescence confocal microscopy analyses of amastigote, epimastigote and trypomastigote forms of *T. cruzi*. These analyses indicate that the TcSCC1 protein is detected mainly in the amastigote forms with distinct nucleus localization. Epimastigote form presented a weak signal for anti-SCC1 antibody and trypomastigote form presented no signal. These results suggest that the SCC1 subunit of the Cohesin complex is present in *T. cruzi* and it is mainly evident in the nucleus of amastigote form of this parasite. Supported by FUB and CAPES.

## BM.070 - FUNCTIONAL CHARACTERIZATION OF mRNA-PROTEIN COMPLEXES (mRNPs) IN *Trypanosoma cruzi*

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Gene regulation is mainly posttranscriptional in trypanosomatids. The stability of mRNA and access to polysomes are thought to be tightly regulated, allowing *Trypanosoma cruzi* to adapt to different environmental conditions during its life cycle. Posttranscriptional regulation requires the association between mRNAs and some proteins to form mRNP complexes. We investigated the dynamic association between proteins and mRNAs, using poli(T) beads to isolate and characterize proteins and protein complexes bound to poli-A+ mRNAs by mass spectrometry. We identified 542 protein components of the mRNP complexes associated with mRNAs. Twenty-four of these proteins were present in all fractions, whereas some other proteins were exclusive to a particular fraction: epimastigote polysomal (0.37%) and postpolysomal (2.95%) fractions; stress polysomal (13.8%) and postpolysomal (40.78%) fractions. We also identified the mRNAs present in each fraction by microarray analysis, showing that proteins expressed mostly in metacyclic form such as mucin II and MASP were present in the postpolysomal fraction from both epimastigote and stressed parasites, hence indicating that these mRNAS that are not being expressed in epimastigotes are stored somewhere in the cytoplasm to be farther expressed in the infective form. We selected five proteins for further characterization: elongation factor 1-alpha (EF1-$\alpha$), zinc finger RNA binding protein (ZF-211.70), RNA binding protein with a cold-shock domain (CD-33.60), prostaglandin F 2 alfa synthase (PF2$\alpha$S) and prostaglandin F synthase (PFS). Immunofluorescence assays, polysome profile in sucrose density gradient and the expression pattern through the parasite's life cycle with the selected proteins allowed a preliminary characterization of specific mRNPs and additional studies will help to elucidate the posttranscriptional regulation mechanisms and dynamics during stress as well as the formation of RNA regulons in *T. cruzi.*
Financial support: CNPq, CAPES (Fiocruz).

## BM.071 - BIOINFORMATIC ANALYSIS OF KDNA MINICIRCLE INTEGRATION SITES IN HUMAN GENOME

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Interspecies DNA transfer is a major biological process leading to the accumulation of mutations among eukaryotes. In a previous study it was demonstrated (Hecht et al, 2010) that *Trypanosoma cruzi* mitochondrial DNA (kDNA) integrated into various human chromosomes, mainly in retrotransposable elements. Most kDNA mutations were found in chromosome X (28%), but any chromosome can be targeted by the minicircle sequences. The kDNA integrations ruptured open reading frames (ORFs) of several genes. Microhomology mediated end-joining of 6-22 AC-rich nucleotide repeats in the minicircles and host DNA seems to mediate foreign DNA integrations. In this work, we performed bioinformatics analysis to investigate the presence of putative chimera proteins in sequenced clones. Sixty-four new ORFs were identified. Most of these putative transcripts bore no similarity with genes/proteins previously described. However, six chimera proteins had already been described in our lab (Nitz et al, 2004; Simões-Barbosa et al, 2006), and other nine chimeras were similar to previously identified proteins. High homology was observed between one chimera protein and the secretory carrier membrane protein SCAMP. The *in silico* analysis suggests that kDNA mutations may generate new genes, pseudogenes and modify pre-existing genes expression. We suggest that resulting genotype-phenotype alterations could be associated with the pathogenesis of Chagas disease. Further functional studies are necessary to r explain the role kDNA mutations play in the intrasignaling events among distant regions in the human genome.

Supported by CNPq and FAPDF.

## BM.072 - MOLECULAR DIAGNOSIS IN THE INFECTIONS BY *TRYPANOSOMA CRUZI* IN PATIENTS CONTAMINATED BY ORAL MODE AND THEIR FAMILY IN THE BRAZILIAN AMAZON REGION

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The epidemiology of Chagas disease is conditioned by the triatomine insect vectors, the parasite *Trypanosoma cruzi* and the mammalian reservoirs. Chagas disease affects about 18 million people in endemic areas in Latin America and 100 million people present a strong potential for contamination by *T. cruzi*. In Brazil, although control measures have succeeded in decreasing the vectorial transmission of the disease seems that there are suspected cases of oral transmission in some areas. The Amazon region, which has been considered low risk area, in the present shows a significant increase in the number of acute and chronic cases of *T. cruzi* infections and Chagas disease. These cases may be related to oral transmission by ingestion of contaminated food typical of the region, for example, the açaí palm fruit. There are few studies describing the morbidity of Chagas disease transmitted orally. The epidemiology of sylvatic *T. cruzi* and oral transmission in Amazon region can cause distinct symptoms in particular cardiac abnormalities with peculiar characteristics. In this report, we investigated 4 families from Breves and Barcarena, Pará State, Brazil, which suspicion of infection seems to be related to ingestion of contaminated food with *T. cruzi*. The objective was to detect the presence of parasite mitochondrial DNA (kDNA) and nuclear DNA (nDNA) in the genomes of Chagas patients and their descendants and correlate with the results obtained by serology.

Supported by Pronex FAPDF, CNPq/MCT

## BM.073 - COMPARATIVE ANALYSIS AMONG THE LEVELS OF PARASITAEMIA, TISSUE DAMAGE AND LYTIC ANTIBODIES CORRELATING WITH THE PRESENCE OF THE *T. CRUZI* COMPLEMENT REGULATORY PROTEIN (TC-CRP) GENE IN BALB/C AND C57BL/6 MICE INFECTED WITH HELENA *T. CRUZI* STRAIN

MARQUES, T[1]; FRARE, JV[2]; SILVA, GC[1]; BRAGA, TT[3]; BORGES, LF[4]; NASCENTES, GAN[1]; HENRIQUE, PM[1]; LAGES-SILVA, E[2]; RAMIREZ, LE[2]; MEIRA, WSF[2]

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The better understanding of the host-*Trypanosoma cruzi* interactions in experimental infections has been extensively studied with genetically different hosts infected by *T. cruzi* strains. Resistant hosts (C57BL/6) present higher production of lytic antibodies (LA), decreased tissue lesions and prolonged survival, whereas the opposite is beheld for susceptible ones (BALB/c). Although the Tc-CRP represents one of the main targets for LA, the antibody levels against this antigen have not been measured during *T. cruzi* experimental infections. So, the aim of this study was to evaluate the presence the Tc-CRP coding gene in different mice organs and its correlation with humoral immune response and the course of infection with Helena strain. A total of fifteen BALB/c and fifteen C57BL/6 mice were infected intra-peritonially with the mentioned strain and their parasitaemia was monitored daily. Five animals of each group were sacrificed in three points of infection; the following organs were collected for histological and molecular analysis: heart, liver, spleen, skeletal muscle, diaphragm, bladder and three gastrointestinal junctions. Sera from these animals were collected for LA evaluation by LMCo and Tc-CRP ELISA tests. Tissues samples were submitted to total genomic DNA extraction followed by the amplification using Tc-CRP specific primers. As expected, C57BL/6 mice produced significantly lower parasitaemia and higher levels of anti-Tc-CRP antibodies during the infection. Moreover, both groups presented higher levels of antibodies in the later phases, which corroborate our previous studies. In the acute phase of infection few alterations were observed only on heart and intestinal junctions' tissues while in the later phases, samples from cardiac, skeletal and smooth muscles presented considerable alterations. The PCR reaction detected the Tc-CRP DNA in five out of nine examined tissues proving the presence of this gene in these samples. Altogether, these findings suggest that either the host or the parasite genetic could influence on the resistance or susceptibility to infection by *T. cruzi* and on the immune response generated against the parasite. Supported by FAPEMIG, CAPES, CNPq, REUNI

## BM.074 - A CLEAN MODEL TO STUDY THE KDNA TRANSFER AND CHAGAS-LIKE INFLAMMATORY CARDIOMYOPATHY

Guimaro, M.C.[1], Hecht, M.M.[1], Nitz, N.[1], Rose, E.C.P.[1], Alves, R.M.[1], Oliveira Neto, O.S.[1], Cordeiro, C.D.[1], Sousa, A.O.[1], Teixeira, A.R.L.[1*]

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Birds are refractory to *Trypanosoma cruzi*, but infection can be established in the embryo during the first week of incubation. The chicks hatched from *T. cruzi* infected eggs retained the kinetoplast DNA (kDNA) in the genome. The PCR assays with specific primer sets revealed kDNA and the absence of nuclear DNA, thus showing the birds were parasite-free. The *Gallus gallus* proved to be a clean model for the study of the pathogenesis of Chagas disease. The identification of the minicircle kDNA sequences integrated in the bird`s genome was made using the tpTAIL-PCR technique, and the chimera sequences showed conserved and variable kDNA regions in several chromosomes. The crossbreedings revealed that the kDNA mutations were vertically transferred to progeny. The kDNA-mutated birds showed cardiomegaly and the histopathology analysis documented typical Chagas disease lesions, whereby parasite-free target heart cells were rejected by the immune system mononuclear cells. These lesions are not present in control non-kDNA mutated birds. These findings highlight the association between the inflammatory heart disease with rejection of the target self-tissue and the genotype alterations resulting from the kDNA integrations in the chicken genome. These results suggest that kDNA mutations in the chicken genome induce autoimmunity and, consequently, the heart lesions in the host. The genetic-driven autoimmune heart rejection would explain the pathogenesis and clinical manifestations of this disease in the parasite-free chicken, which is similar to other genetic-driven idiopathic Chagas-like cardiomyopathies. Supported by CAPES and CNPq.

## BM.075 - GENETIC HETEROGENEITY OF *Trypanosoma cruzi* POPULATIONS ISOLATED FROM STATE OF RIO GRANDE DO NORTE, BRAZIL

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Twenty seven *Trypanosoma cruzi* stocks from the state of Rio Grande do Norte, Brazil, isolated from man and triatomine bugs were studied by random amplified polymorphic DNA. This procedure was performed essentially as described (Steindel *et al*., 1993) and the analysis of the genetic profiles of the *T*. *cruzi* isolates obtained by polymerase chain reaction (PCR) with three primers (L15996, M13-40, and λgt11-F) showed a clear amplification and good reproducibility. The results showed RAPD profiles with an average of 66%, 75% and 80% of shared bands for L15996, λgt11-F e M-1340, respectively. When the RAPD profiles were used to build a phenetic tree by UPGMA and obtained a phenogram consisting of great similarity and grouping between the isolates in the general topology of the trees. The genetic distance analysis revealed three main clusters distinguishing the genetic groups TcI, TcII and TcIII. TcI isolated from the humans; TcII from the humans and *Triatoma brasiliensis* while TcIII only isolated from triatomines-*Panstrongylus lutzi* and *T*. *brasiliensis*. The genetic variability these *T*. *cruzi* populations do not depend on localities origin, but of the host and their genetic group, evidencing that isolates of same group were genetically well correlated. These data reinforce that *T*. *cruzi* populations corresponding to TcI, TcII and TcIII groups are circulating among humans and vector species *P*. *lutzi* and *T*. *brasiliensis* suggesting transmission cycles complexes in different municipalities of this state, and showed a notable genotypic and phylogenetic diversity. Supported by CNPq-Editais Universal e MCT/CNPq/CT-Saúde/MS-SCTIE-DECIT N° 034/2008, FAPERN/PPSUS and DCR/CNPq/FAPERN fellowship.

## BM.076 - CLONING, EXPRESSION AND PURIFICATION OF MEMBERS OF THE MULTIGENE FAMILY MASP OF *Trypanosoma cruzi* DERIVED FROM DIFFERENT SUB-GROUPS

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The surface of pathogens is the major interface with their hosts, and therefore participates in important events related with survival and proliferation. The parasite *Trypanosoma cruzi* has several multigene families encoding surface proteins, many of them already well characterized, such as TcMUC Mucins and Trans-sialidase. A new multigene family, named MASP (Mucin Associated Surface Protein), was identified with the parasite genome sequencing. This family has 1377 genes, 814 of which are full-length genes and 563 are partial and/or pseudogenes. MASP is mainly expressed in the tripomastigote stage of the parasite, has N- and C-terminal conserved domains and a central hypervariable region. These N- and C-terminal sequences encode, respectively, the signal peptide and the GPI anchor addition site, suggesting a surface location of the mature protein. Despite of its extensive variability, this family was divided into 6 sub-groups (Freitas et al., in preparation). Since no member of this family was characterized to date, we decided to generate MASP recombinant proteins representatives of each one of the 6 sub-subgroups to produce monoclonal antibodies and perform structural studies. To this end, MASP sequences were amplified by PCR, cloned in the pGEM-T system, sub-cloned into the pET28a-TEV expression vector and several clones were sequenced to confirm MASP identity. The recombinant proteins were expressed in *Escherichia coli* BL-21 star bacteria and purified by affinity column. To date, three MASPs from distict sub-groups, were already expressed and purified. These three recombinant proteins are already being used to generate monoclonal antibodies and one of them will be used for structural studies.
Supported by: CNPq, FAPEMIG, WHO

## BM.077 - IMPLICATIONS OF *TRYPANOSOMA CRUZI* INTRASPECIFIC DIVERSITY IN THE PATHOGENESIS OF CHAGAS HEART DISEASE

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Chagas disease has a variable clinical course, ranging from symptomless infection to severe chronic disease. Cardiac involvement is the most serious and frequent manifestation of chronic infection and may result from cardiac arrhythmias to sudden cardiac death. Heart transplantation (HTx) is a useful therapy for end-stage Chagas heart disease (CHD), but infection reactivation remains the major complication after the necessary immunosuppression. The factors determining clinical variability and disease reactivation have not been elucidated, but it is reasonably assumed that both host and parasite aspects are involved. Herein, our main goal is to correlate the parasite genetics and the CHD manifestations by molecular profiling of *T. cruzi* populations directly in infected tissues. Retrospective and follow-up studies have been conducted on CHD patients submitted to HTx or pacemaker implantation (PI) in Minas Gerais, Brazil. Parasitological diagnoses were conducted by hemoculture and kDNA-PCR. Fifteen HTx patients were investigated totalizing 43 different samples with 37% of positivity to *T.cruzi* DNA (11/25 fresh cardiac tissues, 2/5 paraffined tissues, 1/8 endomyocardical biopsies after Tx, 1/2 blood samples and 1/1 skin sample from reactivated patients). Blood samples before Tx (2) were negative. *T. cruzi* was detected in 30% of the pericardial fat tissue collected from 10 patients who underwent PI. Positive samples were subjected to strain typing by a triple step assay comprising PCR-RFLP of COII gene, amplification of ITS leader and 24Sα rRNA genes. So far, we detected *T. cruzi* II in all kDNA positive samples. Mixed *T. cruzi* II/VI infection was detected in one PI patient. These results reinforce the current idea that *T. cruzi* II is the major lineage associated to CHD, at least in Minas Gerais. Further studies are ongoing to enlarge the number of analyzed CHD patients and to discriminate the intraspecific variability of *T. cruzi* populations by microsatellites and LSSP-PCR analyses. Supported by FAPEMIG, CNPq and CAPES.

## BM.078 - DIVERSITY AND FUNCTIONALITY ANALYSIS OF GP82 SURFACE GLYCOPROTEIN GENES IN *Trypanosoma cruzi*

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T. cruzi improves the likelihood of invading or adapting to the host through their capacity to present a large repertoire of surface molecules. The metacyclic stage-specific surface glycoprotein gp82 identified by monoclonal antibody (MAb) 3F6 has been implicated in host cell invasion. GP82 is encoded by multiple genes from the trans-sialidase superfamily. However, apart the Mab 3F6-reactive GP82, little information is available on members of this protein family. The central domain of GP82 containing the mammalian cell binding site which is contiguous to and partially overlaps the epitope for MAb 3F6, and appears to be conformational, being possibly formed by juxtaposition of two sequences separated in the linear molecule by a hydrophobic stretch (Yoshida, An Acad Bras Ciênc 78:87, 2006). Here we investigate the genetic divergence and polymorphism among gp82 multigene family members in the strains G, CL, Guatemala and Peru, and clone CL Brener. When we focused on the Mab 3F6 and cell binding motifs, we found differences among the isolates and phylogenetic analysis revealed that sequences from each isolate clustered together, suggesting that GP82 genes may be structured in a strain-specific manner. We are developing a gene expression cassette in Escherichia coli that can be used to readily generate short GP82 peptides carrying the Mab 3F6 and mammalian cell binding sites. This system will reduce time and effort to confirm whether the annotated GP82 genes in the genome are functionally active. A comparison of synonymous (dS) and nonsynonymous (dN) substitutions frequency in GP82 genes detected selective pressure. The reason *dS*/*dN* estimated by SLAC and FEL methods are 0.86 and 0.63 ($\leq$ 0.05 significance), respectively, suggesting a negative selection acting on GP82 genes. Next we will identify specific amino acid sites that are likely targets of selection using both maximum likelihood approaches and patterns of parallel amino acid change.
Supported by FAPESP, CNPq and CAPES.

## BM.079 - CHARACTERIZATION OF TRNA-DERIVED SMALL RNAS IN THE METACYCLIC *TRYPANOSOMA CRUZI*

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Trypanosomatid genes are organized into long policistronic units that are transcribed constitutively, leaving the control of gene expression post transcriptionally. In eukaryotes small non coding RNAs participate in a variety of cellular processes, including gene silencing. Recently, a new subfamily tRNA-derived small RNAs was described in plants, fungi and animals. Experiments revealed that in stressed cells there was an increased amount of small RNAs derived from tRNAs and they were thought to participate in translational repression. When the small RNAs from *Trypanosoma cruzi* epimastigotes were isolated and sequenced, it was noticed that tRNA-derived fragments were not only present but represented 26% of the population of small RNAs. Interestingly, this percentage seemed to increase upon nutritional stress. The objective of this work was to characterize the fraction of short RNAs from metacyclic *T. cruzi*. Not surprisingly, in these infective forms the levels of tRNA-derived fragments increased to 66%, lowering the amount of rRNA-fragments. While fragments of $tRNA^{Asp}$ and $tRNA^{Glu}$ were more significative in epimastigotes, fragments of the last appeared significantly increased in metacyclics. Looking for their location in the cell by FISH using an antisense DNA probe to $tRNA^{Glu}$, the tRNA-fragments were observed in cytoplasmic granules whereas in metacyclics they were dispersed in the cytoplasm. These results confirm the fact that tRNA-derived fragments increase in amount in stressed cells and suggest that the biological function of these fragments may be associated with their location
This project has financial support from CNPq, Fundação Araucária and CAPES

## BM.080 - INTEGRATION OF KDNA MINICIRCLE FROM *TRYPANOSOMA CRUZI* INTO THE MOUSE GENOME

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The lateral DNA transfer (LDT) is now recognized as a major driving force of prokaryotic and eukaryotic genome evolution. Despite the difficulties to document such phenomenon between far distant organisms in the phylum, the growing number of LDT reports in the last five years was driven by robust new sequencing technologies and improved algorithms. The transfer of minicircles of kDNA from *Trypanosoma cruzi* (LkDT) to various vertebrate host genomes has been documented. The insertions of sequences of kDNA minicircles were often associated with retrotransposable LINE-1. In addition, *in vitro* treatment of macrophages previously infected with *T. cruzi* with drugs that block important eukaryote cells signaling pathways was effective to prevent the kDNA integration. We evaluated the impact of treatment of chagasic BALB/C with ciprofloxacin, zidovudine, topoisomerase II and reverse transcriptase inhibitors in association with benznidazole, aiming at to determine the abrogation of minicircle integrations into the animal host genome. To document the structure of the kDNA minicircles integrations at the host's DNA junction sites, the tpTAIL-PCR technique was performed, using sets of primers for *Mus muscullus* LINE-1 ORF2 and for the kDNA minicircles. The investigation revealed that the animals subjected to the therapeutic regimes had the kDNA-insertions in the genomes. A possible decrease in the ratios of kDNA insertions in groups of mice treated with benznidazole awaits further analyses. No difference was observed among groups of mice receiving ciprofloxacin- or zidovudine-benznidazole associations with that obtained in the *T. cruzi*-infected but untreated group. Furthermore, kDNA insertions were always associated with LINE-1 sequences. Due to the huge quantity and similarity of LINEs widely spread in the murine genome, it was not possible to determine yet in which chromosomes the kDNA was integrated. Supported by CAPES and CNPq.

## BM.081 - MOLECULAR DIAGNOSIS IN THE ACUTE PHASE OF CHAGAS DISEASE IN THE PARÁ STATE, BRAZIL

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The *Trypanosoma cruzi* infections and endemic Chagas disease represent a serious public health problem in Latin America. The insect-vector transmitted infection acquisitions via the feces of tritomines are responsible for the endemicity. However, other modes of transmission via blood transfusion and congenitally from mother to offsprings are described. In the northern Pará State, Brazil, oral transmission of the *T. cruzi* infections by contaminated food was suspected. In this work cases of the acute Chagas disease, showing patent parasitemia are reported. The patients with the acute infections often showed nonspecific symptoms, which could be interpreted as any other acute infectious ailment. However in a minority of cases the symptoms were fever, myalgias, arthralgias asthenia, edema and shortness of breath in severely ill people, showing signs of ECG alterations, echocardiograph abnormalities and increased heart silhouette in the X-Rays. The acutely infected cases were subjected to the PCR with specific kDNA and nuclear DNA primer sets to confirming the serological diagnosis by indirect hemagglutination, indirect immunofluorescence and enzyme-linked Immunosorbent assay (ELISA). Our data suggest that PCR can be a solution to the problem related to lack of specificity of serologic tests due to co-infections and/or immunosuppressions in absence of anti-*T. cruzi* antibodies. The discrepancy between the results of serological tests with those obtained by PCR suggests that the onset of acute cases of Chagas disease in the Amazon may well be underestimated. In conclusion, further studies will confirm the importance of genomic technology to performing the diagnosis of the acute *T. cruzi* infections and Chagas disease in the Greater Amazon. Supported by Pronex FAPDF, CNPq/MCT

## BM.082 - *Trypanosoma cruzi* MSH2: MULTIPLE ROLES IN DNA REPAIR AND OXIDATIVE STRESS RESPONSE

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The study of *Trypanosoma cruzi* MSH2, the main protein of DNA Mismatch Repair Pathway (MMR), has led to the discovery of three distinct isoforms, named TcMSH2 A, B and C. Based on the *Tcmsh2* sequence present in their genomes, *T. cruzi* strains can be classified in haplogroups A, B or C. Experimental evidences indicated that strains presenting a TcMSH2 A isoform have a more efficient MMR compared to TcMSH2 B and C. To further characterize the TcMSH2 protein, we generated single knockout parasites and demonstrated that they are more susceptible to hydrogen peroxide treatment and accumulate more 8-oxoguanine in mitochondrial DNA than wild type parasites. This second role of TcMSH2 in *T. cruzi* may explain why it seems to be an essential gene, since it was not possible to generate double knockout parasites. In order to verify whether MSH6 is also involved in the oxidative stress response, we generated *Tcmsh6* knockout, which are currently being characterized. These parasites are being tested for their ability to grow in the presence of hydrogen peroxide. MSH2 and MSH6 proteins have also been expressed in fusion with GFP and RFP, respectively. To investigate its subcellular localization, polyclonal antibodies raised against a recombinant form of TcMSH2 were used in immunolocalization assays, which suggest that TcMSH2 has an unusual cytoplasmic location.
Supported by CNPq, FAPEMIG, HHMI

## BM.083 - RNA-BINDING PROTEINS INTERACTOME IN *Trypanosoma cruzi*

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*Trypanosoma cruzi* is a protozoan parasite that causes Chagas' disease, affecting more than 15 million people in Latin America and having no cure. Besides the problem of public health, this parasite has peculiar molecular aspects which make it an interesting study object for understanding biology; also, it is an ancient eukaryote, and its study can provide clues to cellular mechanisms distinct from those of other model organisms, such as new mechanisms or different controls. One of its major features is the control of gene expression, which occurs post-transcriptionally, and studying the processing, storage and degradation of mRNA is extremely important for understanding the gene expression control mechanisms of this organism. RNA-binding proteins are extremely important in regulating these processes, as through their interaction with specific mRNAs they can define mRNA fate. We are conducting several large scale analyses of *T. cruzi* molecular biology, including microarrays, RNA-seq and mass spectrometry; these results are providing important clues about *T. cruzi* biology, but their integration is expected to provide an even better picture, including new ideas that are not apparent from each dataset. However, integration of distinct types of high throughput data is not trivial; a possible way to improve the initial integration analysis is creating a large map of protein-protein interaction (PPI), over which we can plot the other datasets. Aiming to create an initial map of RNA-binding PPI, we have constructed a *T. cruzi* ORFeome, which consists in cloning all protein coding regions in a suitable vector. Today, we have ~4,000 proteins cloned in a pDONR221 vector (50% of the whole ORFeome), which can be used in downstream applications. We have selected ~300 proteins, consisting of all putative RNA-binding or those that are part of potential interacting functional complexes, as ribosome, splicing and decay machinery. These genes were transferred to appropriate vectors, containing the DNA-binding domain (BD) and activation domain (AD), in order to test their interaction in a yeast two-hybrid (Y2H) system using selective media, as HIS-, URA- and lacZ. Currently, the screening results are being scrutinized using the Cytoscape software and the interaction modules are being used as scaffolds for plotting transcriptomics, proteomics and ribonomics datasets that are available in our Institute. Supported by CNPq, FIOCRUZ, NIH, Fundação Araucária.

## BM.084 - IDENTIFICATION OF CONSERVED SEQUENCES WITHIN THE 3' UNTRANSLATED REGIONS OF CO-EXPRESSED TRANSCRIPTS IN *TRYPANOSOMA CRUZI*

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*Trypanosoma cruzi,* the etiologic agent of Chagas disease, belongs to the Trypanosomatidae family, a group of organisms that has unusual mechanisms of gene expression. There is no identifiable RNA polymerase II promoter, the protein-coding sequences are transcribed as large polycistronic units by through trans-splicing, and cis-splicing is a rare event because only four genes have introns and most mitochondrial mRNAs undergo extensive RNA editing. Because the genes are transcribed constitutively, most of control of gene regulation in these organisms occurs post-transcriptionally. Processing of polycistronic transcripts to generate monocistronic mRNAs involves two coupled co-transcriptional RNA-processing reactions: SL trans-splicing that result in the addition of the splice leader (SL) sequence at the 5'-UTR region and a polyadenylation at the 3'-UTR region of each mRNA. Beside this, mRNA stabilization and translational control are important steps that modulate gene expression in these parasites. It is known that mRNA degradation and translation efficiency may be mediated by the presence of regulatory elements within 3'UTRs of the transcripts. Therefore, the aim of our work is to identify, describe and compare conserved regions within *T. cruzi* stage-specific mRNA sequences that may regulate gene expression in this organism. ESTs from each life stage were downloaded from Genbank and parsed by Seqclean to extract vectors strings, polyA-tail and low-quality sequences. Those parsed-ESTs were aligned against the whole *T. cruzi* genome with Megablast. Perl scripts were developed to select only the best hits from Megablast outputs and to generate a database of 3'-UTR sequences of the parasite. These sequences are been analyzed using clustering methods and compared with data derived from the expression levels of the corresponding mRNAs. Preliminary results using 3´UTR sequences from MASP and trans-sialidases indicated the existence of conserved regions as well as secondary structures that may be related to the trypomastigote-specific expression of those genes. Supported by CAPES, CNPq, HHMI and FAPEMIG

**BM.085 - CARACTERIZATION OF A TPPP/P25 PROTEIN IN *TRYPANOSOMA CRUZI***

Leprevost, F.V.[1], Yamamoto, F.C.[1], Soares, M.J.[1], Rampazzo, R.C.P.[1], Probst, C.M.[1], Krieger, M.A.[1], Pavoni, D.P.[1]

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Proteins from TPPP/p25 (*Tubulin Polymerization-Promoting Protein*) family were initially identified and described as a brain-specific protein. The genome analysis from different organisms made possible the identification of genes homologous to the mammalian TPPP/p25 in ciliated eukaryotes indicating a possible role in function or structural organization of such structures. The proteins from TPPP/p25 family have a strong association to tubulin bundles being capable to induce tubulin polymerization. However, even with their conservation in eukaryote evolution, issues like the function and regulation of TPPP/p25 family of proteins have yet to be proven. Recently our group started the characterization of a gene from *Trypanosoma cruzi* genome annotated as 'hypothetical conserved' gene which sequence analysis revealed to be a member from the TPPP/p25 family. Comparisons between TcTPPP and the mammalian homologous protein revealed similarities like the presence of unordered regions and differences as the absence of the N-terminal region. By DNA microarray analyses and Western blot using polyclonal serum, we observed that both RNA and protein expression patterns reach the highest levels in the metacyclic trypomastigote stage. Strategies based in optical and electron microscopy and the use of transfectant cells porting a GFP-fused protein determined the basal body as the cellular localization of the TcTPPP. Aiming new functional evidences of this protein, we performed the silencing of the ortholog gene in *Trypanosoma brucei* and we observed cells with difficulties in the finalization of the cell division process and cells with an aberrant shape and motility. New evidences about TcTPPP function will be achieved by the gene knockout in *T. cruzi* genome.
Financial Support: CNPq e Fiocruz/Papes V

**BM.086 - CTLA-4 +49 and PD-1.3 polymorphisms can contribute for digestive form in chagasic patients.**

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CTLA-4 and PD-1 molecules are related to T lymphocytes negative regulation, and could constitute a potential mechanism by which *Trypanosoma cruzi* can evade the immune response. Polymorphisms described into CTLA-4 and PDCD1 genes were associated to autoimmune diseases and can be related to magnitude of expression, or associated in their inhibitory function. In this study we analyzed CTLA-4 +49 and PD-1.3 single nucleotide polymorphisms in subjects infected by *T. cruzi* stratified according to clinical presentation and healthy controls by PCR-RFLP. With regard to CTLA-4 polymorphism, AA and AG were more prevalent in both chagasic patients (43.22% each one; GG=13.56%) and healthy controls (AA=39.89%; AG=47.87%; GG=12.24%), thereby suggesting that CTLA-4 +49 polymorphism was not associated with Chagas' disease development susceptibility (p=0,8031). When we compared clinical status with healthy controls, we observed that CTLA-4 gene polymorphism was associated only with digestive form development (p=0.0054) in chagasic patients. The most frequent genotype detected in digestive form group was AA (62.50%), followed by AG (31.25%) and GG (6.25%) genotypes. Genotype frequencies observed in cardiac, indeterminate and mixed forms was similar than in healthy controls. Regarding to PD-1.3 polymorphism, GG was more prevalent in both chagasic patients (AA=2.45%; AG=8.82%; GG=88.73%) and healthy controls (AA=1.58%; AG=15.26%; GG=83.16%), and there was no statistical difference between these groups, suggesting that PDCD1 polymorphism was not associated with Chagas' disease development (p=0,3476). When we compared clinical status with healthy controls, we observed that PDCD1 polymorphism was also associated with digestive form development (p=0.0205) in chagasic patients. The most frequent genotype detected in digestive form group was GG (80.64%), followed by AG (9.68%) and GG (9.68%) genotypes. Genotype frequencies observed in other forms have no statistical difference when compared with healthy controls. Therefore, both polymorphisms analyzed can influence for Chagas' disease digestive form. Supported by CAPES, CNPq and FAPESP.

## BM.087 - GENETIC VARIABILITY OF TRYPANOSOMA CRUZI II BY CORRELATIONS BETWEEN NUCLEAR AND MITOCHONDRIAL GENOME (KDNA) ASSOCIATED WITH CLINICAL MANIFESTATIONS OF CHAGAS DISEASE

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, presents a wide spectrum of clinical manifestations varying between individuals and geographical regions. In the chronic phase, around 70% of the individuals are asymptomatic (IF), whereas ~30% develop the cardiac (CF) or digestive forms of the disease. The factors that determine the outcome of the infection are unknown, but certainly depend on complex interactions amongst the genetic make-up of the parasite, the host immunogenetic background and environment. In this study, we analyzed nuclear and Mitochondrial genes as well as microsatellite markers and the structure of cytochrome oxidase subunit I (*COI*), II (*COII*), III (*COIII*), *Cyb, NADH Dehydrogenase subunit 4(ND4) and 7(ND7)* genes by PCR assays and sequencing in *Trypanosoma cruzi* isolates obtained from 61 chronic patients with well-characterized clinical forms of this disease and belonging to *T. cruzi* II genotype. To analyse our results we decide to group these markers by nuclear and mitochondrial haplotypes to compare and search for correlations between them and their clinical manifestations. For microsatellite analysis we use a software for haplotype reconstruction, and recombination rate estimation from population data (PHASE) and one that generates evolutionary trees and networks (Network). After these analyses we observed that there is no correlation between mitochondrial and nuclear markers, and our network microsatellite tree shows that different isolates from *T.cruzi* II have different steps of mutation. We conclude that there is no correlation between these markers and the clinical manifestation, but we can demonstrate that there are subgroups in *T.cruzi* II genotype which probably are correlated to hybridization events. Supported by CAPES, FAPEMIG and CNPq.

## BM.088 - TCRBP19, A mRNA DOWNREGULATING PROTEIN IN *TRYPANOSOMA CRUZI* AMASTIGOTES?

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In eukaryotic organisms, the relevance of the steps of gene expression regulation that link transcriptional and translational processes is being widely recognized. Though an inactive role has been traditionally ascribed to messenger RNAs along this pathway, it is now clear that its actual fate is defined through the interaction with trans-acting factors. RNA-binding proteins (RBPs) play an important role in controlling gene expression at the post-transcriptional level. Besides, post-transcriptional regulation of gene expression is considered to be the main point for control of transcript abundance and functionality in trypanosomes. For that reason, those parasites are considered proper models for the study of post-transcriptional regulation mechanisms. Here we describe the functional characterization of TcRBP19, an RNA-binding protein from *Trypanosoma cruzi* that is preferentially expressed in amastigotes in association to polysomes. Orthologous genes have only been detected in trypanosome genomes preventing the *in silico* assignment of the functional role. Through ribonomic analysis we assessed that this protein affects the steady state of a set of polysomal mRNA. Further analysis of TcRBP19-RNA interaction network allowed the identification of a group of RNAs containing U rich regions as TcRBP19 targets. Among those messengers, we found TcRBP19 messenger, suggesting an auto-regulation event. In addition, in TcRBP19 over-expressing parasites, a reduced mRNA level of target genes is observed. Globally these data suggest the participation of TcRBP19 in the down regulation of a specific set of genes.
Supported by PEDECIBA, ANII, CSIC.

## BM.089 - MUTAGENESIS EXPERIMENTS AND MOLECULAR SIMULATIONS PROVIDE EVIDENCE OF THE CATALYTIC MECHANISM OF POP Tc80 FROM *Trypanosoma cruzi*

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We have previously demonstrated that the *Trypanosoma cruzi* parasite responsible for Chagas' disease expresses a secreted prolyl oligopeptidase (POP Tc80) and suggested that this proteinase could be involved in the infection process by facilitating *T. cruzi* migration through the extracellular matrix. A 3D model of POP Tc80 was obtained by homology modelling, suggesting a structure formed by two domains: a catalytic α/β hydrolase and a β propeller. The collagen ligand, upon docking on this model, formed an interface between the two domains, promoting a gating access mechanism involving a "jaw opening" of Tc80, for which the collagen binding site encompasses both the hydrolase catalytic triad and a hinge at the domains' junction. To validate the model, three hydrolase residues were identified as candidates for forming a disulfide bridge with propeller Cys255 upon Cys mutation, therefore locking Tc80 into its model predicted closed state, and blocking the enzyme catalytic reactivation. Molecular dynamics simulations of the three Tc80 mutants were conducted in parallel to site-directed mutagenesis and binding assays; the simulation predictions were found in both qualitative and quantitative agreement with experimental results. The Ser591Cys and Asn471Cys mutants showed significantly decreased biological activity (lowest for the former) correlated with the observation of the disulfide bridge (strongest for the former). Surprisingly, the Ala588Cys mutant was more active – this was shown to be related to Cys588 interacting with Arg633 rather than with propeller Cys255, facilitating the interdomain gating mechanism instead of obstructing it. Therefore we consider that our POP Tc80 model structure is validated, and thus provides a strong basis for virtual screening of chemical libraries in parallel with new binding assays. We will now adopt this structure-based drug design strategy in order to detect novel putative anti-Chagas compounds. Supported by: CNPq, CAPES, Finep and FAP-DF.

## BM.090 - EXPRESSION OF ARGININE TRANSPORTER IN *Leishmania (Leishmania) amazonensis* IS SENSITIVE TO THE PRESENCE OF THE AMINO ACID

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In the mammal host, the intracellular form of *L. (L.) amazonensis* needs arginine as substrate for the synthesis of polyamines to support its multiplication. In this study we evaluated the control of gene expression of arginine transporter by the parasite. The transporter is codified by a gene present twice in the genome arranged in tandem. They show identical ORFs, but both 5' and 3' UTR's present distinct regions. Then, targeting those regions by quantitative RT-PCR enables the evaluation of mRNA expression of each copy. We observed that the amount of mRNA of copy B (the second in the tandem array) did not change along a promastigote culture curve. However, copy A presents a large amount of mRNA than copy B, mainly in late log phase, accompanying Meta1, a marker of metacyclogenesis expression. Then, $10^7$ log phase parasites were deprived of nutrients, with the exception of glucose, for 4 hours. After that, the mRNA amount and the arginine uptake were determined after supplementation or not with 400µM arginine. Copy B mRNA number, in the absence of arginine, was similar to the control that received the amino acid, both normalized by GAPDH mRNA. When deprived of nutrients, copy A mRNA number increased, but not in the presence of arginine ($p < 0.05$). The amount of mRNA correlates with amino acid uptake, indicating that the transporter physiological function correlates with mRNA amount. Both Meta1 and arginase mRNA remained constant with or without supplementation. We concluded that parasites have mechanisms to detect arginine and regulate its uptake by the amount of mRNA coding the transporter. Experiments inhibiting mRNA transcription or/and mRNA maturation will determine if the transcription is enhanced, if there is an increase in trans-splicing or a stabilization of the transporter mRNA allowing its translation in a higher level when arginine is at low concentration. Supported by FAPESP and CNPq.

## BM.091 - A SPECIES-DISCRIMINATING DIAGNOSTIC QUANTITATIVE PCR FOR LEISHMANIASIS: PARASITE DETECTION AND ENUMERATION IN HUMAN BLOOD AND IN SAND FLIES

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A rapid method to diagnose leishmaniasis and to discriminate among *Leishmania* species in human blood and environmental samples could be of tremendous use clinically and in assessing the risk of parasite acquisition in endemic regions. Many diagnostic tests depend upon detection of kinetoplast DNA, which contains maxicircle and minicircle DNA sequences unique to the *Trypanosomatidae* family protozoa. There is not a standard systematic application of these methods to distinguish between *Leishmania* species, and to quantify parasite numbers. We developed a qPCR based method to detect, quantify, and distinguish among the different *Leishmania* spp. in human or experimental animal blood and tissue specimens. The method entails first a SYBR green quantitative PCR assay that couples amplification of parasite DNA with the melting temperature of the qPCR product for parasite detection and species screening. A Taqman assay is then employed to validate detection and species identification, using primer sets and Taqman probes specific to the prevalent *Leishmania* species. qPCR assays and a web-based flow chart were developed to detect, quantify and distinguish between *Leishmania* species. DNA extracted from both serum and buffy coat of 2 individuals with symptomatic visceral leishmaniasis (VL) from northeast Brazil tested positive for *Leishmania* spp. Serum from two additional persons with VL tested positive while symptomatic, but resolved to negative after successful treatment. Human serum samples from 5 out of 5 symptomatic VL patients from Bangladesh tested positive. Sand flies were captured from the walls of houses of individuals who had recently contracted VL. Sand flies that were engorged, i.e. had experienced a recent blood meal, were analyzed for Leishmania DNA. 27.2% of sand flies collected from houses in an endemic peri-urban area outside of Natal, Brazil were positive for *L. infantum chagasi* DNA. The range of parasite loads per infected sand fly collected in these endemic households ranged from 17 to 323 parasites per fly. The step-wise diagnostic qPCR assay can address basic questions of parasite species and numbers that are relevant to clinical and environmental studies of leishmaniasis.

## BM.092 - LmHus1 CO-LOCALIZES WITH LmRPA-1 UPON DNA DAMAGE AND ASSOCIATES WITH TELOMERES.

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Leishmania genome stability is constantly challenged by its plasticity as well as by regular DNA damage factors. The ability to deal with such variability denotes the existence of robust machineries evolved to finely control genome maintenance. We have identified the LmHus1 protein, which in other organisms forms the 911 complex with Rad9 and Rad1, involved in DNA damage repair. We raised specific antibodies and observed that LmHus1 is a nuclear protein. In cells submitted to replicative stress by hydroxyurea LmHus1 relocates to the nuclear periphery. Hydroxyurea treatment also promotes the association of LmHus1 to chromatin, a pattern observed for RPA-1, which is known to acts at the early steps of DNA damage repair. We found that LmHus1 co-localizes with LmRPA-1 and hydroxyurea exposure significantly increased this co-localization. Considering that RPA-1 is a telomeric protein in Leishmania, we investigated the possible involvement of LmHus1 with telomeres. We detect telomeric sequences in chromatin immunopreciptation experiments using a-LmHus1 antibody. Also, the overexpression of LmHus1 affected the expression of a resistance marker located at a subtelomere. These data indicate that LmHus1 participates in DNA damage repair and is also a telomere component. We further investigated the existence of the other 911 complex subunits. We were unable to find Rad1 or Rad9 in the genome dataset. However, we found a particular ORF which doubles the size of Rad1 or Rad9 from other organisms and may possess both Rad1 and Rad9 motifs, as showed by secondary structure prediction. This indicates that this protein plays Rad9/Rad1 function. Current work is focused in the generation not only of the antibody a-LmRad9/Rad1, but also the LmRad9/Rad1 overexpressor and KO cell lines in order to investigate the association of this protein with LmHus1 and their potential role in Leishmania telomere maintenance and DNA damage repair. Supported by FAPESP and CNPq.

## BM.093 - MECHANISMS OF ACTIVATION OF p50/p50 NF-κB COMPLEX AND THE MODULATION OF HDACs DURING *Leishmania amazonensis* INFECTION

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*Leishmania* parasites subvert important macrophage signaling pathways involved in the control of the infection. NF-kappaB is an important transcriptional factor which modulates the expression of genes involved in the immunological response. Besides the activation of transcriptional factors, chromatin epigenetic modifications are pivotal regulators of gene transcription. Chromatin remodeling proteins such as deacetylase histones (HDAC) are involved with transcriptional repression while acetyltransferases histones (HAT) are involved with transcriptional activation. In this work, we sought to study the mechanisms of NF-kappaB activation and the participation of HDAC during *L. amazonensis* infection. We have demonstrated by EMSA that *L. amazonensis* infection activates the p50/p50 NF-kappaB complex. We have observed by western blot assay that *L. amazonensis* induces the augment of p50 subunit into the nucleus. Accordingly, we observed a reduction of p105 serine 907 phosphorylation which is thought to be important for p105 stability. In agreement with the activation of p50/p50 NF-kappaB complex, a classic transcriptional repressor, we have demonstrated by gene-reporter assays a dependent-NF-kappaB transcriptional repression of the iNOS promoter due to *L. amazonensis* infection. Consistent with this transcription repression, we have detected an increase of HDAC 1 mRNA and protein levels in early hours of *L. amazonensis* infection. Interestingly, we have observed a reduction of HDAC 11 levels and an increase of lysine 9 acetylated histone 3 in *L. amazonensis* infected macrophages. Our data also revealed that total histone deacetylase activity is increased in *L. amazonensis* infected macrophages. These results suggest a possible mechanism of p50/p50 NF-kappaB activation induced by *L. amazonensis* and indicate that important epigenetic modifications are taking place in infected cells. The relevance of these findings is under investigation.
Financial support: CNPq, INCT-CNPq, FAPERJ.

## BM.094 - INTERGENIC POLYMORPHIC REGION OF THE TCUMSBP LOCUS OF TRYPANOSOMA CRUZI CLBRENER: STUDIES ON THE RNA PROCESSING AND STABILIZATION.

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Gene expression in *Trypanosoma cruzi* is carried out through polycistronic transcripts, which are processed by trans-splicing to mature mRNAs. The control of gene expression is mostly post-transcriptional. In our previous work, we have characterized an intergenic polymorphic region of the TcUMSBP locus, due to an indel of 62 bp, in which the derived transcripts have differential efficiency of processing and two distinct polyadenylation sites. After polycistronic RNA processing the indel remains as the 3'UTR of the beta-5 proteasome subunit (B5PS) gene. Results from the use of chloramphenicol acetyl transferase (CAT) gene in transient constructs corroborated the quantification of endogenous polycistronic RNA, that is, the presence of the 62bp indel in the 3'UTR reporter gene increased production of the CAT enzyme in relation to the absence of the indel. To better characterize this process we have built permanent strains of *T. cruzi* CLBrener in which a plasmid containing the CAT reporter gene is flank by the glyceraldehyde 3-phosphate dehydrogenase (GAPDH) intergenic region at 5' end and the polymorphic intergenic region of TcUMSBP locus at the 3' end. These plasmids are maintained as episomal using G418 selection. We have characterized these strains for further experiments. Assays to confirm the sites of processing of RNAs derived from the transfected plasmid are also being conducted. The process described above may result from increased efficiency of translation or an increased mRNA half-life, resulting from the stabilization of the RNA by the 62pb indel. To address this issue we are measuring the half-life of the mRNA produced by the endogenous polymorphic alleles and the ones originate from the transfection. Supported by FAPERJ and CNPq

## BM.095 - PROLYL OLIGOPEPTIDASE SINGLE-ALLELE KNOCKOUT IN *Trypanosoma cruzi*.

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We have previously demonstrated that the secreted prolyl oligopeptidase of *Trypanosoma cruzi* (POP Tc80) could be involved in the infection process by facilitating *T. cruzi* migration through the extracellular matrix. Since *poptc80* is a single copy gene per haploid genome, its knockout was outlined in order to elucidate the role of this enzyme in the pathogenesis of Chagas disease. G418 (neomycin)-resistant *T. cruzi* epimastigotes (CL-Brener strain) were obtained after transfection and recombination of a fragment containing 470 pb of the 5'UTR and 574 pb of the 3'UTR of *poptc80* interconnected by neomycin phosphotransferase (*neo)* gene. Several PCRs were carried out to verify both the presence of *poptc80* and *neo* in the parasite genome. *poptc80 was* amplified in all G418-resistant parasite. The PCRs using primers of *poptc80* flanking genes corroborate the correct insertion of *neo* gene in the parasite's genome. These results suggest that we were able to produce a *poptc80* single-allele knockout. A detailed analysis of this single-allele knockout is under investigation; however the morphological phenotypes already observed included slow growth and atypical cytokinesis.
Supported by: CNPq, FAP-DF, Finep and CAPES.

## BM.096 - STRUCTURAL PROPERTIES OF OLIGOPEPTIDASE B FROM *Trypanosoma cruzi*

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Oligopeptidase B (OpdB, EC3.4.21.83) belongs to the prolyl oligopeptidase family of serine protease (clan SC, family S9). It is increasingly being implicated as an important virulence factor in trypanosomiasis. The drugs most commonly used in sleeping sickness treatment reduce the activity of *T. brucei* oligopeptidase B. *T. cruzi* oligopeptidase B (OPBTc), the focus of this work, is involved in cell invasion by generating a $Ca^{2+}$ agonist necessary for recruitment and fusion of host lysosomes at the site of parasite attachment. This scenario indicates that further structural and functional characterization of OPBTc should help clarifying its physiological function and lead to the development of therapeutic targets for Chagas' disease. In the present work, we report that OPBTc has a dimeric structure confirmed by different methods: Exclusion size chromatography, Dynamic Light Scattering (DLS) and analytical ultracentrifugation (AU) assays. The dimer association is not due to intermolecular disulfide bonds and it is salt-resistant. The enzyme retains its dimeric structure and it is fully active until 42 ℃. The structural stability of the fully active recombinant OPBTc was investigated through thermal unfolding processes monitored by circular dichroism. Far UV CD experiments showed that OPBTc has a highly stable secondary structure at different pHs and less stable at moderate ionic strength condition. On the other hand, near UV CD spectra demonstrated that the tertiary structure of OPBTc is completely lost when the enzyme is heated at temperatures above 45℃, which correlates well with temperatures-dependent activity assays.
Supported by: CNPq, FAP-DF, Finep and CAPES.

## BM.097 - CONSTRUCTION OF CRE RECOMBINASE SYSTEM IN TRYPANOSOMAS

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Trypanosomatids arouse great interest among researchers, since they use particular mechanisms of controlling gene expression to adapt at different environments and to circumvent the human defenses. To study gene function in these organisms, we decided to develop a system for conditional gene knockout because the RNAi based strategies cannot be applied to all trypanosomes and several genes are encoded by multi copy families. Here, we describe the first steps to transfer a CRE recombinase regulated by ligand (Dimerizable Cre – DiCre) to Trypanosomes. The CRE is a site-specific recombinase that catalyzes the recombination between two sequences called LoxP to excise or invert an intervening sequence or create intermolecular recombinations. Due to its intracellular toxicity and the necessity of a tight regulation of its activity, the DiCre system was chosen to obtain conditional knockouts in *T.cruzi* and *T.brucei*. To create a construct to express DiCre in both parasite species, the DiCre59 and DiCre60 genes from the pCDNA vectors were cloned into pROCK-TK-Hygro generating the pROCK-DiCre-Hygro. A second plasmid, pROCKET-FEKOPur-Neo, was generated to test the DiCre activity in *T.cruzi*. The FEKO Pur cassette from the pyrFEKO-PUR plasmid (Scahill et al 2008) consists in a puromycin resistance gene flanked by two LoxP sites. These linearized plasmids were transfected into *T.cruzi* epimastigotes and the parasite selection is in progress. In parallel, the pROCK-DiCre-Hygro plasmid was also introduced into *T.brucei* procyclic forms, and preliminary PCR analysis of hygromycin-resistant parasites showed that they contain the DiCre gene. Experiments to assess the presence of DiCre activity are being performed. Using this system, the multi copy genes will be deleted by insertion of LoxP sites followed by DiCre excision. Financial support: CAPES/REUNI, CNPq, Fundação Araucária.

## BM.098 - 152 1 MOLECULAR CHARACTERIZATION OF TRYPANOSOMA EVANSI MEVALONATE KINASE

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The synthesis of isoprenoids precursors through mevalonate is an important metabolic pathway in eukaryotes. The biosynthesis of isoprenoids is essential for the viability of trypanosomatids, as part of the formation of glycosylphosphatidyl inositol (GPI)-anchored variant surface glycoproteins (VSGs). *Trypanosoma evansi* is the etiological agent of a disease in horses and other livestock, popularly known as "derrengadera" or “surra”, causing considerable economical losses in endemic regions. This parasite displays only a trypomastigote form, requiring constant modifications to their VSGs coat to protect from attack of the host immune system. Mevalonate kinase (MK) is a ATP-dependent phosphotransferase essential to isoprenoids metabolic route. This study aimed to identify and characterize the mevalonate kinase gene of *Trypanosoma evansi* (TeMK) and purify the recombinant protein for further enzymatic and structural characterization. In order to obtain a purified genomic DNA (gDNA), the blood of a Wistar rat infected with *T. evansi* was first purified by Percoll® gradient and ion exchange chromatography with DEAE-cellulose. The gDNA was then obtained by extraction with phenol-chloroform. The open reading frame encoding TeMK was obtained by using specific degenerated primers. A fragment of 990 base pairs was amplified by polymerase chain reaction, extracted, purified and cloned into a commercial vector. TeMK displays a high homology with *T. brucei* mevalonate kinase (TbMK). The TeMK gene was inserted in pQTEV expression vector (protein structure factory, Berlin), transformed into *E. coli* BL21(DE3) and was induced with 1mM IPTG at 20ºC. A protein band around 35 kDa was observed in SDS-PAGE gels. Western blots assays were performed with *T. evansi* extracts using a polyclonal anti-TbMK, in which a band with the expected molecular weight was observed. Indirect immunofluorescence showed that TeMK colocalized with aldolase in glycosomes, as already observed in other kinetoplastids studied. The recombinant protein is being processed for further biochemical and structural characterizations. Supported by: FAPESP, CNPq, UDESC.

## BM.099 - PROTEOMIC PROFILE OF *TRYPANOSOMA EVANSI*

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*Trypanosoma evansi* is the most widespread of the pathogenic salivarian trypanosomes and affects most livestock and wild animals mainly in endemic regions. The *T. evansi* infections are popularly know as “surra” or “mal de cadeiras” and there are no drugs or vaccines to cure or prevent the disease. Knowledge about molecular and cell biology of the parasite is limited, being most part of the studies linked to *Trypanosoma brucei*, phylogenetically close to *T. evansi*. The study of proteins through proteomics based approach is an important reference for biochemical, molecular and epidemiological studies in order to identificate species-specific proteins, new drugs targets and development of diagnostic tools. Recently the proteomic composition of *T. evansi* was characterized using liquid chromatography (LC/MS). The main goal in the present study is to develop an optimized and reproducible protocol using two-dimensional gel electrophoresis to determine *T. evansi* protein expression profile and post-translational modifications. The parasite was collected from infected Wistar rat and was subjected to purification in Percoll® gradient and ion exchange chromatography on DEAE-cellulose. Trypomastigote protein extracts were obtained with lysis solution containing 7.7 M urea, 2.2 M thiourea, protease inhibitor cocktail (Sigma) and 4% CHAPS. The samples were measured by Bradford method and it was used 220 µg of sample for the elaboration of the 2D gel. The isoeletric focalization was performed using pH 3-11 (GE Healthcare) gradient strips on Ettan IPGphor III (GE Healthcare). The SDS-PAGE was carried out by 15% polyacrilamide gel and was visualized by Coomassie Brilliant Blue G-250 staining. It was observed and marked over 165 spots. The spots were excised from gel and are being processed through the MALDI-TOF mass spectrometer analysis. These preliminar results are the first step towards the generation of proteome profiles for use in future studies on protein expression and comparison with other trypanosomatids. Supported by: CNPq, FAPESC, UDESC.

## BM.100 - 187 1 GENOMIC ORGANIZATION AND EXPRESSION OF MAJOR SURFACE PROTEASES (MSP) IN *TRYPANOSOMA RANGELI*

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Members of Major Surface Proteases (MSP) family were initially described in parasites of the genus Leishmania, and later in other trypanosomatids. MSPs are considered virulence factors in *Leishmania* and have key roles in establishing infection of the host cell. We selected twelve recombinant clones from a *Trypanosoma rangeli* genomic library, which have high sequence identity with *Trypanosoma cruzi* MSPs. The objective of this work is to characterize the recombinant clones carrying the sequences of *T. rangeli* MSPs and determine the genomic organization and expression of the MSPs in different genotypes of the parasite. The sizes of the inserts of recombinant clones ranged from the 2,200bp 3,500bp. The analysis of BLASTX (Basic Local Alignment Search Tool) of the twelve *T. rangeli* sequences showed an identity of 37% to 62% with the sequences of MSPs from other trypanosomatids, and the best results were achieved with the sequences of *T. cruzi*. Of the twelve sequences generated, nine correspond to the carboxy-terminus region of *T. cruzi* MSPs and five sequences to the amino-terminus region, and one of them contains the 5' untranslated region. The multiple sequence alignment showed extensive conservation between sequences of *T. rangeli* and other trypanosomatids, including the catalytic site of MSP (HEXXH), which is represented by three clones in the library. Southern hybridization analysis, at high stringency conditions, revealed the presence of a single band of 4kb in samples of strain P07. The evaluation of the expression of MSP, performed by RT-PCR, employing primers MSP-1 and MSP-2, revealed a fragment of 212bp in the strains PO7 and SO18, representing genotypes KP1 (+) and KP1 (-), respectively. The presence of MSPs in *T. rangeli*, a non-pathogenic human parasite, opens new possibilities for understanding the host parasite relationship, especially in the vertebrate host whose biological mechanisms of parasitism are poorly understood. Supported by: CNPq, FAPEMIG and CAPES.

## BM.101 - CHARACTERIZATION OF RIBOSOMAL P0 PROTEIN OF *Phytomonas serpens*, A TOMATE PARASITE THAT SHARES ANTIGEN WITH *Trypanosoma cruzi*

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Trypanosomatids of the genus *Phytomonas* alternate their life cycle between plants and phytophagous hemipterans. *Phytomonas* spp. can parasitize the phloem, lactiferous tubes, fruits and seeds of some plants with a wide geographic distribution and of great agriculture importance. Previous results from our research group showed that *P. serpens* 15T, a tomato parasite, shares antigens with *Trypanosoma cruzi*, among them, the ribosomal P0 protein (P0ribPs). Humoral immune response against P ribosomal proteins of *T. cruzi* is prevalent in patients with chronic Chagas' heart disease. These antibodies recognize mainly the C-terminal domain of the polypeptides, a highly conserved region between P proteins of eukaryotic organisms. It was also observed that there was partial protection against infection of BALB/c mice immunized with living *P. serpens* 15T by the intraperitoneal or oral route and later challenged with a lethal inoculum of blood trypomastigotes of *T. cruzi*. Taking all together, the aim of this study was to characterize the gene that encodes the ribosomal P0 protein of *P. serpens* 15T. The coding sequence of the gene was cloned and sequenced. Northern blotting analyses, using total RNA of *P. serpens* 15T and specific *P0ribPs* probe, detected only one transcript of 1.28 kb. Recombinant protein was expressed in *Escherichia coli*, purified and inoculated in BALB/c mice to raise antibodies against the P0ribPs. The polyclonal antibodies recognize a polypeptide of approximately 40 kDa in a total protein extract of *P. serpens* 15T. And did not cross-react with other ribosomal P proteins. Indirect immunofluorescense assay using these antibodies showed that the P0 ribosomal protein is localized in the cytoplasm of the parasite. Further studies are warranted to determine the role of P0ribPs during the experimental Chagas' disease, and such investigations are currently underway in our laboratory. Supported by: Fundação Araucária – Paraná and CNPq.

## BM.102 - CHARACTERIZATION OF SINGLE MYOSINS OF *Trypanosoma cruzi*

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Myosins are molecular motors involved with various forms of cell movement like phagocytosis, cytokinesis, muscle contraction, beating of cilia and trafficking of organelles and particles. They use energy derived from ATP hydrolysis to perform these movements and have a common motor domain. The diversity of the family members enables us to classify them in classes. The muscle myosin was defined as conventional (class II), whereas other types are collectively referred to as unconventional myosins (grouped in many classes). Myosins that phylogenetically do not group to any other myosin are termed orphan myosins. *T. cruzi* has, besides the genes coding for 2 myosins (one present in almost all organisms - class I - and the other present only in trypanosomatids), seven other genes coding for orphan myosins, called TrcMyoA, TrcMyoB, TrcMyoC, TrcMyoD, TrcMyoE, TrcMyoF, TrcMyoG, which are the focus of this work. The study of *T. cruzi* myosins, their functions and their interactions within the cell will contribute to the evaluation of the cytoskeleton role in *T. cruzi*. To start the characterization of these myosins, experiments are being conducted to elucidate the cellular localization and the proteins that interact with each myosin, and to achieve this goal it was necessary to produce recombinant proteins for antibody production that will be used for immunolocalization and immunoprecipitation assays. In parallel, transfection of the parasite to the expression of protein fused to GFP and identification of their location is being performed. To date, with the exception of one myosin, all the recombinant proteins were inoculated in mice and the antibodies are being analyzed. The transfection assays are in progress.
Supported by CAPES and Fundação Araucária.

## BM.103 - EXPRESSION LEVELS AND SITES OF A *Trypanosoma rangeli* PROTEIN TYROSINE PHOSPHATASE (*Tr*PTP2)

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Protein tyrosine phosphatases (PTP) play an essential role on the control of crucial cellular processes such as division and differentiation. Kinetoplastid-specific PTPs are associated with these parasites' life cycle and, due to their differences from human PTPs, are considered potential drug targets. The focus of this work was to comparatively characterize the expression sites and levels of a *Trypanosoma rangeli* PTP (*Tr*PTP2). The *Tr*PTP2 gene ORF has 987bp and predicts a protein of 36kDa that revealed to be highly similar to PTPs from phylogenetically related organisms (*T. cruzi*, *T. brucei* and *Leishmania* spp.). *Tr*PTP2 is 72% identical to *T. cruzi Tc*PTP2 and 58% to *T. brucei Tb*PTP1. Among the highly conserved regions between *Tr*PTP2 and these proteins are PTP active sites and trypanosome-specific PTP domains. The *Tr*PTP2 gene was cloned in pET14b, expressed in *E. coli*, purified by electroelution and used for polyclonal antiserum production in Balb/C mice. Western blot assays using anti-*Tr*PTP2 antiserum revealed an ~60kDa protein in the soluble protein extract of *T. rangeli* epimastigote and trypomastigote forms. This unexpected molecular mass is higher than the predicted 36kDa for *Tr*PTP2, possibly due to glycosylation sites observed in its deduced aminoacidic sequence. Immunofluorescence assays indicated a distinct cellular localization pattern for *Tr*PTP2 between *T. rangeli* epimastigotes and trypomastigotes, being spread throughout the cellular membrane in epimastigote forms and concentrated at the flagellar membrane in the infective forms. A disparate distribution for kinetoplastid PTP between biological forms has already been observed in *L. major* and *T. brucei*, in which the association of this protein to the membrane suggests a mean of interaction with these parasites' hosts. Supported by CNPq, FINEP and UFSC.

## BM.104 - COMPARATIVE CHARACTERIZATION OF *Trypanosoma rangeli* GP63 METALLOPROTEASES CODING GENES

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Gp63 is a GPI-anchored $Zn^{2+}$-dependent metalloprotease protein originally described in *Leishmania* promastigotes. In *T. cruzi*, Gp63 is expressed in all stages and has been implicated in the infection of mammalian cells *in vitro*. In this work, Gp63 ORFs encoding for metalloproteases were identified and characterized in *Trypanosoma rangeli* genomic and cDNA libraries. Comparative sequence analysis revealed 13 complete ORFs highly similar to the *T. cruzi* homologous genes. *T. rangeli* Gp63 genes seem to be composed by at least two groups (Gp63-A and Gp63-B) in a family-like organization, reinforcing the phylogenetic proximity to *T. cruzi*. The presence of multiple copies of the Gp63 genes for both groups was confirmed by *Southern blot*, indicating a probable tandem repeat arrangement. Gp63-A and Gp63-B encode deduced proteins of approximately 605 and 587 amino acid (aa) residues (39% identical). Gp63-A showed His and Glu residues in the HEXXH motif, the most important residues associated with metalloprotease activity, while Gp63-B showed a replacement of a Glu residue for an Ala (HAXXH), suggesting the loss of catalytic activity in this group. The most important structural characteristics in both groups are the presence of (i) potencial N-glycosylation sites in Gp63-A (7) and Gp63-B (5), (ii) GPI anchor addition signals of 26aa for Gp63-A and 25aa for Gp63-B on the C-terminal portion of both groups, (iii) a predicted cleavage signal in the N-terminal region, consisting of 20 and 27 amino acids for Gp63-A and Gp63-B, respectively. Since the presence of mRNA for both Gp63-A and Gp63-B gene groups was detected in epimatigote and trypomastigote forms of Choachí and SC-58 strains, we are assessing the expression of Gp63 by *T. rangeli* and the activity of these proteins in both epimastigote and trypomastigote forms. Supported by CNPq, FINEP and UFSC.

## BM.105 - COMPARATIVE PROTEOMIC ANALYSIS OF *Trypanosoma rangeli* EPIMASTIGOTE AND TRYPOMASTIGOTE FORMS

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*Trypanosoma rangeli* is a hemoflagellate parasite that infects humans as well as a variety of domestic and wild mammalian species. The infection in humans is harmless but induces an immune response that leads to serological cross-reactions with *Trypanosoma cruzi*, the agent of the Chagas disease. *T. rangeli* has a complex life cycle alternating between replicative and non-infective (epimastigotes) forms to non-replicative and infective forms (trypomastigotes). In this study, proteomic maps of both *T. rangeli* stages were obtained by unidimensional and two-dimensional (2D) electrophoresis following peptide mass fingerprinting identification by MALDI-TOF mass spectrometry (MS). Resolution of 2D gels using a 3-10 pH range showed that most of the proteins were focused between pH 4-7, where 282 and 205 spots could be identified for epimatigotes and trypomastigotes, respectively. Several proteins revealed multiple isoforms on 2D gels, some displaying differential expression levels between forms. Interestingly, 36% of the spots were found to differ between epimastigotes and trypomastigotes. Despite of the absence of *T. rangeli* genomic database for comparisons, some proteins could be identified by MS based on other trypanosomes databases. Among the identified polypeptides there were heat shock proteins (HSP) like HSP 60, HSP 70, HSP 83 and HSP 85, elongation factors, metabolic pathway enzymes, structural proteins and some virulence factors, including mucin-associated surface proteins (MASP). These results represent the first analysis of the parasite proteome, offering a draft of the protein expression profile of both epimastigotes and trypomastigotes. Upon finishing the ongoing genome sequencing, further studies on identification and molecular characterization of proteins related to the *T. rangeli* metacyclogenesis will allow the understanding of unknown biological aspects of this parasite. Acknowledgement: CDC/CCEHIP/NCEH. Suported by CNPq, FINEP and UFSC.

## BM.106 - THE FOUR TRYPANOSOMATID eIF4E HOMOLOGUES FALL INTO TWO DISTINCT GROUPS, WITH FUNCTIONS IN TRANSLATION AND OTHER CRITICAL PROCESSES

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Translation initiation in eukaryotes requires eIF4E, the cap binding protein, which mediates its function through an interaction with the scaffolding protein eIF4G, as part of the eIF4F complex. In trypanosomatids, four eIF4E homologues have been described but the specific function of each is not well characterized. The functional analysis of these proteins in *Trypanosoma brucei* (*Tb*EIF4E1 through 4) has been initiated and it was seen that, at the sequence level, they can be assigned into two groups; *Tb*EIF4E1 and 2, similar in size to metazoan eIF4E1; and *Tb*EIF4E3 and 4, with long N-terminal extensions. All were constitutively expressed, but whilst *Tb*EIF4E1 and 2 localise to both nucleus and cytoplasm, *Tb*EIF4E3 and 4 are strictly cytoplasmic and are more abundant. *Tb*EIF4E3, despite its inability of binding to 7-methyl-GTP Sepharose, was the only one confirmed to be essential for viability of procyclic form through RNAi assay. For *Tb*EIF4E1, a reduction in the rate of cellular growth was observed, but cells viability was not impaired. We also identified that *Tb*EIF4E3 and 4 were all essential for the bloodstream form. It's worthwhile to note that despite *Tb*EIF4E4 is essential for viability in bloodstream, it is not in the procyclic form. Simultaneous knockdown of *Tb*EIF4E1 and 2 caused cessation of growth and death in procyclics, although with a delayed impact on translation, whilst knockdown of *Tb*EIF4E3 alone or a combined *Tb*EIF4E1 and 4 knockdown led to substantial translation inhibition preceding cessation of growth. Through HA-tagged TbEIF4Es imunoprecipitation and pulldown assays we observed that only *Tb*EIF4E3 and 4 interacted with *T. brucei* eIF4G homologues. *Tb*EIF4E3 bound to both *Tb*EIF4G3 and 4 whilst *Tb*EIF4E4 bound only to *Tb*EIF4G3. These results are consistent with *Tb*EIF4E3 and 4 having distinct but relevant roles in translation initiation while *Tb*EIF4E1 and 2 may be involved in other critical processes.
Supported by CAPES, UFPE, FIOCRUZ and FACEPE.

## BM.107 - MOLECULAR CHARACTERIZATION OF TWO BRAZILIAN STOCKS OF *TRYPANOSOMA EVANSI*

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*Trypanosoma evansi* is the most widespread of the pathogenic salivarian trypanosomes due to its ability to be mechanically trasmitted by blood-sucking flies. Several studies have shown that *T. evansi* is genetically related to *T. brucei* with some differences, such as kinetoplast absence or deletion. In this work, the RAPD technique, hypervariable minisatellite and microsatellite loci were employed to analyze the genetic variability of two brazilian *T. evansi* isolates. The presence of kinetoplast was also verified. To obtain genomic DNA, the blood of a Wistar rat infected with *T. evansi* was purified by Percoll® gradient and ion exchange chromatography with DEAE-cellulose. It was used six primers to RAPD reactions with *T. evansi* stocks and a *T. brucei brucei* strain. Close RAPD profiles, among *T. brucei* and *T. evansi,* were demostrated. The primers showed that the *T. evansi* isolates are highly homologous. Minisatellite and microsatellite markers 292-locus and MORF2 also proved high homology. To detect specific *T. evansi* VSG sequence RoTat 1.2, described primers were employed to assert that both isolates have the VSG sequence. Immunofluorescence staining assay with DAPI was used to observe the Kinetoplast. Primers derived from the sequence of the maxicircle of *T. brucei* encoding NADH dehydrogenase (nadh5) were used to assert the absence of maxicircles. Amplification of 464bp minicircle kDNA sequence were used to certify the loss of minicircles. All methods indicate the total absence of kDNA. The high homogeneity of isolates around the world is characteristic of *T. evansi* stocks, which there is no recombination of the parasite in the vector. The clonal population in the host also contributes to reduce the genetic variability of *T. evansi*. However, some remarkable differences are observed, as presence of kDNA minicircles of african strains and total absence of kinetoplast in american strains. Supported by: CNPq, FAPESC, UDESC.

## BM.108 - IDENTIFICATION OF *Trypanosoma evansi* SELB AND SELD GENES

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Selenoproteins are mainly related to prevent oxidative stress in all kingdoms of life. These proteins contains the amino acid selenocysteine (Sec), encoded by an in frame UGA “stop codon”. In Eukaryotes, there are five main genes involved in the biosynthesis of Sec. In this work, two of these genes in *Trypanosoma evansi* were cloned and characterized: *selB*, a specific elongation factor involved in the translation process; and *selD*, which catalyzes the conversion of selenide and ATP in monoselenophosphate, the active selenium donor compound for Sec biosynthesis. These genes were amplified from genomic DNA, cloned and sequenced. The theoretical pI found was 7.19 and 5.46 for *selB* and *selD*. Analysis of the amino acid sequence showed that both proteins present specific structural characteristics that correlates with their biological function, (i) a Cys residue in position 42 for *selD*; and (ii) EF-Tu domains for *selB.* Since a high similarity in the amino acid composition with *Trypanosoma brucei* was observed, 99.54% for *selB* and 99.74% for *selD*, an antibody of *Trypanosoma* brucei (kindly granted by Dr. Otávio H. Thiemann) was used to determine the presence and cellular localization of *selD*. A cytoplasmatic localization was observed by indirect immunofluorescence and an expected 43 Kda band was detected in a Western blotting assay. Studies have been performed to identify other elements of this metabolic route and their role in *Trypanosoma evansi*. Supported by CNPq, FAPESC, FINEP, UDESC.

## BM.109 - PROTEOMIC DATA SUGGEST A MULTI-PROTEIC COMPLEX RESPONSIBLE FOR SELENOCYSTEINE INCORPORATION IN KINETOPLASTIDS.

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The discovery of new amino acids such as selenocysteine and pirrolisine, resulting in the expansion of the genetic code from the traditional 20 amino acids to the currently recognized 22 amino acids, have attracted the interest of several research groups. The synthesis pathway of selenocysteine (Sec - U) represents the major biological form of selenium, whose incorporation occurs co-translational into selenoproteins and depends is determined by an in-frame stop codon UGA and a tertiary structure in the messenger RNA known as SECIS element (Sec insertion sequence). We identified the existence of the selenocysteine synthesis route in kinetoplastid and characterized the genes of this pathway: SELB (Elongation Factor EFSec), SELD (Selenophosphate sintetase), PSTK (Fosfoseril tRNA kinase), SECSEPS (Selenocysteine Sintase) and SELC ($tRNA^{[ser]sec}$). Furthermore, three selenoproteins were identified, SELK, SELT and SELTryp. The last member has no identity with other mammalian selenoproteins. However, details about composition and structure of the Sec incorporation complex are not known, and the purpose of project is the identification of proteins that participate in the Sec incorporation complex, using techniques of complex purification such as PTP (ProtC – TEV – ProtA)-tag, using trypanosome PSTK as a bait. The PSTK coding-gene was cloned into the pN-Puro-PTP vector, linearized with *Xcm* I and transfected into *Trypanosoma brucei* cells (strain 427). Positive clones were selected with puromycin and western blotting experiments using anti-protein A antibodies confirmed the expression of PSTK fused with PTP-tag. A total *T. brucei* cell protein extract was obtained and immunoprecipitation assays confirmed the ability of PSTK-PTP to interact with IgG sepharose, the first step of PTP-tag purification. After the complete PTP-tag purification process, at least four proteins were observed, with approximately 66, 60, 45 and 29 kDa. These data are awaiting confirmation by mass spectrometry, but strongly suggest the presence a multi-protein complex, responsible for incorporation of selenocysteine in *Trypanosoma* cells. This observation is consistent with previous immunofluorescense experiments using polyclonal antibodies against SELB that indicated the formation of cytoplasmic granules, corroborating a possible protein complex been formed. Supported by: FAPESP and CNPq

## BM.110 - 214 1 CHARACTERIZATION OF A LARGE TFIIA-ASSOCIATED PROTEIN COMPLEX IN *TRYPANOSOMA BRUCEI*

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TFIIA is a basal factor of RNA polymerase (pol) II transcription initiation interacting with the TATA-binding protein (TBP) and stabilizing the TBP-core promoter interaction. TFIIA consists of two subunits in yeast and, due to proteolytic cleavage of the larger protein, of three subunits in higher eukaryotes. In *Trypanosoma brucei*, a clear ortholog of the small TFIIA subunit (TFIIA-2) was characterized as part of a larger complex consisting of three SNAPc subunits, the trypanosome TBP homolog TRF4, and a sixth protein whose sequence conservation is too weak for unambiguous annotation. Interestingly, tandem affinity purification of TFIIA-2 co-purified not only the SNAPc/TFIIA/TRF4 components but specifically also several proteins of minor abundance indicating that TFIIA-2 is assembled into a second protein complex. Since we could identify one of the minor proteins by mass spectrometry, we expressed the protein in trypanosomes as a C-terminal fusion to the composite PTP tag and tandem affinity-purified it from extract. At least eight proteins were co-purified; mass spectrometry identified them as TFIIA-2 and seven *conserved hypotheticals* that lack sequence similarity to proteins outside of trypanosomatids. Since all nine proteins co-sedimented in a sucrose gradient, they appear to form a single complex which we termed TFIIA-associated complex (AAC). While the *AAC-3* subunit is localized in the nucleus, it is, unlike subunits of SNAPc/TFIIA/TRF4, dispensable for RNA pol II transcription of spliced leader RNA genes. Preliminary data indicate that silencing of *AAC-3* is lethal. Interestingly, first RNA analyses revealed that this knockdown affects the abundance of RNA pol II- but not of RNA pol I-synthesized mRNA suggesting that AAC-3 functions specifically in RNA pol II transcription of protein coding genes.

## BM.111 - MOLECULAR CHARACTERIZATION OF A *Trypanosoma brucei* POLY(A)-BINDING PROTEIN.

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Trypanosomatids are a major cause of mortality in tropical regions of the world and are affected by the lack of effective treatments. Several intriguing molecular pathways are found in these parasites also, rendering them particularly attractive for biochemical investigation. This unique eukaryotic cell lacks mechanisms to control gene expression at the transcriptional level, as protein synthesis is mostly controlled by posttranscriptional regulation processes. Hundreds of protein-coding genes of unrelated function have to be separated cotranscriptionally by trans-splicing at the 5' end and polyadenylated at the 3' end. Several RNAs and proteins are central to this process, including poly(A) binding proteins. The poly(A)-binding protein of eukaryotes is the major cytoplasmic mRNA binding protein and also plays a role in polyadenylation of nuclear transcripts. In the cytoplasm, $PAB_1$ has been implicated in translational initiation and termination, and in mRNA turnover. When complexed to mRNA, the poly(A) tail interacts with cap, enhancing translational initiation and stabilizing mRNA. In addition, this protein is important in mRNA decay. In our work a novel poly(A) binding protein ($PABP_1$) was identified in *Trypanosoma brucei*. Transcriptional silencing using the RNA interference technique revealed that the absence of its transcript is lethal to the procyclic form of the parasite. Protein immunolocalization experiments indicate a cytoplasmatic localization. We are presently investigating the domain organization of the protein and its involvement in the polyadenylation/trans-splicing coupled events. Supported by FAPESP, CNPq, NIH

## BM.112 - *Trypanosoma brucei* SELENOCYSTEINE PATHWAY VALIDATION BY INTERFERENCE RNA.

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Selenoproteins, proteins containing the 21st amino acid selenocysteine (Sec – U) are present in the three domains of life and require a complex molecular biosynthesis and incorporation machinery. Genes coding for components of the Sec insertion machinery were identified in the Kinetoplastida genomes of *Trypanosoma cruzi, Trypanosoma brucei* and *Leishmania*. We focused our work on *Trypanosoma brucei*, the etiologic agent of Nagana and Sleeping Sickness. To produce selenocysteine, this organism obtains the biologically active selenium donor compound through the catalysis of selenide and ATP by Selenophosphate Synthetase (SPS2). RNA interference experiments of SPS2 in procyclic *T. brucei* form shows significant growth inhibition and an apoptotic-like phenotype. Also, an increased susceptibility to oxidative stress induced by hydrogen peroxide suggests a possible role of selenoproteins on oxidative pathways and protection to oxydative stress. The selenoproteome of these flagellated protozoa consists of distant homologs mammalian SelK and SelT, and a novel selenoprotein designated SelTryp, that has neither Sec- nor cysteine-containing homologs in the human host, appearing to be a Kinetoplastida-specific protein (Lobanov, 2006). Since these proteins have predicted redox motifs, we cloned them into a vector for RNAi ($p2T7^{TABlue}$) to individually investigate their function in different life stages of *T. brucei.* Complementary, polyclonal antibodies are been used to estimate the expression levels of these proteins, giving a better understanding of the role of selenoproteins in this organism.
Supported by FAPESP.

## BM.113 - IMPROVEMENT OF THE HETEROLOGOUS GENE EXPRESSION IN *Trypanosoma brucei* USING POST-TRANSCRIPTIONAL AND POST-TRANSLATIONAL MECHANISMS

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The functional genomics is a growing field due to the increasing number of published genomes, a fact which tends to improve with the advent of new sequencing platforms. To elucidate the gene function in *T.brucei*, there was a large range of techniques and methodologies to be employed. One of these is the heterologous expression using inducible promoters regulated by tetracycline repressor. However, some reports have shown that there is some weakness on controlling gene expression, and the leak in the expression of toxic gene products in the absence of the inductor limits the advances of various post genomic approaches. On this assumption, we designed a *T.brucei* expression vector (based on pLEW100) regulated by exogenous molecules: transcriptionally by GPEET promoter with tetracycline (tet) operators, post-transcriptionally by a riboswitch molecule (Ribo), and post-translationally by fusioning the target protein to FKBP destabilizing domain (DD). The constructs were created using pLEW100 as a parental vector. Our first attempts, using procyclics transfected with two pLEW100 vectors containing *Renilla* luciferase controlled by the inducible promoter and a tet-sensitive riboswitch at 3'UTR, or an always active ribozyme, showed that this new system regulates over a thousand times the luciferase activity in the presence of the ligand in relation to its absence. To obtain a higher efficiency of gene regulation, we are generating constructs carrying GFP fused to DD polypeptide wich will be also regulated by tet repressor and riboswitch.
Financial support: Fundação Araucária, CAPES/REUNI, FAPEMIG and CNPq

## BM.114 - STANDARDIZATION OF A SINGLE MOLECULE ANALYSIS OF REPLICATED DNA (SMARD) OF *Trypanosoma brucei* CHROMOSOME I

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DNA replication is a crucial step during the cell cycle and little is known so far about the DNA replication of *Trypanosoma brucei*. Single Molecule Analysis of Replicated DNA (SMARD) allows the visualization by fluorescence microscopy of single molecules of replicated DNA stretched on microscope slides. Using this method is possible to determine the number of replication origins, the fork direction and the DNA replication rate (kb/min) of a DNA fragment. Here we want to define how replication occurs on chromosome 1 from *T. brucei* (1.85 and 3.6 Mbp). Since the technique limits the molecules analyzed to a maximal length of 500 kbp, two different approaches are being developed: the analysis of chromosome 1 (1.85 and 3.6 Mbp) fragments smaller then 500 kb and the entire chromosome 1. For the fragment analysis, DNA was digested with two different enzymes, Fse I and Asc I. After running the pulsed field gel electrophoresis, the fragments were analyzed using specific probes and they showed length differences compared to the prediction because the strain sequenced (*T, brucei* TREU 972) was different from that used in this experiment (*T. brucei* 427). Although the fragments were still smaller then 500 kbp and they can be identified on slides through specific probes that are being developed. In order to analyze the entire chromosome 1 (1.85 Mbp), a PFEG program will be developed to separate and extract chromosome 1 from the genome. Probes are also being developed to determine the chromosome 1 orientation (5' – 3'). Once all these probes are available, chromosome 1 and the fragments can be analyzed and then it will be possible to know how many origins are present in chromosome 1, and also the fork direction and speed. Supported by FAPESP.

## BM.115 - **A NEW *T. BRUCEI* CELL LINE THAT IS USEFUL FOR ACCESSING FUNCTION OF PROTEINS INVOLVED IN TRANSLATION**

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*Trypanosoma brucei* is a privileged model for studies of gene expression mainly because of its feasible cultivation and the development of several tools involving genetic manipulation and inducible expression. Many plasmids developed for *T. brucei* are based on tet-responsive elements; however the evaluation of individual proteins involved in basic processes, such as translation, would require the use of a constitutive gene reporter that could be easily detected in these parasites. We aimed to generate new *T. brucei* cell lines expressing the luciferase as a monocistronic reporter to access, in a direct manner, the translation-related functions of some trypanosomatid initiation factor (eIF) homologues. The constructs used in transfection experiments were derived from pLew20 and pLew82. The modifications were designed for a constitutive expression of firefly luciferase and a puromycin drug resistance marker under the action of a single promoter. Cultures of procyclic host cell line 29-13, coexpressing T7RNAP e TetR, were harvested and transfected with *Not*I-linearised plasmids pLEW20ΔtetOpac and pLEW82ΔtetOpac. Puromycin-resistant clones were evaluated for constitutive luciferase expression and the expression driven by an endogenous (pLew20) or a T7 promoter (pLew82) was also compared. Initially the clones examined expressed continuous levels during a growth curve, with T7RNAP leading to luciferase activities exceeding those produced by the endogenous promoter. The cell lines were then designated 29-13-20m and 29-13-82m. However, after several passages, the cell line 29-13-82m showed much reduced levels of expression, similar to the parental 29-13, but it maintained the puromycin-resistance showing that the cells turned into revertant forms. The cell line 29-13-20m maintained high levels of luciferase expression and it proved to be useful for new rounds of transfection with tet-responsive plasmids for functional assays involving the eIF homologues and luciferase activity which will be able to serve as parameter to determine their influence in translation.
Supported by CAPES and CNPq

## BM.116 - IDENTIFICATION OF POST-TRANSLATIONAL MODIFICATIONS ON HOMOLOGUES OF TRANSLATION INITIATION FACTORS IN *Trypanosoma brucei*

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*Trypanosoma brucei* is a unicellular protozoan which in humans causes the sleeping sickness and whose life cycle alternates between two hosts, its procyclic form being found in insect vectors and its bloodstream form in susceptible mammals. Genomic analysis identified a considerably large number of protein kinases, leading to speculation that protein phosphorylation, among the post-translational modifications, may also be a key mechanism for such regulation events. In eukaryotes, the eIF4F complex (formed by subunits eIF4A, eIF4E and eIF4G), assisted by the poly(A)-binding protein (PABP), works in translation initiation and has its activity regulated by modifications such as phosphorylation. This study sought to analyze the expression of selected eIF4E, eIF4G and PABP homologues in *T. brucei* (*Tb*EIF4E1, 3 and 4; *Tb*EIF4G4 and 5) and to investigate the occurrence of post-translational modifications which may be associated with control of their function. First, growth curves were generated and protein extracts made from cells derived from selected time points. All the chosen proteins were observed to be expressed throughout the curves in both forms of the parasite's life cycle, but with distinct expression patterns. *Tb*EIF4E1 and 3 are the least expressed and the most expressed proteins, respectively, in both procyclic and bloodstream forms. *Tb*EIF4E3 and 4 and *Tb*EIF4G4 were represented by more than one isoform, unlike the other proteins studied, and the three proteins were found in phosphorylated forms. Through two-dimentional electrophoresis it was possible to visualize 2, 5 and 4 isoforms for *Tb*PABP1, *Tb*EIF4E3 and *Tb*EIF4E4, respectively, whilst only one non-phosphorylated isoform was observed for the *Tb*EIF4AI. In addition, it was shown that phosphorylation of *Tb*EIF4G4 does not prevent its interaction with *Tb*EIF4E3. These results indicate that phosphorylation is a mechanism that may have a predominant role in regulating the function of the translation factors studied.
Supported by FACEPE and CNPq.

## BM.117 - EVALUATION OF THREE METHODS FOR DNA EXTRACTION FROM STOOL SAMPLES FOR DETECTION OF *ENTAMOEBA* BY REAL-TIME PCR

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Amebiasis is an infection caused by *Entamoeba histolytica.* Differentiation between *E. histolytica* and *E. dispar*, a morphologically identical species, is fundamental for precise diagnosis in order to base public health and therapeutic conducts to prevent and control the occurrence of invasive disease. Parasite demonstration by microscopy continues to be the technique more frequently used for the diagnosis of amebiasis. However, this method cannot differentiate *E.histolytica* from *E. dispar*. Real-time Polymerase Chain Reaction (RT-PCR) was shown to be a high specific and sensitive method for diagnosis and species differentiation. DNA extraction is required for an efficient PCR reaction and should be adequate to each type of clinical sample and microorganism. In this study, the efficiency of three different methods for extraction of DNA from stools was evaluated: two commercial kits, FastDNA® (MP Biomedicals) and QIAamp® DNA Stool (QIAGEN), and one "in house" technique. The SYBR Green reaction was used for all the tests. Stool samples negative for the *E. histolytica/E. dispar* complex were spiked with 5.5 x $10^7$ trophozoites of *E. histolytica* and stool samples with cysts of *E. dispar* were used for DNA extraction. Amplified DNA sequences of 132 base-pairs (bp) and 96 bp were specific for *E.histolytica* and *E.dispar*, respectively. Four different concentrations of DNA obtained from samples containing trophozoites were assess. The cycle numbers ($C_t$) detected were: 19.0, 21.0, 24.3 and 28.1 for the "in house" extraction; 19.9, 24.2, 26.4 and 29.0 for the QIAamp® method, and 22.1, 24.6, 27,5 and 28.4 for the FastDNA® method. The results indicate that the "in house" extraction method was the best to detect for trophozoites, while detection of cysts was better achieved by using the FastDNA® method. Supported by FAPERJ and CNPq.

## BM.118 - MULTIPLEX REAL-TIME PCR FOR THE IDENTIFICATION AND DIFFERENTIATION OF *ENTAMOEBA HISTOLYTICA* AND *ENTAMOEBA DISPAR* BY USING THE SYBR GREEN TECHNOLOGY

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*Entamoeba histolytica* is a protozoan that causes amoebiasis, a infection that can result in serious disease. The microscopic examination of fresh or fixed stools is the method more frequently used for *Entamoeba* detection. Two morphologically indistinguishable species, the potentially pathogenic *E. histolytica* and the non-pathogenic *E. dispar*, are established. Then, new diagnostic methods are necessary for the identification and differentiation of these two species. Polymerase Chain Reaction (PCR) - based methods have been proposed by several groups. The development of real-time PCR contributed to circumvent sensitivity and contamination problems observed with conventional PCR, improving these techniques in order to be used in clinical laboratories. The objective of this study was to standardize a multiplex real-time PCR protocol using the SYBR Green technology to identify and differentiate the species belonging to the *E. histolytica*/*E. dispar* complex. A standard curve was obtained using 10 1:2 DNA serial dilutions. DNA amplification was detected for all the dilutions, expressing the sensitivity of 0.0143 pg for *E. histolytica* and 0.5156 pg for *E. dispar.* In order to differentiate the *Entamoeba* species with the SYBR Green technology, the dissociation stage after the cycling conditions was included. This additional step produces a melting curve, which indicates specific melting temperatures (Tm) for each species sequences. The mean melting temperature (Tm) observed for the DNA sequence amplified from *E. histolytica* was 73.6ºC while *E. dispar* DNA sequence was 70.6ºC. The results demonstrate that the SYBR Green multiplex real-time PCR offers a new and improved alternative method for the differentiation of the *E. histolytica*/*E. dispar* complex.
Supported by CNPq and FAPERJ.

## BM.119 - CHARACTERIZATION OF THREE APPLE CONTAINING DOMAINS PROTEINS FROM THE APICOMPLEXAN *Neospora caninum*

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*Neospora caninum*, an Apicomplexan parasite, has been increasingly recognized as an important cause of bovine fetal loss worldwide due to the abortion it causes in cattle. The host cell invasion process of this obligatory intracellular parasite is very conserved among the phylum and involves secretory organelles like micronemes, roptries and dense granules. Pan/Apple domains, which are known to be related to adhesion functions, were found in microneme proteins from other Apicomplexan parasites, like EtMIC5 from *Eimeria tenella* and TgMIC4 from *Toxoplasma gondii*, probably mediating binding interactions between parasite and host cell. The aim of this work is the identification and characterization of proteins containing Apple domains in *N. caninum*. Based on ESTs containing Apple domain sequences, three flanking fragments were found in a single contig after screening the non-annotated genomic database of *N. caninum.* Sequencing data revealed a high level of similarity among the three fragments and each of them was predicted to encode one protein containing three Apple domains (PAN1), and one of these fragments also had one Apple-like domain (PAN3). Two fragments were chosen to be cloned in pET28 and expressed in *E. coli* BL21 cells, resulting in two recombinant proteins with molecular weight of 37 KDa and 41 KDa, called PAN domain-containing protein1 (PANdcp1) and PANdcp2. Two New Zealand rabbits were immunized with each recombinant protein and sera raised were collected after each of four immunizations. ELISA assays were made to verify serum conversion and recognition of native and recombinant Apple proteins. We are currently investigating by 2D Western Blot for the Isoeletric point and Molecular Weight of the native proteins in total extract from *N. caninum*. Next steps include *in vitro* invasion assays and localization of these Apple proteins in the tachyzoite of *N. caninum*. Supported by CNPq (project grant 480039/2009-7) and CAPES (fellowship to LPO).

## BM.120 - TRANSIENT CONTROL OF EXPRESSION BY TETR/TETO SYSTEM ADAPTED FOR *N.CANINUM*

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*Neospora caninum* is an Apicomplexan protozoan, obligatory intracellular parasite that has the dogs as definitive hosts and especially cattle as intermediate hosts, causing in the first ones encephalopathy and the last ones abortion with fertility impairment. Because of its intracellular cycle, several strategies have been developed to characterize the invasion process in the phylum, such as knock-out or expression control of proteins involved in active invasion. The operon TetR/tetO system is present in gram-negative bacteria that gain the ability to expulse tetracycline from periplasmic region. The system is composed by TetR, a dimmer protein that bind to the tetO nucleotide sequence, repressing expression on tetracycline operon. The expression is activated when tetracycline interact to dimmer TetR liberating it from tetO sequence and allow the expression of operon. An inducible system based on TetR/tetO in *Toxoplasma gondii*, where TetR is expressed by a tubuline vector and tetO is downstream a RPS13 promoter controlling the expression of β-galactosidase. Thus, TetR can be incorporated in the parasite genome by a chloramphenicol cassette resistance flanked by the promoter and the 3' region of *T. gondii* SAG gene (TgSAG1). In our work we have substituted the *T. gondii* promoters to *N. caninum* in TetR and tetO (tubulin 1990 pb and RPS13 651pb, respectively) and the promoter and 3' region of the *Tg*SAG to *N. caninum* SAG1 gene. In a transient assay the NctetO vector expressed β-galactosidase 700% over control (for 10 days). Tachyzoites were transiently transfected with NcTetR followed by NctetO and treated with 0,1; 1 and 10 μg/ml of tetracycline and the presence of 1 μg/ml improved the expression of β-galactosidase by 14% The experiments with stable transfection and control of β-galactosidase expression are being performed aiming to future functional assays with *N. caninum* genes involved with invasion.
Supported by CAPES (fellowship) and FAPESP (project grant 2005/53785-9).

## BM.121 - GENOMIC AND PROTEOMIC CHARACTERIZATION OF *TOXOPLASMA GONDII* BRAIN CYSTS FROM MICE INFECTED WITH GENETICALLY DISTINCT STRAINS

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Toxoplasmosis, caused by the coccidian *Toxoplasma gondii,* is transmitted by ingestion of raw or undercooked meat or by ingestion of food and water contaminated with oocysts from infected cat faeces. Although asymptomatic in most cases, the agent causes ocular disease or severe disease in immunocompromised patients or fetus. Several strains of *T.gondii* have been described in recent years, with variable virulence that could be associated with severity of human disease, including the reinfection of a previously immune host. The primary infection by *T.gondii* usually confers reinfection protection by a new strain but there are anecdotal reports of successful reinfection. We devised to study reinfection and co-infection models in experimental mouse toxoplasmosis using genetically defined strains for study specific cyst survival in the brain, looking for evidence of recolonization after primary infection. We used two genetically defined strains, ME49 (genotype II) and VEG (genotype III), which promotes cyst brain infection. Brain cyst genotype was determined by SAG2 gene RFLP on infected brains. Whole anti-*T.gondii* IgG and peptide specific strain IgG was determined by ELISA. Strain specific immunohistochemistry of infected brains was performed with anti-peptide antibodies. ME49 primary infection give higher cysts number than VEG primary infection both with strain specific IgG production. Co-infection with both strains presented early predominance of ME49 cysts with subsequent VEG cysts with both strains specific IgG. Primary infection with subsequent challenge with another strain showed that the primary infection colonizes the brain and block subsequent challenge strain. Specific strain antibody production is also from primary strain despite evidence of virulent infection. Immunohistochemistry confirms those data. Our findings show that primary strain infection protects the brain from subsequent colonization from a new strain of *Toxoplasma* but without evidence of adaptive specific immune response to the new strain. Supported by CNPq and LIM49HCFMUSP.

## BM.122 - IMMUNOLOCALIZATION BY ELECTRON MICROSCOPY OF PROTEINS INVOLVED IN *Toxoplasma gondii INVASION*

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The parasite *Toxoplasma gondii* belongs to the Phylum Apicomplexa and is able to invade all types of nucleated cells. This process is mainly coordinated by the parasite, and depends on three types of secretory organelles: rhoptries, micronemes and dense granules. It is common sense that at the region of entry in host plasma membrane, parasite secreted proteins give rise to the moving junction. That is crucial for the formation of the parasitophorus vacuole, a “safe heaven” inside the host cell for the parasite’s development. In this work, we have used high resolution scanning and transmission electron microscopy to immunolocalize toxoplasma’s secreted proteins involved in the moving junction. Our model is *T. gondii* RH strain and as host, the epithelial cell line LLC-MK2. The interaction was for 15 minutes at 4$^{o}$C and 5 minutes at 37$^{o}$C before the fixation. Standard protocols for immunolocalization were employed for visualization by scanning and transmission electron microscopy and imunoflurescence (IFA). IFA assays for RON-4, a protein from rhoptry neck, had a ring shape distribution, and was a marker for the moving junction region. However, not all invasion points presented this stain. Using scanning electron microscopy, we confirmed the presence of moving junction, and the hourglass shape of the parasites but the labeling at this point had only a few Au particles. The immunocytochemical labeling by TEM confirmed these data. Comparing this model with macrophage invasion, the same pattern was observed using both FE-SEM and IFA assays. These results indicate that invasion of host cells may result from more than one process of interaction, and that the moving junction may not be present in all of them. The RON-4 labeling in moving junction was visualized at first time by SEM.
SUPPORTED BY CNPq and FAPERJ.

## BM.123 - CONGENITAL TOXOPLASMOSIS IN MINAS GERAIS, BRAZIL: ISOLATION, VIRULENCE AND MOLECULAR DIAGNOSIS OF *Toxoplasma gondii*

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*Toxoplasma gondii* is a widely distributed apicomplexan parasite of great medical importance. Most primary infections are asymptomatic in immunocompetent hosts. However, immunosuppressed individuals like fetuses, organ graft recipients or patients with acquired immunodeficiency syndrome develop a severe disease. The objective of this work was to carry out a study of congenital toxoplasmosis in the State of Minas Gerais, Brazil. In an attempt to samples isolate and characterize *T. gondii*, blood from 220 newborns were collected. Of the total children screened, 81% (178/220) had the diagnosis confirmed with toxoplasmosis congenital by the persistence of IgG in ELISA test after the 12th month of life. *T. gondii* was isolated from peripheral blood of 27 infected newborns by mouse bioassay. Our experiments showed that 15.2% (27/178) of the newborns with congenital toxoplasmosis showed parasitemia. Isolates were divided into three groups according to the virulence phenotype. Ten isolates (38%) were characterized into virulent for mice (LD100 equivalent to a single viable tachyzoite). Two (8%) were characterized into non-virulent, as all mice survived after the 30-day-period of observation. All surviving mice showed to have anti-*T. gondii* antibodies. Fourteen isolates (54%) showed to have intermediate virulence (virulence phenotype varying among those described above). Gene amplification was performed for B1 gene in all blood samples verifying a total of 64/220 (29.0%) children were positive by PCR (sensitivity 31.46% and specificity 80.85%). Our research corroborates that nested PCR using the primer B1, is not sufficiently sensitive and specific to be a useful diagnostic tool for congenital toxoplasmosis diagnosis in peripheral blood samples from newborns.
Supported by: FAPEMIG, SES-MG, CNPq, NUPAD-UFMG

## BM.124 - LABORATORIAL DIAGNOSE OF CRYPTOSPORIDIOSIS USING REAL-TIME PCR

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*Cryptosporidium spp.* is an important diarrhea-causing parasitic protozoan found in both humans and animals. *Cryptosporidium hominis* and *Cryptosporidium parvum* are responsible for the most cases of cryptosporidiosis in human beings. In developing countries, *Cryptosporidium spp* infections occur mostly in children younger than 5 years of age. In immunodeficient human (HIV / AIDS), cryptosporidiosis can be associated with chronic potentially life-threatening diarrhea. The conventional method for detecting oocysts in fecal specimens in HUAP laboratorial routine, involve microscopy and safranin-methylene blue staining technique. However, this method cannot identify *Cryptosporidium* at the species level. Real-time PCR with specific primers and probes represents an alternative to conventional PCR for increasing the sensitivity, specificity and speed of sample analysis. The objective of this study was to evaluate the TaqPCR in relation to the safranin-methylene stain in clinical specimens examined in the HUAP laboratory. From June of 2009 to July of 2010, 19 stool samples from HIV serology-positive patients examined for *Cryptosporidium*. Of these stool samples, 4 were positive for *Cryptosporidium spp.* by microscopy and by TaqPCR (3 - *C. hominis* and 1 - *Cryptosporidium spp.*). One sample was positive only by microscopy and 4 samples were positive only by TaqPCR (2 - *C. hominis* and 2 - *Cryptosporidium spp.*). These results showed the advantage in the association of these two techniques which allowed the detection of *Cryptosporidium* in nine samples. Species discrimination is important for molecular epidemiological purposes in order to evaluate potential sources of infections.
Supported by FAPERJ.

## BM.125 - CHARACTERIZATION OF GLYCERALDEHYDE-3-PHOSPHATE DEHYDROGENASE FROM *Phytomonas serpens* 15T

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The enzyme glyceraldehyde-3-phosphate dehydrogenase (GAPDH) catalyses the oxidative phosphorilation of glyceraldehyde-3-phosphate to 1,3-biphosphoglycerate. This reaction plays an important role in controlling ATP production in pathogenic parasites. GAPDH enzymes from human pathogenic trypanosomatids species have been extensively studied at both the enzymological and gene level. Overall, at least two isoenzymes, glycosomal and cytoplasmic, of GAPDH have been described for these trypanosomatids. The glycosomal enzymes are encoded by two tandemly arranged genes of identical sequence and encode polypeptides with approximately 38 kDa which present around 55% identity with the amino acid sequences of cytosolic enzymes. In the case of other trypanosomatids there are little informations about the GAPDH enzymes. In view of this, here we describe the characterization of glycosomal GAPDH from *P. serpens* 15 T, a heteroxenic tomato parasite. Northern blot employing total RNA from *P. serpens* exponential promastigotes was hybridised with *gapdh* probe, and a transcript of around 1.5 kb was observed. GAPDH was overexpressed in *E. coli* BL21(DE3) strain carrying the recombinant plasmid pET28a-GAPDH and the recombinant protein was used as antigen to immunize BALB/c mice. Western blot analysis of log-phase promastigotes with this antiserum recognized a protein with an estimated molecular weight of 38 kDa. The localization of GAPDH was further investigated. The protein was detected mainly at the cytoplasmic region of *P. serpens* promastigotes by confocal immunofluorescence microscopy. Transmission electron microscopy showed the localization of GAPDH gold particles in membrane-bound cytoplasmic structures of the plant parasite. No background staining was observed when secondary antibodies were incubated alone with the *P. serpens* promastigotes in both microscope assays. Supported by: Fundação Araucária – PR and CAPES.

## BM.126 - 422 1 DOCKING STUDIES OF NADH ANALOGUES AS POTENTIAL INHIBITORS OF *PLASMODIUM FALCIPARUM* LACTATE DEHYDROGENASE

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The enzyme lactate dehydrogenase from *Plasmodium falciparum* (*Pf*LDH) is considered a potential molecular target to the development of new chemotherapics against malaria based on its importance in the final step of the parasite energy obtention process. It has been suggested that the mechanism of action of the quinolinic antimalarials could be related to the inhibition of this enzyme by competition with its binding pocket for NADH (Read et al, J. Bio. Chem., 1999). Based on this assumption, we have now performed a screening on the Drug Bank for analogues of NADH and further computational studies on the binding mode of these compounds in the *Pf*LDH active site. The docking energies of each compound were calculated with the software Molegro Virtual Docker (MVD)® which permitted the selection of 50 compounds with interactions on the active site similar to that of NADH. In addition, we also analyzed the main interactions between the *Pf*LDH active site and the selected compounds in order to evaluate if the residues involved are specific of *Pf*LDH, as compared to human LDH. Among the compounds studied three (posaconazole, itraconazole and atorvastatin) were chosen for further experimental studies on *Pf*LDH. Since they presented very good theoretical results and, also, because are commercially available for use in human beings, these compounds were forwarded to be tested in experimental protocols (see abstract by Penna-Coutinho et al.).
Supported by FAPERJ, CNPq, CAPES, Fundação Estudar (fellowship to W.A.C.).

## BM.127 - THE KINASES SECRETED IN SALIVARY GLANDS FROM TRIATOMINAE (HEMIPTERA: REDUVIIDAE)

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The transmission of Chagas disease by triatominae insects across South and Central America has imposed enormous economic and health burdens to the human populations. The kissing bugs in the subfamily Triatominae rely on the chemical properties of the saliva constituents to inhibit the host's blood homeostasis and to prevent harmful proliferation of microorganisms. The investigations about the saliva contents from various triatomine insects suggested the presence of protein kinases in the insect's saliva. This existing enzymatic function, which had never been reported on triatomines saliva, could elucidate some unexplained peculiarities noticed during triatomines blood feeding. Mass spectrometry LC-MS/MS assays performed with salivary gland proteins from *Panstrongylus megistus* and *Rhodnius brethesi* revealed a gamut of different proteins with reported kinase activities in salivary glands from those triatomine species. The analyses of transcript from salivary gland of *Rhodnius robustus* and *Triatoma infestans* also indicated the presence of three main kinases, two of which had been identified by LC-MS/MS assays. The databank searches showed that some of the kinases involved with the blood feeding possibly evolved from genes acquisitions in the course of natural selection. In this regard, kinase genes differentiated to display different functions. Our results suggest that protein kinases in the kissing bugs salivary glands might play a role in hematophagy. Further experiments will determine the functional roles of the secreted protein kinases in the triatomines salivary glands.
Supported by CAPES ans CNPq.

## BQ.01 – *TRYPANOSOMA CRUZI* Y STRAIN EPIMASTIGOTES PROTEOMIC MAP: NEW INSIGHTS IN POST-TRANSLATIONAL MODIFICATIONS

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Chagas' disease caused by protozoan *Trypanosoma cruzi*, is an endemic neglected illness in Latin America, responsible for considerable human mortality and morbidity. Parasite's life cycle involves vertebrate and invertebrate hosts, and three evolutive forms. In this study, the proteomic profile of *T. cruzi* epimastigotes (Y strain) was evaluated, to identified post-translational modifications. 2D-PAGE was employed, and the gels were stained with colloidal Coomassie for total protein or ProQ-Emerald or Diamond for glyco- or phospho-proteins, respectively. All peptides identification was performed in MALDI-TOF/TOF. Epimastigotes' proteins were extracted by freezing-thawing in a lysis solution (8M urea, 4M thiourea, 4% CHAPS, 40 mM Tris, 60 mM DTT, 1% ampholytes and protease inhibitor cocktail). Two-dimensional electrophoresis (500 μg) was performed using 4-7 IPG strips followed by 12% SDS-PAGE. Approximately 617 spots were detected, being 160, 24 and 35 identified in the gels stained with Comassie, Pro-Q-Emerald and Pro-Q-Diamond, respectively. Glycoprotein content embrace chaperones, beta-tubulin, prostaglandin F2a synthase, ATPase, tyrosine aminotransferase, sterol 24-c-methyltransferase, tryparedoxin peroxidase, among others. Phosphoproteins included glutamine synthetase, pyrroline-5-carboxylate synthetase, 14-3-3, spermidine synthase, initiation factor 5a, chaperones, cytoskeleton proteins, as well as five hypothetical proteins. Our data could provide new insights in the signaling networks in *T. cruzi*, supplying additional information for the development of alternative drugs for Chagas' disease.
Supported by PDTIs, CNPq, IOC/FIOCRUZ and FAPERJ.

## BQ.02 – A PROTEOMIC APPROACH TO UNDERSTAND TGF-β EFFECT IN *T. CRUZI* BIOLOGY

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TGF-β is a molecule involved in the development and maintenance of acute and chronic chagasic cardiopathy. This cytokine is also intimately associated with *Trypanosoma cruzi* as a regulator of different stages of the parasite´s life cycle, such as parasite survival, invasion, proliferation and differentiation. This raises the question about which *T. cruzi* molecules could be involved in these cellular processes stimulated by TGF-β. This work aims to characterize TGF-β responsive molecules through a phosphoproteomic approach using two-dimensional non-linear pH 3-10 gels, differential fluorescent protein staining and mass spectrometry (MALDI-TOF-TOF). We determined that 5 ng/ml was the optimal TGF-β dose for the induction of phosphorylation events, under our experimental conditions. A kinetic study was performed by incubating or not *T. cruzi* epimastigotes (Y strain) with TGF-β in four different time periods (1, 5, 30 and 60 minutes). Phosphoproteins were stained with ProQ Diamond and total proteins with Sypro Ruby (both from Invitrogen), images captured using Typhoon Trio (GE Healthcare) and differential profiles analysed with PDQuest software (BioRad). A number of 30 proteins had their phosphorylation and/or expression pattern altered in response to TGF-β and were identified through mass spectrometry. Their putative functions were correlated with their possible roles in intracellular signaling triggered by TGF-β, in agreement with the phosphorylation and/or expression behavior observed in our analysis. Moreover, we assessed the effect of TGF-β on parasite proliferation. We found that TGF-β addition to epimastigote cultures led to a 36% increase in parasite growth after 72 hours ($p<0,05$). The data presented here contributes to the elucidation of the molecular mechanisms related to TGF-β signaling in *T. cruzi*, providing a source of new potential therapeutic targets against Chagas disease.
Supported by IOC (Fiocruz), CNPq and PAPES V.

## BQ.03 – PROTEOMIC ANALYSIS OF POPULATIONS OF *Leishmania braziliensis* AND *Leishmania infantum chagasi* RESISTANT TO POTASSIUM ANTIMONY TARTRATE.

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The emergence of drug-resistance *Leishmania* species is a relevant problem in several countries. Although several mechanisms of drug-resistance are known, there are few studies concerning drug resistance on New World *Leishmania* species. Recently, we selected *in vitro* populations of *Leishmania braziliensis* and *L. infantum chagasi* that are 20 and 4-fold more resistant to potassium antimony tartrate Sb III, respectively (LbSbR/LcSbR) than theirs susceptible counterparts (LbWTS/LcWTS) (Liarte & Murta, 2010). In the present study, proteomic analysis of *L. infantum chagasi* and *L. braziliensis* susceptible (WTS) and SbIII-resistant (SbR) populations was performed using two-dimensional gel electrophoresis (2-DE). The protein extracts (600µg) from four *Leishmania* samples were applied to immobilized pH gradient (IPG) strips (17cm, pH 4-7 linear) and then submitted to isoelectric focusing. Subsequently, IPG strips were transferred to a 12% polyacrylamide gel and after electrophoresis, the gels were stained with colloidal Coomassie Blue G-250. For each pair of samples, proteins from three independent experiments were obtained, and gels in duplicate wer analyzed for each cultivate. The differential expression analysis was performed comparing the quantity of matched spots in each pair of sample. A protein was considered differentially expressed when the ratio between the intensities of S (susceptible) and R (resistant) spots were ≥ 2.5-fold. The analysis of the gel images showed significant difference of the protein profiles between the two *Leishmania* species analyzed. The average number of spots from *L. braziliensis* (353 spots) was higher than *L. infantum chagasi* (320 spots). Analysis of the gel images showed that *L. braziliensis* presented 92 and 26 spots more expressed in the susceptible and resistant populations, respectively. On the other hand, *L. infantum chagasi* presented higher number of spots overexpressed in the resistant population (101 spots) than in its susceptible pair (26 spots). Further, these spots will be submitted to mass spectrometry for protein identification. Financial Support: CNPq, FAPEMIG, CPqRR, UNIMONTES and UNICEF/UNDP/World Bank/WHO/TDR.

## BQ.05 – *TRYPANOSOMA CRUZI* REDOX- INTERACTOME: *IN VIVO* IDENTIFICATION OF PROTEINS TARGETED BY TRYPAREDOXIN I

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During infection, *Trypanosoma cruzi* is subjected to the host oxidative environment. Its antioxidant responses are critical for successful infection. Trypanosomatids have a unique hydroperoxide detoxification system dependent on the thiol trypanothione. Tryparedoxin peroxidases catalyze the reduction of peroxides and they are regenerated by tryparedoxins (TXN), which in turn are reduced by trypanothione. In previous work we have functionally and structurally characterized the *T. cruzi* tryparedoxin peroxidases (cytosolic and mitochondrial) which detoxify hydroperoxides and peroxynitrite in a very efficient way. Moreover, when parasites are transfected with these peroxidase genes, they become more infective. Tryparedoxins belongs to the thioredoxin superfamily of proteins, which are oxidoreductases involved in antioxidant defences. They have been implicated in distinct cell functions such as hydroperoxide detoxification cascades, DNA synthesis and kinetoplast replication due to their ability to reduce target proteins. However, only a few target proteins of tryparedoxins are currently known. The aim of this work was to extend our knowledge of *T. cruzi* tryparedoxin interactome *in vivo*. Tryparedoxin reaction mechanism is based in two conserved cysteines. Cys40 residue and the oxidized target form a transient disulfide bridge, which is further resolved by Cys43 of the tryparedoxin, releasing the oxidized tryparedoxin and the reduced protein target. Based on the reaction mechanism, we generated a mutation at the active site of TXNI, by replacing the resolving cysteine residue (Cys43). The mutant TXNC43S gene was transfected and expressed in *T. cruzi* with an additional 6xHis tag. Protein extracts from these parasites were obtained, and purified with magnetic beads that bind His-tagged proteins. The eluted proteins were separated through one and two-dimensional electrophoresis, and analysed by mass spectrometry. Our *in vivo* approach led to the discovery of several putative redox-regulated proteins belonging to unexpected pathways, indicating that tryparedoxin plays a relevant role as a "redox hub" in *T. cruzi*.

## BQ.06 – MDL28170, A POTENT CALPAIN INHIBITOR, AFFECTS *TRYPANOSOMA CRUZI* METACYCLOGENESIS, ULTRASTRUCTURE AND ATTACHMENT TO THE LUMINAL MIDGUT SURFACE OF *RHODNIUS PROLIXUS*

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*Trypanosoma cruzi* is the etiological agent of Chagas' disease. During the parasite life cycle, several molecules are involved in the differentiation process and infectivity, but less is known about which of them are key molecules. Peptidases are relevant for crucial steps of *T. cruzi* life cycle, as such, it is conceivable that they may participate in the metacyclogenesis and interaction with the invertebrate host. In this paper, we have investigated the effect of the calpain inhibitor MDL28170 on the attachment of *T. cruzi* epimastigotes to the luminal midgut surface of *Rhodnius prolixus*, as well as on the metacyclogenesis process and ultrastructure. In addition, we have analyzed the effect of anti-calpain antibodies on the interaction of epimastigotes to the midgut surface of the insect. MDL28170 treatment was capable of significantly reducing the number of bound epimastigotes to the luminal surface midgut of the insect. Once the cross-reactivity of the anti-*Dm*-calpain was assessed, it was possible to block calpain molecules by the antibody, leading to a significant reduction in the capacity of adhesion to the insect guts by *T. cruzi*. Moreover, the *in vitro* metacyclogenesis process was impaired by the calpain inhibitor presenting a significant reduction in the number of metacyclic trypomastigotes. The calpain inhibitor also demonstrated a direct effect against bloodstream trypomastigotes. Ultrastructural analysis of epimastigotes treated with the calpain inhibitor revealed disorganization in the reservosomes, Golgi and plasma membrane disruption. The presence of calpain and calpain-like molecules in a wide range of organisms suggests that these proteins could be necessary for basic cellular functions. Herein, we demonstrated the effects of MDL28170 in crucial steps of the *T. cruzi* life cycle, such as attachment to the insect midgut and metacyclogenesis, as well as in parasite viability and morphology. These results help to shed some light on the functions of *T. cruzi* calpains. Considering the potential roles of these molecules on the interaction with both invertebrate and vertebrate hosts, it is conceivable to explore them as an alternative target to treat Chagas' disease.
Supported by MCT/CNPq, FUJB, CEPG/UFRJ, FAPERJ and FIOCRUZ.

## BQ.07 – HETEROLOGOUS EXPRESSION AND PURIFICATION OF THE PROTEIN ECTO-NUCLEOSIDE TRIPHOSPHATE DIPHOSPHOHYDROLASE (E-NTPDASE 2) OF *LEISHMANIA MAJOR*

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The Leishmaniasis is a parasitic disease caused by protozoaries of the kinestoplatida order, Trypanosomatidae family and Leishmania gender. Nowadays, these diseases represent a great problem concerning the Brazilian Public Health System and also in some parts of the world. The E-NTPDases or apyrases are proteins that can be found in these parasites and they also have the major function of extracellular nucleotide degradation, which in turn allows the parasite to modulate purinergic signling and "dribble" the immunological system of its host. The aim of the present work is to obtain the heterologous expression of *Leishmania major* E-NTPDase-2 protein for future studies. Initially, a specific pair of primers was designed in order to remove the amino-terminal transmembrane coding region. A PCR reaction was conducted and subsequently, the cloning procedures in the amplification vector (pJET-blunt) and in the expression vector (pET-21b) were obtained. The NTPDase-2 of recombinant *L. major* was expressed through a heterologous bacterial system and then purified through affinity chromatography in nickel agarose (Ni-NTA). The samples were analyzed by SDS-PAGE 10% and quantified through the Bradford method. The confirmation of the correct expression of an e-NTPDase protein was obtained by a Western Blot. The results show that the *primers* were able to amplify the coding region of the protein without the N-terminal transmembrane coding region. The events of cloning were successfully conducted and in addition, it was possible to obtain the heterologous expression for the E-NTPDase-2 protein of *L.major* which was properly confirmed through the Western Blot method.
Supported by CNPq and FAPEMIG.

## BQ.08 – HETEROLOGOUS EXPRESSION AND BIOCHEMICAL CHARACTERIZATION OF *Leishmania chagasi* NUCLEOSIDE TRIPHOSPHATASE (NTPDase) AS A GENUÍNE APYRASE

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Visceral Leishmaniasis is a serious tropical neglected disease, responsible for around 57,000 deaths per year worldwide. The protozoa *Leishmania chagasi* is the mainly pathogenic agent of this disease in the New World.This parasite is classified in the Tripanosomatidae family, which is unable to synthesize purines. It is believed that the Nucleoside Triphosphate Diphosphohydrolases (NTPDases) participate in the process of uptake of purines in "purine salvage pathway" and in the modulation of host immune responses induced by extra cellular nucleotides in the purinergic signaling pathway, acting as facilitators of parasites infections. In this work we cloned the putative nucleoside phosphatase gene predicted in *L. chagasi* genome and expressed it in *Escherichia coli* pET21-b system. The recombinant active NTPDase was purified from inclusion bodies, renaturated and was biochemically characterized according to nucleotidase activity. The recombinant enzyme (LcNTPDase) showed preference for GTP, UDP and ADP as substrates and was active only in presence of magnesium as cofactor. The use of calcium as cofactor completely abolished nucleotidase acitivty. The pH dependent activity showed that the optimum pH range between 7.0 and 8.0. The known apyrase partial inhibitors: ARL 67156, Suramin, sodium azide and Gadolinium were tested. The presence of Gadolinium 300μM showed 97% enzyme inhibition. These results showed that LcNTPDase is a genuine apyrase and presented for the first time an active *Leishmania* recombinant apyrase. LcNTPDase ADPase and UDPase activities could be related with the host immune system modulation. This enzyme can be used in biological assays to study its participation in *L. chagasi* infection and can be applied in biotechnological applications such as rational drug design to be used in Leishmaniasis chemotherapy. Supported by UFV, CNPq, FAPESP and CAPES

**BQ.09 – BIOCHEMICAL AND BIOLOGICAL STUDIES WITH A RECOMBINANT E-NTPDase from *Leishmania infantum chagasi***

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An putative GDPase, from E-NTPDase family, was identified in genome of *Leishmania infantum chagasi*. This enzyme family seems to be related with trypanosomatids virulence molecules, In this work the full-length coding region of this putative E-NTPDase, with 2043 bp was cloned into pJET vector (Fermentas). To further characterize the GDPase, we performed the heterologous expression of the active recombinant enzyme using pET21b system (Novagen). In silico analyses predicted a possible aminoterminal signal peptide cleavage sitebetween amino acid positiosn 28-29, immediately following an amino-terminal predicted transmembrane segment, thus suggesting that GDPase could be produced as a soluble exported protein. Using this information we design a strategy to express the soluble GDPase. Full-lenght coding regioncloned in pJET vector was used as template to amplify a 1953 bp DNA fragment that was transferred to pET21b vector (that codes for Hexa-HIS at the carboxy terminal of the recombinant fusion protein). This construction was used to transform *E. coli* BL21-codonplus RIL (Estratagene) cells. Recombinant protein was expressed after 2 hours of induction. Insoluble recombinant GDPase was solubilized, renaturated and purified by Ni-NTA affinity chromatography in the AKTA PURIFIER UPC10 system (GE Healthcare). Nucleotidase activity assays showed preference for GTP hydrolysis (0,186 nmol Pi/ug $xmin^{-1}$) than GDP 0,083 nmol Pi/ug x $min^{-1}$. The GTPase activity was higher in pH 7,4 than pH 8. Now we are performing another biochemical characterizations and producing polyclonal antibodies anti-GDPase in rabbit to do immunolocalization assays in *L. infantum chagasi*
Supported by: UFV and FAPEMIG.

**BQ.10 – Quercetin induces death in *Leishmania amazonensis* by mitochondrial dysfunction and reactive oxygen species production**

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Leishmaniasis, a parasitic disease caused by protozoa of the genus *Leishmania*, affects more than 12 million people worldwide. Quercetin has generated considerable interest as a pharmaceutical compound with a wide range of therapeutic activities. One such activity is exhibited against the bloodstream parasite *Trypanosoma brucei* and amastigotes of *Leishmania donovani*. However, the mechanism of protozoan action of quercetin has not been studied. In the present study, we report here the mechanism for the antileishmanial activity of quercetin against *Leishmania amazonensis* promastigotes. Quercetin inhibited *L. amazonensis* promastigote growth in a dose- and time-dependent manner beginning at 48 hours of treatment and with maximum growth inhibition observed at 96 hours. The $IC_{50}$ for quercetin at 48 hours was 31.4 µM. Quercetin caused mitochondrial dysfunction due to collapse of mitochondrial membrane potential. In addition, mitochondrial reactive oxygen species (ROS) production was increased in *L. amazonensis* treated with quercetin. Pre-incubation of promastigote forms of *L. amazonensis* with reduced glutathione (GSH) or N-Acetyl-cysteine (NAC) inhibited quercetin activity. Recently, the effects of several drugs that interfere directly with mitochondrial physiology in parasites such as *Leishmania* have been described. The unique mitochondrial features of *Leishmania* make this organelle an ideal drug target while minimizing toxicity. Taken together, our results demonstrate that quercetin exerts its antileishmanial effect on *L. amazonensis* promastigotes by generating ROS and affecting parasite mitochondrial function.
*Supported: FAPERJ, IOC/FIOCRUZ*

## BQ.11 – A MITOCHONDRIAL PROLINE DEHYDROGENASE OF *TRYPANOSOMA CRUZI* (*Tc*PRODH) PARTICIPATES OF RESPIRATION CHAIN AND IS REGULATED DURING LIFE STAGE

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*Trypanosoma cruzi* is capable of using carbohydrates and amino acids as carbon and energy sources. Preferentially, epimastigotes catabolize glucose and, after carbohydrate exhaustion, a shift to amino acid metabolism occurs. The amino acid L-proline participates in *T. cruzi*'s bioenergetic metabolism and also supports the differentiation processes, resistance to oxidative metabolic and osmotic stresses. Besides, it was recently shown that proline is a relevant metabolite to supply the energy that powers host cell invasion. Biochemical evidences supports the hypothesis that *T. cruzi* oxidizes L-proline through a proline dehydrogenase (*Tc*PRODH) (EC 1.5.1.2) and a $\Delta^1$pyrroline-5-carboxylate dehydrogenase (*Tc*P5CDH) (EC 1.5.1.12). Here, we describe the biochemical activity, mitochondrial localization and the role of *Tc*PRODH in the mitochondrial respiratory chain. The mRNA analysis, profile protein expression and specific activity showed that this enzyme is up-regulated in the intracellular epimastigote, a stage that requires external supply of proline. It was also showed by two methods indirect immunofluorescence and partial permeabilization that *Tc*PRODH is located in the mitochondrial membrane. These data, together with the fact that this enzyme uses FAD as cofactor indicates that *Tc*PRODH can also contribute with reduced equivalents to the respiratory chain. No significant differences between succinate and proline supported oxygen consumption rates were observed. Experiments are being carried out in order to see how *Tc*PRODH interacts with the other components of the mitochondrial respiratory chain.

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## BQ.12 – INOSITOL PHOSPHORYLCERAMIDE EXPRESSION KINETIC DURING *L. (L.) amazonensis* DIFFERENTIATION

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In eukaryotes, sphingolipids (SLs) are important membrane components and powerful signaling molecules. In *Leishmania*, the major group of SLs is inositol phosphorylceramide (IPC), which is common in yeast and Trypanosomatid but absent in mammals and, thus, the IPC metabolical pathway could be considered a good target for new therapy drugs. In order, to analyze the *L. (L.) amazonensis* sphingolipid expression in amastigotes and promastigotes, indirect immunofluorescence assay were carried on with mAb LST-1, directed to IPC. Amastigote forms were isolated from hamster footpad lesions (0h) and cultivated in LIT medium at 23°C for 6h, 24h, 48h and 72h. Parasites were fixed and the reactivity with LST-1 was analyzed by indirect immunofluorescence using of confocal microscopy. 0h and 6h Amastigotes are not recognized by LST-1. On the other hand, after 24h, LST-1 reactivity was observed in all plasmatic membrane. After 48h and 72h the LST-1 reactivity was also identified in parasites vesicles structures. Quantitative RT-PCR for sphingolipids metabolic enzymes are under investigation during the differentiation of amastigote to promastigote.

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## BQ.13 – LYSOPHOPHATIDYLCHOLINE (LPC) EFFECT ON THE PROLIFERATION AND DIFFERENTIATION OF *TRYPANOSSOMA CRUZI*

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*Trypanosoma cruzi*, etiological agent of Chagas disease, is an heteroxenous species that is submitted to morphological and physiological changes during its life cycle. Parasite epimastigote forms replicate and differentiate into infective metacyclic trypomastigotes at the Triatominae insect vector midgut. They are released with kissing bug feces during blood feeding, leading to vertebrate infection. Our experimental model is *Rhodnius prolixus*, Chagas disease vector at Central and South Americas. *R. prolixus* saliva contains lysophosphatidylcholine (LPC), a lysophosphatidyl lipid resulted of phosphatidylcholine hydrolysis by phospholipase $A_2$ enzyme. Once this bioactive multisignaling ubiquitous lipid is found in human plasma ingested by the insect during blood feeding, the goal of the work is determining the role of LPC in the proliferation and differentiation of *T. cruzi*. We analyzed the effect of LPC (1nM, 1µM, 10µM, 50µM) on parasite proliferation (Y and Dm28c strains) grown in LIT medium supplemented with 10% fetal bovine serum for 7 days. We observed a discrete increasing in LPC-treated-parasite growth, mainly in Y strain. In the presence of 10% delipidated fetal bovine serum and of 10% purified human low-density-lipoprotein (LDL), the effect was more evident with the growth rate of 10µM LPC/ LDL-treated group 3 fold higher at the $6^{th}$ day post-treatment than control. LPC effect on parasite differentiation was performed by assaying Dm28c strain metacyclogenesis *in vitro*. Parasites were incubated in a nutritional poor medium TAU, that mimetizes the composition of kissing bugs urine, in the absence or presence of LPC (1nM, 1µM, 10µM), and the percentage of trypomastigotes was obtained at each day post-treatment for 7 days. We observed no difference between untreated and treated groups. From now on, we intend to identify the signaling pathways that are activated by LPC in *T. cruzi* and that lead to parasite increased proliferantion. Supported by: CNPq, FAPERJ& IFS.

## BQ.14 – CHARACTERIZATION OF A SIR2-RELATED PROTEIN 1 FROM *LEISHMANIA (L.) AMAZONENSIS* (LaSir2) AS A CYTOPLASMIC, GLYCOSYLATED $NAD^+$-DEPENDENT DEACETYLASE.

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*LaSir2* was obtained by a PCR-based cloning using primers designed from the *LmSir2* sequence. *LaSir2* nucleotide sequence analysis revealed an 1122pb gene that encodes for a 373 amino acid protein with a predicted MW of ~40kDa. Amino acid sequence analysis showed that LaSir2 shares identity with other Kinetoplastid Sir2 orthologues (92% for *L.infantum* and 49% for *T.brucei)* and similarities of 53% and 59% with the catalytic site of yeast Sir2 and human Sirt1, respectively. The catalytic site of LaSir2 presents all of the elements essential for the transcriptional silencing, deacetylase and ribosyltransferase activities and, additionally, LaSir2 presents an N-terminal signal anchor, a putative C-terminal NES and it is predicted to be O-glycosylated and phosphorylated. The recombinant 6xHisLaSir2 was purified to homogeneity and was monomeric (41kDa±1%) as investigated by SEC-MALS. Spectroscopic studies agreed with tridimensional models from orthologues as 6xHisLaSir2 presented a characteristic alpha-helix rich Circular Dichroism spectrum, with minima at 208 and 222 nm, and fluorescence emission spectrum with $\lambda_{max}$ at 335nm suggesting that the tryptophan residue is buried. Also, the 6xHisLaSir2 displayed $NAD^+$-dependent deacetylase activity, being able to accept as acetylated substrates, a p53-derived peptide and a ~50kDa protein from promastigote extract. A serum raised against 6xHisLaSir2 detected a major ~50kDa protein in extracts of both promastigotes and lesion-derived amastigotes. Analysis of subcellular fractioning of promastigotes revealed that LaSir2 is excluded from cytosol and mitochondria. Indirect immunofluorescence of promastigotes showed LaSir2 distributed into cytoplasmic granules and in secretory-like vesicles. Furthermore, by Western blot probed with the anti-6xHisLaSir2 serum, LaSir2 was detected in both promastigotes and amastigotes secreted material. A concanavalin A-affinity purification of either promastigotes or amastigotes extracts, followed by LaSir2 immunodetection, revealed a major protein of ~66kDa indicating that, according to *in silico* predictions, LaSir2 could be highly glycosylated *in vivo*.
Supported by FAPESP and CNPq.

## BQ.15 – EFFECT OF PLATELET-ACTIVATING FACTOR (PAF) ON THE INFECTION OF PERITONEAL MOUSE MACROPHAGES BY *LEISHMANIA CHAGASI*

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In the New World, the visceral form of leishmaniasis is caused by *Leishmania chagasi*. Platelet-activating factor (PAF) is a potent phospholipid mediator of several cellular functions in diverse biological and pathophysiological processes, such as cell differentiation, inflammation and allergy. Previous study from our group showed that PAF modulates the interaction of peritoneal mouse macrophages with *Leishmania amazonensis*. PAF also stimulates cell differentiation of *Trypanosoma cruzi* and *Herpetomonas muscarum muscarum*, triggering a signal transduction pathway that activates a protein kinase CK2 in *H. m. muscarum*. PAF effects in trypanosomatids seem to occur through membrane receptor and intracellular signaling, via protein kinase C (PKC). In this work, we describe the effects of PAF on the interaction of *L. chagasi* with peritoneal mouse macrophages. Prior to the infection, *L. chagasi* promastigotes and/or the macrophages were treated for four hours with PAF and/or one of the following modulators: WEB 2086 (antagonist of PAF receptor), BIS I (PKC inhibitor), TBB (CK2 inhibitor), KT 57 and H89 (PKA inhibitors). The interaction was inhibited when the macrophages or both the promastigotes and the macrophages were treated with PAF. On the other hand, when only the promastigotes were treated with PAF, a two-fold increase in the association indices was observed. The antagonist of PAF receptor, WEB 2086, as well as the protein kinase inhibitors abrogated all PAF effects. We also showed that PAF stimulated nitric oxide production when the macrophages or both the parasites and the macrophages were treated with this phospholipid. Corroborating these results, we demonstrated that PAF can modulate some protein kinases of *L. chagasi*. When the promastigotes were treated with PAF, the activity of PKC, PKA and CK2 increased 57%, 60% and 50%, respectively. This set of results suggests that PAF triggers key intracellular signaling pathways in *L. chagasi* that may lead to an increase in parasite infectivity. Supported by: CNPq, FAPERJ, CAPES and INCT-EM.

## BQ.16 – COMPARATIVE ANALYSIS OF METALLOPROTEASE SECRETED BY *L. (V.) braziliensis* IN TWO-DIMENSIONAL AND THREE-DIMENSIONAL COLLAGEN MATRICES

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*Leishmania (V.) braziliensis* is the main causative agent of American Tegumentary Leishmaniasis (ATL) in Brazil and it is responsible for heterogeneous clinical manifestations ranging from cutaneous to mucosal lesions. Previous work of our group showed that proteases secreted by *L. (V.) braziliensis* strains isolated from patients with distinct clinical manifestations of ATL display different pattern of metalloproteases. In addition, we showed that the proteolytic profile is a stable phenotypic character. Currently, studies are being developed to better explore the interaction of the cells with the extracellular environments using collagen matrices. Previous works showed the important role of the extracellular matrix in the signaling and activation of immune cells during *Leishmania* infection. So, in this work, we aimed to determine the profile of secreted proteases of *L. (V.) braziliensis* strains, isolated from patients with mucosal, cutaneous and disseminated clinical manifestations, during interaction with both two-dimensional (2D) and three-dimensional (3D) collagen matrices in co-culture with macrophages host cells. Zymographic assays were performed using SDS-polyacrilamide gels (10%) copolymerized with 0.2% porcine gelatin. Enzymes were characterized according to their pH range of activity, and sensitivity to distinct protease inhibitors. We observed that proteolytic activities were due to enzymes belonging to the metalloprotease class and present optimal activity between the pH range 5.5 and 10.0. Zymographic assays also revealed that proteolytic profile is similar in 2D and 3D matrices and remains unchanged in co-culture with macrophages. However, when we compare the interaction of *L. (V.) braziliensis* strains (mucosal, cutaneous and disseminated) with the collagen, we observed significant differences regarding the affinity, interaction and invasion of the collagen matrices, as revealed by scanning electron microscopy. Supported by CAPES

## BQ.17 – METALLOPEPTIDASE ZYMOGRAPHIC PROFILES TO DISTINGUISH *LEISHMANIA (VIANNIA) BRAZILIENSIS* AND *LEISHMANIA* (*VIANNIA*) *PERUVIANA* ISOLATES FROM PERU

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American Tegumentary Leishmaniasis (ATL), a disease caused by protozoa of the *Leishmania* genus, comprises a broad range of clinical manifestations ranging from mild skin ulcers that may spontaneously heal to disfiguring mucosal lesions that imply parasite dissemination from the primary cutaneous lesion. *L.* (*V.*) *peruviana* and *L.* (*V.*) *braziliensis* from Peru, are species genetically so related that their characterization as distinct species have been intricate. Despite such genetic similarity, these species are associated to different clinical manifestations of ATL: *L.* (*V.*) *peruviana* only causes cutaneous leishmaniasis, whereas *L.* (*V.*) *braziliensis* can cause both cutaneous and mucocutaneous leishmaniasis. Since the primary cutaneous lesions caused by the infection with these species are indistinguishable, it is necessary to develop a suitable method to differentiate the infecting *Leishmania* species in order to prevent possible metastasis to oropharingeal mucosa. A different electrophoretic pattern in the mannose phosphate isomerase isoenzyme was the first approach that allowed distinguishing these two species. However, such isoenzymatic distinction depends on the electrophoretic system used for the characterization, is time-consuming and requires high-tech facilities that are not always available in the regions where the infection is endemic. In the present study, we investigate the proteolytic profile from *L.* (*V.*) *peruviana* and *L.* (*V.*) *braziliensis* by zymographic analysis. Enzymes were characterized according to their pH range of activity, and sensitivity to distinct protease inhibitors. We observed that *L. (V.) peruviana* isolates displayed three proteolytic bands with molecular mass ranging from 55 to 80 kDa whereas *L. (V.) braziliensis* isolates showed five proteolytic activity between 55 and 130 kDa. Using specific inhibitors we determined that proteolytic activities are due to metallopeptidases enzymes and present optimal activity between the pH range 5.5 and 10.0. Finally, we propose the metallopeptidase profile as a potential phenotypic marker to distinguish between these species.
Supported by FIOCRUZ CNPq

## BQ.18 – TRANSLATION FACTOR eIF5A OF *TRYPANOSOMA CRUZI*

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In trypanosomatids the post translational modifications is particularly important because these organisms do not use transcription initiation as a regulatory step to control gene expression. In our laboratory, phosphoproteomic studies revealed that a protein known as eukaryotic translation initiation factor A (eIF5A) is highly phosphorylated in exponentially growing cells and that phosphorylation is decreased in stationary *T. cruzi* cells. The protein eIF5A is a small, acidic and highly conserved protein in eukaryotic cells. It's unique as it contains a hypusinated lysine residue. eiF5A is involved in multiple cellular functions including translation initiation, mRNA decay, cell cycle progression, cell survival, and in translation elongation. Therefore, we identified that the serine 2 is the phosphorylated modified residue by using mass spectrometry analysis. By overexpressing wild type and serine 2 eIF5A we found that this serine is possible related to the hypusination, which is probably required for protein in the translation elongation.
Supported by FAPESP and FINEP

## BQ.19 – ANALYSES OF INHIBITORS CANDIDATES FOR THE *Leishmania (Leishmania) amazonensis* ARGINASE

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To survive inside the host macrophage organisms from *Leishmania* genus escape from several microbicidal mechanisms such as the production of NO and superoxide radicals (Bogdan et al., 1996). The NO synthesis by inducible nitric oxide synthase (iNOS) requires L-arginine as substrate, the same aminoacid used by arginase to produce urea and L-ornithine. The synthesis of L-ornithine is fundamental for the production of polyamines essencial for the proliferation of *Leishmania* (Camargo, 1979). Moreover, the arginase from the parasite can compete with iNOS for the same substrate, modulating L-arginine levels, decreasing the production of NO and reducing the host microbicidal response (Boucher, Moali et al. 1999). Previously in the laboratory the *Leishmania*'s arginase was characterized and a purification method was described for a recombinant enzyme produced by *E.coli*. The molecular model based on the rat arginase crystal also enable an *"in silico"* search for arginase inhibitors looking for a compound that could present a higher affinity for the parasite arginase than for the mammal enzyme. We selected three of the screened compounds - L-norvaline, L-citrulline and L-lysine - that showed the inhibition rates of 83,6%, 52,8% and 97,5%, respectively, in assays using 80 mM of each and the same concentration of the substrate L-arginine. After this we tested the inhibitors effect on the growth of cultured *L. (L.) amazonensis* promastigotes and observed that L-norvaline and L-lysine were capable of inhibit the parasite growth by 75% and 89%, respectively. Finally we realized "*in vitro*" infections of murine macrophages treated with those compounds and found out that with 40 mM of L-lysine, 20% of the cells were infected, while using 150 mM of L-norvaline only 10% of the macrophages were infected but when we used the same concentration tested for L-lysine no significant inhibition of the infection was detected. So the analyzes of the results allowed to conclude that L-lysine would be the best inhibitor among the selected ones in respect of potency and effect on the parasite growth and infection capacity. Supported by FAPESP and CNPq

## BQ.20 –BIOCHEMICAL ANALYSYS OF THE PROLINE DEHYDROGENASE IN *TRYPANOSOMA CRUZI*

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In the flagellated parasite *Trypanosoma cruzi,* the amino acid L-proline is involved in energy metabolism, differentiation processes and resistance to oxidative, metabolic and osmotic stresses. Biochemical evidences supports the hypothesis that *T. cruzi* oxidizes L-proline through a proline dehydrogenase (*Tc*PRODH) (EC 1.5.1.2) and a $\Delta^1$pyrroline-5-carboxylate dehydrogenase (*Tc*P5CDH) (EC 1.5.1.12) producing glutamate. In the present work, we describe the experimental conditions for the expression and purification of the active enzyme, which was used to determine its biochemical characteristics. The recombinant *Tc*PRODH was expressed in *Escherichia coli* BL21 (codon plus) strain using pAE vector. The apparent molecular weight for *Tc*PRODH fused with a 6-histidine tag (N-termus), was of 66 kDa as verified by SDS-PAGE. The recombinant protein was purified by affinity chromatography using NTA-$Ni^{2+}$ resin and eluted with 250 mM of imidazole. A policlonal serum anti- *Tc*PRODH was produced by mice immunization and used for protein detection in western blot assays. Specificity parameters were obtained from the recombinant *Tc*PRODH showed a highest specific activity for L-proline than 4-hydroxyl-L-proline or D-proline. In addition, the activity of recombinant *Tc*PRODH resulted in a reduction of $FAD^+$ cofactor instead of $NAD^+$ and $NADP^+$. *In silico* protein analyses showed the presence of a mitochondrial address signal, the active site recognition signature and intriguingly an EF-hand domain, which was not previously described for any PRODH. Since EF-hands are putative $Ca^{2+}$ or $Mg^{2+}$ binding sites, we evaluated its functionality. Our results showed that *Tc*PRODH has a functional EF-hand domain which confers to the enzyme the ability of having its activity regulated by $Ca^{2+}$ or $Mg^{2+}$, an unique feature for these enzymes.
Supported by FAPESP, USP AND CNPq

## BQ.21 – AN ECTO-PYROPHOSPHATASE ACTIVITY FROM PROCYCLIC PROMASTIGOTE FORMS OF *LEISHMANIA AMAZONENSIS.*

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*Leishmania amazonensis* parasites are intracellular protozoa and the etiological agent of cutaneous and diffuse cutaneous leishmaniasis. The flagellated metacyclic promastigote forms are transmitted to vertebrate hosts by sandfly bites. The promastigotes develop into aflagellated amastigotes in the vertebrate host and multiply by binary division, inside macrophages. Membrane interactions between parasites and hosts are crucial for its survival, from both physiological and immunological viewpoints. Cytoplasmic membranes contain enzymes whose active sites face the external medium rather than the cytoplasm. The activities of these enzymes, referred to as ecto-enzymes, can be measured using intact living cells. During its life cycle, the protozoa pass through different compartments of insect, presenting different pH values. So, it is of great importance the study of proton pumps, like the $H^+$-PPases, the integral membrane protein that utilizes the energy released by hydrolysis of pyrophosphate (PPi) to transport protons across the membrane, against the electrochemical potential gradient. In this context, we have characterized a pyrophosphatase activity on the external cell surface of procyclic promastigote forms of *L. amazonensis*. This activity is stimulated by $MgCl_2$ in a dose-dependent manner, with $S_{0.5}$ value of 2,0 mM $MgCl_2$, while the *p*-nitrophenylphosphatase and β-glycerophosphatase activities described in these parasites are not stimulated by $MgCl_2$. Furthermore, sodium orthovanadate, an acid phosphatase inhibitor, and levamisole, an alkaline phosphatase inhibitor, did not inhibit the ecto-pyrophosphatase activity. However, sodium fluoride, an inhibitor of pyrophosphatases, inhibited the ecto-pyrophosphatase activity in a dose-dependent manner with $IC_{50}$ value of 2,5 µM reaching maximal inhibition of 15% in 30 µM NaF. The pyrophosphatase activity of *L. amazonensis* was stimulated in pH 7.5-8.5, presenting optimum pH at alkaline range. These data suggest that this enzyme probably is a pyrophosphatase of family I, specially because its activity is stimulated by $MgCl_2$, but not by $MnCl_2$. Supported by CNPq, FAPERJ and UFRJ/PIBIC.

## BQ.22 – ECTO-PHOSPHATASES ACTIVITIES IN *BLASTOCRITHIDIA CULICIS* AND *AEDES AEGYPTI* SALIVARY GLAND

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*Blastocrithidia culicis* is a monoxenous trypanosomatid that inhabits mosquitoes. Using the mosquito *Aedes aegypti* as an experimental model to exploit monoxenous life cycle we demonstrated that *B. culicis* colonizes mosquito midgut and reaches its hemocoel. Indeed, we show that *B. culicis* interacts and invade mosquito salivary glands *in vitro* and *in vivo*. Ecto-phosphatases are enzymes capable to hydrolyze phosphorylated substrates in the external side of cells. Phosphorylation/dephosphorilation events have several biological roles and ecto-phosphatase activity has been implicated in microorganism differentiation, proliferation and parasite-host interaction. In order to investigate if mosquito or protozoa ecto-phosphatases are involved in protozoa-insect interaction, we first characterize these enzymes present in both, protozoa and *A. aegypti* organs. In order to characterize the hydrolytic activity of ecto-phosphatases, *B. culicis* or mosquito salivary glands were incubated with *p*-nitrophenylphosphate (*p*-NPP) for 1h in different pHs or in the presence of phosphatase inhibitors. The variation on pH range shows that *B. culicis* ecto-phosphatases present an acid activity while mosquito salivary gland has a neutral activity. Vanadate (1mM), molybdate (1mM) and sodium fluoride (1mM) inhibited both activities, as well as, inorganic phosphate (1mM). The samples were either incubated in the presence of divalent cations to evaluate its involvement on ecto-phosphatases activity. Our results show that $MgCl_2$ (5mM) enhanced significantly the *B. culicis* ecto-phosphatase activity, but inhibited the salivary gland activity. Interestingly, the aposymbiotic strain of *B. culicis*, that is unable to colonize *A. aegypti* midgut,,has a ecto-phosphatases activity 64% lower than the symbiont-containing cells and $MgCl_2$ have no had effect on its activity. Collectively, these results will be useful to elucidate the participation of ecto-phosphatases in *B. culicis* mosquito colonization. Supported by CAPES, CNPq and FAPERJ.

## BQ.23 – IDENTIFICATION AND CHARACTERIZATION OF AN ECTO-PYROPHOSPHATASE ON EXTERNAL SURFACE OF PLASMA MEMBRANE OF *TRYPANOSOMA RANGELI* AND ITS POSSIBLE ROLE IN INTERACTION WITHIN THE VECTOR *RHODNIUS PROLIXUS*

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We have identified by PCR a putative $H^+$-PPase in epimastigotes of *T. rangeli* that is recognized by antibodies raised against the enzyme of *A. thaliana*. Its location in the plasma membrane was confirmed by immunofluorescence microscopy technique. The optimum pH for the ecto-$H^+$-PPase activity is 7.5. The activity is inhibited by about 75%, 80% and 90% by NaF, Pi and AMDP, respectively. There are two sites of hydrolysis of PPi, one of high affinity with Km of 0.32 mM PPi, and another of low affinity, with Km of 1.69 mM PPi. The ecto-$H^+$-PPase of *T. rangeli* is stimulated by $MgCl_2$, and $Mg^{2+}$-dependent ecto-$H^+$-PPase activity is inhibited by $CaCl_2$. The ecto-pyrophosphatase activity is modulated by the extracellular concentration of Pi, reaching about twice as much activity when the cells are maintained in culture medium depleted of Pi. Cells grown with PPi have their ecto-$H^+$-PPase activity increased by about three times compared to that of cells grown in culture medium control, cells grown in the presence of NaF for 2 days, about twice, and cells grown for 6 days in the presence of the inhibitor showed the same activity of cells grown in control medium. Oddly enough by feeding insects *Rhodnius prolixus* 5th instar with such parasites, cell density of parasites found in crops of these insects three days after blood meal obeys exactly the same descending order of activity: grown with PPi > grown with NaF for 2 days > grown with NaF for 6 days = control. We have also found ecto-pyrophosphatase activities in other protozoa of Trypanosomatidae family, such as *T. cruzi*, *Leishmania chagasi* and *L. amazonensis*, and in *Euglena gracilis*. These enzymes presented different biochemical characteristics suggesting that this protein could be a differential marker for these protozoa.
Supported by: CAPES, CNPq and FAPERJ.

## BQ.24 – ECTO-ATP DIPHOSPHOHYDROLASE FROM *Leishmania (Leishmania) amazonensis*: A POSSIBLE NEW BIOCHEMICAL TARGET FOR THE DEVELOPMENT OF THERAPIES

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Chemotherapy remains the mainstay for the control of leishmaniasis. We characterize a novel ATP diphosphohydrolase (apyrase) in *L. (L.) amazonensis* promastigote forms. By Western blots, rabbit polyclonal antibodies against potato apyrase reacted with bands de 50-63 kDa from enriched plasma membrane, flagellum and microssomal fractions. By ultrastructural cytochemical, ATP diphosphohydrolase activity was found distributed as an electron-dense lead phosphate deposit at the plasma membrane surface, flagellar pocket, and flagellar membrane of promastigote forms when they were incubated in the cytochemical medium containing ATPases, nucleotidase and phosphatase inhibitors plus ADP or ATP as substrate. The *in vitro* effect of cisplatine, 6-mercaptopurine, thionicotinamide, N-27, T-27 or allopurinol, which has antileishmanicidal, antitumoral, antibacterial and/or antifungal activity, was evaluated. The measurement in duplicate (n= 5) was performed in reaction medium containing 50 mM MOPS buffer, pH 7.4, 1 mM CaCl2, 1 mM MgCl2, 100 µM ortovanadate, 1 mM NaN3, and plasma membrane fraction (0.03 mg protein/ml), plus 100 µM of the compound. After preincubation for 30 min at room temperature, the hydrolytic assay was initiated by addition of 3 mM ATP or ADP, and allowed to proceed for 30 min at 37°C. Inorganic phosphate liberated was determined spectrophotometrically according to Taussky&Shorr. Control samples, preincubated in the presence of 1% (v/v) dimethyl sulfoxide, hydrolyzed ATP or ADP in the range (mean±SD) of 112±55 and 102±10 nmol Pi.mg-1.min-1, respectively. Cisplatine significantly inhibited 90% ATPase and 80% ADPase activities; mercaptopurine inhibited approximately 35% of these activities, whereas thionicotinamide has lower effects. N-27 and T-27, two nicotinamide derivatives, inhibited significantly ADPase activity, 50% and 30% respectively, whereas allopurinol inhibited 70% of the ATPase activity. These results appointed the *L. (L.) amazonensis* ATP diphosphohydrolase as a new target for therapies development. In addition, these inhibitors may contribute for further functional assays that may elucidate the role(s) of this enzyme. Supported by FAPEMIG, CNPq, CAPES, IOC/FIOCRUZ and UFJF

## BQ.25 – $\Delta^1$-PYRROLINE-5-CARBOXILIC ACID (P5C) METABOLISM IN *TRYPANOSOMA CRUZI*

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P5C is a metabolic node for proline, ornithine and glutamate pathways, linking the Kreb´s and urea cycles in eukaryotes. Regulation of P5C metabolism and the relative sizes of P5C pools in the cytosol and mitochondria are important in controlling flux between these pathways, and may prevent futile cycles among these metabolites. In *T. cruzi,* P5C is generated from proline and might be generated also from ornithine. The proline-glutamate pathway in *T. cruzi* (being characterized by our group), is mediated by two enzymatic steps: the proline oxidation by the proline dehydrogenase (*Tc*ProDH), rendering P5C, and the subsequent oxidation of P5C into glutamate by P5C dehydrogenase (*Tc*P5CDH). Previously we reported the functionality of both genes by yeasts complementation assays. In this work, the expression during the life cycle of *T. cruzi* is analyzed. The mRNA and protein levels were similar in most of stages, with the only exception of intracellular epimastigote stage, in which both duplicated those in other stages. The subcellular location was analyzed by immunolocalization and digitonin permeabilization assays in epimastigote forms, being predominantly mitochondrial. However, a relevant P5CDH activity was also detected in the cell cytoplasm. For biochemical analysis, the recombinant *Tc*P5CDh was expressed in the active form fused to a his-tag in the C-termus in *Escherichia coli* (64 kDa), and P5C was synthesized, isolated, and characterized. The enzymatic activities for P5CR, OAT and P5CDH detected in crude extracts from *T. cruzi* showed the ability of *T. cruzi* to synthesize proline and glutamate from $\alpha$-ketoglutarate (Krebs cycle) or ornithine. The fact that *Tc*ProDH localizes only in the mitochondria and *Tc*P5CDH in the mitochondria and cytosol also suggest that P5C could be a $H^+$ acceptor in an electron shuttle system operating between the mitochondria and the cytoplasm, with a physiological role in the regulation of the cellular redox status. Supported by FAPESP.

## BQ.26 – *PHYTOMONAS SERPENS*: PYRUVATE/INDOLEPYRUVATE DECARBOXYLASE GENE AND PRODUCTION AND FUNCTIONALITY OF INDOLE-3-ACETIC ACID PHYTOHORMONE

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A gene encoding a putative pyruvate/indolepyruvate decarboxylase (PDC/IPDC) is present in the plant trypanosomatid *Phytomonas serpens*. This gene shares high similarity with a *Leishmania* spp. gene. Pyruvate decarboxylases (PDCs) convert pyruvate to acetaldehyde, a key step in the alcoholic fermentation, whereas indolepyruvate decarboxylases (IPDCs) participate in the conversion of indolepyruvate into the phytohormone indole-3-acetic acid (IAA). Both enzymes display high sequence and structural similarities, which preclude unequivocal functional assignment based solely on sequence data. The goal of this study was to investigate the functionality of *P. serpens PDC/IPDC* gene. HPLC and GC-FID analyses of *P. serpens* conditioned medium indicates the production of IAA (4 µg/$10^8$ cells) and ethanol (40 mg/$10^8$ cells). PDC activity was evaluated in semi-purified parasite lysates. The enzyme shows a typical Michaelis-Menten behavior with a $K_M$ of 1.1 mM for the substrate pyruvate. Addition of 2 mM indolepyruvate, the substrate of IPDC, promoted a ~10-fold increase of the $K_M$ to pyruvate, without alteration of $V_{max}$. Such behavior indicates that indolepyruvate is a competitive inhibitor of PDC activity, suggesting that PDC/IPDC is a bifunctional enzyme determining the production of IAA or ethanol, according to the substrate availability. Work is in progress to clone the *PDC/IPDC* gene to assay both enzymatic activities in the recombinant protein. The phytohormone produced by *P. serpens* is biologically active since it promoted typical curvature responses and the elongation of tomato hypocotyls (~15% size increase compared with control), with an effect analogous to that obtained with the synthetic auxin. In addition, we observed an increase in the amount of auxin conjugated with amino acids and sugars in tomato fruits infected with *P. serpens.* Taken together the data indicate that the *PDC/IPDC* gene is active *in vivo* and may play an important role in the association plant-trypanosomatid. Support: FAPESP; CNPq.

## BQ.27 – IDENTIFICATION OF A CATALYTICALLY ACTIVE ATP DIPHOSPHOHYDROLASE ISOFORM FROM *Leishmania (Leishmania) chagasi* PROMASTIGOTES

Maia, A.C.R.G. [1], Porcino, G.N. [1], Emídio, N.B. [1], Fessel, M.R. [1], Detoni, M.L.[1], Mendes, R.G.P.R.[1], Marques, M.J.[2], Diniz, V.A.[3], Corte-Real, S.[3], Vasconcelos, E.G.[1]

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A putative protein identified as NDPase, homologous to the members of the ATP diphosphohydrolase (or apyrase) family, was found in the genome of *L. infantum* (sin. *L. chagasi*). In order to search for an active ATP diphosphohydrolase, phosphohydrolytic activity was assayed in promastigote preparations from *L.* (*L.*) *chagasi* (MHOM/BR/1972/BH46 strain) using standard reaction medium. These preparations presented a phosphohydrolytic activity that was equally effective at hydrolysis of either ATP or ADP, in the range of 65 $\pm$ 38 or 59 $\pm$ 37 nmol Pi.mg-1.min-1, respectively. CTP, UDP and GDP were also hydrolyzed. $Ca^{2+}$, $Mg^{2+}$ or $Mn^{2+}$ was activating metal ion for both ATPase and ADPase activities. No significant hydrolysis of AMP, inorganic pyrophosphate or *p*-NPP could be observed discarding 5'-nucleotidase, pyrophosphatase or phosphatase activity, respectively. Several inhibitors of classical ATPases were tested. Sodium orthovanadate (100 $\mu$M; P-type ATPase inhibitor) showed a slight effect, and inhibited 27% of the ATPase activity. No significant interference was observed when DCCD (100 $\mu$M; mitochondrial ATPase inhibitor), bafilomicyn A (1 $\mu$M; vacuolar ATPase inhibitor) or Ap5A (100 $\mu$M; adenylate kinase inhibitor) was tested. On the other hand, sodium azide (1 mM), an known inhibitor of either *L. amazonensis* or *L. braziliensis* ATP diphosphohydrolase isoforms, inhibited partially (24%) and almost totally (85%) the ATPase and ADPase activities, respectively. Additionally, rabbit polyclonal anti-potato apyrase antibodies recognized a polypeptide of approximately 50 kDa in Western blots and, when immobilized on Protein A-Sepharose it was capable to immunoprecipitate 97% and 75% of the ATPase and ADPase activities, respectively, from a C12E9-solubilized promastigotes preparation. Ultrastructural cytochemical microscopy showed ATP diphosphohydrolase activity at plasma membrane, flagellar pocket and flagellum of the promastigote. All together, these results confirmed the presence of an active ATP diphosphohydrolase isoform in *L. (L.) chagasi* promastigotes that shares conserved epitopes with potato apyrase. Supported by FAPEMIG, CNPq, CAPES, IOC/FIOCRUZ and UFJF

## BQ.28 – CYTOCHEMICAL LOCALIZATION OF ATP DIPHOSPHOHYDROLASE FROM *Leishmania (Viannia) braziliensis* PROMASTIGOTES

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We identified an ATP diphosphohydrolase activity (EC 3.6.1.5) in the preparation of *L. (V.) braziliensis* promastigotes. Localization of the ATP diphosphohydrolase activity in *L. (V.) braziliensis* promastigote forms was obtained by ultrastructural cytochemical techniques. The promastigote forms were fixed in 1% paraformaldehyde and 1% glutaraldehyde in 0.1 M Na-cacodylate buffer, pH 7.4, for 30 min. Subsequently, the cells were washed with the cacodylate buffer and incubated in the cytochemical medium, which contained 50 mM MOPS, pH 7.4, 1 mM $CaCl_2$, 100 µM orthovanadate (P-type ATPase inhibitor), 100 µM DCCD or 1 mM sodium azide (mitochondrial ATPase inhibitors), 100 µM ammonium molybdate (nucleotidase inhibitor), 1 mM levamisole (phosphatase inhibitor), 2 mM $CeCl_3$ and 3 mM of either ATP, ADP or GDP for 1 h at 37$^o$ C. Post-fixation was done in 1% $OsO_4$ in Na-cacodylate buffer for 1 h at 4$^o$ C. Dehydration was done in acetone and inclusion in Epon. Stained and unstained thin sections were observed in a JEM-1011 electron microscope. Enzyme activity was found distributed as an electron dense cerium phosphate deposit at the surface of the plasma membrane, and at flagellar pocket and flagellum of *L. (V.) braziliensis* promastigote forms when they were incubated in the cytochemical complete medium containing ATP, ADP or GDP as substrate. Besides its ecto-localization, the hydrolytic activity appeared to be identically distributed at outer surface of the mitochondria. In the absence of nucleotides, controls showed no electron dense deposits. This enzyme activity is possibly associated with purine recuperation and/or as a protective mechanism against the host organism under conditions that involve nucleotides. Furthermore, this subcellular location suggests the participation of this enzyme in metabolic pathways still not investigated, possibly essential for parasite survival. Effects of leishmanicidal compounds on the ATP diphosphohydrolase activity is the present subject of study of our laboratory. Supported by FAPEMIG, CNPq, CAPES, IOC/FIOCRUZ and UFJF.

## BQ.29 – ISOLATION OF AN ACTIVE ATP DIPHOSPHOHYDROLASE ISOFORM FROM *Leishmania (Viannia) braziliensis* PROMASTIGOTE FORMS

Porcino, G.N.[1], Rezende-Soares, F.A.[1], Carvalho-Campos C.[1], Faria-Pinto, P.[1], Mendes, R.G.P.R.[1], Souza, M.A.[2], Marques, M.J.[3], Vasconcelos, E.G.[1]

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Isolation of an active ATP diphosphohydrolase (EC 3.6.1.5) isoform from *L. (V.) braziliensis* (Lb) was obtained by cross-immunoreactivity with polyclonal anti-potato apyrase antibodies. Rabbit polyclonal anti-potato apyrase antibodies recognized bands of approximately 48 and 43 kDa in Western blots of the promastigotes preparation (50 μg of total protein). Anti-potato apyrase antibodies (serum diluted 1:500) were tested for their ability to immunoprecipitate ATP diphosphohydrolase from a detergent-homogenized *L. (V.) braziliensis* promastigotes preparation. After homogenization with non ionic detergent $C_{12}E_9$ and centrifugation, significant parasite enzyme activity was maintained in the high-speed supernatant (ADPase activity, 249 nmol Pi.mg-1.h-1; ATPase activity, 206 nmol Pi.mg-1.h-1). No significant difference was observed in control assays with either pre-immune serum (ADPase activity, 242 nmol Pi.mg-1.h-1; ATPase activity, 230 nmol Pi.mg-1.h-1) or Protein A-Sepharose (ADPase activity, 312 nmol Pi.mg-1.h-1; ATPase activity, 252 nmol Pi.mg-1.h-1) added alone in the absence of serum. On the other hand, rabbit polyclonal anti-potato apyrase antibodies immobilized on Protein A-Sepharose immunoprecipitated approximately 83% of the ATPase (30 nmol Pi.mg-1.h-1) and 87% of the ADPase activities (42 nmol Pi.mg-1.h-1) corresponding to depletion of an ATPase/ADPase activity ratio of approximately 1. The immunoprecipitated resin-rabbit antibody-antigen complex was washed and subjected to electrophoresis and Western blots. The rabbit polyclonal antibodies against potato apyrase immobilized on Protein A-Sepharose immunoprecipitated the same bands of approximately 48 kDa and, in lower amount, the band of 43 kDa, which were recognized by mouse polyclonal anti-potato apyrase antibodies (serum diluted 1:500). This band of approximately 43 kDa could be result of proteolysis of the band of 48 kDa, as a consequence of experimental conditions or even a natural occurrence *in vivo*. These results confirmed the identity of an active ATP diphosphohydrolase isoform in *L.* (*V.*) *braziliensis* promastigote forms, which shares conserved epitopes with potato apyrase. Supported by FAPEMIG, CNPq, CAPES, UFJF

## BQ.30 – 5261 NELFINAVIR, AN HIV ASPARTYL PEPTIDASE INHIBITOR, IS EFFECTIVE IN INHIBITING THE MULTIPLICATION AND ASPARTYL PEPTIDASE ACTIVITY OF SEVERAL *LEISHMANIA* SPECIES

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There is a general lack of effective and non-toxic chemotherapeutic agents for leishmaniasis and there is yet no study about the effect of HIV peptidase inhibitors on *Leishmania*-HIV co-infected patients. In the present work, we performed a comparative analysis of the spectrum of action of HIV peptidase inhibitors on different *Leishmania* species, including strains obtained from $HIV^+$ patients under antiretroviral treatment or not. The effect of HIV peptidase inhibitors, nelfinavir and saquinavir, on *Leishmania* promastigotes proliferation was assessed by means of a colorimetric assay (MTT). Subsequently, the effect of nelfinavir on aspartyl peptidase activity of *Leishmania* species was assessed by a cathepsin D fluorogenic substrate MCA-Gly-Lys-Pro-ILe-Leu-Phe-Phe-Arg-Leu-Lys(DNP)-Arg. Nelfinavir was capable of significantly reducing the multiplication of *Leishmania amazonensis*, *Leishmania braziliensis*, *Leishmania donovani*, *Leishmania major*, *Leishmania chagasi*, *Leishmania chagasi* from $HIV^+$ patient under antiretroviral treatment or not, with a strong growth inhibition of at least 50% to *Leishmania major* and 94% to other *Leishmania* species including strains isolated from $HIV^+$ patients. In addition, Nelfinavir was also capable of inhibiting aspartyl peptidase activity of the *Leishmania* strains tested at either 1μM or 10 μM. Present data may contribute to the study of the effect of HIV peptidase inhibitors on *Leishmania* infection and add new in vitro insights into the possibility of exploiting aspartyl peptidases as promising targets to treat leishmaniasis. Supported by Fiocruz, FAPERJ and CNPq.

## BQ.31 – MOLECULAR CHARACTERIZATION OF THE MEVALONATE KINASE FROM *TRYPANOSOMA CRUZI*

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In eukaryotic cells, sterols are important determinants of membrane fluidity and permeability and serve as precursors for bioactive molecules, which function as regulators of cell cycle and development. The importance of sterols for trypanosomatids has been proved and there are some inhibitors that act at different points of the pathway. Mevalonate Kinase (MK) plays a central role in the cholesterol biosynthesis pathway, catalyzing the phosphorylation of mevalonic acid to form mevalonate 5-phosphate. Recently,a MK study showed that when trypanosomatids invasion occurs in the host, this enzyme is overexpressed. This suggests the importance of this enzyme as a target for the development of future drugs. Although structural characterization of some MK from trypanosomatids is known, but there have been no studies that characterize the MK from *Trypanosoma cruzi* (TcMK). Herein, we report biochemical and structural preliminary studies of the TcMK. TcMK was cloned into pet28a(+) vector, overexpressed in *E. coli* at 22 °C in auto-induction medium (ZYM 5052) and purified by affinity chromatography. Size exclusion chromatography (SEC) was performed in order to determine the oligomeric state. The SEC result revealed that the protein has two different oligomeric states. These oligomers of TcMK protein were used for activity assays suggesting that the enzyme is active mainly in the dimeric form. Crystallization experiments are been realized aiming to obtain suitable crystals for diffraction measurements.
Supported by CNPq.

## BQ.32 – EXPRESSION ANALYSES OF *T. CRUZI'*S ANTIOXIDANT ENZYMES ALONG THE GROWTH CURVE

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One of the difficults to find therapeutics targets to Chagas's disease is the huge biochemistry and molecular variation in *T. cruzi* different strains. We evaluated the expression of enzymes that act in parasite's antioxidant system, i.e., cytosolic and mitochondrial tryparedoxin peroxidases and superoxide dismutase A and B (TcCPx, TcMPx, SODA and SODB, respectively) on two different strains of *T. cruzi* (Tulahuen 2 and Y) along the growth curve; logarithmic, early and late stationary phase (3, 5 and 7 days, respectively). TcCPx, in Tulahuen 2, had an increase of expression in approximately 50% after 5 days and 105% after 7 days, in relation to the log phase. For Y, the TcCPx showed a similar expression pattern, except by a decrease of 16% after 5 days. TcMPx had its expression increased after fifth day (~633% in Tulahuen 2 and ~202% in Y) and decreased to lower levels than the previous one in the late stationary phase for both strains. In SODA, its expression increased in 12 and 15% in early and the late stationary phase (comparing with the logarithmic phase) for Tulahuen 2, whereas for Y reduced in 16 and 49%, in this same phases. The results of SODB expression for Y were approximately 97% higher than Tulahuen 2, in the logarithmic phase. However, its expression decreased 6 and 16% after 5 and 7 days for Y, whereas for Tulahuen 2 this expression enhanced in ~85% in the stationary phase and reduced 29% in the late one comparing to 5 days. This results suggests that TcCPx increases its expression toward the late stationary phase and TcMPx presents high expression in the stationary phase, whereas a low one in the late phases.
Supported by FAPESP, CAPES and SAE-UNICAMP.

## BQ.33 – *TRYPANOSOMA CRUZI*'S ANTIOXIDANT ENZYMES PROFILE UNDER OXIDATIVE STRESS

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The enzymes involved in *T. cruzi's* antioxidant system of play a fundamental role in parasite survival against oxidative stress. We evaluated cytosolic and mitochondrial tryparedoxin peroxidases and superoxide dismutase A and B (TcCPx, TcMPx, SODA and SODB, respectively) expression on control (pTEX), cytosolic and mitochondrial tryparedoxin peroxidases (pTEX-TcCPx and pTEX-TcMPx, respectively) overexpressing cells treated or not with 200μM $H_2O_2$ (lethal dose). TcMPx expression in non treated cells increased in pTEX-TcMPx (138%) and interestingly in pTEX-TcCPx (46%) in relation to pTEX. TcCPx had an increase of 42 and 24% on pTEX-TcCPx and pTEX-TcMPx, respectively, in relation to pTEX under no treatment. Similar levels were observed when those cells were treated with $H_2O_2$, leading to an increase of 43 and 16% in pTEX-TcCPx and pTEX-TcMPx, respectively in relation to pTEX $H_2O_2$-treated. The treatment with $H_2O_2$ led to a higher protein expression in all cells in relation to their respective control. SODA expression decreased in overexpressing cells under both conditions compared to their respective controls. On the other hand, SODB levels in non-treated overexpressing cells increased whereas upon $H_2O_2$ treatment a decrease was observed. These results suggest a collaboration between cytosolic and mitochondrial tryparedoxin peroxidase, since the overexpression of one increased the expression of the other. Experiments are being carried out to clarify SOD expression under oxidative stress. Supported by FAPESP, CAPES and SAE-UNICAMP.

## BQ.34 – BIOCHEMICAL CHARACTERIZATION OF THE BIOSYNTHESIS OF VITAMIN E IN INTRA-ERYTHROCYTIC STAGES OF *P. falciparum*.

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The study of isoprenoid biosynthesis in *Plasmodium falciparum* by 2C-methyl-D-erythritol-4-phosphate pathway (MEP) is presented as a therapeutic target once that it is absent in humans. Our group found in intra-erythrocytic stages of *P. falciparum* the biosynthesis of isoprenoids by the MEP pathway.This pathway was described exclusively in plants, fungi or bacteria. The shikimate and MEP pathways are the precursors of biosynthesis of vitamin E and both pathways have already been described in *P. falciparum*. It is suggested that the biosynthesis of vitamin E might occur in the parasite, representing a possible target for developing new antimalarial drugs. Using metabolic labeling with [$^3$H]farnesil-PP or [$^3$H]geranilgeranil-PP, three different methods of RP-HPLC and mass spectrometry analyses confirmed the biosynthesis of vitamin E in the three stages of parasites (ring, trophozoites and schizonts). The treatment with usnic acid, an inhibitor of hydroxyphenylpyruvate dioxigenase - enzyme responsible by the biosynthesis of vitamin E - showed an inhibition of this biosynthesis (53,5 ± 7%) and of the growth of parasite ($IC_{50}$ 24,6 ± 4μM). We are trying to demonstrate by means of a fluorescent probe, the acid Parinaric, that vitamin E acts as a lipophilic antioxidant protecting the membrane of lipoperoxidation. These findings not only contribute to the current understanding of **P. falciparum** biology but shed light on a pathway that could serve as a chemotherapeutic target. Supported by FAPESP and CNPq.

## BQ.35 – ASCORBATE PEROXIDASE PROTEIN EXPRESSION IN *Trypanosoma cruzi* IS MODULATED BY STRESS GENERATED BY HYDROGEN PEROXIDE

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Ascorbate peroxidases (APXs) are Class I heme-containing peroxidase enzymes. They catalyze the reduction of hydrogen peroxide to water. They are not present in mammals what make them potential target for chemotherapy of Chagas disease. Literature data have shown the regulation of ascorbate peroxidase gene by $H_2O_2$. Here we have investigated the levels of TcAPX protein in *T. cruzi* populations susceptible and resistant to benznidazole (BZ) after $H_2O_2$ exposure. *T. cruzi* populations with *in vitro*-induced (17LER) and *in vivo*-selected (BZR) resistance to BZ and their susceptible counterparts 17WTS and BZS were used in this study. Epimastigote forms of these populations were incubated in the absence or presence of different concentrations of $H_2O_2$ at room temperature for 1 h. Following exposure, cells were harvested, washed and the pellet submitted to protein extraction for Western blot analysis using rabbit anti-TcAPX polyclonal antibody. The results showed that the levels of TcAPX protein increased in a dose-dependent manner only when the resistant strains were treated with $H_2O_2$. Such modulation does not happen with the susceptible strains. We also observed that $H_2O_2$ concentration higher than 400 µM and longer incubation period are lethal for *T. cruzi* parasites. The results suggest that TcAPX protein expression was up-regulated in response to cellular $H_2O_2$ levels and that parasites use APX to overcome the oxidative stress. Interestingly, *Leishmania major* and soybean cells also overexpress APX in the presence of $H_2O_2$. Supported by CNPq, FAPEMIG, CPqRR/ FIOCRUZ

## BQ.36 – INVASION OF HOST CELLS BY *TRYPANOSOMA CRUZI*: A NEW PUTATIVE RECEPTOR

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Trypomastigotes of *Trypanosoma cruzi*, express at the cell surface glycoproteins known as Tc85 belonging to the gp85/trans-sialidase superfamily. Several members of the superfamily have been implicated in the invasion of host cells by *T. cruzi* and components of the extracellular matrix, as fibronectin and laminin, were described as their ligands. Using the phage display technique, a sequence (pp7) was identified that specifically binds in a dose-dependent manner to H3.3p, a recombinant protein corresponding to an internal fragment of a cloned member of Tc-85. Alignment analysis identified the prokineticin receptor 2 (PKR2), as a putative candidate. Prokineticin receptors (PKR1, PKR2) are expressed in many tissues, structurally are members of the rhodopsin family, with seven transmembrane domains and putative post-translational modifications. The ligands, prokineticins 1 and 2, are peptides involved in a variety of biological processes. In order to verify whether PKRs may be a Tc85 receptor, MCF10A, a human mammary epithelial cell line, which expresses PKR2, as assessed by immunofluorescence and RT-PCR, was employed as host cell. We showed that: 1. H3.3p binds to the surface of MCF10A, detected by immunofluorescence, employing anti-His antibody or G1/G8 monoclonal antibody (raised against H3.3p); 2. The synthetic peptide pp7 inhibits the binding of H3.3p to a ~45 kDa band in a nitrocellulose blot of MCF10A extract and the same region is recognized by anti-PKR2 antibody; 3. The antigens recognized by anti-PKR2 antibody and by anti-pp7 antibody colocalize at the cell surface of MCF10A, as evaluated by confocal microscopy; 4. Anti-PKR2 antibody inhibits by ~60% the infection of MCF10A by *T. cruzi*; 5. The peptide pp7 (0.2 mM) inhibits by ~40% the infection of MCF10A by *T. cruzi*. Altogether, the data indicate a possible role of PKR2 as a ligand for *T. cruzi* infection, in particular the amino acid sequence pp7. Suported by FAPESP and CNPq.

## BQ.37 – THE INTERACTION OF *TRYPANOSOMA CRUZI* MEVALONATE KINASE PROTEIN WITH HOST CELL MEMBRANE MAY MODULATE THE INVASION OF EXTRACELLULAR AMASTIGOTE (EA) FORMS

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*Trypanosoma cruzi* extracellular amastigotes (EA) forms, which are generated by the extracellular differentiation of trypomastigotes are able to invade cultured cells. EAs of the G strain are more infective than CL strain parasites. EA microarray analysis has demonstrated that mevalonate kinase (TcMVK) has higher expression in G than in CL parasites, suggesting an important role of the MVK pathway in EA infectivity. Interestingly, bioinformatics tools have shown that *Trypanosoma cruzi* is the only trypanosomatid studied that has two MVK isotypes, one of which contains a signal peptide, indicating that MVK is secreted into the extracellular medium. This was confirmed by Western blot assay. Furthermore imunofluorescence assays showed that MVK colocalizes with aldolase, a glycosomal protein which is also present in other trypanosomatids. We cloned and obtained a purified form of TcMVK protein (MVKR). A kinase activity assay demonstrated that MVKR is functional in both monomeric and dimeric forms. The secreted isoform of MVKR dose-dependently binds to HeLa cells membrane, suggesting an interaction between MVKR and cholesterol at the host cell. MVKR added on the invasion assay led to an increased rate of EA invasion**.** Preliminary studies showed that MVKR increases the phagocytic ability of host cells. Taken together, these data suggest that TcMVK may be an important modulator in EA invasion and could become an important chemotherapeutic target.
Support: FAPESP

## BQ.38 – MECHANISM OF TUNICAMYCIN RESISTANCE AND EVALUATION OF N-GLYCOSYLATION INHIBITION OVER VIRULENCE IN *LEISHMANIA* PARASITES

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Leishmaniasis is spectral disease caused by 20 species of *Leishmania* and the clinical manifestation profile is specie-dependent. The molecular mechanism of virulence remains largely unknown, but it is known that glycoproteins on the surface of the parasite are often implicated in evasion of host immunity and invasion of host cells to establish parasitism. Glycosylation of proteins usually lead to changes in their biological function and the regulation of this event may be essential to determine the parasite's virulence pattern. Inhibition of protein glycosylation of several parasitic protozoans by drugs, such as tunicamycin (TM), as well as parasites made resistant to them are useful for host-parasite interaction studies.Herein we report our success to generate and evaluate TM resistant *L. braziliensis* variants. Tunicamycin-resistant variants were produced by gradual acclimatization of cells to increasing concentrations of the drug, up to 40 μg/mL ($IC_{50}$= 0.3 μg/mL). Four clones were recovered from TM-resistant population by limiting dilution but only one of them showed phenotype stability after one cycle of promastigote-amastigote-promastigote differentiation (the amastigote stage occurring in BALB/c mice under drug-free conditions). Transmission and scanning electronic microscopy revealed morphological modifications in the mutant and *in vitro* immune response survey indicates remarkable changes in cytokine and nitric oxide production in comparison with the parental wild type line. *In vivo* assays have been conducted to evaluate whether virulence and cytokine production pattern is altered in murine model infected with this mutant. To further delineate the mechanism of tunicamycin resistance and elucidate how this affects immune response and host-parasite interaction we have used a proteomic approach to identify proteins that may be involved in this phenomenon. These results highlight the relevance of glycosylation and shed light on host-parasite interaction issues revealing possible targets to pharmacological intervention. Supported by: FAPESP and CNPq.

## BQ.39 – SITE-DIRECTED MUTATIONS OF MACROPHAGE MIGRATION INHIBITORY FACTOR (MIF) OF LEISHMANIA MAJOR

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The macrophage migration inhibitory factor (MIF) is considered an important factor in the control of infection by parasites. Studying the structure and function of proteins of *Leishmania* allows the understanding of the molecular host-parasite interaction and the presentation of new targets to use in the development of therapies against the parasite, which is responsible for several different forms of Leishmaniasis. The aim of this study is to investigate the effect of site-directed mutations of the coding sequence of MIF *L. major* residues, involved in the tautomerase enzymatic activity and the maintenance of its quaternary structure. The site-directed mutagenesis was performed in 3 steps of PCR reactions, the fragments obtained were purified, sequenced and cloned to the pET21b vector using NdeI/HindIII restriction sites. The recombinant MIF and the mutants, all of them containing a His6-tag, were expressed as soluble form in E. coli and subsequently highly purified from the cell lysate by affinity chromatography using a Ni-NTA resin. With the purified proteins had been performed experiments gel filtration to check the status of oligomerization of MIF and its mutants in solution. The preliminary results and next experiments may contribute to the understanding of the state of oligomerization and the structural mechanism involved in *L. major* MIF protein interactions during the modulation of the host immune response.
Supported by FAPESP

## BQ.40 – THE ROLE OF SELENIUM IN INTESTINAL MOTILITY DISTURBANCE CAUSED by *Typanosoma cruzi* INFECTION

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Infection with *Trypanosoma cruzi* causes megasyndromes of the gastrointestinal (GI) tract in humans and animals. In the present study, we employed magnetic resonance imaging to non-invasively monitor the effect of selenium supplementation on alterations in the GI tract of *T. cruzi*-infected mice. CD1 mice infected with *T. cruzi* (Brazil strain) exhibited dilatation of the intestines similar to that we recently reported in infected C57Bl/6 mice. The average intestine lumen diameter increased by 65% and the increase was reduced to 29% in mice supplemented with 2 ppm selenium in the drinking water. When supplemented with 3 ppm selenium in chow the lumen diameter was also significantly reduced although the difference between the infected and infected supplemented mice was smaller. Intestinal motility in infected mice fed with selenium-enriched chow was increased compared with infected mice fed with normal unsupplemented chow and was not significantly different from intestinal motility in uninfected mice. We suggest that Se may be used to modulate the inflammatory, immunological, and/ or antioxidant responses involved in intestinal disturbances caused by *T. cruzi* infection.
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## BQ.41 – INHIBITORY ACTIVITY OF RISEDRONATE AGAINST PLASMODIUM PARASITES *IN VITRO* AND *IN VIVO*

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The biosynthesis of several end products of isoprenoid pathway in *Plasmodium falciparum* was recently described. Interestingly, the intermediates and final products biosynthesized by this pathway in mammals differ from those biosynthesized in *P. falciparum.* Thus, the intermediates and some end products of the isoprenoids pathway, could be a potential drug target in *P. falciparum*. Recent studies have shown that bisphosphonates containing nitrogen blocked the biosynthesis of isoprenoid and inhibit protein isoprenylation in humans. Risedronate showed an $IC_{50}$ of 20 µM on cultures of the intraerythrocytic stages of *P. falciparum.* We have also demonstrated that risedronate treated or untreated parasites, labeled with either [$H^3$] farnesyl pyrophosphate or [$H^3$] geranylgeranyl pyrophosphate showed a decrease of intensity of the band corresponding to farnesylated protein and, on the other hand, an increase in intensity in geranylgeranylated protein. Using Ras and Rap-specific monoclonal antibodies, putative Rap and Ras proteins of **P. falciparum** schizont stages were immunoprecipitated upon treatment with risedronate. Another time, we confirmed the results shown by SDS-PAGE. Risedronate showed strong inhibitory activity on the biosynthesis of the menaquinone and phylloquinone in the schizont stage. By thin-layer chromatography, we showed that isoprenoids attached to the proteins are partially modified confirming the activity of risedronate on the isoprenoid metabolism in *P.falciparum*. Additionally, we have shown that treatment of BALB/c mice infected with *P.berghei* at 25 mg/Kg i.p. for 7 days with risedronate decreased the parasitemia in approximately 90%.Supported By: FAPESP e CNPQ (Brazil)

## BQ.42 – EFFECT OF MYRIOCIN IN *Leishmania (V.) braziliensis* GROWTH, SPHINGOLIPID SYNHESIS AND MORPHOLOGY

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Sphingolipids have been related with several biological processes, such as intracellular transport, modulation of signal transduction and apoptosis. Inositol phosphorylceramide (IPC) is the major sphingolipid expressed in promastigote forms of *Leishmania.* In this study it was investigated the effect of myriocin, inhibitor of serino palmitoyltransferase, the first step of sphingolipid synthesis, in promastigote forms of *L. (V.) braziliensis*. Parasite growth, morphology and sphingolipid/phospholipid expression were analyzed in cultures treated with myriocin and supplemented or not with ethanolamine (EtN) and 3-ketodihidrosphingosine (3-KDS). We observed that *L (V.) braziliensis* treated with 1µM of myriocin showed a 52% inhibition of growth, the promastigotes presented a more round form when compared to control parasites. Aberrant cell phenotypes were observed after parasite incubation with 1 µM and 5 µM of myriocin. After 72 hours, a defective cytokinesis was observed by optic microscopy and confirmed by transmission electron microscopy, resulting in giant cells with multiples nucleus and flagella. Addition of exogenous EtN and 3-KDS on myriocin-treated promastigotes did not revert myriocin growth inhibition effect, and also addition of EtN did not revert morphologic alterations, suggesting that the myriocin effect is not due to blockage of synthesis of ethanolamine phosphate necessary for Kennedy pathway as verified in *L.major* (Zhang *et al.*, 2007). Addition of exogenous 3-KDS on 1 µM and 5 µM myriocin-treated parasite reduced the number aberrant cell phenotypes, and partially reverted the expression of parasite IPC. These results suggest that sphingolipids are important for completion of cytokinesis and may also act as signals for *L (V.) braziliensis* proliferation.
Supported by FAPESP, CAPES, CNPq.and FADA.

## BQ.43 – INHIBITION OF STEROL BIOSYNTHESIS IN *LEISHMANIA* IS COUNTERACTED BY A SHIFT ON INTRACELLULAR CHOLESTEROL ENDOCYTOSIS AND METABOLISM

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The development of more effective and selective drugs is a priority for the treatment of leishmaniasis and the identification of exclusive metabolic pathways of the parasite that can be used as a target could be an interesting starting point. In spite of leishmania parasites synthesize their own sterols, they accumulate significant percentage of exogenous cholesterol, reaching in some cases to be the major sterol at all, indicating a biological role for this molecule. This work aims to study the importance for *L. amazonensis* of the use of cholesterol from the serum in various situations, assessing the potential of this system as a possible drug target. The activity of the ergosterol biosynthesis inhibitors (ketoconazole, miconazole, terbinafine and simvastatin) was evaluated in the presence of normal or delipidated serum. It was observed that the deprivation of serum lipoprotein potentiates the effect of inhibitors of ergosterol. The promastigotes treated with these inhibitors showed differences in their lipid composition, with accumulation of cholesterol in treated cells, mainly with ketoconazole and miconazole, suggesting a compensation mechanism in leishmania, which may overcome ergosterol inhibition. Experiments with LDL-$I^{125}$ indicated that leishmania promastigotes increase uptake of LDL upon pressure with either ketoconazole or simvastatin, two sterol biosynthesis inhibitors with different mechanism of action. Suramine, an inhibitor of the uptake of LDL, showed a decrease in cholesterol content of the leishmania and in combination with simvastatin, had a synergistic effect, showing that content of LDL, especially cholesterol, could be involved in maintaining the cell membrane integrity. Taken together, these results suggest that cholesterol plays an important role in the activity of ergosterol inhibitors biosynthesis and that the blocking of its use by leishmania may be a possible drug target. Support by FAPERJ and CNPq/PAPES.

## BQ.44 – INVOLVEMENT OF GP63 MOLECULES IN *BLASTROCRITHIDIA CULICIS* ADHESION TO THE INSECT MIDGUT

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*Blastocrithidia culicis* is a insect trypanosomatid that harbors an endosymbiotic bacterium in its cytoplasm. The major surface peptidase of *Leishmania* spp. is the best characterized metallopeptidase in the Trypanosomatidae family and homologues of these enzymes have been described in several monoxenic trypanosomatids. Here, the gp63 expression of endosymbiont-harboring was assessed by flow cytometry analysis using a panel of anti-gp63 antibodies. It was shown that the wild strain expresses gp63 with homology to *Leishmania* and *T. cruzi* gp63 molecules. We also analyzed the effect of the pre-treatment of *B. culicis* with these anti-gp63 on the parasite adhesion to *Aedes aegypti* midgut, and showed that anti-Tc-gp63 promoted the strongest inhibition of the parasite adhesion (75%). Finally, the pre-treatment of *A. aegypti* midgut with purified metalloproteinase also promoted a significant adhesion on parasite binding, suggesting the involvement of insect receptors to this molecule in the binding process. Taken together, these results suggest a role for gp63 molecules in *B. culicis* adhesion to the insect host midgut.
Supported by CNPq, Faperj and Fiocruz.

## BQ.45 – CRUZIPAIN PROMOTES *TRYPANOSOMA CRUZI* ADHESION TO *RHODNIUS PROLIXUS* EXPLANTED MIDGUTS

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Cruzipain is the major lysosomal cysteine peptidase of *Trypanosoma cruzi*, which is the causative agent of Chagas' disease. This enzyme is expressed at variable levels in all developmental forms and strains of the parasite. Cruzipain is required for parasite infectivity and intracellular growth in mammalian cells, however, its role in parasite interaction with the vector has been overlooked. Here, we have analyzed the effects of the treatment of *T. cruzi* with anti-cruzipain antibodies and a panel of different cysteine peptidase inhibitors on the parasite adhesion to *Rhodnius prolixus* posterior midgut. In parallel, we have analyzed the adhesion rate using genetically manipulated *T. cruzi,* which superexpresses chagasin, an endogenous cruzipain inhibitor (pCHAG). Brielfy, the parasites were treated for 1 hour with iodoacetamide, leupeptin, antipain, Ca074me or E-64 at 10 µM or cystatin at 1 µg/ml and allowed to bind to *R. prolixus* explanted guts for 15 minutes. The interaction rate of the parasites treated with the cysteine peptidase inhibitors was on average 70% lower in comparison to the untreated parasites, except for Ca074me (a cathepsin B inhibitor), which showed no significant alteration. In addition, anti-cruzipain antibodies (1:1000) reduced the adhesion to the insect posterior midgut in 64%, corroborating, pCHAG parasites adhered to the insect gut 73% less than control. In vivo infection assays, performed with pCHAG or control parasites revealed that pCHAG parasites were virtually unable to colonize *R. prolixus* rectum. Furthermore, the expression of surface cruzipain in *T. cruzi* cells was drastically enhanced after passage in *R. prolixus*. Collectively, these results suggest that cruzipain somehow mediates actively the interaction between *T. cruzi* and epithelial cells from the invertebrate host.
Supported by: MCT/CNPq, FAPERJ and FIOCRUZ.

## BQ.46 – IDENTIFICATION OF PROTEINS MODIFIED BY PHOSPHORYLATION IN *TRYPANOSOMA CRUZI* DURING THE INVASION OF HOST CELLS

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We aimed to identify proteins from the infective stage of *T. cruzi* (trypomastigotes) that were modified as a consequence of parasite adhesion to components of the extracellular matrix (ECM), as it is well established that these are involved in host cell invasion. Furthermore, members of the gp85/trans-sialidase superfamily of the parasite bind to ECM elements (laminin, fibronectin, collagen and heparin sulphate). Additionaly, approximately 190 kinases and 86 phosphatases have been identified in the genome of *Trypanosoma cruzi* (CL Brener strain). For this reason, phosphoproteomes from different stages of *T. cruzi* have been published recently. In order to address this question, trypomastigotes (Y strain) were incubated with laminin-, fibronectin- or BSA-coated surfaces for 2h, followed by 2D-PAGE analysis pattern after staining with Pro-Q Diamond Phosphoprotein Gel Stain (Invitrogen). Proteins with significant differences in stained intensity when compared to the control (BSA) ($p$<0.05) were selected for further identification by LC-MS/MS. From the 64 spots presenting modification to the degree of phosphorylation on incubation with laminin, 16 corresponded to cases of novel phosphorylations, 26 corresponded to cases of dephosphorylation and 22 presented alteration to the level of phosphorylation. Only 9 of the latter showed variation at a level greater than 2.0-2.5 fold. **After incubation with fibronectin:** from the 78 spots selected, 8 corresponded to novel phosphorylation, 44 to dephosphorylation events and 26 to variation in the level of phosphorylation. Of the latter, 5 spots showed variation greater than 2.0 to 2.5 fold when compared to the control. Currently the full identification of the proteins is under way. For example, tubulin (alpha and beta) showed an increase in the phosphorylation when trypomastigotes were incubated with laminin. So the identification of proteins modified by phosphorylation during the interaction of trypomastigotes with ECM may help in understanding the signaling events within the parasite during the invasion. Supported by FAPESP and CNPq.

## BQ.47 – INTRAERYTHROCYTIC *Plasmodium falciparum* STAGES SYNTHESIZE MENAQUINONE AND PHYLLOQUINONE AND MODIFIES ITS UBIQUINONE/MENAQUINONE POOL DEPENDING ON OXYGEN AVAILABILITY

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During its intraerythrocytic development in the human host, the malaria parasite *P. falciparum* is submitted to considerable changes in the oxygen concentration due to intermittent cytoadherence in the deep vasculature, with consequences for the energy metabolism of the parasite. Facultative anaerobic organisms such as *E. coli* employ two types of electron carriers, ubiquinone and menaquinone which are tightly regulated depending on the oxygen supply in the environment. Herein, we show that intraerythrocytic stages of *Plasmodium falciparum* have an active pathway for biosynthesis of menaquinone (vitamin K2) and phylloquinone (vitamin K1), as well as, ubiquinone. Kinetic assays confirmed that plasmodial menaquinone acts at least in the electron transport. Similarly to *E. coli*, we observed increased levels of menaquinone in parasites kept under very low oxygen pressures. Additionally, parasite growth is strongly inhibited when menaquinone synthesis was blocked by Ro 48-8071, an inhibitor of 1,4-dihydroxy-2-naphthoate prenyltransferase. Due to its absence in humans, the menaquinone biosynthesis can be considered an important drug target for malaria. Supported by FAPESP and CNPq.

## BQ.48 – PEPTIDASE ACTIVITY CHARACTERIZATION OF LYSATE AND LIVE CELLS OF *LEISHMANIA (L.) AMAZONENSIS* AMASTIGOTES AND PROMASTIGOTES

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Proteases (proteinases, peptidases or proteolytic enzymes) are enzymes present in all organisms, from viruses to vertebrates. Peptidases in parasites are involved in invasion of host cells and tissues, parasite nutrition, modification of host proteins and also in immuno evasion. The aim of this study was to identify the proteinase activity of *Leishmania amazonensis* amastigotes in lysates and live cells in of amastigote and promastigote forms. Fluorescence Resonance Energy Transfer (FRET) peptides and MCA substrates (Z-Phe-Arg-MCA, Z-Arg-Arg-MCA, D-Pro-Phe-Phe-MCA, ε-$NH_2$-caproyl-Cys(SBzl)-Cys(SBzl)-MCA) were used to perform inventories of the peptidase activities. The pH and temperature profiles of peptidase activities of the lysates were done in the presence of inhibitors (E-64, TLCK, PMSF, Pepstatin and EDTA). The peptidase activity in live cells were observed and quantified with confocal microscopy. The lysates presented peptidase activity in wide range of pH, with higher activity around pH 5.0 and pH 8.0. The confocal microscopy confirmed intracellular peptidase activity in pH 5.0, in agreement with the cellular environment of amastigotes, that was also inhibited for E-64 and TLCK, similar with the lysates. Proteolytic activities of live cells were not observed with hydrophobic substrates ε-$NH_2$-caproyl-Cys(SBzl)-Cys(SBzl)-MCA and D-Pro-Phe-Phe-MCA, indicating selectivity of the parasite. All together we observed a large peptidase activity linked to cysteine proteases but we also observed a significant peptidase activity similar to oligopeptidase B that has particular preferences for basic sequences of amino acids.
Supported by CNPq and FAPESP.

## BQ.49 – LEISHMANICIDAL ACTIVITY OF SPECIFIC CERAMIDASE AND GLUCOSYLCERAMIDE SYNTHASE INHIBITORS AND IMPLICATIONS FOR *LEISHMANIA AMAZONENSIS* CERAMIDE METABOLISM

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Introduction. Sphingolipids (SLs) are essential components of biological structures and biological recognition processes between pathogen and host cells. The biosynthesis of SLs in Trypanosomatidae has been only partially characterized. In *Leishmania,* serine-palmitoyil transferase and inositol-phosphoryl ceramide synthase have been identified and glucosylsphingolipids were detected in *L. amazonensis* amastigotes. These molecules appear to be involved in signaling events that mediate metacyclogenesis and host cell-parasite interactions. (1S,2R)-D-erythro-2-(N-myristoylamino)-1-phenyl-1-propanol (MAPP) and 1-phenyl-2-decanoylamino-3-morpholino-1-propanol (PDMP), are inhibitors of eukaryotic ceramidase and glucosylceramide synthase, respectively and their activities against trypanosomatids have not been described. Objectives. We aimed to evaluate the activity of MAPP and PDMP against *L. amazonensis* and investigate the interference of these inhibitors in the incorporation of ceramide by promastigotes. Methodology. MAPP and PDMP effects were observed after incubating *L. amazonensis* promastigotes with increasing concentrations of the inhibitors. After 24h, the viability was assessed by the MTT test. Morphology and ultrastructural aspects were evaluated in treated promastigotes using optical and electron microscopy. C6-NBD-ceramide incorporation and metabolism in the presence of the inhibitors were investigated by HPTLC. Results. The EC50% values for MAPP and PDMP were 112.6 and 62.5 microM, respectively. Parasites treated with MAPP did not present significant alterations on cellular morphology. In contrast, parasites treated with PDMP became rounded with elongated flagella and, by ultrastructural analysis, showed unusual cytoplasmic multilamellar bodies. The labeling of MAPP or PDMP treated-parasites with C6-NBD-ceramide followed by the analysis of lipid extracts showed that drug treatment induced an accumulation of ceramide accompanied by changes in the biosynthesis of other SL. Conclusions. Inhibitors of eukaryotic ceramidase and glucosylceramide synthase are active against *L. amazonensis* promastigotes and induce modifications in ceramide metabolism, suggesting that these enzymes are also present in *Leishmania.* These results open new perspectives for the study of these metabolic pathways in *Leishmania*. Support: FAPESP, CNPq.

## BQ.50 – LIPID METABOLISM in *Herpetomonas megaseliae*

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*Herpetomonas megaseliae* is a monoxenic trypanosomatid isolated from the phorid fly *Megaselia scalaris*. During its lifecycle, this parasite remains in the insect gut lumen. Despite this is not pathogenic to humans, such protozoa represents a safe models for initial studies on lipid metabolism. Lipids are hydrophobic molecules that play a variety of cellular functions of great importance to all organisms. In this work we are investigating the ability of *H. megaseliae* to incorporate and synthesize lipids. $^{3}$H-palmitic acid was added to the culture medium and after 24 h of incubation, the radioactivity incorporated by the parasites was determined by scintillation counting. We observed that *H. megaseliae* were able to incorporate $^{3}$H-palmitic acid added to the culture medium and to use it for *de novo* lipid synthesis. Triacylglycerol and phospholipids were the major lipids found. Also we tested for the ability of this parasite to incorporate the main lipoprotein present on vector hemocel, lipophorin (Lp). Parasites were incubated in the presence of radioactively labelled lipophorin either on the phospholipid ($^{32}$P-Lp) or in the protein moiety ($^{125}$I-Lp). We observed that parasites were able to incorporate Lp in both experiments. In order to localize the incorporated lipids parasites were incubated with fluorescent tagged lipids such as BODIPY-fatty acid (BODIPY-FA) and phosphatidylethanolamine-Texas Red (Texas Red-PE) for 24 h. The results again showed the capacity of incorporation of lipids free in cell medium and their intracellular fate. This work was supported by CNPq, FAPERJ and IFS

## BQ.51 – RECEPTOR-MEDIATED ENDOCYTOSIS OF LDL BY *Leishmania amazonensis* AND LOCATION OF THIS RECEPTOR IN PARASITES LIPID MICRODOMAINS

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DRM (detergent-resistant membranes) or *lipid rafts* are lipid-enriched microdomains in which cholesterol is the principal component. Recent studies showed that it can be isolated from different cell types and are involved in cholesterol transport, endocytosis and signal transduction. Other studies showed the existence of a scavenger receptor for LDL oxidized, CD36, located in lipid rafts of Chinese Hamster Ovary cells. The aim of this work is to study the LDL endocytosis by *Leishmania amazonensis* and localize the LDL-receptor in parasites membranes microdomains. In order to verify the receptor-mediated endocytosis of LDL, the parasites were incubated in the presence of LDL-$I^{125}$ at 28ºC and 4ºC. After 24h, cells were collected, washed and the radioactivity determined by gamma counting. The LDL was found associated with cells and endocytosis was significantly inhibited in assay with fold-excess of LDL or Transferrin. To observe the existence of a LDL-receptor like protein, we isolate *L.amazonensis* membranes. Cells were lysed with glassbeads (1:4 w/w) and separeted in a ultracentrifugation gradient. The membranes were subjected to eletrophoresis gel with a poliacrylamide gradient, using calf hepatocytes membranes as positive control. We found a 38 kDa protein that corresponds to hepatocytes lipoprotein receptor. To examine the LDL-receptor localization in DRM, we first isolated *L. amazonensis* DRMs. The parasites were lysed, homogenated, incubated at 4°C in Triton X-100 1% for 20 minutes and subjected to sucrose density gradient ultracentrifugation. The gradient fractions were subjected to a dot-blot using different agents for investigate the presence of DRM components (GM1 and Flotillin-1). We also used the antibody against the human LDL-receptor. We identified a large Flotillin, GM1 and LDL-receptor content in the fractions from 4 to 7 of sucrose gradient. These results could provide better tools for understand the interactions between parasites and their hosts. Supported by CNPq, FAPERJ, IFS.

## BQ.52 – PURIFICATION OF PROTEINS LIBERATED FROM *TRYPANOSOMA CRUZI* IN RESPONSE TO CALCIUM STIMULATION

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Cell invasion by *Trypanosoma cruzi* is a multi-step process that includes host cell attachment, cell signaling events, host cell lysosome recruitment to the site of parasite entry, host cell microtubule reorganization, lysosome survival, lysosome escape and differentiation into the replicating amastigotes. The first measurable change observed in *T. cruzi* after the parasite binds to the surface of a potential mammalian host cell is an increase in intracellular calcium. Calcium is an important secondary messenger for controlling cellular responses to cell signaling events, including exocytosis in other cell types. Considering the importance of cell invasion to the life cycle of *T. cruzi*, we predict that this initial rise in calcium controls the release of proteins involved with cell invasion from internal sources. To identify these hypothetical proteins, a differential labeling protocol was designed using reversible and irreversible biotin in conjunction with a calcium stimulation using a calcium ionophore. After the labeling with biotin, marked proteins were purified using streptavidin-agarose for analysis by SDS-PAGE and western blots. Analysis consisted of identifying proteins present in the samples stimulated with calcium compared to control samples without calcium stimulation. In addition, western blots were performed using streptavidin and sera from patients diagnosed with Chagas disease. A major objective of this project is to identify potential biomarkers for diagnosis of *T. cruzi* infections and prognosis for the progression to Chagas disease. Proteins that demonstrate a differential with regards to calcium stimulation and are recognized by antibodies in the sera of patients are of the greatest interest.
Supported by FAPERJ, CNPq and Fiocruz

## BQ.53 – SODIUM-DEPENDENT UPTAKE OF INORGANIC PHOSPHATE IN *TRYPANOSOMA RANGELI*

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*Trypanosoma rangeli* is a protozoan that infects a great number of mammals and triatomine vectors. In this work we demonstrated, for the first time, that living cells of *T. rangeli* are able to transport the extracellular inorganic phosphate (Pi) to cytosol through a carrier-mediated process. The dependence on $Na^+$ concentration revealed a normal Michaelis-Menden kinetics for the uptake of Pi and the values of apparent $K_m$ and $V_{max}$ were 1.2 ± 0,3 mM and 22.0 ± 1.2 *p*mol × $min^{-1}$ ×$10^7$ $cells^{-1}$, respectively. Addition of $Na^+$ ionophore, monensin, reduced the Pi accumulation into the cells in the presence of NaCl to the level observed in absence of $Na^+$. Pi-influx showed a sigmoidal dependence on the Pi concentration with a Hill coefficient of 2.2 ± 0.3, and the values of $K_{0.5}$ and $V_{max}$ were 0.05 ± 0.004 mM and 23.6 ± 1.0 *p*mol × $min^{-1}$ ×$10^7$ $cells^{-1}$, respectively. Treatment with FCCP or by v($H^+$)-ATPase inhibitor bafilomycin A1 resulted in a significant decrease in Pi influx, consistent with the predicted electrogenicity of transport. Parasites maintained at Pi-supplemented culture medium (50 mM Pi) presented a decreased Pi-influx, suggesting that this transport is stimulated by Pi-starvation in medium culture. Furthermore, these parasites showed a decrease in *TrPho89*, a $Na^+$, Pi symporter homologue, mRNA levels when compared to parasites maintained at Pi-depleted culture medium (2 mM Pi). Altogether, these results suggests the presence of a $Na^+$/Pi cotransporter present in *T. rangeli*, contribute to inorganic phosphate acquisition by epimastigotes development. Supported by CNPq, CAPES and FAPERJ.

## BQ.54 – HEME UPTAKE IN *TRYPANOSOMA CRUZI* EPIMASTIGOTES INVOLVES ATP-BINDING CASSETTE (ABC) TRANSPORTERS.

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*Trypanosoma cruzi*, the etiological agent of Chaga's disease, takes up heme from the environment to supply their nutritional needs, since it is not synthesized in epimastigotes. Heme is a porphyrin (Fe-protoporphyrin IX) that plays a critical role in several biological reactions such as oxygen transport and cell respiration. However, the mechanisms involved in its uptake across biological membranes are poorly understood. Indeed, in these parasites, no heme transporter has yet been characterized. Thus, here we evaluated the heme import by epimastigotes. Our results showed that when parasites were pre-loaded with other porphyrins such as Sn-protoporphyrin IX, Pd-mesoporphyrin IX and Zn-mesoporphyrin IX the heme uptake decreased drastically. The same effects were observed when these parasites were treated with ATP- binding cassette (ABC) transporters blockers, verapamil, cyclosporin and indomethacin. Furthermore, we evaluated the effect of these drugs in epimastigotes growth kinetics and observed that, in contrast with heme, in the presence of heme plus these drugs the parasites growth decreased. These results suggest the existence of a mutual porphyrin carrier in *T. cruzi* epimastigotes, possibly an ABC-like transporter. Supported by CAPES, CNPQ and FAPERJ

## BQ.55 – THE MAJOR NEUTRAL HEXAMERIC LEUCYL AMINOPEPTIDASE OF *Trypanosoma cruzi* (LAPTc) BELONGS TO THE PEPTIDASE FAMILY M17

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Pathogens depend on peptidase activities to accomplish many physiological processes, as well as to interact with their hosts, highlighting parasitic peptidases as virulence factors and, thus, potential drug targets. In this study, a major leucyl aminopeptidolytic activity was identified in the kinetoplastid *Trypanosoma cruzi*, the aetiological agent of Chagas' disease. It was isolated from epimastigote forms of the parasite by a two-step chromatographic procedure and associated with a single 320-kDa homo-hexameric protein as determined by sedimentation velocity. Interchain disulfide bonds do not take part in the oligomeric assembling of the active peptidase. Molecular identity of the enzyme was revealed by peptide mass fingerprinting as the predicted *T. cruzi* aminopetidase EAN97960. Molecular and enzymatic analyses indicated that this leucyl aminopeptidase of *T. cruzi* (LAPTc) belongs to the peptidase family M17 or leucyl aminopeptidase family. Its sequence shares identity to sequences of other M17 family members, including assigned and unassigned leucyl aminopeptidases of kinetoplastid parasites such as *Leishmania* ssp. and African trypanosome. The enzyme is expressed by all *T. cruzi* forms and localizes within vesicles in the cytoplasm of the parasite. LAP-Tc has a strong dependence on neutral pH, is mesophilic and retains its oligomeric form up to 80 °C. Conversely, its recombinant form, like other LAPs, is thermophilic and requires alkaline pH. The activity of this metalloaminopetidase is inhibited by bestatin and metal chelants such as 1,10-phenanthroline, restored by $Zn^{+2}$, and potentiated by $Mn^{2+}$ or $Ca^{2+}$. Since biosynthetic pathways for essential amino acids, including leucine, are lacking in *T. cruzi* and other kinetoplastid parasites, LAPTc could have a function in nutritional supply. Furthermore, the peptidase activity could also play a role in peptide and protein processing.
Supported by: CNPq, Finep, FAP-DF, UnB

## BQ.56 – MODULATION OF CELL CYCLE AND PROTEIN EXPRESSION OF *LEISHMANIA (VIANNIA) BRAZILIENSIS* BY DEPLETION OF IRON

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Iron is an essential element for *in vitro* and *in vivo* survival of microorganisms, acting as a cofactor of several enzymes and playing a critical role in host-parasite relationship. Only the more virulent strains, endowed with effective mechanisms to acquire iron from healthy hosts can invade, colonize, multiply and establish infection. Endosomes and lysosomes of macrophages are gradually depleted in iron by transporters such as Nramp1 that pump the ion into the cytosol reducing the amount accessible to pathogenic microorganisms. In *Leishmania amazonensis* iron transporter LIT1 is essential for the growth of intracellular amastigotes and development of lesions in mice. *L. (V.) braziliensis* is a parasite widespread in the new world and considered to be the major etiological agent of American Tegumentary Leishmaniasis (ATL). Despite this fact, the role of iron on the growth and virulence of this species is still unclear. In this scenario, the identification of proteins regulated by iron in *L.(V.) braziliensis* can provide important information about their mechanisms of pathogenicity contributing to the development of new therapies for the ATL. In the current work, we aimed to analyze the effect of iron depletion on the growth and protein expression of *L. (V.) braziliensis*. Promastigotes from cultures which had reached the logarithmic phase of growth were inoculated into iron-supplemented and iron-depleted medium. Iron depletion was carried out by the addition of different concentrations of 2,2-dipyridyl ranging from 25 µM to 300 µM. It was observed that parasite growth is affected by both concentration of 2,2-dipyridyl and time of culture in depleted medium. Whole extracts of promastigotes obtained from parasites growth in iron-rich and iron-depleted medium were submitted to fractionation by two-dimensional electrophoresis (2DE). Preliminary data suggest that there are several proteins involved in proteolysis and signaling suffering up- or down-regulation by iron. Supported by: CAPES

## BQ.57 – OPTIMIZATION OF EXPRESSION OF A 21 KDA PROTEIN FROM *TRYPANOSOMA CRUZI*

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Chagas disease is caused by the intracellular protozoan *Trypanosoma cruzi.* It is estimated that 13 million people are directly affected in Central and South American. The current available drugs are limited and ineffective. To overcome these limitations, the identification of new macromolecular targets is very important. This work aim to conduct crystallography studies with a 21 kDa protein (P21) from *T. cruzi*, P21 shows high probability of being secreted and interacts in the mammalian cell invasion process. The recombinant P21 was expressed in *Escherichia coli* BL-21 containing pET-28a (+) vector. P21 was expressed mostly in insoluble fraction and in order to obtain soluble fraction we tested two culture media (LB end LB 2x), three temperatures to expression (37, 20 and 16°C**)**, three concentrations of isopropyl b-D-thiogalactopyranoside-IPTG (1mM, 0,5mM, 0,1mM) and three methods of bacterial lysis (French press, sonication and ***Freeze***-Thawing), the fractions soluble and insoluble were analyzed by Coomassie blue staining SDS-polyacrylamide gels. P21 showed more soluble in the following conditions: LB 2x media, 16°C of expression temperature***,*** 0,1mM of IPTG and sonication lysis method.
Suported by: FAPESP, CAPES, CNPq

## BQ.58 – E-NTPdases: PREDICTION OF FAMILIES AND CLASS DESCRIPTORS

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Enzymes of E-NTPDase family hydrolyze ATP and other nucleosides tri and diphosphates. Extracellular nucleotides act as signaling molecules in immune response of mammalian hosts. The ATPe hydrolysis plays a role on infectivity and virulence of parasites (*Trypanosoma*, *Leishmania*, *Trichomonas*, *Toxoplasma* and *Entamoeba*). All the enzymes belonging to this family have five highly conserved regions named Apyrase Conserved Regions. The mechanism of nucleotides hydrolysis is clear, but little is known about what determines the distinct nucleotide preferences. In this study we analyzed all complete E-NTPDase sequences, showing that it's possible to divide them in sub-families based on sequence similarities and aminoacid positions conservations that could be related with nucleotide preferences. For this, 566 UniProt sequences were filtered, leaving 366 threads. All reviewed proteins of this family, deposited in the SwissProt (42) were detailed analyzed through alignment and literature annotations. Statistical analysis of conserved and correlated mutations was applied to this final alignment. A matrix was constructed containing the correlation value of each correlation between the statistically significant perturbations with other alignment positions. This matrix has undergone a procedure of hierarchical clustering and removal of low signals, resulting in small groups of highly correlated positions. We showed that there is a trend in substrate preference between subfamilies. There are four positions that appear to be strongly correlated and which seem to be good descriptors of the distinct subfamilies. Based on known structural information's, we can suggest why some of these positions could act as keys to substrate specificity. Furthermore we can predict the substrate preference of tripanosomatides E-NTPDases (*T. cruzi* and *Leishmania infantum*) and confirmed many of then using enzymatic activities of recombinant expressed proteins. These data could be explaining the influence of pathogen´s E-NTPDases on hosts purinergic signaling and may be applied in new approaches to rational drug design. Supported by: INBEQMeDI, CNPq, Capes, FAPESP, UFV.

## EP.01 - ANALYSIS OF INTESTINAL PARASITES CAUSED BY PROTOZOA IN THE POPULATION OF CONDE, PARAÍBA

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Infectious and parasitic diseases are a serious public health problem worldwide, which reaches alarming levels in many Latin American countries. Considering the scarcity of studies on these disorders in Brazilian municipalities, this study aimed to conduct a survey on intestinal parasitic infections caused by protozoa present in individuals served by the Clinical Laboratory of the town of Conde, Paraíba, during the months of January 2009 to January 2010. It was analyzed the results of 1759 fecal examinations, which were obtained by the Hoffman technique, Pons & Janer, stored in a laboratory log book, being separated by gender. The data showed that 42.07% of the sample was infected by at least one intestinal parasite and 77.3% of the patients were infected by protozoa. The most frequent parasites in the positive results were *Endolimax nana* (13.59%), *Entamoeba coli* (12.67%) and *Entamoeba histolytica* (10.46%). It was also noticed that the protozoan *Giardia lamblia* (6.46%) was more frequent in men, while *Endolimax nana* (15.25%) was more prevalent in women. Therefore, it can be concluded that the high incidence of intestinal parasitism caused by protozoa is a reality to be minimized in the population of Conde.

## EP.02 – GENES INFLUENCE SEROPOSITVITY FOR *T. CRUZI* INFECTION AND EKG MEASURES OF CHAGAS DISEASE PROGRESSION

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We searched for genes influencing seropositivity for *T. cruzi* infection and EKG traits related to Chagas disease progression in a Brazilian population using large extended kindreds. Whole genome STR genotyping information was available for 1350 individuals. The prevalence of *T. cruzi* infection in these individuals (who were born in 1975 or earlier) was 61.2%. Using a standard threshold liability model, the heritability of *T. cruzi* infection was 0.614 ($p=4.6 \times 10^{-9}$). We observed significant evidence for polygenic genotype x sex interaction ($p<0.05$), and consequently allowed for genotype x sex interaction in our linkage analyses. We identified two significant loci influencing seropositivity for *T. cruzi* infection. The first one was localized to chromosome 2 at 27cM with a lod score of 3.40 (genome-wide p = 0.046). This locus showed larger effects in males (p-value for genotype x sex interaction = 0.00012). The second locus was on chromosome 15 at 23 cM (lod score = 3.37, genome-wide p = 0.049) and exhibited greater effects on females (genotype x sex interaction p = 0.0019). These loci represent the first significant evidence for specific genomic regions influencing seropositivity for *T. cruzi* infection found by unbiased genome-wide searching. We also searched for genes influencing cardiovascular parameters that differentially respond to *T. cruzi* infection. The QT interval obtained from EKG measures showed pronounced prolongation in infected individuals ($p=1.9 \times 10^{-10}$). Using a quantitative trait genotype x infection interaction model, we localized a gene influencing the QT interval on chromosome 1 at 18cM (p=0.00045) that showed strong evidence for differential response to *T. cruzi* infection (genotype by infection interaction p=0.0018). Infected individuals exhibited larger effects due to this locus than uninfected individuals. To our knowledge, this is the first strong evidence of genetically based differential response of cardiovascular parameters relevant to Chagas disease.

## EP.03 - ANALYSIS OF RESTRICTION FRAGMENT LENGTH POLYMORPHISM (RFLP) OF ISOLATES OF *TRYPANOSOMA CRUZI* IN THE STATE OF AMAZONAS, BRAZIL.

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The Brazilian Amazon is considered non-endemic area for Chagas disease due to a small number of records of triatomines that invade homes and of sporadic human cases that usually occur by oral infection. This scenario makes it difficult to clarify the epidemiological situation of transmission of *Trypanosoma cruzi* in the region. This study aimed to analyze the restriction fragment length polymorphism (RFLP) of isolates of *T. cruzi* from different municipalities in the state of Amazonas. We analyzed 44 samples, 31 isolated from humans, 6 from wild animals and 7 from different species of triatomines. The samples were grown in LIT medium and DNA was extracted and amplified with the primers Tcmit-10 and Tcmit-21 generating a fragment of approximately 400bp of the gene for subunit 2 of cytochrome oxidase (COII). This product was digested with the enzyme *Alu* I and RFLP was determined in polyacrylamide gel at 4.5%. The isolates from humans, two were classified as DTU (*Discrete Typing Units*) TcI and 29 had profiles patterns compatible with bands observed in DTU TcIII or TcVI. The isolates from opossums (*Didelphis marsupialis* and *Philander opossum*) belong to TcI and 4 isolates from vectors belong to TcIII ou TcVI and 3, the TcI. The definition of the isolates from humans and triatomines belonging to more than one DTU will be made by sequencing, since the visualization of the gel bands of RFLP does not allow to differentiate DTUs TCIII and TcVI. However, with these preliminary results it is assumed that these isolates belong to TCIII, since there are no reports yet of the presence of TcVI in Amazon, which is consistent with the DTUs found in this region. Additional epidemiological and biological studies with these isolates may give subsidies to propose measures of prevention and control of Chagas disease in Amazonia.
Supported by Fundação Araucária, CNPq, PPG/UEM, CAPES, PROAP-CAPES

## EP.04 – PHYSICAL THERAPY COMBINED WITH A LAXATIVE FRUIT DRINK FOR TREATMENT OF CHAGASIC MEGACOLON

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Chagasic megacolon is characterized by intestinal constipation and frequent abdominal distension. The treatment of Chagas' disease colopathy is limited to clinical management in the initial phases of the process, and for patients for whom surgery is not indicated or is not possible, anti-constipation diets are used, along with judicious administration of laxatives and enemas. The objective of this work was evaluate over time the effects of physical-therapy interventions combined with daily ingestion of a laxative fruit drink in the treatment of chagasic megacolon. In a quantitative, prospective, and comparative study, 12 patients of both sexes and with a mean age of 67 ± 12 years were clinically evaluated to receive 12 sessions of physical therapy twice a week, along with a daily fruit drink, and were evaluated for intestinal constipation before and after treatment. A significant difference ($p<0.0022$) was observed in the constipation scores before and after 6 weeks of intervention in 91.7% of the patients, and in 72.7% after 12 months, with reduction of laxative medications, softer stools, and increased number of bowel movements, indicating improvement. With respect to gender, age, and whether or not the patient had received surgical treatment, there was no significant difference ($p>0.05$) in the results of the physical-therapy treatment combined with the fruit drink. We conclude that the proposed protocol is easy to implement, safe, non-invasive, and low-cost, with the potential to be deployed in health care by providing benefits independent of gender, age, or whether the participant has undergone reconstructive surgery, improving the condition of patients with chagasic megacolon. Supported by PPG/UEM, PROAP-CAPES.

## EP.05 – AUTOCHTHONOUS CHAGAS' DISEASE IN ESPÍRITO SANTO: CASE REPORT

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*Trypanosoma cruzi*, the etiologic agent of Chagas' disease, is widely distributed on the American continent where 16–18 million inhabitants are infected. In Espirito Santo (ES) there is a high diversity of etiologic agents: *Cavernicula pilosa, Panstrongylus diasi, Panstrongylus geniculatus, Panstrongylus megistus*, *Triatoma tibiamaculata*, *Triatoma vitticeps* and *Rhodnius domesticus.* None of these species has acquired the capacity of domiciliation, but some are captured into domestic localities and transmitted to the residents. *Triatoma vitticeps* is the most important species present in ES. The objective of this work was to report a Chagas' disease case in a patient residing in Iconha localized in southern region of ES. Following the initial clinical diagnosis, the patient complaining of low fever, fatigue, edema and pruride. These symptoms appeared two weeks after she smacked a bug, and the intestinal material sprayed in her eyes. Thirty days after contamination, the patient was evaluated at the Hospital Universitário Cassiano Antonio de Moraes (HUCAM/UFES), by Indirect immunofluorescence (RIFI) and xenodiagnosis tests for Chagas' disease. The RIFI was positive (1:320) and the xenodiagnosis induced the infection in all triatomines, confirming acute phase of Chagas' disease. Benznidazole was prescribed 200 mg daily for 60 days. Three months after the treatment, serological tests were performed and the results showed non reactive Indirect Hemaglutination (HI) and indeterminate RIFI. Every six months, in a period of two years, new serological tests were performed by the same methodology, showing negative results, demonstrating possible parasitological cure. After six years, the patient returned to the hospital and complained of fatigue and recurrent chest pain. She performed the serological tests (RIFI, HI and ELISA) and all were positive for Chagas' disease. The serological tests were positive probably because of the intracellular amastigotes reactivation.
Supported by UFES and Secretaria de Estado da Saúde (SESA/ES).

## EP.06 – SEROLOGIC PREVALENCE OF Trypanosoma cruzi IN DOGS FROM THE NORTH ZONE (TEMPORAL) FROM THE DEPARTMENT OF COCHABAMBA-BOLIVIA, FROM THE YEARS 1997 AND 2007.

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Chagas disease is a major public health problem in Latin America. At present, Bolivia has the highest rate of vector and congenital transmission, and the old rural profile of the disease is changing rapidly into urban. Recent epidemiologic data indicates that all the capital cities of the 9 Bolivian departments still have new cases of Chagas disease in children under fifteen years. However, good news came from Bolivia: the Chagas control program has reduced importantly the rate of infection. This work is designed to expand research studies on the epidemiological situation of infection with ***T.cruzi*** in domiciliary dogs from the North (NZ) Zone "Temporal" a place with high socially, and economically situation of the Cochabamba department of Bolivia. We drawing a comparison to the years 1997 and 2007. Samples of the year 1997 indicate a result of 449 serum dog samples indicated that 64,15% are seropositive and 35,85% are seronegative. In 2007 we returned to the same place and we obtained 445 serum dogs samples, when we did the analyse of them, 6,74 % were seropositive for Chagas disease and 93,26% are seronegative with indirect hemaglutination IHA analysis Polychaco-Argentine kit, positive and negative controls provided in the kit were used with all samples. Probably, This low positivity is due to better preventive health management is now mostly housing, improving housing, the use of insecticides in good campaigns planned.

## EP.07 – INFECTIVITY FOR MICE OF *TRYPANOSOMA CRUZI* ISOLATES OF DIFFERENT HOSTS FROM THE STATE OF AMAZONAS, BRAZIL

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Chagas disease is emerging in the Amazonian region of Brazil with 600 acute cases recorded. *Trypanosoma cruzi* isolates from this region exhibit genetic and biological peculiarities. Infectivity for mice of 11 *T. cruzi* isolates from the state of Amazonas was determined and compared to one isolate from the state of Parana. Eight isolates of human acute cases from the municipality of Coari, three of *Didelphis marsupialis* (Manaus) and one of chronic patient from Maringa (PR). Groups of 10 mice, males, 21-28 days, were inoculated intraperitoneally (1.05 to 17.5 x $10^3$ blood trypomastigotes (BT)/animal). Parasitemia was assessed by fresh blood examination, blood culture and PCR. The rate of infectivity (% INF) was determined by the number of animals that displayed at least one positive test/total. The %INFs ranged from 40-100% and 0-90%, respectively, for isolates from Coari and *D. marsupialis*. Was 100% for the isolated of chronic case. The prepatent period ranged from 5.3 to 9.5 days (Coari). It was 12.8 days for the isolate of chronic case. All isolates of *D. marsupialis* showed sub-patent parasitemia. The patent period ranged from 2.2 to 9.5 days for isolates from Coari and was 19.9 days for the isolate of chronic case. The peak of parasitemia (Pmax) varied from 280-8200 BT/0.1 mL (Coari). It was 1900 BT/0.1 mL for isolate of chronic case. All isolates from acute cases showed early peaks of parasitemia (7-8$^o$ di). For the isolate of chronic case the Pmax was late (25$^o$ di). The mortality rates ranged from 0-13% (Coari) and 0-20% (*D. marsupialis*). It was 0% for the isolate of chronic case. The infectivity varied with both the host and with the geographical origin of the isolate. Supported by CNPq, UEM and Fundação Araucária.

## EP.08 – INFECTION WITH *TRYPANOSOMA* SP. (KINETOPLASTIDA: TRYPANOSOMATIDAE) IN ARMORED CATFISH (*PTERYGOPLICHTHYS PARDALIS*) IN FREE MARKETS OF MANAUS, AMAZONAS, BRAZIL

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The trypanosomatids hemoflagellates are widely distributed in all continents, parasites from different hosts. Currently there are 140 described species of *Trypanosoma* in freshwater fish worldwide, with about 40 registered fish in natural river basins in Brazil and at least 18 recorded in catfishes. The *Pterygoplichthys pardalis*, known as the Acari-bodo is too consumed by the Amazonian population, which has gotten used to buy this fish alive, by showing a rapid degradation process after his death. Has detritivorous feeding habits, with a distribution in floodplain environments, where a very large amount of particulate organic material, formed by plants and decomposed remains of dead animals. The aim of this study was to determine the rate of infection of *Trypanosoma* sp. in armored catfish (*P. pardalis*) and describe the morphology of the flagellate. 62 live fish were purchased in street markets of Manaus, recorded, photographed, examined and made observations and external evaluation of the cap to search for ectoparasites. The blood was collected by cardiac puncture, liver and caudal to carrying out fresh tests. Regardless of positive blood smears sewn up for morphometric studies. The infection rate was 88.7% in the examination of fresh blood, confirmed by stained smears. These flagellates have cytoplasm with granules and light purple. With tapered ends, the front is located in the kinetoplast, that are round, with pink. The nucleus is usually oval and may be slightly circular and the most often occupies the entire width of the body, same color as the kinetoplast. The undulating membrane is conspicuous and in the majority of specimens presented a few undulations. Supported by: FAPEAM

## EP.09 – THE EPIDEMIOLOGY OF THE ACUTE CHAGAS DISEASE IN THE STATE OF PARÁ, BRAZIL

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Chagas disease remains a highly relevant public health problem in the Amazon. The disease now advances to new regions without previous recognition of the hyperendemicity of the *T. cruzi* infections. Seasonal outbreaks of acute Chagas disease are usually detected in the months of September and October. The State of Pará Chagas Disease Program notified 351 acute cases of the disease in the last two years. The acute cases were originated from different geographic regions of the State, but a majority of cases were from the small cities in archipelago of Marajó. The epidemiological findings for patients treated at the Clinical Hospital revealed that a majority of acute symptomatic cases of Chagas disease occurred as outbreaks suggestive of infection acquisitions by the oral route. Interestingly, most acute cases were seen in people residents in the urban area, although some were from rural areas neigboring the cities. There was no age or gender preference, and no professional activity favored contamination. The gathering of specimens of triatomines inside the houses was sporadic, and triatomine colonies were not found in the intra-domicile. Randomized epidemiological studies should be conducted in the region, aiming at to identifying environmental and epizooetiological factors associated with constant outbreaks of acute Chagas disease in the Greater Amazon.

## EP.10 – SYMPTOMATIC ACUTE CHAGAS DISEASE IN THE AMAZONIAN STATE OF PARÁ, BR. CLINIC-EPIDEMIOLOGIC STUDY OF CASES SEEN IN THE HOSPITAL GASPAR VIANNA, BELÉM, 2009

Almeida AJB*.[1,2], Souza DSM.[2], Coelho de Souza TA, Rodrigues ED, Tibério CT, Reis CF, Nitz N.[1], Hecht, M.M.[1], Teixeira, A.R.L.[1]

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The clinic manifestations of the acute phase Chagas disease have been reported in about 5% of cases. However, the acute illness in cases from the northern State of Para, Brazil, showed a majority of acute cases with various specific symptoms related to severe cardiomyopathy. In this study, we assessed all acute cases that were notified to the Secretary of Health of Pará during 2009. Each acute case yielded clinical histories, and clinical examinations were performed. The laboratory analysis included blood cell counts and biochemistry, and urinalysis. ECGs, chest X-rays and echocardiography were recorded. The relevant clinical findings were prolonged fever, malaise, anorexia, myalgia, headache and swelling of the face and of lower limbs. Some cases presented acute myocarditis with increased cardiac area on chest X-rays, pulmonary congestion and pericardial effusion. Leukocytosis, increased ALALT and ASALT transaminases, creatine kinase, anemia and alterations in the lipid profile were recorded. The ECGs showed atrial fibrilation, diffuse changes of repolarization, ventricular, QT prolongation. Doppler echocardiography, revealed pericardial effusions and various degrees of heart insuficiency, and blood regurgitation in the left ventricle. The variability of signs and symptoms requires long lasting field studies to determine the progress of acute phase lesions to chronic phase Chagas disease in the Greater Amazon.
Funded by the FAPDF/HCFGV

## EP.11 – INFECTIVITY FOR MICE OF *TRYPANOSOMA CRUZI* ISOLATES FROM THE STATE OF AMAZONAS INOCULATED BY INTRAGASTRIC ROUTE

Dias, G.B.M. [1], Meza, S.K.L. [1], Monteiro, W.M. [2], Magalhães, L.K. [2], Bossolani, G.D.P. [3], Teston, A.P.M. [1], Gomes, M.L. [1,3], Araújo, S.M. [1,3], Barbosa, M.G.V. [2], Toledo, M.J.O. [1,3,*]

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Experimental and field observations have demonstrated the transmission of *Trypanosoma cruzi* to man and various mammals by oral route. In the last decades, at least 600 cases of acute Chagas disease were reported in the Brazilian Amazon, most linked to oral transmission. In this study, it was determined the infectivity for mice of four *T. cruzi* isolates from the state of Amazonas. Three of human acute cases from municipalities of Coari and Santa Isabel do Rio Negro and one of *Rhodnius robustus* captured in Apuí. Groups of 20 male Swiss mice, 21-28 days, were inoculated (5.6 to 11.2 X $10^3$ blood trypomastigotes (BT)/animal): 10 intragastrically (IG) and 10 by intraperitoneal route (IP). Parasitemia was assessed by fresh blood examination and blood culture. The rate of infectivity (%INF) was determined by the number of animals that had at least one positive test/total X 100. The %INF ranged 30-80% in mice inoculated IG and 90-100% in those inoculated IP. The medium prepatent period was 9.8 to 11.7 days (IG group) and 3.6 to 7.1 days (IP). The medium patent period was 1.7 to 4.3 days (IG) and 5.2 to 6.3 days (IP). The medium peak of parasitemia (Pmax) varied from 1,506-14,000 BT/0.1 mL (IG) and 5,911-43,960 BT/0.1 mL (IP). The day of Pmax was later for the IG group (11° - 12° days after inoculation-di) than for the IP group (6.4° - 9° di). The mortality rate was lower for the IG group (0 - 20%) compared with IP group (0 - 40%). With the parameters analyzed we can conclude that IG infection with Amazon *T. cruzi* was less virulent for mice compared to the IP route, with the inocula used. Supported by CNPq, UEM, and Fundação Araucária.

## EP.12 – INFECTING POTENTIAL, PATHOGENICYTY AND GENETIC VARIABILITY OF *Trypanosoma cruzi* I IN DIFFERENT MICE LINEAGES

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The *T. cruzi* (Tc) populations are grouped in six main lineages (TcI, TcII, TcIII, TcIV, TcV and TcVI).The influences of these genotypes in the evolution of experimental infections and human clinical manifestations are little studied. TcII is associated with pathogenicity but controversy exists regarding TcI pathogenicity. In this research, the molecular and biological behavior of TcI triatomines strains ALVANI, MUTUM (*Panstrongylus megistus* - Minas Gerais) and AQ1-7 (*Triatoma sordida* - Bahia) were evaluated in three mice lineages (Swiss, Balb/c and C57BL/6). Fifteen animals of each lineage were infected with 10.000 trypomastigotes from MK2 tissue culture. The parasitemia was evaluated during 30 days by microhematocrit (MH), fresh blood examination (FE), blood cultures (BC) and PCR .Animals were sacrificed after 35 days for histopathology analysis and *T. cruzi* tissue PCR (121 and 122 primers).The parasite genetic characterization by *Low Stringency Single Specific Primer* was performed to kDNA of *T. cruzi* in culture, blood and tissues. All TcI strains presented low virulence followed by a variation on the pathogenic potential detected by inflammatory process (IP) and amastigotes nests. The parasitemia, in all isolates in all animals lineages, was 100% subpatent (MH and FE).The survival rate was of 100%. MUTUM showed blood parasitism (BC+ and PCR+) and low pathogenicity (low positive rate of tissue PCR without nests). ALVANI and AQ1-7 presented low blood parasitism (only PCR+) but intense tissue parasitism (PCR+ and amastigotes nests) and pathogenicity with severe IP. All strains showed smooth and striated muscle tropism independent of mouse strains and the kDNA genetic characteristics between inoculum, culture and blood were maintained. MUTUM and AQ1-7 were genetically associated. TcI strains differences could be attributed to the polar populations associated with and without the capacity to produce lesions. These facts may explain the clinical controversies found in TcI human infections in different endemic regions.
Supported by CAPES, CNPq (Universal 2008), FAPEMIG and FUNEPU.

## EP.13 – CONTRIBUTION IN LABORATORY DIAGNOSIS OG CHAGAS DISEASE WHEN THE TRANSMISSION BY FOOD IN THE AMAZON AFFECTS THE STATE OF PARÁ.

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The detection of cases of chagas disease (DCh) in the Amazon is different from other regions in the country due to the investigation of suspected form of transmission from contaminated food through deposition of faeces from infected tick. In 2007 outbreaks were investigated in the state where there was an association of consumption of fresh food, especially the açaí juice. From the implementation of surveillance actions at the state leyel, results in higher integration and structuring of the network diagnostic in Pará. The aim of this paper is to analyze the contribution from the aspect of serological reactivity in samples from municipalities in Pará sent to the Laboratory of State Reference (LACEN/PA) in the period from January 2008 to December 2009. We conducted a retrospective descriptive study by analyzing the results of serum samples from suspected cases of acute DCh. 3158 samples were examined, using standard techniques (RIFI-IgG, ELISA and HAI), considering the title reagents with samples of least 1/40 in two tests and analyzed the probable mode of infection through the records of investigation. The reagents were 166 samples (5,2%) in the period, these 25 (15%) by probable oral way, 129 (78%) ignored, 08 (4,8%), vectorial, 02 (1,2%) transfusion and 02 (1,2%) accidental. The data obtained imply that there are questions to be clarified such as: the paucity of scientific studies on the contamination of açaí juice would influence the investigation; and other foods would be in the food chain and transmission of *T. cruzi* in the region, there would be concomitant pathologies. In summary, the elucidation of the mechanism of transmission is of really important for the control of the infection.

## EP.14 – PREVALENCE AND PARASITEMIA OF TRYPANOSOMES FOUND IN ARMOURED CATFISH

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Fish trypanosomes are transmitted by bloodsucking aquatic leeches. Natural fish trypanosomiasis is widespread with high prevalence in the wild. The objective of this study was to report the prevalence and intensity of infection of trypanosomes and leeches in armoured catfishes from Rio Pomba, MG, investigating the dynamics of natural infections caused by trypanosomes. Armoured catfishes were collected from Rio Pomba, (21º21'07"S, 43º02'49" O) Guarani City, MG, Brazil during the years 2008 - 2009. The leeches found infesting fishes were counted and removed from the body. The blood samples were obtained by cardiac puncture and analyzed for the presence of hemoparasites. The prevalence was determined by the analysis of the stained blood smears and the parasitemia was estimated counting trypanosomes in 50 fields (10 Ocular X 20 Objective). Leeches were found during all the seasons infesting armoured catfishes, located mainly in the mouth. In the winter sample the intensity of infestation reached 10 leeches per fish and in the summer it presented a low number, 1 leech per fish. The prevalence was high in armoured catfishes with trypanosomes detected in all fishes collected. The parasitemia ranged throughout the seasons, in the spring and summer the parasitemia was 1 parasite/$cm^2$ and 1.2 parasites/$cm^2$, respectively. In autumn the parasitemia was 2.3 parasites/$cm^2$ and in the winter it showed a peak of 4.5 parasites/$cm^2$. These data showed that the elevation of the parasitemia coincides with elevation of intensity of leeches. Other studies will be conducted for complementary analyses on the dynamics of infection caused by those trypanosomes.
Supported by CNPq, FAPERJ

## EP.15 – LEISHMANIASIS: SOCIAL REPRESENTATIONS ABOUT THE DISEASE AMONG TEACHERS OF PRIMARY AND SECONDARY EDUCATION FROM DIVINÓPOLIS CITY, MINAS GERAIS, BRAZIL

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Leishmaniasis is a serious public health problem in Brazil. One of the reasons for the increase of the disease's dissemination is the lack of public information and poor knowledge among health and educational professionals. This study aimed to investigate the social representations about leishmaniasis by using semi-structured interviews among science and biology teachers of the primary and secondary education system in Divinópolis, an endemic area. Out of 139 teachers eleven were randomly selected, and after 10 having been interviewed the criterion of saturation was met. The interviews were submitted to content analysis according to Bardin (1977) and revealed that teachers consider leishmaniasis of little importance. Furthermore they were never trained on this subject, confounding the disease with others such as schistosomiasis. Nine professionals associated disease transmission to mosquito bites and three considered humans as source of infection for sandflies. In relation to the parasite, one classified it as virus and another one as bacteria. The knowledge about the vector, the biological cycle and symptoms of the disease also proofed to be fragmented; some professionals named the vector incorrectly and stated that the vector breeding is standing water. Two considered the reservoirs as propitious environment for the disease; eight associated these to dogs. All of them reported that there are treatment and cure for humans, although they do not know the medicine. The prevention and control were associated with: putting down dogs (5); treatment of these animals (5) or vaccination (2); vector combat (6); avoid standing water (3); treatment of patients (4), sanitation (2), information for people (7). In conclusion, the teachers' conceptions do not favor the Leishmaniasis prevention. These results will be helpful for a contextualized health education program for these professionals promoting a meaningful learning. Supported by FAPEMIG and CNPq.

## EP.16 – CANINE LEISHMANIASIS: SEROPREVALENCE IN AN ENDEMIC AREA OF MINAS GERAIS

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Leishmaniasis are zoonoses with wide geographic distribution in Brazil and it is an increasing public health concern. It is known that the canine cases of the illness precede the human cases; therefore, high prevalence in these reservoirs leads to the emergence of epidemic outbreaks in many Brazilian regions. The purpose of the present work was to evaluate the seroprevalence of leishmaniasis in domestic dogs in Divinópolis city, MG. Blood samples from 151 dogs were collected in six veterinary clinics from 2008/ 2009. The blood sera were tested by ELISA and RIFI and the cells was frozen for further molecular studies. The main signs and symptoms of leishmaniasis were evaluated by a veterinary physician and the data were stored. The results demonstrated an increasing seroprevalence of canine leishmaniasis in Divinópolis city. Out of 77 dogs evaluated in 2008, 29.9% were serological positive for *Leishmania* sp. In 2009, the seroprevalence increased 6.6%, from 29.9, to 36.6%. Out of 151 dogs evaluated during this period, 50 seropositive dogs (68%) showed signs and symptoms of leishmaniasis and were classified as symptomatic. Correlation analyses showed an agreement, ranging from 0.57 to 0.69, between ELISA positive tests and the following clinical signs: dermatitis, nodules and hepatosplenomegaly, in addition to a mild correlation (0.21 - 0.43) with alopecia, conjunctivitis, loss of weight, prostration and lynfadenopathy ($P>0.05$). The increase of canine leishmaniasis seroprevalence observed from 2008 to 2009 demonstrated the importance to implement control and prevention measures with the health authorities of Divinópolis city, These measures are crucial for leishmaniasis to avoid spread of disease and the occurrence of human cases. Supported By FIOCRUZ, FUNEDI, CNPq.

## EP.17 – EPIDEMIOLOGICAL ASPECTS OF THE AMERICAN TEGUMENTARY LEISHMANIASIS (ATL) IN TABATINGA AMAZONAS STATE, TRÍPLICE FRONTIER REGION: A RETROSPECTIVE STUDY

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The Tabatinga, southwestern of Amazon, is a region of triplice frontier with others Amazonian countries.This situation allows the migration of foreigners for the place in search of better conditions of life and differentiated health, initiating a process of deforestation for occupation of the area, thus causing a propitious environment for man infection. To characterize the epidemiological situation of the ATL, 49 human cases (77,5%♂ and ♀22,5%) notified in the data base of FVS, had been analyzed (2005-2009). The data was treated statistically (EPIINFO 3.5.1). All of the ages were grouped and the greater percentage of ATL occurrences was given in that correspondent to the biggest activity in the work (38,7%, 16 - 29 years). A total of 6% had more than 70 years. Respect to the level of patients education it showed that 53% had at least 4 to 11 years of study, 28% were illiterate or it was not informed, 15% had only one to the three years of study and 4% of them had more than 12 years studying. The patients were mainly from Tabatinga 80%, and remain of the cases distributed accordance with the origin of the cities proximity as Atalaia do Norte 10%, Santo Antonio do Içа 4%, São Paulo de Olivença, Jutaí and Benjamin Constant 2%, respectivelly. It had predominance of deriving patients of the urban zone with 61.2%, followed of 38.8% of the agricultural zone. The most frequent occupation was farmer (37,5%). Other groups that had important prevalence had been the students and military, both with 25%. The majority of the cases was cutaneous with 97,9%, while only one case (2,1%) with mucocutaneous clinical form. The occurrence of ATL in the 62 Amazonas municipality in the last five years, Tabatinga is placed as the 26° in number of cases registered.

Supported by FAPEAM.

## EP.18 – LEISHMANIASIS: THE LEVEL OF HEALTH PROFESSIONALS KNOWLEDGE IN ENDEMIC AREA

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The lack of health professionals' information contributes to spread of various diseases, including leishmaniasis. Thus, this study aimed to: investigate the level of knowledge among health professionals in Divinópolis city through an epidemiological basic concept of leishmaniasis inquire. Out of 228 professionals were interviewed: 95 zoonoses agents (AZ), 83 community health agents (CHA), 18 doctors, 17 nurses, 8 dentists and 7 veterinarians. Analyses were performed using the SAS program, which showed the highest individual average accuracy in veterinarians (8.3) and physicians (8.1). The averages in these groups were higher than in CHA (6.7). We observed doubts related to preventive measures (42.5% error), clinical manifestations (25.9%), transmission (20.18%) and popular names of the disease (20.61%), (the last three showed $p<0.05$). The affirmative "Cover the water containers and do not keep water pools" was a preventive measure showing the best answer for CHA (21.7%), dentists (50%), nurses (43.8%), physicians (38.9%) and veterinarians (28.6%). As for the forms of the disease, the response "symptomatic and asymptomatic" was 7.4% and 20.5% respectively, the responses of AZ and ACS, while 12.5% of dentists responded forms "classical and hemorrhagic". The transmission was answered incorrectly by the dentists (12.5%), CHA (10.8%) and physicians (11.1%) using the affirmative "the disease is transmitted by the bite of *Aedes aegypiti* vector". Another incorrect affirmative answered by AZ (8.42%), CHA (20.48%), dentists and nurses (12.5%) and vets (28.57%) was: "The control of leishmaniasis is based in dogs vaccination, treatment of patients and elimination of standing water". The conceptual gaps are evident, supporting the necessity to better prepare health professionals and increase the information about leishmaniasis. Supported by: FIOCRUZ and FUNEDI.

## EP.19 – USE OF HUMAN BIOPSIES FROM CUTANEOUS LEISHMANIASIS LESIONS FOR PCR DIAGNOSIS: COMPARISON WITH CLASSICAL LABORATORY METHODS.

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The diagnosis of cutaneous leishmaniasis (CT) is time consuming, and do not discriminate the leishmania species implicate in the pathological process, one of the actual clinical questions for directing a better therapy. The technique of polymerase chain reaction (PCR) using specific primers (as the ones direct to kDNA) has been reported as a useful tool for CT diagnosis. Here we present a pilot study comparing three well known methods for CT diagnosing: search of amastigotes in a stained-slide, parasites in vitro isolation and kDNA-PCR. This pilot study is part of a project where PCR amplifications with five different and prospective primers will be compared aiming at a specie-specific CT diagnosis. Biopsies were collected from patients presenting typical clinical manifestations at the leishmaniasis ambulatory of the IIER, São Paulo, Brazil. Slides were made with biopsy imprinting, stained and microscope examined. The remaining patient biopsy was transported to IMT-USP, imbedded in antibiotic solution, then was sliced for: DNA extraction and parasite isolation by culture media incubation. After DNA extraction, DNA samples were PCR amplified with kDNA primers. From the 52 patients analyzed, 39 (75%) were positive at parasitological examination, and 47 (90,38%) by kDNA PCR. All samples positive for parasitological examination were positive for PCR. As expected, the kDNA-PCR was able to confirm the disease in patients not positive by others methods, demonstrating the better sensibility and PCR successful from human biopsies. Previous comparison among kDNA PCR and techniques of parasite isolation shown 90,38% positivity for PCR (47 patients), against 29,82% by both isolation methods (only 17 samples). The PCR was easier to execute, more efficient and faster than culture of parasites from human biopsy. The data demonstrated that kDNA-PCR technique could be applied as an alternative laboratory method, and must be validated as a specific tool for CT diagnosis. Supported by CNPQ and LIM48-FMUSP.

## EP.20 – EVALUATION OF PCR TECHNIQUE FOR THE DIAGNOSIS OF CANINE VISCERAL LEISHMANIASIS IN DIFFERENT TISSUES OF SEROPOSITIVE DOGS

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The classical diagnosis of canine visceral leishmaniasis (CVL) basically consists in the isolation of parasite *in vitro,* its identification by microscopic analysis and/or serological tests. It is possible to infer that these methods are difficult to be implemented, time-consuming and present low level of specificity when compared to diagnosis of related parasitic diseases. With the development of molecular methods such as Polymerase Chain Reaction (PCR), the diagnosis of CVL became more accurate. In order to improve the efficacy of this reaction, we analyzed four different tissues samples from dogs with CVL aiming at the highest sensibility after PCR amplification using specific DNA primers directed from the minicircle kinetoplast DNA (kDNA). We compare samples collected from tissues such as blood, spleen, lymphonode and intact skin of 26 seropositive dogs from the Serviço de Vigilância Epidemiológica of Embu das Artes (located on São Paulo boundary). Afterwards, PCR results were compared to the ones stemmed from serology test, *in vitro* parasite isolation and stained-slide analysis. Previous analysis shown spleen samples as the more appropriate tissue to be used, since 96% confirmed the parasitological diagnosis (23 positive and 2 negative), while intact skin, lymphnode and blood samples shown respectively 92%, 88%, 80% of concordant results. Interestingly, at least 2 dogs from the 26 diagnosed by serology as CVL positive, presented here, parasitological and PCR test negative, suggesting that both dogs could be kept alive. Besides spleen samples presented the best results, we are working towards to use a less invasive sample (as blood) in the final diagnosis. In addition, a more specific primer, capable to discriminate the leishmania species by PCR analysis, must be developed.
Supported by: CNPq, and LIM-48, FMUSP.

## EP.21 – EVALUATION OF PARASITE RECOMBINANT PROTEIN AS ANTIGENS FOR CUTANEOUS LEISHMANIASIS SERODIAGNOSIS.

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Serological tests with crude *Leishmania* antigens are important tool for the diagnosis of leishmania infection. However, crude *Leishmania* antigens despite being very sensitive some of the antigens exhibit cross-reacting epitopes shared by other pathogens and not always present reproductive results. The aim of this study is to develop diagnosis of Tegument Leishmaniasis using some parasite recombinants antigens. In this study ELISAS were performed using a series of intracellular leishmania recombinant proteins to screening antigenic proteins for Cutaneous Leishmaniasis (CL) and Mucosal Leishmaniasis (ML) patients' serum. To identify the effectiveness of the recombinant proteins we used ROC curves and correlation analysis to select possible optimal antigens and to discard crude *Leishmania* antigens. In the first series of experiments, HSP70 and nucleosomais histones recombinant proteins were selected in order to combine good sensitivity and/or specificity. In further experiments we identified the best effectiveness of HSP70 protein and HSP70 plus H3 for ML and CL patients´ serum respectively. In the last step of preliminary experiments, using sera from patients with different responses to anti-leishmania IgG, we observed that some proteins were more specific for different ranges of antibody titers. Ours results show the high antigenicity of some *Leishmania* recombinant proteins, suggesting the possibility of using recombinant proteins to probe for Tegument Leishmaniasis serodiagnosis. Supported by CNPq, FIOCRUZ and CYTED.

## EP.22 – FIELD RANDOMIZED DOUBLE-BLIND TRIAL TO EVALUATE THE EFFICACY OF A VACCINE AGAINST CANINE VISCERAL LEISHMANIASIS IN PORTEIRINHA MUNICIPALITY, SOUTH-EASTERN BRAZIL: I - PREVALENCE OF BASELINE OF ASSAY, 2008.

Tibúrcio, A. M. L.[1]; França-Silva, J. C.[2]; Regina-Silva, S.[3]; Cruz, A. C. S.[4]; Almeida, S. N.[4]; Campos, L. M.[1]; Ferreira, D. G.[1]; Dias, E. S.[3]; Machado-Coelho, G. L. L.[5*]

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Brazil is an endemic country for zoonotic visceral leishmaniasis (ZVL) and that regularly conducts prophylactics and epidemiologic control programs that involving treatment of human cases, insect vector control and the removal of seropositive infected dogs. Dogs have a fundamental role as much in that urbanization as in the maintenance of the disease in urban or rural areas. In the urban and rural areas of municipal of Porteirinha, a city near of Montes Claros, were accomplished a survey to estimate the prevalence of Canine Visceral Leishmaniasis (CVL). The serology was accomplished using eluate of dried blood in filter paper, confirmed by serum blood of the negative samples. Both were processed by Enzyme Linked Immunosorbent Assay – ELISA and Immunofluorescence antibody test - IFAT. Blood samples were collected of 2,629 dogs distributed in all 14 urban areas (2,015 dogs) and six of 11 rural districts (614 dogs). The prevalence of CVL were 20.6% (9.1% - 55.3%) and 73.3% (35.9% - 92.8%) in the urban and rural areas, respectively. The prevalence gray zone values of ELISA were 9.4% (5.7% - 25.0%) and 1.3% (0.0% - 4.7%) in the urban and rural areas, respectively. After serologic survey, a sample of 865 negative dogs by ELISA and IFAT were selected to receive three doses and one booster of Leish-Tec® vaccine. In highest infection force areas 150 negative, pre-vaccinated beagle dogs were domiciled for serve as sentinels' dogs. In function of high prevalence, Porteirinha was considered as an active high infection transmission area, being appropriate for follow-up of dogs in a field assay to evaluate the Leish-Tec®. Supported by Hertape Calier Saúde Animal S.A.

## EP.23 – A PROTEOMIC APPROACH TO THE "RECOGNOME" OF THE *PLASMODIUM FALCIPARUM* INFECTED RED BLOOD CELL SURFACE

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After multiple *Plasmodium falciparum* infections, some individuals become asymptomatic carriers, protected from clinical malaria. While cellular immunity is required, antibodies that recognize proteins localized on the merozoite and at the red blood cell surface seem to play a major role to control the infection but the identity of many antigens is still elusive. In the Amazon, the repertoires of variant antigens in *P. falciparum* populations is very low, offering the opportunity to investigate the antigens involved in protection without having to sample a large number of parasite isolates. Herein, we apply a proteomics strategy to identify unknown antigens differentially recognized by the antibodies present in the serum of symptomatic ("unprotected") and asymptomatic ("protected") patients. Erythrocyte ghosts infected with a *P. falciparum* Amazon isolate were fractionated from the parasites and the proteins extracted with SDS page buffer. After 1D electrophoresis separation, western blotting was applied to check the presence of antibodies in the serum of symptomatic and asymptomatic patients from the Amazon. Neither sera from symptomatic or asymptomatic patients reacted with uninfected ghost preparations. Sera from patent malaria patients poorly reacted with infected erythrocyte proteins, while oligosymptomatic patients – without fever – strongly reacted with at least 9 proteins of molecular weights above 80 kDa. The reactivity of sera varied and the strongest reaction was observed in sera from oligosymptomatic carriers. The reaction of asymptomatic and oligosymptomatic sera also varied in dependence of the isolate analyzed. Several protein species were differentially recognized in different isolates, however, the observed protein sizes do not coincide with the sizes of expected major variant antigens such as RIFIN, STEVOR, or PfEMP1. Present efforts are now guided to mass spectrometry identification of the identified proteins. Supported by PRONEX, FAPESP and CNPq.

## EP.24 – TOXOPLASMOSIS: EPIDEMIOLOGIC ASPECTS AND SEROPREVALENCE OF *T. GONDII* IN PREGNANT WOMEN AT THE HEALTH CARE CENTER (SUS) IN DIVINÓPOLIS CITY, MG, BRAZIL

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Toxoplasmosis is a zoonotic disease of worldwide distribution. Infection with *Toxoplasma gondii* can cause several clinical syndromes including encephalitis, chorioretinitis, congenital infection and neonatal mortality. In pregnant women can develop fatal disease. The objective of this work was to evaluate the prevalence of toxoplasmosis by serological screening in pregnant women at the Municipal Health Care Center from October 2007 to September 2008 in Divinópolis city. The quantitative inquiry provided information about toxoplasmosis infection in 200 pregnant women and 1823 samples were analyzed. The results showed that 49% and 3.4% were positive for IgG and IgM, respectively. We observed an increase number of acute toxoplasmosis in pregnant women from 25 - 30 years old. Only 4.16% from 432 women showed IgM positive. A comparative study showed an irregular distribution from 11 regions ($p<0.01$). However, we observed a significant difference between the total numbers of pregnant women in each region. The results unfortunately demonstrated that 93% of pregnant unknown about toxoplasmosis, 24% had contact with the parasite and none of them showed acute form. We observed the correlation ($p=0.0455$) of IgG positive and presence of animal. Some characteristics increase the risk of toxoplasmosis transmission: presence of rodents and cats, contact with soil and mainly by ingesting tissue cysts in raw or undercooked meat. Moreover, low level of acknowledgments can contribute to maintenance the disease. Therefore, preventive measures may reduce the risk of primary infection during pregnancy and control the increase number of toxoplasmosis cases. Such data are essential to elucidate the relative importance of the various sources of infection for humans, to control disease, and to prevent reduction in quality of human life caused by this parasite.
Supported by FUNEDI, FIOCRUZ and FAPEMIG.

## EP.25 – THE KNOWLEDGE OF PROFESSIONALS IN BASICS HEALTH UNITS ABOUT PREVENTIVE ACTIONS OF TOXOPLASMOSIS IN PREGNANT WOMEN, MARINGÁ, PARANÁ, BRAZIL

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Toxoplasmosis can be transmitted to humans by ingestion of cystis/oocysts present, respectively, in tissues of hosts that serve as food and in water, fruits and vegetables contaminated, in adittion to tachyzoites on congenital way. This study evaluated the knowledges of professionals of health belonging to basic Health Units of Maringá (BHU), in relation with control of toxoplasmosis in pregnant women. A questionnaire valid was administered to health professionals who work with pregnant women on 21 from 26 BHU who exists, containing dates about contamination sources and prevention in pregnant women seronegatives. After statistical calculating the sample (expected frequency of 50%, confidence level of 95%) From 379 professionals were interviewed 214 (72/118 doctors, 25/68 nurses and 117/193 technicals/nurse assistants). The faeces of young cat and their presence were considered important sources of transmission for 42,2% and 29,8% of doctors, 56% and 32% of nurses and 68,7% e 47,3% of technicals/nurse, respectively. The consumption vegetables raws can be important source according 32.2% doctors, 60% nurses and 50.4% technicals/nurse. The ingestion of fresh sousages was considered source according only 17,4% of doctors, 16% of nurses and 18,3% of technicals/nurse, as well as the ingestion of beef, pork, lamb or chicken raw meat or undercooked to 22,3%, 12,4%, 14,9%, 2,5% of doctors, 40%, 32%, 32%, 28% of nurses and 25,9%, 27,5%, 30,5%, 13,7% of technicals/nurse, respectively. Were considered as important preventive actions the hands washing/surfaces of boards/knives after handling raw meats for 44,6% of doctors, 56% of nurses and 58% of technicals/nurse; the hands washing after handling soil/sand and avoid the ingestion of unpasteurizing milk for 47,1%, 22,3% of doctors, 76%, 32% of nurses and 68,7%, 41,2% of technicals/nurse, respectively. Is necessary the regular realization of workshops for these three professional classes, because exist an expressive lack of knowledge about sources of contamination and actions of prevention.
Financial support: Araucária Foundation.

## EP.26 – NOTIFICATION OF ANTI-*TOXOPLASMA GONDII* IGM ANTIBODIES CASES THE EPIDEMIC SURVEILLANCE OBTAINED OF PREGNANT WOMEN IN THE PERIOD OF 2007-2009

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The parasitic apicomplexa obligate *Toxoplasma gondii* induce toxoplasmosis a disease that affects millions of people in the world. The infection occurs by ingestion of oocysts from water or food contamined excreted in faeces of felines or ingestion of undercooked meat containing cysts. Most of infected people don't present symptoms, but some patients have only lymphonodular febrile acute syndrome or chronic retinochoroiditis. In certain groups induce severe disease, as congenital toxoplasmosis in pregnant women with fetal complications and toxoplasmic encephalitis in immune-deficient patients. Several laboratory methods are used for toxoplasmosis detection, mainly serology. In this study we investigated notified cases of positive IgM antibodies the epidemic surveillance in the period of 2007-2009 obtained of pregnant women with 17 and 43 years old during prenatal care accomplished by public health system. The serologic diagnosis was established by enzyme-linked immunosorbent assay (ELISA) and avidity test according to instructions at the Municipal Laboratory of Cascavel, Paraná. The annual serology profile was determined by frequency of anti-*Toxoplasma gondii* IgG, IgM antibodies and avidity of IgG where during this period were notified 58 cases in 2007, 31 cases in 2008 and 9 cases in 2009. The prevalence in 2007 was: 49 IgG and IgM positives cases (84,48%) where 13 (26,53%) accomplished IgG avidity assay; 6 IgG positive and IgM inconclusive cases (10,34%) where 3 with avidity assay (50%); 3 IgG negative and IgM positive cases (5,18%) where 1 accomplished IgG avidity assay (33,33%). The prevalence in 2008 was: 25 IgG and IgM positives cases (80,65%) where 22 (88%) accomplished IgG avidity assay; 6 IgG positive and IgM inconclusive cases (19,35%) where all patients with avidity assay (100%). In 2009 the prevalence was: 9 IgG and IgM positive cases where all accomplished IgG avidity assay (100%). The epidemic surveillance is indispensable to monitoring the IgM positive cases with evidence acute infection and risk of fetal damage. It was observed that in the last 3 years the municipal diagnosis centre incorporated in the routine the avidity test to discriminate infection recent and last reducing congenital toxoplasmosis and this proceeding came to improve the toxoplasmosis diagnosis in Cascavel pregnant women.
Financial support: LAP-UNIOESTE-PR.

## EP.27 – NOTIFIED CASES OF ANTI-*TOXOPLASMA GONDII* IGM ANTIBODIES IN SERA FROM PREGNANT WOMEN IN THE PERIOD OF 2006-2008

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Toxoplasmosis is a worldwide protozoan infection who affects 30% of the human population. In most of the cases is non-symptomatic, but the protozoan obligate parasite *Toxoplasma gondii* induce severe fetal disease, when infected during pregnancy for the first time, causing acute infection. In this case their surveillance is mandatory during prenatal care, usually by serology, especially uninfected women at risk of contracting the disease must be followed by monthly serology, for therapy of seroconverted reducing fetal lesions. For this purpose several assays are used as ELISA and others in large centers and smaller cities, but the diagnosis became difficult due high sensibility of commercial assays. In our study we investigated only notified cases of positive IgM antibodies the epidemic surveillance in the period of 2006-2008 obtained of pregnant women during prenatal care. These pregnant women are assisted in UBS's (Basic Units of Health) of public health system and the serologic diagnosis was established by enzyme-linked immunosorbent assay (ELISA) according to instructions, at the Municipal Laboratory of Cascavel, Paraná. Most of the pregnant women had among 19 and 29 years old (60,46%). According their anti-*T.gondii* serology 86 cases were notified and the frequency of anti-*Toxoplasma gondii* IgG and IgM antibodies in sera samples from these pregnant women were: 75 (87,21%) IgG and IgM positive; 1 (1,16%) IgM negative and 10 (11,63%) IgG negative; 10 (11,63%) IgM inconclusive and 1 (1,16%) IgG inconclusive. The sera-prevalence showed that the presence of 10 negatives anti- *T. gondii* IgG antibodies cases e 10 inconclusives anti-*T. gondii* IgM antibodies cases in the gestacional period can had resulted in false negatives affecting the medical conduct and adequate treatment. The careful search of anti-*Toxoplasma gondii* IgG and IgM performed commercial assays and also avidity test must be established for improvement in the congenital toxoplasmosis diagnosis in this diagnosis centre. Financial support: LAP-UNIOESTE-PR.

## EP.28 – OCCURRENCE OF *Giardia sp* IN HUMANS AND ANIMALS IN THE MUNICIPALITY OF ANGULO, PARANA STATE, BRAZIL

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*Giardia intestinalis*, also known as *G. lamblia* and *G. duodenalis* is found in several species of mammals, including humans. This parasite causes giardiasis, infection with or without symptoms like diarrhea, abdominal pain, bloating and malabsorption. Due to the number of giardiasis cases detected in the last months by Municipal Health Department of the Angulo, Parana/Brazil, this study aimed to verify and confirm the occurrence of *Giardia* in humans and animals in this municipality. Stool samples were collected from March to abril/2010 of 134 individuals (adults and children from day care center (67), from municipality (30) and state (37) schools, including family members of positive cases) and four dogs. The individuals interviewed were aged between 2 and 56 years, 77 females and 57 males. Analysis of fecal material was performed using Lutz and Faust methods. Twenty-three (17.2%) samples obtained from humans were positive for *Giardia*. Of these, 60.9% were from the day care center, 13.1% of the municipality school and 26.0% of the state school. Of the dog samples, two (50%) were positive. Total positivity in humans is close to the rate observed in Brazil (20-30%), but the individuals of day care center showed positive results in high percentage. The age and the hygienic and sanitary conditions may be the determining factors for this high rate. Among infected individuals had a school food server of municipality school with high positivity, which is a risk factor for the students. The positivity of animals can also be considered a risk factor, because the homes of positive dogs had positive individuals. To investigate further the dynamics of transmission of this parasite between humans and animals and control infection greater number of samples will be analyzed and typed by molecular markers and etiologic treatment and educational activities will be proposed.
Supported by SETI/Fundo Paraná, CNPq, CAPES, PROAP/CAPES.

## EP.29 – OCCURRENCE OF *BLASTOCYSTIS* SP IN HUMANS AND ANIMALS OF THE MUNICIPALITY OF ANGULO, PARANA, BRAZIL

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*Blastocystis sp* is a single-celled protozoan belonging to the Chromista Kingdom, one of the parasites most found in the intestinal tract of animals, including humans. Can be found in the feces in diverse forms, being the most common the cystic. Little is known about this parasite, which has aroused interest in detecting it and study it. The blastocystosis is influenced by age and immune status of the host, often occur in children and immunocompromised individuals who may or no produce clinical manifestations. Like other intestinal parasites, it is believed that infection by *Blastocystis sp* occurs through fecal-oral route, by water, vegetables and animals. As a result of giardiasis cases detected by the Health Department of city of Angulo/PR, especially in children, we have proposed in this work verify the occurrence of *Blastocystis* sp in school children, their families and dogs from peridomestic from this municipality. One hundred and thirty-eight fecal samples were collected from march to abril/2010, being 134 from humans and 4 from dogs. The ages of patients ranged between 2 and 67 years, being 77 females and 57 males. The samples were examined using saline wet mount, Lutz and Faust. Thirteen (9.7%) samples from humans and one (25%) from dog were positive for *Blastocystis sp*. In the house where was the positive dog also had a patient positive for this parasite, suggesting the involvement of this animal in the transmission chain. In this study, positive results for humans were lower than that observed in other studies conducted in the state of Parana (18-28%). However, larger number of samples will be analyzed by giving high attention to the diagnosis of *Blastocystis sp*, as well as to study the transmission dynamics of this parasite between humans and animals, since it is present in several hosts and associated with poor hygiene.

Supported by Fundo Parana and CNPq.

## IM.01 – IDENTIFICATION OF NOVEL *Leishmania infantum chagasi* ANTIGENS THAT HELP THE SERODIAGNOSIS IN VISCERAL LEISHMANIASIS

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Visceral leishmaniasis (VL) is a chronic and potentially fatal disease caused by the *Leishmania* genus. Rio Grande do Norte is endemic for VL, where the etiological agent is *Leishmania infantum chagasi* (*Lic*). Treatment requires toxic drugs and diagnosis depends of parasitological confirmation on bone marrow. There is neither vaccine nor gold standard for immunodiagnosis, therefore, purified antigens are essential to be used like infection marker. Thus, our aim was to test the effectiveness of four *Lic* amastigote antigens in the detection of specific antibodies in humans and dogs resident in Rio Grande do Norte. The antigens were expressed, purified, tested by ELISA and Western blot, and compared to *r*K39 (Burns, 1993) and soluble *Leishmania* antigens (SLA). Antigens termed 314, 319, 503 and 648 had sensitivity and specificity, respectively: 95.24%-19.35%, 90.48%-51.61%, 95.24%-22.58%, 28.57%-90.32% when compared to SLA, and 90.48%-11.54%, 80.95%-30.77%, 95.24%-15.38%, 33.33%-84.62% when compared to *r*K39. With human serum, the antigens showed sensitivity and specificity, respectively, 76.14%-78.52% (314), 90.91%-57.78% (319), 69.32%-71.11% (503), 95.40%-58.54% (648) when compared to *r*K39, and 46.56%-75.76% (314), 62.96%-45.45% (319), 46.56%-63.64% (503), 69.68-39.39% (648) when compared to SLA. The relationship between the presence of infected dogs in the neighborhood and human infection shows that, the diagnosis with 503, 64.44% of asymptomatic dogs that had positive serology were related with positive humans too. This data illustrate the necessity to monitor the dogs for prevent the parasite spread. Western blot with human serum showed a better recognition of 648 antigen, whereas with the canine serum 503 stood out. The Western blot showed to be a more sensitive test for the diagnosis, and 648 and 503 the best antigens for diagnosis of human and canine infection, respectively.
Supported by CNPQ, CAPES, NIH.

## IM.02 – IMMUNOLOGICAL PROFILE INDUCED BY *LEISHMANIA MAJOR* INFECTION IN A MODEL OF PARAFFIN IMPLANTATION

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A model of monocytic inflammation using a subcutaneous implantation of paraffin tablets is able to produce a chronic inflammatory reaction. When paraffin implantation is coupled with *Leishmania major* infection, both genetically susceptible (BALB/c) and resistant (C57BL/6) strains of mice have large amounts of parasites associated with inflammatory macrophages at 21 days post infection. At the present study we have determined cytokines produced in inflammatory capsule. This model was carried out by implantation of paraffin tablets under the dorsal skin of Balb/c or C57BL/6 mice. Mice were then infected with *L. major* and sacrificed 21 days after infection. Inflammatory capsule was collected for histopathology and cytokines measures by RT PCR. Cytokines IL-12, TNF-alfa and IFN-gamma associated to resistant phenotype and IL-4 and IL-10 associated to susceptive phenotype to *L. major* infection were determined. Otherwise, chemokines related to monocyte-macrophage and lymphocyte recruitments were also investigated. RT-PCR analysis has shown that BALB/c mice showed strong IL-4 and IL-10 mRNA expression than controls with very little expression of IFN-gamma. In contrast, both IFN-γ and IL-10 mRNA was found in higher levels in C57BL/6 animals. Moreover, in C57BL/6 mice the expression of chemokines mRNA of CCL3/MIP-1 alpha was more highly expressed than CCL2/MCP-1, suggesting differential and important role in recruitment and activation of distinct immune cells which can define a permissive profile at the site of *L. major* infection. We conclude that the Th1 immune response C57BL/6 did not change to a Th2 response. Supported by CAPES

## IM.03 – EVIDENCE FOR ENDOGENOUS INTERLEUKIN-10 DURING NOCICEPTION IN THE EAR MODEL OF *L. MAJOR* INFECTION

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Cutaneous leishmaniasis (CL) has been experimentally reproduced by inoculating low dose parasite loads into an intradermal site (ear). *L. major* infected BALB/c mice presented severe lesions and Th2 response. IL-4-/- C57BL/6 and BALB/c mice showed healing lesions and Th1 response. Th1 and Th2 cells secrete pro- and anti-inflammatory cytokines, respectively. Classical description of syndromes produced by CL does not include pain complaints, and some studies reported painless lesions. Using the ear model, we investigated variations in nociception along 12 weeks post infection and its relationship to IL-10 production in BALB/c susceptible mice and in IL-4 -/-C57BL/6 and BALB/c resistant mice. The infection induced hyponociception in BALB/c after wk 9, followed by a decrease of IL-10tissue levels. C57BL/6 showed a short-lived hypernociception in wk 2, followed by an IL-10 local increase. IL-4 -/- BALB/c mice showed a sustained hypernociception from wk 1 associated to an IL-10 increase at wk 12. Recently, we have showed that cytokines such as IL-6, TNF-α and KC contribute to afferent nerve sensibilization. However, exogenous IL-10 has been demonstrated to prevent the development of dynorphin-induced allodynia, presumably by inhibiting pro-inflammatory cytokines. In the other hand, our results suggest that endogenous IL-10 may be involved in nociception induction in this infection model. In support to our observations, recent data indicate the involvement of endogenous IL-10 in nociception. Regardless the mechanisms, these results suggest that further studies on IL-10 and other cytokines in nociception are needed. Financial Support: FAPEMIG(CBB-1048/05), CAPES and CNPq.

## IM.04 – EFECTIVENESS OF IMMUNOENZYMATIC METHOD FOR RESEARCH INTO PROTOZOAN CYSTS IN HIV PATIENTS

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Diarrheal diseases associated with parasitic infections are highly prevalent in HIV patients. Among the protozoa, *Cryptosporidium* and *Giardia duodenalis* are major etiological agents of diarrheal infections. The enzyme immunoassays have replaced the microscopic routine procedures in hospitals and health services. These tests are sensitive and specific and allow analysis of numerous samples. The aim of this study was to evaluate the efficiency of the immune ELISA method to detect antigens of *Giardia duodenalis* and *Cryptosporidium* spp in fecal specimens of HIV patients in comparison with conventional techniques of microscopy, to demonstrate the importance of employment in a routine laboratory method more sensitivity from a single stool sample. Methods were employed to parasitological flotation, Ziehl-Neelsen modified and enzyme immunoassays for *Giardia* and *Cyptosporidium* Test II. The samples were collected in three alternate days and stored in 10% formalin and sent to the Laboratory of Parasitology /DBS/UEM where they were examined. We collected 132 samples of faeces. 45 (34%) were positive for some intestinal parasite. Through the parasitological methods have been diagnosed *Entamoeba coli* (40%), *Giardia duodenalis* (20%) *Strongyloides stercoralis* (13%), *Cystoisospora* sp (13%), *Blastocystis hominis* (6,6%) and detection of *Cryptosporidium* sp negative. The enzyme immunoassays were negative for *Cryptosporidium* and *Giardia* sp (63%) positive. It is observed in this sample there is no correlation of parasitological methods and immunoassay for the diagnosis of *Giardia* sp and there may be cross reactivity between commensal protozoa. For the diagnosis of *Cryptosporidium* concordance of methods suggesting that the immunoenzymatic method could be used routinely because it is easy to perform and interpret compared to the method of Ziehl-Neelsen modified. Supported by Fundação Araucária

## IM.05 – INCREASED TRL2 AND TLR4 EXPRESSION IN MONONUCLEAR CELLS FROM DOGS NATURALLY INFECTED BY LEISHMANIA SP. AFTER IMMUNOMODULATOR TREATMENT

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Visceral leishmaniasis in dogs causes immune system unbalance. In some advanced stages of visceral leishmaniasis there is an increase in the production of anti-leishmania antibodies and leishmania antigen-specific lymphoproliferative unresponsive; there is a decrease of gama IFN production with a concomitant increase of IL-10. The chemotherapy is not effective and reoccurrence is often observed after treatment due to imperfect elimination of the parasite. Furthermore, the use of antimony in dogs can select resistant strains to these drugs. It is therefore, essential to study new alternatives for the treatment of infected dogs, which may reduce the incidence of the disease in epidemic areas. The immunmodulator, P-MAPA, a proteinaceous aggregate of ammonium and magnesium phospholinoelate-palmitoleate anhydride derived from Aspergillus Oryzae, has been shown to induce immunity. However, the process in innate immunity is unknown. Mononuclear blood cells of infected dogs were assessed for cell surface expression of TRL2 and TRL4 following incubation with 2,5; 5; and 10 ug/ml of P-MAPA, these receptors were measured using monoclonal antibody by flow cytometry. P-MAPA 2,5 and 10 ug/ml showed higher TRL2 surface expression when compared with the baseline expression (65,42% baseline ; 75,45% P- MAPA -2,5 µg/ml; 81,10% P- MAPA -10 µg/ml). Similarly, 10ug/ml P-MAPA increased TRL4. Our findings showed that P-MAPA increased TRL2 and TRL4, which enhanced innate immune response. The sequential effect of P-MAPA is being investigated. Financial Support FAPESP

## IM.06 – REAL TIME PCR FOR DETECTION OF LEISHMANIA SPP IN SPLEEN TISSUE FROM DOGS NATURALLY INFECTED

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*Leishmania* spp. is intracellular protozoan parasites that cause a wide spectrum of diseases in humans and dogs worldwide. The performance of the less expensive SYBR-Green-based PCR assay, for quantifying *Leishmania chagasi* in spleen tissue samples was to monitor the efficacy of antileishmanial drugs or vaccines. The assay was performed with the LightCycler system using SYBR Green and primers 150 and 152 that amplifying a 120-bp fragment from minicircles of the kinetoplast DNA (kDNA). Twenty dogs with clinical manifestation of visceral leishmaniasis and positive serology for anti-Leishmania antibody were included in study group. DNA was extracted from spleen samples using Hight Pure PCR Template Preparation Kit (ROCHE). There was no evidence of PCR inhibition when the DNA was isolated from spleen sample. The standard curve designed for quantitation of parasite showed linearity with a correlation coefficient of 0,99 and slope -3,058. All dogs with symptoms and positive serology showed positive real-time PCR. Real Time PCR based on SYBR Green may therefore be an appropriate and inexpensive alternative for detection Leishmania spp in dogs.
Financial Support : FAPESP

**IM.07 – APOPTOSIS IN THE SPLEEN AND PERIPHERAL BLOOD IN DOGS NATURALLY INFECTED BY *LEISHMANIA (L.) CHAGASI***

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Dogs are the main domestic reservoirs of *L. (L.) chagasi*. In naturally infected dogs changes in the white pulp of the spleen and decrease of lymphocytes in peripheral blood are frequently observed. To investigate whether if apoptosis is responsible for such features T cell apoptotic were quantified from the spleen and peripheral blood of dogs naturally infected with *L. (L.) chagasi*, with clinical manifestation and compared with healthy dogs. A total of 13 adult symptomatic dogs, were serum positive for *L. (L.) chagasi* by indirect ELISA. A group of 6 healthy dogs, from a non-endemic area were included in the study as negative controls. These animals were serum negative for *L. (L.) chagasi*, by indirect ELISA. Samples of spleen from both groups were removed by surgical excision after sedation. The mononuclear cells were simultaneous labeled with CD3 monoclonal antibody (Serotec, UK) and apoptosis (Anexin V kit and Mutilcaspase kit - Guava, Hayward, CA). The procedure of the test was in accordance with the manufacturer's instructions. Data were acquisition in EasyCyte mini ® (Guava, Hayward, CA), the analysis of the data was held in the Software Express Plus ® Guava. Data clearly indicated that T lymphocytes from PBMC and spleen tissue of infected dogs showed a significantly higher level of apoptosis compared with that observed in healthy controls ($p<0.05$, Wilcoxon test). We showed that the apoptosis level in T cell from the spleen and peripheral blood were higher in infected dogs when compared to that of healthy ones showing that the presence of *L. (L.) chagasi* induces apoptosis in T cell. Since the progression of infection is related to the impairment of cell mediated immunity the detection of T cell apoptosis could contribute to inefficient cell immune response during *L. chagasi* infection.
Financial Support FAPESP

**IM.08 – QUANTITATION OF REGULATORY T CELL IN SPLEEN AND PERIPHERAL BLOOD IN *L. (L.) CHAGASI* NATURALLY DOGS INFECTED**

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Dogs are the main domestic reservoirs of *L. (L.) chagasi*. Once in the vertebrate host, the parasite may cause visceral leishmaniasis, which can also be transmitted to humans. The infected dogs showed increase of anti-Leishmania antibodies and decrease of cellular immunity. Regulatory T cells (T reg) have been shown to be involved in the direct induction of immunosupression of effector cellular immune response. To investigate the possible involvement of T reg cells during *Leishmania* infection, the presence of T reg from the spleen and peripherical blood mononuclear cells (PBMC) of dogs naturally infected with *L. (L.) chagasi*, with clinical manifestation, were quantified. A total of 15 adult dogs from the Zoonosis Control Center of Araçatuba, S.P Brazil, and serum positive for *L. (L.) chagasi* by indirect ELISA and positive rK39 and 05 adult healthy dogs from non endemic area were included in the study. Samples from spleen and PBMC were used for quantification of T reg by flow cytometry using monoclonal antibodies for CD4 and Foxp3. In the spleen T reg levels in infected dogs were lower than in control groups ($P<0.05$), whereas in PBMC no differences were observed between two groups. These results suggest that T reg population is involved in *Leishmania* infection and may have a possible role in promoting parasite persistence and establishment of chronic infection. Financial Support FAPESP

## IM.09 – CCL2, CCL4 AND CCL5 CHEMOKINES INDUCE THE MONOCYTES AND PLAMOCYTES MIGRATION IN DERMAL OF DOGS NATURALLY INFECTED BY *LEISHMANIA INFANTUM*

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The immune response in the skin tissue of dogs infected by *L. infantum* and its association with the clinical progression during canine visceral leishmaniasis (CVL) is poorly understood and limited studies are available. In this work, a detailed analysis of the chemokines expression (CCL2, CCL4, CCL5, CCL13, CCL17, CCL21, CXCL8 and CCL24) using Real-Time PCR, as well as, the histopathology study focusing in the dermal inflammatory infiltrate (neutrophils, eosinophils, macrophages, basophils and lymphocytes) of 35 naturally infected dogs presented different clinical status of CVL. Infected dogs were subdivided according as follows: Asymptomatic (AD;n=10), Oligosymptomatic (OD;n=10) and Symptomatic (SD;n=15). Sixteen non-infected dogs (CD) were used as control group. Our results demonstrated that severe forms (OD and SD) of the CVL are characterized by the appearance of numerous clinical signs in the skin (localised or generalized alopecia, dermatitis and cutaneous lesions) and a positive correlation with skin parasite density was observed ($r$=0.4409/$p$=0.0080). Enhance of parasite load also was detected in the skin dogs showing the maximum clinical score (SD) when compared with AD ($p$<0.05). Skin of the SD group presented increase of CCL2 and CCL4 expression when compared with CD ($p$<0.05). Moreover, OD and SD presented increase of CCL5 expression in relation CD ($p$<0.05). Assessment of the skin inflammatory cells revealed increase of macrophages (%) and reduction of lymphocytes, eosinophils and basophils according to the clinical progression of CVL ($p$<0.05). In agreement with these results, increase of cellular infiltrate composed mainly by mononuclear cells was correlated with clinical evolution ($r$=0.5400/$p$=0.0004). In conclusion, our date indicate that higher expression of CCL2, CCL4 and CCL5 chemokines is associated with the severe disease and contribute for the orchestrate the cell migration (monocytes and plamocytes) to compose the intense dermal inflammatory infiltrate trying to control the infection. Supported by: Pronex 2007 (CNPq/FAPEMIG); CAPES; IRR/FIOCRUZ and UFOP

## IM.10 – FUNCTIONAL ALTERATIONS OF MACROPHAGES CULTURED IN HYPOXIA AND INFECTED WITH *LEISHMANIA AMAZONENSIS*

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Regions of low oxygen tension (hypoxia) are common features of inflamed/infected tissues. Macrophages exposed to hypoxia and infected with *Leishmania amazonensis* amastigotes are able to reduce intracellular parasitism. However, the mechanisms contribute to the resistance of macrophages to *L. amazonensis* infection under hypoxia are not known. In this study we investigated modifications of infected macrophages in hypoxia, such as NO and ROS synthesis, cytokines production, exo/phagocytosis, ATP release, HIF-2α expression, and whether apoptosis occurred in intracellular amastigotes. Our results indicate that hypoxia does not induce the synthesis of NO in macrophages infected with *L. amazonensis* as well as iNOS expression, and iNOS knockout macrophages lacking NO synthesis are still able to reduce infection when cultured in a hypoxia. Although noninfected macrophages produce more ROS in hypoxia than in normoxia, *L. amazonesis*-infected macrophages show similar levels of ROS in normoxic and hypoxic conditions. Antioxidants NAC (ROS scavenger) and Ebselen (glutathione peroxidase mimic) inhibit the leishmanicidal effect of hypoxia, indicating that ROS is important to the effect of hypoxia on leishmanial infection. The cytokines TNF-α, IL-12 and IL-10 releases are similar in normoxia and hypoxia by infected macrophages. Although hypoxia inhibits the phagocytosis of inert particles or fixed parasite, it does not affect *L. amazonensis* entry into macrophages. Hypoxic treatment does not induce the exocytosis of internalized particles by macrophages.Also Infected macrophages show similar levels of ATP in normoxia and hypoxia and apoptosis-like death in intracellular amastigotes does not occur in hypoxic conditions. HIF-2α immunoreactivity is elevated in nuclei of macrophage infected with *L. amazonensis.* Thus, with exception of ROS, NO, cytokines, phago/exocytosis and energetic metabolism of macrophages are not related to the anti-*Leishmania* activity of hypoxia. Furthermore, we can speculate if HIF-2α could be involved in the phenotype changes of infected macrophages in hypoxia. Supported by FAPESP and CNPq

## IM.11 – *LEISHMANIA INFANTUM* INFECTION IN THE GOLDEN HAMSTER MODEL

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The hamster is considerate a good experimental model to study visceral leishmaniasis (VL), considering the parasite visceralization and the disease progression that is similar to observed in humans and dogs. However, little is known in relation the evolution of the natural history during the experimental infection by *L. infantum* in hamsters as well as the use of this model to evaluate vaccine and/or drugs in pre-clinical therapeutic study. In the present work, different infection routes (intradermal, intracardiac and intraperitoneal) were tested to evaluate the disease progression. The outcome of the infection was assessed by clinical signs, serum levels of total-IgG, nitric oxide and parasite load in spleen tissue during 1, 3 and 6 months post infection with *L. infantum* promastigotes. Our majors results demonstrate that the infection culminate in the parasites dissemination regardless of the infection route. Splenomegaly was observed in 50% of intracardiac group and the histopathology analysis of the spleen reveled hyperplasia and hypertrophy in the white and red pulp, red pulp congestion and higher reactivity with exacerbation of white pulp in three groups during 1, 3 and 6 months after infection. These findings were most pronounced in the animals inoculated by intracardiac route. The increase of NO levels was detected in three groups in the months (1, 3 and 6) when compared to control group, regardless of the infection route. All groups of hamsters experimentally infected produced higher levels of IgG after infection when compared with the control. The animals infected by intracardiac route developed an intense polyclonal activity resulting higher production of IgG levels in sixth months after infection. Given the parallelism existing between the outcomes of *Leishmania* infection in hamsters, dogs and humans, we believe that our data illustrate that the hamster presented similar disease evolution confirming their capability as experimental model to study VL. Supported by: CAPES, FAPEMIG, FIOCRUZ and UFOP

## IM.12 – 1631 Murine infection by *Leishmania amazonensis* after oral treatment with pyrazole carbohydrazide derivatives: Histopathology study of cutaneous lesion

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Leishmaniasis is found in group of diseases known as Neglected Tropical Diseases. Since there is no antileishmanial vaccine in clinical use, control of Leishmanisis relies almost exclusively on chemotherapy. Synthetic new compounds pyrazole carbohydrazide were tested and produced no toxicity on peritoneal cells of mice and demonstrated *in vitro* activity in promastigotes of *Leishmania sp*. Furthermore, we observed significant therapeutic effect in experimental murine infection with *L. amazonensis* resulting in lower parasite load and reducing size of a skin lesion without causing any toxic effect. Our objectives were to analyze the inflammatory cells and evaluate the histology of skin lesions of mice infected with *L. amazonensis* and treated with pyrazole carbohydrazide. CBA mice were infected in the foot with *L. amazonensis*. The animals were orally treated from the second to sixth week after infection with 1.5mg/Kg/day of pyrazole carbohydrazide. At 12 weeks post-infection the animals were anaesthetized and sacrificed for histological examination. In immunohistochemistry were used monoclonal antibodies to identify B and T lymphocytes, macrophages and neutrophils. Histopathological study revealed that changes in the dermis are correlated to the macroscopic size of the lesion. In footpad of mice infected with *Leishmania* was observed an intense presence of vacuolated macrophages infected with richly diverse intracellular and extracellular amastigotes. In addition to dermal macrophages, we observed a mixed inflammatory infiltrate containing lymphocytes and neutrophils. CBA mice infected and treated with pyrazole carbohydrazide minimized skin lesions and the structures of the epidermis and dermis were found preserved with little inflammatory infiltrate. Although the dermis of treated animals were found vacuolated macrophages with intracellular parasites, they were far less numerous than in untreated animals. In conclusion, therapeutic activity of pyrazole carbohydrazide influence in reducing infiltration of macrophages, neutrophils, B and T lymphocytes in skin lesions during experimental murine cutaneous leishmaniasis. Supported by PROPPI/UFF, FAPERJ, CAPES, FIOCRUZ

## IM.13 – COMPARTMENTALIZED IMMUNE RESPONSE IN SPLEEN OF DOGS IMMUNIZED WITH LBSAP AND LBSAPSAL VACCINES AFTER EXPERIMENTAL CHALLENGE WITH *LEISHMANIA CHAGASI*

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The spleen provides an appropriate environment for the priming/activation of T cells by antigen presenting cells and microbicidal mechanisms by *Leishmania*-infected macrophages. Herein, we evaluated a detailed immunological analysis of spleen trying to understand the involvement of the cellular immune response, particularly in these vaccines phase I and II trials. In this context, two new vaccines against canine visceral leishmaniasis (CVL) were tested. LBSap vaccine, composed of *Leishmania braziliensis* antigen (LB) and saponin (Sap), and LBSapSal vaccine, composed of LB, Sap and sand fly gland extract (Sal). We evaluated the cellular immune response in spleen considering *ex vivo* and *in vitro* analysis by immunophenotyping and cytokine levels in the supernatant of *Leishmania*-stimulated cultures at 885 days after *Leishmania chagasi*-challenge (dac). Our major results showed that LBSapSal vaccine elicited increased levels of $CD5^+$ and $CD4^+$ T-splenocytes. In addition, *in vitro* immunophenotypic analysis of splenocytes in non-stimulated cultures showed higher counts of $CD8^+$ T-cells in dogs vaccinated with LBSap vaccine and also in splenocytes stimulated with soluble *L. chagasi* antigen (SLA) of dogs vaccinated with LBSapSal. Furthermore, the LBSap group exhibited a negative correlation between $CD8^+$ T-cells and IL-10 when stimulated by SLA ($P$=0.0075; r=–0.9286). Additional the analysis in LBSap group displayed a negative correlation ($P$=0.0074; r=–0.9287) between $CD4^+$ T-cells and TNF-$\alpha$ following stimulation with SLA. Similarly, LBSapSal group exhibited a negative correlations between $CD4^+$ T-cells and IL-10 ($P$=0.0038; r=–0.6929) and between $CD4^+$ T-cells and TNF-$\alpha$ ($P$=0.0033; r=–0.7074). In conclusion, our data suggested that the potential organ-specific resistance profile elicited in splenocytes in dogs immunized by both vaccines (LBSap and LBSapSal) at 885 dac. This immunological feature observed in spleens of LBSap or LBSapSal immunized dogs is compatible with effective control of the etiological agent of CVL.
Supported by PRONEX–FAPEMIG, CNPq, PAPES-V and UFOP.

## IM.14 – CYTOKINES LEVELS AND PARASITE LOAD IN THE BONE MARROW OF DOGS IMMUNIZED WITH LBSAP VACCINE AND CHALLENGED WITH *LEISHMANIA CHAGASI*

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A dog vaccine may be the most practical and effective method by which to reduce the incidence of human visceral leishmaniasis. In this sense, the vaccine composed by *Leishmania braziliensis* promastigotes protein plus saponin as adjuvant has been investigated as a pre-requisite to understanding the mechanisms of immunogenicity against canine visceral leishmaniasis (CVL). In this study, dogs were immunized with saline (C); *L. braziliensis* promastigotes protein (LB), Saponin (Sap), *L. braziliensis* promastigotes protein and saponin (LBSap). Cytokines (IL-10 and TNF-$\alpha$) in the supernatants of peripheral blood mononuclear cells (PBMC) cultures and parasite load in bone marrow were evaluated at times 90, 435 and 885 days after challenge (dac). Our major results demonstrated that LBSap group displayed significant decrease of IL-10 in the presence of both stimuli (vaccine soluble antigen-VSA or soluble *L. chagasi* antigen-SLcA) during 885 dac. Parasitological analysis in bone marrow was not displayed parasite load until 885 dac. In conclusion, the results of this study encourage the continuation of investigations related to parasitological and immunological events after challenge of this new vaccine against canine visceral leishmaniasis and the establishment of new biomarkers of immunogenicity. In this context, we are analyzing IL-4, TGF-$\beta$ and IFN-$\gamma$ cytokines and the *Leishmania* DNA load by Real Time PCR in different tissues for efficacy vaccine evaluation. Supported by PRONEX–FAPEMIG, CNPq, PAPES-V and UFOP.

## IM.15 – NEUTROPHILS ARE REQUIRED TO THE EARLY CONTROL OF *LEISHMANIA AMAZONENSIS* INFECTION IN BALB/C MICE

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Neutrophils provide the first line of defense against infection and contribute to the initiation of inflammation. However the role of neutrophils during infection with *Leishmania* is not clear. Moreover, most studies were performed in *Leishmania major* model of infection. Hence the aim of this work was to investigate the role of neutrophils during infection with *Leishmania amazonensis*. Our results showed that during the first hours after infection there was a massive migration of neutrophils to the site of infection in both BALB/c and C57BL/6 mice, however the presence of neutrophils was more prominent in BALB/c mice. We also demonstrated that the presence of neutrophils at the site of infection was essential for the expression of IL-1β on the first 24 hours post-infection. In the absence of neutrophils there was an exacerbation of lesions during the first week of infection in BALB/c mice, but not in C57BL/6 mice. However, the final outcome of the disease was not affected. The larger lesions were associated with higher activity of arginase at the infection site and higher parasite loads in the ears and draining lymph nodes one week post-infection. Also, there was increased secretion of IL -10 by draining lymph node cells of infected mice depleted of neutrophils. Neutrophil have also proven to be important for the migration of cells to the site of infection and draining lymph nodes. There was an increase in the numbers of dendritic cells in the ears one day post-infection followed by a reduction in the percentage of B cells and regulatory T cells and an increase in T cells at seven days after infection in depleted mice. In conclusion, our results indicate that neutrophils are involved in the early control of *L. amazonensis* infection in BALB/c, but not in C56BL/6 mice. Supported by CAPES, CNPq and FAPEMIG.

## IM.16 – MODULATION OF DENDRITIC CELL RESPONSE BY DIFFERENT SPECIES OF *Leishmania* AND THE PARTICIPATION OF EXTRACELLULAR-ATP AND ADENOSINE ON THIS PROCESS

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Dendritic cells (DC) play an essential role in the modulation of innate and adaptive immune response and several studies have evaluated the interaction between *Leishmania* and DC. Extracellular-ATP exhibits pro-inflammatory properties whereas adenosine is an important anti-inflammatory mediator. In this work, we investigated the effects of *Leishmania sp* infection on DC response and the participation of ATP and adenosine on this process. C57BL/6 bone marrow DC infected with metacyclic promastigotes of *L. amazonensis*, *L. braziliensis* or *L. major* showed decreased expression of CD86 and MHCII, and increased expression of 5'-nucleotidase. *L. amazonensis* was more infective than other species. In addition, we examined the proliferation of T CD4+ cells of *L. amazonensis* infected C57BL/6 mice as well as T cells from BALB/c mice (MLR) after co-culture with infected DC. *L. amazonensis* infected DC presented a reduced ability to induce cellular proliferation in both situations. On the other hand, *L. braziliensis* or *L. major* infections had no such effect. In order to evaluate the mechanisms by which these parasites can modulate the DC response, we used the most infective specie as model. IL-10 production was not altered after *L. amazonensis* infection. DC infection in the presence of suramin (an inhibitor of CD39 activity) enhanced CD86 and MHCII expression. Treatment with adenosine receptor antagonists was also able to increase the expression of the activation markers. Moreover, we demonstrate that the presence of suramin or adenosine receptor antagonists at the time of infection recovered DC ability to induce T cell proliferation. In conclusion, *Leishmania* promastigotes impair DC response and this process may be influenced by ATP hydrolysis and activation of adenosine receptors.
Supported by FAPEMIG and CNPq.

## IM.17 – RESTRICTION OF *LEISHMANIA AMAZONENSIS* INFECTION BY THE INFLAMMASOMES

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Pattern recognition receptors such as Nlrc4, Naip5, Nlrp3 and Nlpr1, belongs to the family of the Nod like receptors (NLRs), and plays a critical role in the activation innate immune cells in response to pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs). Once activated, these receptors trigger activation of a molecular platform composed by the protease caspase-1, so-called inflammasome. Although it is well established that inflammasomes play a critical role in inflammation and resistance against bacterial infections, there is virtually no information on the role of inflammasomes for recognition and restriction of protozoan parasite infections. Here, we investigated the role of the inflammasomes in the recognition and restriction of the infection by the protozoan parasite *Leishmania (L) amazonensis*. By investigating different inflammasomes, we found that Nlrc4-triggered inflammasome is important for recognition and restriction of *L. amazonensis* infection in macrophages and in vivo. Macrophages obtained from Nlrc4$^{-/-}$ and caspase-1$^{-/-}$ -deficient mice showed a diminished leishmanicidal activity in response to IFN-γ or IFN-γ+TNF-α activation. Furthermore, Nlrc4$^{-/-}$ and caspase-1$^{-/-}$ infected-mice developed more severe lesions containing higher parasite burdens. These features were accompanied by an impaired production of IL-1β in the spleen and draining lymph node and a diminished NOS2 expression in the lesion and lymph node. By searching for the mechanisms involved in the Nlrc4/caspase-1-dependent restriction of *L. amazonensis* infection, we found that IL-1β production was key for NO-dependent restriction of *L. amazonensis* multiplication in macrophages. Conversely, *L. amazonensis*-infected Nlrc4$^{-/-}$ and caspase-1$^{-/-}$ macrophages showed a reduction of NOS2 expression and NO production. Collectively, our data shows that inflammasome-derived IL-1β is a key mediator in the induction of effector immune responses toward *L. amazonensis* infection, primarily via NOS2 expression and nitric oxide generation. Importantly, this study shows for the first time that the inflammasomes effectively participate of the recognition and induction of innate immune responses against a protozoan parasite. Supported by FAPESP, CNPq, PEW and WHO/TDR.

## IM.18 – LBSAP-VACCINE; *IN VITRO* IMMUNE RESPONSE IN VACINATED DOGS AFTER CHALLENGE THROUGH INTRADERMAL INOCULUM USING PROMASTIGOTES OF *LEISHMANIA CHAGASI* PLUS SAND FLY SALIVA

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The control of *L. chagasi*/*L. infantum* infection in dogs is essential to interrupt the current spread of human visceral leishmaniasis. In this context, a vaccine against canine visceral leishmaniasis (CVL) would be an important tool in the control of human visceral leishmaniasis (VL). Recently our group developed and evaluated the immunogenicity of a vaccine against CVL composed by *L. braziliensis* antigens plus saponin (LBSap). Herein we show the results of the LBSap immunogenicity until 435 days after challenge (dac) by intradermal inoculum using 1x10$^7$ late-log-phase promastigotes of *L. chagasi* and saliva of *Lutzomyia longipalpis*. Dogs vaccinated with LBSap showed increased levels of numbers of both CD4$^+$ and CD21$^+$ lymphocytes and higher counts of circulating CD14$^+$ monocytes. Moreover, the circulating CD5$^+$ and CD8$^+$ T lymphocytes presented positive correlation with MHC-II expression by lymphocytes in the LBSap group. The evaluation of the *in vitro* immune response into LBSap vaccinated dogs including: lymphoproliferative reaction and immunophenotyping of peripheral blood mononuclear cells (PBMC) after antigenic stimuli using VSA (vaccine soluble antigen) and SLcA (soluble *L. chagasi* antigen). Our major results displayed in PBMC VSA-stimulated intense cell proliferation and an increased count of SLcA-CD4$^+$T-lymphocytes at 90dac in the LBSap group. Furthermore, higher cells proliferations were observed at 435dac in PBMC of LBSap vaccinated dogs after both VSA and SLcA stimuli. These results indicate an effective *Leishmania*-specific immune response even after challenge. In addition, the LBSap group showed at 90dac, higher expression of MHC-II in the lymphocytes cells populations following *in vitro* stimulation with SLcA. LBSap group exhibited positive correlations between CD4+ T-cells and MHC-II expression in cultures stimulated by SLcA or VSA. Taken together, these findings supported the hypothesis that the vaccination using LBSap vaccine elicited strong immunogenicity after challenge with *L. chagasi* maintaining potentially and compatible effective control of the etiological agent of CVL. *Supported by:* FAPEMIG, UFOP, FIOCRUZ, CNPq and CAPES.

## IM.19 – THE ROLE OF *LEISHMANIA*'S NTPDASES IN THE PROCESS OF PARASITE ADHESION TO MACROPHAGES

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ENTPDases are enzymes that have the ability to hydrolyze di and triphosphate nucleotides under the stimulus of bivalent ions. Recent studies demonstrate the important role of this enzyme in the process of parasite adhesion and internalization into host cells. Our study aims to understand more deeply the mechanism by which this enzyme favors infection by parasites of the genus *Leishmania*. To achieve the objectives of this study, experiments were performed in order to evaluate adherence rate (30 minutes) and internalization (3 hours) of *Leishmania amazonensis* by peritoneal macrophages using optical microscopy. After treatment of macrophages with recombinant ENTPDase, we noted a reduction in the percentage of cells with adhered parasites and the rates of infection after three hours. This result indicates that the macrophage may present a ligand responsible for adhesion of the enzyme that is important in the process of parasite uptake. The same results were obtained after incubation of parasites with a polyclonal antibody anti-ENTPDase for 30 minutes. Furthermore, the increased expression of the enzyme induced by growth of the parasite with suramin (ectonucleotidases inhibitor) improved the rate of adherence and internalization of the parasites. On the other hand addition of adenine to culture medium reduced the expression of the enzyme also reducing the uptake rates. Interestingly, in all cases there were no differences in numbers of adhered parasites or the number of amastigotes per macrophage, which suggests a limited number of the ligand on the macrophage membrane and an "all or nothing" expression of this molecule by the host cell. In conclusion, our results suggest that parasite ENTPDase is an important molecule in the adhesion of *Leishmania amazonensis* promastigotes to host cells. We are currently investigating the macrophage ligand involved in this association. Supported by FAPEMIG and CNPq

## IM.20 – 2271 DISTINCT PATTERN OF IMMUNOPHENOTYPIC FEATURES OF INNATE AND ADAPTIVE IMMUNITY AS A PUTATIVE SIGNATURE OF THE CLINICAL AND LABORATORIAL STATUS OF PATIENTS WITH LOCALIZED CUTANEOUS LEISHMANIASIS

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**Introduction and Objectives:** American Tegumentary Leishmaniasis is a protozoan disease with distinct clinical manifestations depending on the infecting *Leishmania* species and on the pattern of the host immune response. In this study, we analyzed the phenotypic features of innate and adaptive immunity in patients with localized cutaneous leishmaniasis (LCL), categorized according to their clinical/laboratorial status, including: the number of lesion (L1 and L2-4), the days of illness duration (≤60 and >60) and the reactivity in the Montenegro skin test ($MT^-$ and $MT^+$), aiming to identify immunological biomarkers applicable in clinical studies. **Results:** Our findings highlighted several phenotypic features observed in all LCL patients (↑HLA-DR in neutrophils, ↑$CD8^+$HLA-$DR^+$/$CD4^+$HLA-$DR^+$ T-cells and ↑HLA-DR in B-lymphocytes, ↑CD23 in neutrophils, monocytes and B-cells and ↑seric $NO2^-$+$NO3^-$ levels). Selective changes were associated with distinct clinical/laboratorial status, with L1 displaying enhanced cellular immunity (↑HLA-DR in neutrophils, ↑$CD8^+$HLA-$DR^+$/$CD4^+$HLA-$DR^+$ T-cells and ↑seric $NO2^-$+$NO3^-$ levels) and L2-4 mostly marked by increased humoral immune response (↑$CD5^-$ and $CD23^+$ B-cells). Patients from ≤60 presented mixed profile of innate and adaptive immunity (↓CD28 in neutrophils and ↑$CD4^+$ T-cells, without compensatory leishmanicidal mechanisms) whereas patients from >60 showed most changes in the adaptive compartment with prominent activation of $CD8^+$ T-cells and ↑seric $NO2^-$+$NO3^-$ levels. Patients from $MT^+$ displayed increased putative leishmanicidal capacity including (↑HLA-DR and ↑CD23 in neutrophils, ↑CD23 in monocytes, ↑$CD8^+$HLA-$DR^+$/$CD4^+$HLA-$DR^+$ T-cells and ↑seric $NO2^-$+$NO3^-$ levels). In conclusion we summarize a range of immunological biomarkers (↓CD28 in neutrophils, ↑CD23 in monocytes, ↑HLA-DR in CD8+ T-cells and ↑seric $NO2^-$+$NO3^-$ levels) and discuss their significance in clinical studies of LCL. **Financial Support:** CAPES, FAPEMIG, CNPq and FIOCRUZ.

## IM.21 – STUDY OF THE INTERACTION BETWEEN CANINE NEUTROPHILS AND *LEISHMANIA CHAGASI*

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Visceral leishmaniasis (VL) is one of the most important emerging diseases with high prevalence in Latin American. Dogs have an important role in public health, been the principal reservoir for this intracellular parasite in the urban zone. Neutrophils are the major population of circulating leukocytes and are quickly recruited to an inflamed site participating in pathogens killing by diverse mechanisms. One of such killing mechanism, NETosis, occurs with the release of neutrophil chromatin associated to granule proteins in a web shape. Here we study NET release by *L. chagasi* promastigotes in neutrophils of healthy dogs. Our results showed that *L. chagasi* induce NET release in dogs' neutrophils and these structures are composed of histone, elastase and DNA. Quantification of NETs showed 3 times more NET released after parasite interaction than control canine neutrophils [NØ = 2.653 ± 1893 ng/mL; NØ + parasite = 6.031 ± 2.386ng/mL]. Incubation of *L. chagasi* with neutrophils resulted in 44% of promastigote killing compared with parasites alone. We also measured myeloperoxidase (MPO), another NET component. Diminished MPO activity was seen in neutrophils after parasite interaction [NØ = 0.472 ± 0.6 mU/$10^5$céls.; NØ + parasites = 0.413 ± 0.581 mU/$10^5$céls; NØ + *E. coli* = 2.427 ± 1.906 mU/$10^5$céls]. Our results demonstrate that canine neutrophils are able to release NETs after *L. chagasi* interaction and that these webs are toxic to the promastigotes. Interestingly, our results suggest that promastigotes modulate MPO in the NETs.

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## IM.22 – ECTO-NUCLEOTIDASIC ACTIVITIES IN PROMASTIGOTES OF *Leishmania (Viannia) braziliensis* STRAINS

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*Leishmania braziliensis* is responsible for the majority of cases of human cutaneous leishmaniasis in Brazil. This parasite has been associated with a broad range of clinical manifestations ranging from a simple cutaneous ulcer to a very destructive form of leishmaniasis with mucosal involvement. In this context, ecto-nucleotidases have been implicated in a crucial role in metabolism of extracellular nucleotides, which can be correlated to parasitic adhesion to target cells and parasite virulence. In this study, the ecto-nucleotidase activity of various *Leishmania (V.) braziliensis* strains, isolated from lesions of patients from different locations in Brazil presenting diverse clinical manifestations were characterized and compared using whole-promastigotes. Moreover, we also examined if parasite ecto-nucleotidase activity can be correlated with infectivity in C57BL/6 mice. For enzyme activity evaluation, we measure the amount of inorganic phosphate released after incubation of live parasites with ATP, ADP and AMP. Furthermore, we evaluated lesion size in C57BL/6 mice after inoculation of promastigotes forms of *L. braziliensis* isolates in the footpad. Tissue parasitism in the footpad and draining lymph node was evaluated by limiting dilution assay. The isolates obtained from patients with mucocutaneous leishmaniasis (MCL) hydrolyze higher amounts of adenine nucleotides than isolates obtained from patients with cutaneous leishmaniasis (CL). Corroborating the hypothesis about the correlation between ecto-nucleotidase activity and virulence, the lesions caused by PPS6 (MCL isolate) in the footpad of C57BL/6 mice were larger and persisted longer than most of the CL isolates. Increased lesion development was accompanied by increased parasite load in both footpad and draining lymph node. We suggest that the enzymes involved in metabolism of extracellular nucleotides may have an important role in the clinical manifestations of patients with mucocutaneous and cutaneous leishmaniasis in the same or different geographical region. Financial Support: CNPq, CAPES, FIOCRUZ, UFOP, PRONEX/FAPEMIG, FAPEMIG

## IM.23 – PHENOTYPIC FEATURES OF THE IMMUNE RESPONSE IN SERONEGATIVE DOGS NATURALLY INFECTED BY *LEISHMANIA INFANTUM* WITH INAPPARENT DISEASE CONFIRMED BY PCR

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Herein, the canine visceral leishmaniasis was re-classified clinically in asymptomatic dogs naturally infected by *L. infantum* using to serological and molecular diagnosis in two subgroups: Asymptomatic Dogs I (AD-I) with negative serological tests, but presenting positive *Leishmania* molecular diagnosis and Asymptomatic Dogs II (AD-II) animals with positive serology and molecular diagnosis for *Leishmania*. Detailed analysis of immune response including humoral (IgG, IgG1, IgG2, IgM, IgA and IgE) and cellular T-lymphocytes ($CD5^+$, $CD4^+$ and $CD8^+$), B-cell ($CD21^+$) and monocytes ($CD14^+$) in *ex vivo* context was performed in comparison with the Symptomatic Dogs (SD) and Control Dogs (CD). The results demonstrated that AD-I presented similar immunophenotypic features as those detected in CD group including isotype profile as well as the number of monocytes ($CD14^+$) cells. Moreover, equivalent biomarkers in AD-II and SD groups was observed such as higher levels of IgG, IgG2, IgM and IgA immunoglobulins and elevated number of eosinophils. High frequency of T ($CD5^+$) lymphocytes and the $CD4^+$ T-cells was observed in both AD-I and AD-II groups in comparison to the SD group, whilst ($CD8^+$) T-cells was higher only in AD-II in comparison to the SD group. The analysis of B-lymphocytes revealed an increased frequency of this cell-type only in AD-II animals in comparison to the SD group. Overall, the results supported the hypothesis that the asymptomatic dogs have a dichotomous clinical spectrum able to influence the immunological status and this finding may be decisive in controlling the infection or promoting the clinical evolution of canine visceral leishmaniasis. Supported by PRONEX–FAPEMIG, CNPq and UFOP, DECIT/MS.

## IM.24 – *IN VITRO* IMMUNOGENICITY IN DOGS VACCINATED WITH LBSAPSAL VACCINE AFTER CHALLENGE WITH *LEISHMANIA INFANTUM*

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The development of new vaccines is a priority for the control of canine visceral leishmaniasis (CVL) since therapy dogs is ineffective and no vaccine against CVL is accepted by the Ministry of Health (Brazil). We investigated the *in vitro* immune response in dogs immunized with LBSapSal vaccine and challenged intradermally with 1 x $10^7$ promastigotes of *L. (L.) infantum plus* salivary gland extract of *Lutzomyia longipalpis* following by 435 days after challenge (dac). Previous studies showed that LBSapSal vaccine was able to increase the absolute number of $CD5^+$T-lymphocytes circulating, reflected by higher $TCD4^+$ and T $CD8^+$ counts in 435dac, besides an increase of $CD8^+$T-lymphocyte during throughout the period after the challenge, indicating the establishment of protective immunity against *Leishmania* infection. Consistent with this hypothesis, our results showed an increase lymphoproliferative activity after antigenic stimulation with SLcA (soluble *L. chagasi* antigen) in 90 and 435dac as well as an increase frequency (stimulation index) of SLcA-specific $CD5^+$ and $CD8^+$T-lymphocytes during 435dac in the LBSapSal group. We also observed an increase in the rate of proliferation MHC-II-lymphocyte stimulation with VSA (soluble vaccine antigen) accompanied by a positive correlation between $CD21^+$B cells and $CD8^+$T-lymphocytes with MHC-II expression in 90dac. The correlations between cell proliferations in the LBSapSal group 435dac showed a similar profile in both stimuli employing VSA or SLcA. In this sense, positive correlations were observed between MHC-II expression with $CD8^+$T and $CD5^+$T-lymphocytes VSA/SLcA-specific, and negative correlations with $CD21^+$B-lymphocytes VSA/ SLcA-specific in 435dac.Thus, this study indicated that LBSapSal vaccine presents an *in vitro* profile consistent with immune protection against CVL. Further investigation focusing the parasitological and molecular analysis to evaluate the efficacy of dogs immunized with the LBSap vaccine has been assessed in our lab. Supported by: FAPEMIG, CNPq, FIOCRUZ and UFOP.

## IM.25 – RELATIONSHIP BETWEEN DENDRITIC AND CD4$^+$/CD8$^+$T CELLS IN THE SKIN OF BALB/C MICE INFECTED WITH *L. (L.) AMAZONENSIS* AND *L. (V.) BRAZILIENSIS*

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The role of the Langerharns and dermal dendritic cells in the development of cellular immune response is still contradictory. In BALB/c mice, *L. amazonensis* infection is characterized by an uncontrolled replication of parasites, whereas *L. braziliensis* infection is characterized by self-healing lesions with reduction in the number of parasites. In order to evaluate the relationship between the cellular immune response and dendritic cells in the skin, BALB/c mice were inoculated into the hind footpads with $10^6$ promastigotes of both parasite species, control was inoculated with PBS. The infection was monitored during 8 weeks. At 4$^{th}$ and 8$^{th}$ weeks PI, biopsies of skin inoculation site were collected to determine the parasite load by limiting dilution and the density of CD207$^+$, CD11c$^+$, CD4$^+$ and CD8$^+$ cells by immunohistochemistry. Concerning to the density of Langerharns (CD207$^+$) and dermal dendritic (CD11c$^+$) cells, BALB/c infected with *L. amazonensis* showed significant increasing in density of positive cells at 4$^{th}$ week PI compared with control group and BALB/c mice infected with *L. braziliensis* at 8$^{th}$ week of infection. Already, the density of CD4$^+$ cells in BALB/c infected with *L. amazonensis* increased at 4$^{th}$ and 8$^{th}$ weeks PI in relation to the control; and BALB/c infected with *L. braziliensis* at 8$^{th}$ week PI in relation to control and *L. amazonensis* group. By the other side, the density of CD8$^+$ cells was higher only in BALB/c infected with *L. braziliensis* at the 8$^{th}$ week PI. The results showed that an efficient CD4 and CD8 immune response occurs in BALB/c mice infected with *L. braziliensis* at 8$^{th}$ week of infection when a significant increase on the number of dendritic cells was observed, suggesting differences on the antigen presentation to the host immune system between parasite belong to the sub-genus *Leishmania* and *Viannia.* Supported by FAPESP, CAPES and LIM-50 HC-FMUSP.

## IM.26 – THE PATTERN RECOGNITION RECEPTORS NOD1 AND NOD2 ARE RECRUITED TO *LEISHMANIA*-CONTAINING VACUOLES AND ACCOUNT FOR THE RESTRICTION OF *LEISHMANIA AMAZONENSIS* MULTIPLICATION IN MACROPHAGES

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Leishmaniasis, caused by parasites of the *Leishmania* genus, is the second-largest parasitic induced disease in the world. The immunity against leishmaniasis is based on the development of IFN-γ-producing Th1 lymphocytes and on the production of nitric oxide by activated macrophages. Thus, an early generation of a Th1 response is a key event for an effective immune response. Th1 differentiation requires effective recognition of the parasites by professional phagocytes such as macrophages and dendritic cells. These cells express a series of pattern recognition receptors (PRR), such as toll-like receptors (TLRs) and nod-like receptors (NLRs). Among NLRs are Nod1 and Nod2, which are cytosolic proteins, know to participate of the recognition of intracellular bacteria. Up to date there is no information regarding the recognition of *Leishmania* by Nod1 and Nod2. Here we evaluated the role of Nod1 and Nod2 receptors for recognition and killing of *L. amazonensis in vitro* and *in vivo*. Initially, we used chinese hamster ovary (CHO) cells transfected with vectors encoding Nod1 or Nod2 to evaluate the recruitment of Nod1 or Nod2 proteins to the *Leishmania*-containing vacuole (LCV). By confocal microscopy we demonstrated that both Nod1 and Nod2 are readily recruited to the LCV. To evaluate the role of these proteins in macrophage resistance we infected macrophages obtained from C57BL/6 (wild-type), Nod1$^{-/-}$, Nod2$^{-/-}$ or Rip2$^{-/-}$ mice and found that the NLR-deficient macrophages presented higher proportion of *L. amazonensis* infected cells and high amounts of intracellular parasites per cell. By performing *in vivo* infections, we confirmed the importance of Nod/Rip2 pathway for restriction of the infection as Nod1$^{-/-}$, Nod2$^{-/-}$ or Rip2$^{-/-}$ mice show a robust lesion development. These data suggest that Nod1 and Nod2 receptors are important for sensing of *L. amazonensis* infection and may act as key components of the innate immune response against leishmaniasis. Supported by FAPESP, CNPq, PEW and WHO/TDR.

## IM.27 – TREATMENT WITH BONE MARROW CELLS INFLUENCE IN CHRONIC CUTANEOUS LESION OF MICE (TNFRp55$^{-/-}$ BL/6) INFECTED BY *LEISHMANIA MAJOR*

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Data from our group suggest that TNFRp55$^{-/-}$ C57BL/6 mice might be a good model to study chronic lesions caused by *Leishmania* infection and to test therapies, for example, cell therapy with stem cells. We showed that when infected with *L. major*, TNFRp55$^{-/-}$ mice develop chronic lesions and can control parasite growth at the site of infection when compared to wild type mice, but maintain the intense inflammatory infiltrate. The aim of this study was to verify the efficacy of using preparations of purified mononuclear bone marrow cells (MO-BMC) as a treatment for chronic lesions in TNFRp55$^{-/-}$ mice infected with *L. major*. After 15 weeks of infection groups of mice were treated with MO-BMC (intravenously) and analyses were performed 4 weeks after the treatment. After the treatment with purified MO-BMCs the lesions were reduced and the histological analysis showed evidence of healing in the treated mice in comparison with animals treated with PBS. MO-BMC GFP$^{+}$ cells were transferred to infected mice and after 24 hours or 7 days, no GFP$^{+}$ cells were located at the site of infection but were found in the draining lymph nodes. The analysis of cytokines in the draining lymph nodes of treated mice showed increased levels of IL-10, after recall response with soluble antigen of *L. major,* when compared to animals treated with PBS. Our work suggests that the treatment with MO-BMCs can influence the course of chronic cutaneous lesions in TNFRp55$^{-/-}$ mice infected with *L. major*. Supported by CAPES, CNPq and FAPEMIG.

## IM.28 – 2871 THE BALANCE BETWEEN ARGINASE I AND iNOS EXPRESSION IS RELATED TO THE INITIAL CONTAINMENT OF THE LESION AND PARASITE REPLICATION IN IFN-γ DEFICIENT MICE DURING LEISHMANIA AMAZONENSIS INFECTION

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The resistance to *Leishmania major* is associated with the development of a Th1 immune response, with IFN-γ as the key effector cytokine activating macrophages to kill intracellular parasites, via the induction of NO production. The susceptibility to *L. major* is due to the development of Th2 response. Although this Th1-Th2 dichotomy is well established in the *L. major* infection model, it may not adequately explain the pathogenesis of infection by *L. amazonensis*; for which susceptibility is not associated with a polarized Th2 response. Surprisingly, IFN-γ can induce the replication of these parasites *in vitro*. In addition, IFN-γ-/- mice present higher susceptibility at later time points after infection. Since data on the role of this cytokine *in vivo* is lacking, the aim of this work was to investigate the role of IFN-γ during infection by *L. amazonensis*. Our results showed that lesions in the footpads of IFN-γ-/- mice displayed less parasites 8 weeks after infectionas compared to the same size of lesionin the footbeds of as C57BL/6 mice. The decreased parasite numbers were associated with lesser expression of arginase I in the footpad and IL-10 in the lymph nodes. Interestingly, these mice showed the same expression of iNOS in the lesion, suggesting an IFN-γ-independent mechanism of induction of this enzyme. After 16 weeks of infection, IFN-γ-/- showed exacerbated lesions and higher parasite loads. Also, there was an increase in arginase I activity, and a dramatic decrease in iNOS expression. We also demonstrated that IFN-γ is essential for the development of immunity conferred by Leishvacin. In conclusion, our results indicate that it is possible to induce iNOS in the absence of IFN-γ and this induction maybe important for the initial containment of the lesion progress.
Supported by CAPES, CNPq, FAPEMIG and INCT/Redoxoma.

## IM.29 – SIGNALING PATHWAYS INVOLVED ON NEUTROPHIL EXTRACELLULAR TRAPS TRIGGERED BY *LEISHMANIA,* PHORBOL ESTER AND N-*FORMIL*-METIONIL-LEUCIL-FENILALANIN

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A new mechanism of neutrophil death, NETosis, was recently described where neutrophils die releasing fibrous traps of DNA, histones and granule proteins, named NETs (Neutrophil Extracellular Traps), which can kill bacteria and fungi. Moreover, NETosis is activated by microorganisms, synthetic and microbial products such as PMA (Phorbol Myristate Acetate) and fMLP (N-*formil*-metionil-leucil-fenilalanin). Our group demonstrated that the protozoan parasite *Leishmania* stimulate neutrophils to release NETs, and are killed by these structures. NETs were also detected in biopsies of patients with cutaneous leishmaniasis. Since then, our group is interested in studying, comparatively, the cell signaling pathways involved on NETs induction by three different inducers – PMA, fMLP and promastigotes of *L.amazonensis.* Thus, human neutrophils, isolated by a density gradient from blood of healthy donors, were incubated with inhibitors of protein kinase C (Bisindolylmaleimide I, BIS), phospholypase C (U73122), protein G (Pertussis Toxin), or NADPH oxidase (diphenylene iodonium, DPI), before the addition of PMA, fMLP or parasites. After 2 hours, NETs released in the supernatant were quantified by the Picogreen assay, which detects NETs DNA. Our results demonstrated that NET induced by PMA and fMLP were inhibited (ranging between 10 and 50%) by all inhibitors tested. DPI and U73122 inhibited 50% of the NET induction by fMLP. The highest inhibition of PMA induced NETs was obtained with BIS (35% inhibition). Interestingly, NETs triggered by *L. amazonensis* were not affected by none of the tested compounds. Our results suggest that different from *Leishmania,* the signaling pathways involved in NET release by PMA and fMLP are linked to protein G, activation of PKC, and dependent of reactive oxygen species. We thank the Hemotherapy Service of Hospital Clementino Fraga Filho (UFRJ), and the support from FAPERJ, CNPq, PIBIC-UFRJ.

## IM.30 – HIGH NOS2 EXPRESSION IN MACROPHAGES IS ASSOCIATED WITH LOW PARASITE BURDEN IN SPLEEN OF DOGS NATURALLY INFECTED WITH *L. (L.) CHAGASI*

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*Leishmania* is intracellular parasite of macrophages responsible for visceral leishmaniasis in dogs. Dogs are the main reservoir for human leishmaniasis. Recent studies suggest that asymptomatic dogs develop a Th1 immunological profile. In protective immune response, amastigotes are phagocytosed and destroyed by activated macrophages, in which in vivo studies suggest iNOS plays an important role as regulating and effector molecule preventing the multiplication of amastigotes. The aim of this study was evaluate the expression of NOS2 in macrophages of spleen from dogs with visceral leishmaniasis in order to identify a possible involvement of this molecule on the parasite control. Twenty *Leishmania* positive dogs, 10 symptomatic and 10 asymptomatic from the Center of Zoonosis Control of Araçatuba city were submitted to euthanasia and biopsies of spleen were collected and processed by immunohistochemistry using mouse anti-*Leishmania* and rabbit anti-human NOS2 (Santa Cruz) polyclonal antibody and LSAB kit (DAKO). Quantitative analysis was performed using the image analysis system in order to quantify the number of amastigotes and the number of NOS2+ cells. Biopsies of dogs from non-endemic area of visceral leishmaniasis were used as control. Correlation between the number of parasites and the number of cells expressing NOS2 was assessed using Spearman test. High expression of NOS2 in macrophages was related with low number of amastigotes in all cases ($r= -0.508$, $p= 0.04$). The results suggest that NOS2 expression by macrophages plays an important role in the control of *Leishmania* parasites in the dog tissue.Supported by FAPESP and LIM50 HC-FMUSP

## IM.31 – SERIAL STUDY OF HUMAN CASES OF CUTANEOUS LEISHMANIASIS: CLINICAL AND EPIDEMIOLOGICAL ASPECTS

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The northern region has the highest incidence of leishmaniasis in the country, estimated to be about more than 2,000 new cases a year only in the state of Amazonas. The clinical manifestations are related to different species of *Leishmania* and the host immune response. The objective of this preliminary study was to correlate the clinical human cutaneous leishmaniasis with cellular response by analysis of the cells population by hemogram and detection of the peripheral T-cells (CD4+ and CD8+ lymphocytes). The select cases diagnosed as ATL were analyzed. This study of serial cases was conducted from June 2009 to June 2010, coming from five cities of Amazonas State. Eleven patients with skin lesions and aged between 21 and 58 years, evolution time of the injury about a month in 83% of cases, with predominance of activities in the forest area, presented lymphocytosis in 73% of cases, monocytosis 63%, eosinophilia 45%, neutropenia 45% and anemia 9% in the patients before the treatment with pentavalent antimony N-methylglucamine. Two patients had a low platelet values.The predominant parasite species was Leishmania (V.) guyanensis in 36% of cases, L.(L.) amazonensis and L.(V.) naiffi in 9,5% of cases, respectively. Was observed high levels of CD4+ T-cells (increase of 82%) and decreasing of 91% CD8+ in all of the cases studied. The presented data contribute to understanding the epidemiology of leishmaniasis, immune status and clinical form that the mechanisms involved in the infectious process can be better understood. The determination of Th1 and Th2 response can also be influenced by the strains of *Leishmania*, parasite inoculated dose, by the inoculation site and vector sand fly saliva, besides the immunological aspects and genetic predisposition of the host. This work is in progress. Supported by: Capes, Fapeam/PPSUS.

## IM.32 – VACCINATION WITH THE LEISHMUNE®´S NUCLEOSIDE HYDROLASE MAPS THE C-TERMINAL DOMAIN AS THE TARGET OF THE PROTECTIVE IMMUNE RESPONSE

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Nucleoside hydrolases (NHs) of protozoa emerged vital protagonists of pathways for parasite replication and establishment of early infection. Immune protection against NHs would prevent disease at the early infection of several pathogens. We identified the domain of the NH of *L. donovani* (NH36) responsible for its immunogenicity and protective efficacy as a potential candidate of a multivalent synthetic vaccine. Methods: three recombinant proteins of NH36 [aminoacids 1-103 (F1), 104-198 (F2) and 199-314 (F3)] were generated in the pET28b system and used with saponin for vaccination of Balb/c mice further challenged with *Leishmania chagasi or Leishmania amazonensis*. The protective response was evaluated by a cytokine-ELISA assay, inhibition of antibody binding by synthetic predicted epitopes, intracellular staining (ICS), DTH to leishmanial antigen, *in vivo* depletion with anti-CD4+/anti-CD8+ antibodies, parasite load evaluation, increase of footpads lesions and RTPCR. Results: protection against *L. chagasi* is related to a mainly CD4+ T cell driven response with a lower contribution of CD8 + T cells and to antibodies directed to its C-terminal domain of NH36 (amino-acids 199-314). This was mediated by an increase in specific antibodies, DTH and the ratios of IFNγ/IL-10 and TNFα-IL-10 CD4+ and CD8+ producing cells and confirmed by *in vivo* depletion with monoclonal antibodies, algorithm predicted CD4 and CD8 epitopes and a pronounced decrease in parasite load (90.5-88.23%; p=0.011). No decrease in parasite load was detected after vaccination with the N-domain of NH36, despite the induction of IFN-γ/IL-10 expression by CD4+ cells after challenge. Both peptides reduced the size of footpad lesions, but only the C-domain reduced the parasite load of mice challenged with *L. amazonensis* (p=0.039). Conclusions: The identification of the target of the immune response to NH36 represent a basis for the rationale development of a bivalent vaccine against leishmaniasis and for multivalent vaccines for NHs-dependent pathogens.
Support: by CNPQ and FAPERJ

## IM.33 – COMPARISON THE PROPHYLACTIC POTENTIAL OF THE NUCLEOSIDE HYDROLASE GENETIC VACCINE ON VISCERAL LEISHMANIASIS ADMINISTERED IN MICE THROUGH THE INTRAMUSCULAR OR THE INTRANASAL ROUTE

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In this work we assayed the potential development of a "needle free" vaccine using the Nucleoside hydrolase gene of *Leishmania donovani* cloned in the VR1012 plasmid (VR1012-NH36). Methods: 100µg of either the VR1012NH36 vaccine or empty plasmid were administered through the intramuscular (*im*) or the intranasal (*in*) mucosal route in BALB/c mice further challenged with *L. chagasi*. Antibodies were measured in an ELISA assay against the recombinant NH36. The cell immune response evaluated by DTH and intracelluar staining of splenocytes and the parasite load evaluated in LDU of liver. Results: After immunization, the DNA vaccine *im* induced a signifficant decrease of IgG1 and the vaccine *im* and *in* determined an increase of IgG3 antibodies. After infection only the im vaccine induced a significant increase of IgG2b antibodies. The DTH response against leishmanial lysate was significantly increased by both vaccines, both before and after infection. Before infection both vaccines were equally potent in DTH induction. This response was 70% higher for the *im* group after infection (ANOVA p=0.000;Tukey´s HSD p<0.05). The ICS analysis disclosed no differences in proportions of IFN-gamma CD4 T cells (p=0.166) . The TNF-alpha-CD4_ T cells (p=0.012) and IFN-gamma-CD8+ T cells were significantly increased (p=0.036) only in the *im* group and the IL-10-CD4+ cells were increased for the empty plasmid *im* control group (p=0.012). The reduction of parasite load showed significant variations (ANOVA P=0.002; Kruskall Wallis p=0.0069). We observed a 72,01% reduction generated by the vaccine administered through the *im* route in contrast with a 31.90% reduction by the vaccine administered by the *in* route (ANOVA p=0.002; p<0.05). Both vaccine protected more than the saline and empty plasmid controls. Despite the lower protection, a needle free vaccine using the gene of Nucleoside hydrolase might be developed with the aid of adjuvants.
Support: by CNPQ and FAPERJ

## IM.34 – EFFECT OF ADMINISTRATION OF A LIPOSOME FORMULATION OF MEGLUMINE ANTIMONIATE BY INTRAPERITONEAL OR INTRAVENOUS ROUTES AS A TREATMENT OF VISCERAL LEISHMANIASIS

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New treatments for visceral leishmaniasis are very important because of disease severity. Some conventional therapies are not completely efficient, and there are various cases of drug resistance. The aim of this study is to test the best route of administration of a new medicine formulation, meglumine antimoniate-containing liposomes. The effect of this drug is attributed to the sustained release of liposomes and to their natural tendency to be cleared from the circulation by the fixed macrophages of the liver, spleen and bone marrow, which are the major sites of parasite infection. Therefore the liposome formulations improve the use of antimonials, enabling a reduction in drug dose and therapy duration. In this study Balb/c mice were infected by *Leishmania (Leishmania) chagasi*, and treated with drug by two routes: intraperitoneal and intravenous administration, in a unique dose of 30mg Sb/Kg. The liposome formulation was prepared by the dehydration–rehydration method, allowing the encapsulation of 40% of meglumine antimoniate in lipid vesicles with a 400 nm mean diameter. A quantitative limiting dilution assay was performed to determine its impact on the reduction of parasitic load, and the hematoxilin-eosin staining technique was performed to analyze the histological alterations. We have observed a significant reduction of liver and spleen parasite burdens in animals treated with this drug by both routes, when compared to animals treated with free meglumine antimoniate, empty liposomes or PBS. In some cases the parasite was not found after treatment. The histological changes were observed by the reduction of hepatic granuloma formation or complete absence of them. In conclusion we can affirm that both routes were effective to reduce the parasite load and to prevent granuloma formation. Supported by CAPES.

## IM.35 – CD1a$^+$, FACTOR XIIIa$^+$ Dermal Dendrocytes, CD4$^+$ And CD8$^+$ T-cells EXPRESSION IN AMERICAN CUTANEOUS LEISHMANIASIS

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The dendritic cells, including Langerhans cells (LCs), have been regarded as a pivotal link between T lymphocytes and macrophages, presenting *Leishmania* antigens and stimulating specific CD4$^+$ and CD8$^+$ T-cells proliferation. The role of LCs in the immunopathogenesis of human and experimental cutaneous leishmaniasis has been well studied, however, there are few articles addressing the involvement of Factor XIIIa$^+$ dermal dendrocytes (FXIIIa$^+$ DD) in such diseases. Factor XIIIa$^+$ DD is a bone marrow monocytic lineage derived cell and member of the skin immune system. The aim of this study was to determine the CD1a$^+$, Factor XIIIa$^+$ DD, CD4$^+$ and CD8$^+$ T-cells expression in the cellular infiltrate of skin lesions of twenty-two cases of localized cutaneous leishmaniasis (LCL) from Buriticupu municipality, pre-Amazonian region of Maranhão State, Brazil. In addition, the skin biopsies performed for immunohistochemistry were also submitted to a Polymerase Chain Reaction (PCR) for characterizing the *Leishmania* species. The paraffin-embedded biopsies were submitted to immunohistochemistry using monoclonal antibodies for CD1a$^+$ (1/20, clone 010[1]/DAKO), Factor XIIIa$^+$ DD (1/100, CM377C/Biocare Medical), CD4 (1/400, OPD4/DAKO) and CD8 (1:100, CD8/144 DAKO) T-cells. For amplification and visualization of the reaction Novolink max polymer was used. The immunostained cells were counted in 5–10 fields (400x) in each section by using an image analysis system (Zeiss). The Factor XIIIa$^+$ DD (546 mm$^2$) expression was higher ($P$< 0.05) than that of CD1a$^+$ (296 mm$^2$) and the CD8$^+$ (2374 mm$^2$) was also higher ($P$< 0.05) than that of CD4$^+$ (1268 mm$^2$) in the lesions of these patients. The PCR results confirmed that all parasites associated to these LCL cases were classified as *Leishmania* (*Viannia)* spp. Considering that all these LCL cases came from a typical *L. (V.) braziliensis* endemic area and that this *Leishmania* parasite is a good modulator of a cellular immune response, we conclude that our findings are strongly suggesting a correlation between the Factor XIIIa$^+$ DD expression with that of CD8$^+$ T-cells, which might explain the high delayed-type hypersensitivity reaction found in this group of patients. Supported by: LIM-50/HCFMUSP, FAPESP, CAPES.

## IM.36 – *LEISHMANIA MAJOR* ENCODED INHIBITORS OF SERINE PEPTIDASES (ISPS) MODULATE THE UPTAKE AND RELEASE OF PARASITE FROM MURINE NEUTROPHILS

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The successful establishment of Leishmania infection is associated with the capability of the parasite to evade microbicidal responses by professional phagocytes. Neutrophils are the first cells to be recruited to the site of infection and to be parasitized. *Leishmania* is able to avoid killing by neutrophils, and the phagocytosed parasites reside temporarily as viable metacyclics, before being released to infect macrophages. Alternatively, parasitised apoptotic neutrophils are taken up by standby macrophages. In a previous work, our group showed that *L. major* has three genes similar to bacterial ecotins, which are inhibitors of trypsin-fold serine peptidases, termed ISPs. Recombinant ISP2 inhibits neutrophil elastase with moderate affinity and *L. major* mutant lines lacking *ISP2* and *ISP3* (Δ*isp2/isp3*) are more efficiently phagocytosed by peritoneal macrophages than wild type (WT) parasites, in a mechanism dependent on neutrophil elastase activity. On the other hand, approximately half of intracellular Δ*isp2/isp3* die within the first 15 hours. Since neutrophils produce large amounts of tryspin-fold serine peptidases that contribute to their microbicidal activity we set out to evaluate the possible role of ISPs in the interaction of neutrophils with *L. major* by using Δ*isp2/isp3* null mutants as a tool. We show that Δ*isp2/isp3* are internalized more efficiently by bone-marrow purified neutrophils from C57/BL6 mice when compared to WT parasites and are also released in higher numbers within 12 hours after phagocytosis, contrary to the observed in macrophages. The passage of parasites by neutrophils influences their subsequent infectivity to macrophages. Moreover, peritoneal macrophages infected with Δ*isp2/isp3* were able to recruit neutrophils in vitro more efficiently than those infected with WT or with parasites re-expressing *ISP*2 and *ISP*3. Our results suggest that the regulation of host SP activity by *L. major* ISPs influences the parasite interaction with professional phagocytes, possibly contributing to the establishment of the infection. Supported by Wellcome Trust, FAPERJ and CNPq.

## IM.37 – COLLAGEN, FIBRONECTIN AND LAMININ ALTERATIONS OF THE CERVICAL LYMPH NODE IN CANINE VISCERAL LEISHMANIASIS

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The aim of this work was study the extracellular matrix alterations in liver, spleen and cervical lymph nodes in dogs naturally infected with *Leishmania (Leishmania) chagasi* correlating with clinical aspects, histological, parasitological and immunological. This study was carried out with 30 dogs, divided at three groups: ten not infected animals (group control) and twenty infected animals. All them was mongrel dogs with undefined age, obtained from the municipality of Belo Horizonte, MG, metropolitan area. Infected animals were divided in two groups: asymptomatic group composed by ten animals without clinical signs of the disease; group denominated symptomatic: composed by ten animals with classical clinical signals of the disease as skin lesions (alopecia, eczemas and ulcers), loss weight and lymphopathy. During necropsy cervical lymph nodes fragments were collected and fixed in buffer formaldehyde solution to 10% for histological analyses. Paraffined sections were stained by Hematoxylin-Eosin (HE); Gomori's Ammoniacal Silver staining for reticular fibers and strepto-avidin peroxidase Immunohistochemical method for tissue *Leishmania* amastigotes detection. Frozen tissue sections were stained by strepto-avidin peroxidase Immunohistochemical method for laminin (LN) tissue characterization and immunofluorescence technique for fibronectina (FN). The tissue images were transferred to a computer video screen by means of the software KS300 and relayed to a computer-assisted image analysis system (Kontron Elektronic/Carl Zeiss, Germany) for morphometrical analysis. Significant increase collagens deposition in cervical lymph nodes of infected dogs when compared to controls animals. There was significant difference between symptomatic and asymptomatic dogs collagen deposition in organs. Positive correlation between the parasite load and collagen deposition in cervical lymph nodes of infected animals. In fact, symptomatic animals showed increase collagen deposition in these organs, it's can be associate to parasite burden. Adhesive fibers LN and FN expression in cervical lymph nodes was higher in symptomatic animals than in asymptomatic. No significant statistical difference when we compare LN expression in cervical lymph nodes between symptomatic and asymptomatic groups. Our results demonstrate that in canine visceral leishmaniasis induces fibrogenesis in lymph nodes attached the parasite load and degenerative processes. **Key words**: Lymph node, extracellular matrix, dog, canine visceral leishmaniasis

## IM.38 – IDENTIFICATION OF AN ANTIGENIC TARGET ASSOCIATED WITH THE PROGRESSION OF THE EXPERIMENTAL *Leishmania* (*Leishmania*) *amazonensis* INFECTION

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Previously we isolated an active and antigenic ATP diphosphohydrolase isoform from *Leishmania* (*Leishmania*) *amazonensis* promastigotes, and described cross-immunoreactivity with potato apyrase, suggesting that the parasite and vegetable proteins share antigenic conserved epitopes (Parasitology 135:327, 2008). Seven domains were identified as highly conserved among plant apyrases and putative NDPases from *L. major*, *L. infantum* and *L. braziliensis* found in the genomes of these parasites (Parasitology 135:943, 2008). A recombinant fragment (rDomA) designed based on domain A was cloned into pQE30 for heterologous expression in *E. coli* BL21 as a 6xHis fusion protein. The rDomA was used as a probe to evaluate, along a period of 90 days, the reactivity of sera from BALB/c mice (n= 5) subcutaneously infected by injecting $10^4$ *L.* (*L.*) *amazonensis* amastigotes in the left hind footpad. The primary lesion kinetics in amastigote-infected mice showed progressive large lesions at 20, 40, 60 and 90 days after infection. Histopathological analyses of additional animals showed, at 20 days after infection, discreet inflammatory infiltrates at the site of inoculation that increased progressively during the course of the infection and, at 90 days post-infection, the dermis presented necrotic tissue. Analysis by ELISA, using rDomA as coating antigen and sera diluted 1:100, showed significantly higher ($P<0.001$) IgG antibody levels at 20 ($0.084 \pm 0.027$) and 40 ($0.754 \pm 0.227$) days post infection, when compared to the levels found prior to infection ($0.033 \pm 0.017$). At 60 ($0.269 \pm 0.179$) and 90 ($0.354 \pm 0.171$) days post-infection, IgG antibody levels significantly ($P<0.001$) decreased as compared to the levels found at 40 days post-infection. Taken together, these results suggest that an antigenic NDPase in *L. amazonensis* species contributes to modulate the host immune response, and appoint the rDomA as a new biomolecule to be tested in protocols for investigation in murine *Leishmania* models. Supported by FAPEMIG, CNPq, CAPES, IOC/FIOCRUZ and UFJF.

## IM.39 – *L. amazonensis* NDPase: A NOVEL MEMBER OF THE ATP DIPHOSPHOHYDROLASE FAMILY WHICH SHARES A HIGHLY CONSERVED ANTIGENIC DOMAIN

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A particular domain A was shown as a conserved functional region in plant apyrases and putative NDPases found in the Leishmania genomes, suggesting a clear association between structure and antigenicity (Parasitology 135:943, 2008). These results prompted us to search for the NDPase gene from *L. (L.) amazonensis*. Genomic DNA was extracted from promastigotes (MHOM/BR/1973/M2269 strain) and used as template in a PCR amplification with oligonucleotides that were designed based on the nucleotide sequence of both putative *L. major* and *L. infantum* NDPases. The amplified 1152 pb fragment (LaNDPase) obtained by PCR was cloned into pCRII vector. Positive clones were sequenced using M13 universal primers and the sequences of nucleotides were identical between them. Analysis of the multiple alignments between the deduced protein sequence and its orthologues revealed that LaNDPase displayed the five conserved regions described for the ATP diphosphohydrolase family and it is highly homologue (79-87% identity and 85-92% similarity over 361 amino acids) to the putative NDPases found in the *L. infantum*, *L. major* and *L. braziliensis* genomes. High homology was also found between the domain A from the LaNDPase and its counterpart within putative *Leishmania* NDPases (80-90% identity and 85-97% similarity) and potato apyrase (52% identity and 60% similarity). High level of homology exists between the predicted 3-dimensional structures of these proteins, and the domains A are exposed and have high score for antibody binding. IgG antibody from promastigote *L. amazonensis*-infected BALB/c mice had high reactivity against a recombinant fragment (rDomA), designed based on this domain A and obtained as a 6xHis fusion protein. These results identify, for the first time, an NDPase gene in *L. amazonensis* species and revealed that it codifies to a protein that maintains the same functional antigenic domain A, which could be explored in future studies of the leishmaniasis. Supported by FAPEMIG, CNPq, CAPES and UFJF.

## IM.40 – IDENTIFICATION OF A NEW TOOL FOR THE STUDY OF CANINE VISCERAL LEISHMANIASIS

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We identified an active ATP diphosphohydrolase (EC 3.6.1.5) isoform, which shares conserved epitopes with potato apyrase, in promastigote of *L. (L.) chagasi*, the causative agent of canine visceral leishmaniasis. Thus, sera (dil. 1:100) of healthy (HEA) and infected (INF) dogs domiciled in endemic area were analyzed by ELISA, using potato apyrase as antigen. The IgG antibody level in HEA (n=18; 0.154 ± 0.058; 78% seropositivity) or INF (n=38; 0.159 ± 0.057; 76% seropositivity) was significantly ($P<0.001$) higher than that found in healthy dogs domiciled in non endemic area (Control; n=30; 0.049 ± 0.030), suggesting that HEA group was pre-sensitized with the same epitopes. The IgG antibody seropositivity was elevated in the INF group clinically classified as asymptomatic (AD; n=11; 73%), oligosymptomatic (OD; n=12; 75%), and symptomatic (SD; n=15; 80%), and no significant difference was observed among them. Analysis of the amino acid sequence from a *L. infantum* putative NDPase (ATP diphosphohydrolase) revealed high identity with a conserved domain (DomC) belonging to potato apyrase. A recombinant fragment (rDomC) was designed based on this domain and cloned into pQE30 for heterologous expression in *E. coli* BL21. The rDomC was obtained as a 6xHis fusion protein. By ELISA, the IgG antibody reactivity (serum diluted 1:100) against rDomC from INF (0.366 ± 0.083) was elevated, and significantly ($P<0.001$) higher than that found in HEA group (0.271 ± 0.044). When compared to the HEA, the total IgG antibody level was significantly higher in the AD (0.311 ± 0.045; $P<0.05$), OD (0.354 ± 0.073; $P<0.01$) and SD (0.414 ± 0.087; $P<0.001$) groups. These results suggest that in visceral leishmaniasis the conserved domain is a target for the host immune response. Additionally, this work appointed the rDomC as a new biomolecule to be tested in protocols of studies of the visceral leishmaniasis. Supported by FAPEMIG, CNPq, CAPES and UFJF

## IM.41 – INFLUENCE OF ECOTIN-LIKE SERINE PEPTIDASE INHIBITORS OF *LEISHMANIA MAJOR* IN PARASITE INTERNALIZATION AND SURVIVAL IN MURINE DENDRITC CELLS

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Survival of *Leishmania major* in professional phagocytes is associated with the downmodulation of microbicidal and inflammatory responses. We described three genes in *L. major*, *ISP*1, *ISP*2 and *ISP*3, that share similarity to ecotin, a bacterial inhibitor of serine peptidases (SP) such as trypsin, cathepsin G and neutrophil elastase (NE). We reported that *L. major* mutants deficient in *ISP*2 and *ISP*3 (Δ*isp*2/3) are internalized more efficiently by macrophages but have diminished capacity to survive and multiply inside those cells. Increased phagocytosis of Δ*isp*2/3 requires the CD11b, a sub-unit CR3, TLR4 and unregulated activity of NE, present at the surface of macrophages. Infected dendritic cells are very efficient in initiating the parasite specific T-cell response in *L. major* infections and this is related to the ability of the parasite to modulate the parasithophorous vacuole-lysosome fusion. In this work, we investigated if ISPs influence the interaction of *L. major* purified metacyclic promastigotes with bone marrow-derived dendritic cells (BMDC) from BALB/c mice. Δ*isp2/3* mutants were internalized by BMDC less efficiently than wild type (WT) parasites, a phenotype that was reversed by the re-expression of both genes in the mutant. The uptake of Δ*isp2/3* by BMDC returned to WT levels upon addition of the serine peptidase inhibitor aprotinin, of recombinant ISP2 or upon pre-incubation of BMDC with neutralizing antibodies to TLR4. Internalized parasites survive inside BMDCs for at least 24 h post-infection. In contrast to the observed in suceptible BALB/c, Δ*isp2/3* were internalized more efficiently than WT by BMDC of resistant C57/BL6 mice, and this was likewise reversed by aprotinin. Those results suggest that host SP and TLR4 influence the interaction of *L. major* with DCs, which is subject to modulation by parasite ISPs. Furthermore, the consequences of SP inhibition by ISPs to parasite uptake by DCs might vary between susceptible and resistant mice. Supported by CNPq, Wellcome Trust, FAPERJ.

## IM.42 – IDENTIFICATION OF A DOMAIN FROM *L. braziliensis* NDPase AS TARGET FOR THE HUMAN IMMUNE RESPONSE

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Domains of high identity between potato apyrase and the putative *L. braziliensis* NDPase found in the genome of this parasite were observed by alignment of their primary amino acid sequences and by hypothetical three-dimensional models, suggesting that these conserved domains may be exposed and available for antibody binding. These results prompted us to test the antigenicity of a specific homologue domain. Potato apyrase was purified from *Solanum tuberosum* and Lb preparation was obtained from *Leishmania (V.) braziliensis* promastigote forms (MHOM/BR/1975/M2903 strain). Synthetic peptides belonging to the conserved domain from both *L. braziliensis* NDPase (LbB1LJ) and its potato apyrase counterpart (potB1LJ) were obtained by solid-phase synthesis. Patients with American cutaneous leishmaniasis (ACL) were diagnosed by positive parasitological examination, Montenegro skin test and polymerase chain reaction (DNA standard obtained from MHOM/BR/1975/M2903 strain). Potato apyrase was recognized in Western blots by IgG antibody from ACL patients, suggesting that the parasite and vegetable proteins share antigenic conserved epitopes. Serum samples (dil. 1:50) from healthy individuals from non-endemic area for leishmaniasis (n= 10) and ACL patients (n= 20) were tested by ELISA ($OD_{492\ nm}$). The IgG antibody levels against Lb (C, 0.095 ± 0.027; ACL, 0.193 ± 0.078; cutoff, 0.149), potato apyrase (C, 0.111 ± 0.022; ACL, 0.198 ± 0.049; cutoff, 0.155), LbB1LJ (C, 0.045 ± 0.050; ACL, 0.214 ± 0.111; cutoff, 0.145) and potB1LJ (C, 0.108 ± 0.040; ACL, 0.202 ± 0.103; cutoff 0.188) were significantly ($P < 0.01$) higher than that found in healthy individuals, with 65%, 90%, 80% and 50% seropositivity, respectively. These results are in accordance with the existence of shared antigenic epitopes between potato apyrase and *L. braziliensis* NDPase, and demonstrate that in leishmaniasis infection the conserved domain is a target for the human immune response.
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## IM.43 – EVALUATION OF CD11B, MHC CLASS II AND TLR-2 IN PERIPHERICAL BLOOD MONONUCLEAR CELLS OF NATURALLY INFECTED DOGS WITH *LEISHMANIA (LEISHMANIA) CHAGASI* TREATED WITH MEGLUMINE ANTIMONIATE ENCAPSULATED IN NANOMETRIC LIPOSOMES AND ALLOPURINOL

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Novel liposome formulation of meglumine antimoniate (LMA) associated with allopurinol are being evaluated in mongrel dogs with visceral leishmaniasis. Group I (n=16) was treated with six doses of LMA at 6.5mg $Sb^{5+}$/kg, dose given with 4-day intervals; Group II (n=16) received six doses of antimony-free lipossomes (FL) given at the same dose as that for group I; Group III (n=8) and IV (N= 12) received six doses of saline. On the first day of protocol, eight dogs in groups I, II and III were also co-treated with allopurinol, 20 mg/kg/s.i.d., for 180 days. Group IV remained as control. Thus, to study the activation state of monocytes, peripheral blood of dogs (20ml) was collected from the jugular vein into EDTA tubes. Density gradient separation was used to enrich for peripheral blood mononuclear cells. The analysis was performed selecting a region of side scatter (SSC) *versus* forward scatter (FSC) (gate R1) in flow cytometer. Within in R1, the mean fluorescence intensity (IMF) of each of the markers was determined in a second histogram. The analysis made 60 days after initiation of treatment revealed that the IMF of CD11b, in $CD14^+$ monocytes, was significantly higher in dogs treated with FL associated with allopurinol compared to group LMA associated with allopurinol ($p<0,01$). For the IMF of TLR2, within the population of monocytes $CD11b^+CD14^+$, the values were statistically higher in FL group associated with allopurinol ($p<0,01$); allopurinol ($p<0,01$) and control ($p<0,05$) in comparison to LMA. When comparing the IMF of MHC Class II wasn't observed statistical difference between the groups. In the literature, the role of empty liposomes in the immune response has been reported. Thus, we are looking forward to analyze these results. So far the data obtained is the preliminary results of the period of 60 days after initiation of treatment protocol assays. Supported by CNPq and FAPEMIG and CAPES

## IM.44 – HISTOPATHOLOGICAL, PARASITOLOGICAL AND LYMPHOCYTES $TCD4^+FOXP3^+$, $CD8^+FOXP3^+$ STUDY OF THE JEJUNE AND COLON OF INFECTED DOGS WITH *Leishmania (Leishmania) infantum*

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Canine visceral leishmaniasis is a worldwide zoonosis. Gastrointestinal tract disorders occur in response to human and canine visceral leishmaniasis. The aim of this study was to provide a systematic immunopathological study. Populations of T lymphocytes $CD4^+FOXP3^+$ and $CD8^+FOXP3^+$ of seven dogs naturally infected with *L. infantum* were characterized. Samples of skin ears, liver, spleen and lymph nodes were obtained to confirm *Leishmania* infection. Samples of gastrointestinal tract - jejune and colon were prepared for histological, morphometrical analysis and immunological assays. Suspensions cells obtained form the lamina propria were obtained and analyzed by flow cytometry. Macroscopic observation revealed no severe alterations of the mucosa however, 42% of animals contained hyperemia. A chronic cellular exudate was mainly observed in the lamina propria layer. It was composed plasma cells, lymphocytes and macrophages parasitized or not with amastigotes forms of *Leishmania*. Correlation between inflammatory reaction and parasitism was higher in the colon ($r^2=0,68$) and skin ($r^2=0,59$). In comparison to these parameters between the organs of single dog, the inflammatory reaction was statistically different ($p<0,0016$), where the abdominal lymph nodes and jejune were the anatomical site more reactive. However in the parasitic load was not verified difference statistics. The analysis of lymphocytes demonstrated that the population of $LTCD4^+FOXP3^+$ in colon, jejune e lymph node mesenteric it is greater that of $LTCD8^+FOXP3^+$ ($p=0,0411$; 0,0085 and 0,0115, respectively). However, we did not observe statistical difference between the population of $LTCD4^+FOXP3^+$ and $CD8^+FOXP3^+$ of organs of one same dog, (p=07165 and 0,7562, respectively). The correlation between parasitism and $LTCD4^+FOXP3^+$ was only positive ($r^2=0,5545$) in the colon. In conclusion, we observed a high parasite burden throughout the mucosa jejune and colon without severe tissue alterations.
Support by CNPq and FAPEMIG

## IM.45 – ACTIVATION OF PLATELETS BY *LEISHMANIA MAJOR* PARASITES TO ATTRACT A POPULATION OF KILLER MONOCYTES TO THE SITE OF INFECTION

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*Leishmania spp* are intracellular parasites that reside in macrophages where they can multiply and cause disease. During steady state, resident tissue macrophage homeostatically arise from local tissue-resident progenitor stem cells which constitutively give rise to mature tissue macrophages. During inflammation, however, monocytes from the blood represent the major source of macrophages. Monocytes rapidly exit the blood and within a few days transform into cells that are morphologically and functionally indistinguishable from macrophages. There are two major sub-populations of monocytes, based on their morphology, physiology and by differential surface "marker" expression. One of the populations expresses the granulocyte marker called GR1 and it is considered as the inflammatory subset. In this study we examined the kinetics of leukocyte recruitment into lesions following *Leishmania major* infection of mice, and observed the rapid migration of a population of F4/80+CD11b+GR1+ monocytes that rapidly engulf and efficiently kill *L. major*. We demonstrate that platelet activation by complement-opsonized parasites is responsible for the recruitment of these killer monocytes into the lesions. Activated platelets adsorb to *L. major* in the presence of complement and secrete PDGF. PDGF induces the rapid release of MCP-1 from mesenchymal cells to recruit GR1+ monocytes. In conclusion, this work shows the role of platelets in host defense, which involves the rapid recruitment of a sub-population of inflammatory monocytes from the blood to tissue where they rapidly engulf and kill intracellular parasites. Supported by National Institutes of Health (NIH) –USA.

## IM.46 – THE EFFICACY DURATION OF THE INTRANASAL pCI-neo-LACK VACCINE AGAINST LEISHMANIA CHAGASI INFECTION IS CONCOMITANT WITH THE PERIPHERAL EXPRESSION OF LACK TRANSCRIPTS.

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We previously demonstrated that intranasal (i.n.) immunization with a plasmid DNA encoding the p36/LACK leishmanial antigen (pCI-neo-LACK) effectively protects susceptible mice against both cutaneous and visceral leishmaniasis. In the present study, systemic expression after nasal uptake, and the duration of protective immunity was addressed. By using reverse transcriptase (RT)-PCR, we detected the expression of mRNA-LACK transcripts in the spleen, brain, cervical and popliteal lymph nodes of BALB/c mice on day seven through 3 months after vaccination with two i.n. doses of 30 μg of pCI-neo-LACK, declining afterwards. RNA expression coincided with an enhanced cutaneous hypersensitivity to skin-injected parasite antigens, and to protection against visceral leishmaniasis. Mice that were infected with *Leishmania chagasi* after 7 days or 3 months, but not 6 months of vaccination had significantly lower parasite loads than non-vaccinated controls. An examination of the responsiveness of their spleen cells to parasite antigens revealed an enhanced blastogenesis and increased production of IFN-γ and IL-4 cytokines, but decreased IL-10. On the other hand, in animals infected after 6 months of vaccination, the IL-10 response was as high as in non-vaccinated controls. Together, these data show that the 3-month duration of the protective immunity against *L. chagasi* infection conferred by intranasal pCI-neo-LACK is associated with the concomitant systemic expression of mRNALACK. Financial support: CNPq. *Key Words:* Leishmaniasis; *Leishmania chagasi*; intranasal; vaccine; LACK.

## IM.47 – EFFICACY OF INTRANASAL VACCINATION WITH LACK-DNA AGAINST VISCERAL LEISHMANIASIS IN EXPERIMENTAL HAMSTER MODEL

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LACK (*Leishmania* analogue of the receptor kinase C) is a conserved protein in the protozoan of the genus *Leishmania*, that is associated with the immunopathogenesis and susceptibility of BALB/c mice to *L.major* infection. Previously, we demonstrated that intranasal immunization with a plasmid carrying the LACK gene of *Leishmania infantum* (LACK-DNA) promotes protective immunity in BALB/c mice against *Leishmania amazonensis* and *Leishmania chagasi.* In the present study, we investigated the protective immunity given in hamsters intranasally vaccinated with 2 doses of LACK-DNA (30 µg). Compared with the controls (PBS and pCI-neo), animals vaccinated with LACK-DNA showed a significant reduction in parasite load in the spleen and liver, increased lymphoproliferative response and increased nitric oxide (NO) production by splenocytes stimulated with parasite antigens. Furthermore hamsters vaccinated with LACK-DNA showed high IgG and IgG2a serum levels as compared to control animals comparable not only predictive of clinical outcome following vaccination, but also with the protection observed. Our results showed that intranasal vaccination with LACK-DNA promoted protective immune response in hamsters and shows the broad spectrum of intranasal LACK DNA in different host species as previously demonstrated in murine visceral leishmaniasis. Financial support: CNPq. Key words: Leishmaniasis; *Leishmania chagasi*; hamster model; intranasal administration; LACK-DNA; mucosal vaccine

## IM.48 – PAF RECEPTOR IS REQUIRED TO ANTI-LEISHMANIAL ACTIVITY MILTEFOSINE MEDIATED

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Visceral leishmaniasis, which can be caused by *Leishmania donovani* in India or *L. chagasi* in Brazil, is considered by WHO of the six main public health problems worldwide and is fatal if untreated. Miltefosine (hexadecylphosphocholine), which was first used as treatment in cancer patients has been used as an effective oral drug in visceral leishmaniasis. There is a structural similarity between Miltefosine and platelet activating factor (PAF). PAF acts by binding to the receptor of PAF (PAFR) present in target cells. The mechanism by which the drug works is not well established, our hypothesis is that due to structural similarity between Miltefosine and PAF, the drug uses this receptor to enter in the cell. To test this hypothesis, BALB/c and $PAFR^{-/-}$ mice were orally treated from the 14th day after infection for 7 consecutive days at doses of 20mg/kg/day and killed on day 28. Our data showed that treatment of BALB/c led to a reduction in parasite load in the liver and spleen of these mice. Interestingly, we did not find the same treatment effect in $PAFR^{-/-}$ mice. Therefore, these mice continued to show higher parasite load in these organs. To investigate an immunomodulatory function of the drug, we measured the levels of IL-4, IL-10, IFN-γ and TNF-α in the serum of these mice. We found no differences in the amount of released cytokines between BALB/c mice treated or not. However, peritoneal macrophages from wild type mice infected and treated showed reduction in Arginase I activity. Thus, our results indicate that the drug uses the PAF receptor to enter in target cells where, in addition to direct cytotoxic effect on Leishmania, showed immunomodulatory mechanism by inhibiting the activity of Arginase I in macrophages. Supported by CAPES, CNPq and FAPEMIG.

## IM.49 – EVALUATING THE EFFECTIVENESS OF TRANSGENIC TOBACCO (NICOTIANA TABACUM L.) EXPRESSING THE PARASITE ANTIGEN LACK IN ORAL VACCINATION AGAINST CUTANEOUS LEISHMANIASIS IN MICE

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LACK (analogue of the receptor for activated protein kinase C (PKC)) is a well conserved parasite protein. Our group has shown that oral and nasal vaccination with whole parasite antigen LaAg or nasal vaccination with its component LACK in the form of DNA confer protection against visceral and cutaneous leishmaniasis. In this work, we evaluated the potential of a transgenic tobacco expressing the LACK antigen (LACK +/+) to serve as an edible vaccine against cutaneous leishmaniasis. The fresh leaves were crushed to powder in liquid nitrogen and then lyophilized for one day. BALB/c mice received 2 doses of the vaccines with a 1 week interval: a) tobacco LACK+/+ or control LACK-/- (20 mg,); b) LaAg (100 ug). One week after the second dose, mice were s.c. infected with $2x10^6$ promastigotes of *L.amazonensis*. The growth of the lesions was followed for three months, when the animals were sacrificed for quantification of parasite load in the footpad by Limiting Dilution Assay. The results showed a slower development and lower parasite burden compared to PBS, LACK-/- tobacco and LaAg. Vaccination with the LACK -/ - tobacco did not affect growth of the lesion, however, the parasite load in this group was significantly lower than PBS. These results indicate that the immunomodulatory effect of oral vaccination with the transgenic tobacco is reflected in protection, even if partial, against infection by *L.amazonensis*, proving to be even more effective than LaAg. Ongoing experiments are comparing the effect of intragastric gavage versus ad libitum intake, as well as evaluation of the use of transgenic tobacco LACK +/+ in protection against visceral leishmaniasis. Supported by CNPq.

## IM.50 – SALIVARY GLAND HOMOGENATE FROM *PSYCHODOPYGUS WELLCOMEI* DID NOT ENHANCES *LEISHMANIA (VIANNIA) BRAZILIENSIS* INFECTION IN BALB/c MICE.

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The promastigote forms of *Leishmania* are transmitted by sand flies during blood feeding. Some reports have shown that vector's saliva plays an important role in the modulation of immune response, helping the establishment of the infection. But the most of these reports used *L. longipalpis* or *P. papatasi* laboratory-reared as a source of saliva to infect different models with different species of parasite; and conflicting data have been described when the natural vector/parasite binomium is used. Thus, the present work has the main aim evaluate, in the natural vector/parasite binomium, the effects of wild-caught vector saliva of *Psychodopygus* **wellcomei *in* L. (V.) braziliensis *promastigotes*** infection in BALB/c mice***. The animals*** were inoculated into the hind footpads with $10^6$ *L. (V.) braziliensis* promastigotes without (P group) or with salivary gland homogenate from wild-caught *P.* **wellcomei** (P+W-SGH group). Control groups received only salivary gland homogenate or PBS in the hind footpads. After 10 weeks post infection, fragments from skin and lymph nodes were collected to analyze the main histopathological changes, parasite burden, as well as cytokine and nitrate amounts in supernatant of draining lymph nodes cells cultures. Both groups, P and P+W-SGH, showed similar footpad swelling with a peak at 5 weeks pi, followed by progressive regression. In addition, no significant difference has been found in histological pictures as well as in parasite load in skin and lymph nodes. Concerning to the cytokines production in the supernatant of the lymph nodes cells culture, IL-4 did not show significant differences between the groups, however P+W-SGH group produced decreased IFN-$\gamma$ levels compared to P group ($p<0.05$). NO levels were similar between the groups. The results presented herein indicated that *P. wellcomei* salivary gland homogenate from wild-caught vector does not promote the enhancement of the *L. (V.) braziliensis* infection in BALB/c mice.
Supported by LIM50 HC-FMUSP.

## IM.51 – VITAMIN A-DEFICIENCY IMPAIRS INTESTINAL CD4$^+$ FOXP3$^+$ REGULATORY T CELL EXPANSION AND THE EFFICACY OF THE ORAL LAAG VACCINE AGAINST *LEISHMANIA AMAZONENSIS* INFECTION

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Oral immunization with *L. amazonensis* promastigotes total antigen (LaAg) induces protection in mice against *L. amazonensis* infection (Pinto *et al*, 2003). Retinoic acid (RA), a vitamin A metabolite, is a cofactor required for efficient TGF-β-mediated naive T cell differentiation to Foxp3$^+$ regulatory T cells in the intestinal mucosa (Sun *et al*, 2006). In this study, the RA influence on the oral LaAg vaccine immunogenicity and efficacy was investigated in vitamin A-deficient mice. Pregnant BALB/c were fed with vitamin A-containing (Vit A+) or vitamin A-free (Vit A-) pelleted food from days 7-10 of gestation and during lactation. After weaning, pups were kept under their mother's diet throughout the experiment. At six weeks of age, they were orally vaccinated with two doses of LaAg (100 μg) with seven days interval. Two days after the last dose, Foxp3 expression on CD4$^+$ mesenteric lymph node (MLN) cells was assessed by flow cytometry. To evaluate the vaccine efficacy, 7 days after the last dose, mice were infected subcutaneously in the footpad with *L. amazonensis* and lesion development was monitored for 70 days. On day 70 post infection, the parasite burden and the cytokine profile were evaluated in the infection site. Vaccination increased the percentage of CD4$^+$ Foxp3$^+$ cells only in Vit A+ mice (from 5.9% to 10.9%). The faster lesion development and increased parasite burden indicated that Vit A- are more susceptible to infection than Vit A+ animals. Oral LaAg was only effective in Vit A+ animals, increasing IFN-γ and decreasing IL-4 production in the infected footpads. These results show that vitamin A/RA is required for an efficient response against peripheral *L. amazonensis* infection and suggest that vitamin A/RA–dependent expansion of CD4$^+$ Foxp3$^+$ regulatory T cells in the intestinal mucosa dictates the efficacy of the oral LaAg vaccine. Supported by CNPq.

## IM.52 – TGF-β *VERSUS* ENZYME INDUCIBLE OXIDE NITRIC SYNTHASE (INOS) EXPRESSION IN AMERICAN CUTANEOUS LEISHMANIASIS DUE TO *LEISHMANIA (VIANNIA) BRAZILIENSIS* AND *LEISHMANIA (LEISHMANIA) AMAZONENSIS*

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TGF-β is a potent regulatory cytokine that suppresses the expression of iNOS and IFN-γ. In contrast, little is known about the expression of iNOS in human ACL. The aim of this study was to determine the TGF-β and iNOS expression in lesions of ACL due to the major pathogenic leishmanial parasites found in Brazil: *Leishmania (Viannia) braziliensis* and *Leishmania (Leishmania) amazonensis*. 26 patients were examined: anergic diffuse cutaneous leishmaniasis (ADCL) and borderline disseminated cutaneous leishmaniasis (BDCL), both due to *L. (L.) amazonensis* and with DTH$^-$ (five cases); localized cutaneous leishmaniasis (LCL) also due to *L. (L.) amazonensis* with DTH$^-$ (five cases) and DTH$^+$ (three cases) and, LCL due to *L. (V.) braziliensis* with DTH$^+$ (eight cases). Paraffin-embedded biopsies were submitted to immunohistochemistry using the polyclonal anti-TGFβ1 (sc146) and anti-iNOS (N-20, sc-691) antibodies. For amplification and visualization of the reaction Novolink max polymer was used. The immunostained cells were counted in 5–10 fields (400x) in section by using an image analysis system (Zeiss). The TGFβ1+ cellular expression (mm$^2$) has shown a progressive increase from the reactive LCL central form due to *L. (V.) braziliensis* to the non-reactive ADCL and BDCL extremity forms due to *L. (L.) amazonensis* (LCL/*Lb*$^{DTH+}$[492] < LCL/*La*$^{DTH+}$[562] < LCL/*La*$^{DTH-}$[600] < BDCL$^{(DHT-)}$[944] < ADCL$^{(DHT-)}$ [1534]). In contrast, the iNOS expression has shown a decreasing profile in the same way of this spectrum (LCL/*Lb*$^{DTH+}$[1284] > LCL/*La*$^{DTH+}$[946] > LCL/*La*$^{DTH-}$[606] > BDCL$^{(DHT-)}$[460] > ADCL$^{(DHT-)}$[243]), characterizing an inverse association between these modulators of cellular immune response. In conclusion, our results allow a logical understanding on the role of TGF-β and iNOS expression in the genesis of these different clinical forms of ACL, emphasizing the importance of the antigen-specific ability of *L. (V.) braziliensis* or *L. (L.) amazonensis* as primordial factor for the development of these clinical forms of ACL.

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## IM.53 – STUDY OF REGULATORY T CELLS (TREGS) FUNCTION IN HUMAN CUTANEOUS LEISHMANIASIS DUE TO *LEISHMANIA BRAZILIENSIS* INFECTION

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In *L. braziliensis* cutaneous leishmaniasis (CL), despite the patients present strong Th1 responses, related to protection, they develop the characteristic skin ulcers. However, sub-clinical patients, that control parasite replication without developing lesions, have milder Th1 responses and higher immunoregulatory cytokines production. This suggests that excessive or non-modulated Th1 responses may be related to tissue damage. Since Tregs are important modulators of Th1 responses in murine leishmaniasis, our objective is to characterize the role and participation of Tregs in human infection with *L. braziliensis*. PBMC from CL patients or noninfected donors (ND) were phenotyped by flow cytometry. $CD4^+CD25^+$ populations from ND and CL patients' PBMC were isolated and their suppressive functions on PBMC evaluated. Also, patients' Montenegro skin reaction measurements were correlated with FoxP3, IL-10 and IL-10R mRNA expressions in the lesions. CL patients' frequency of cells with Treg characteristics was higher, although not significantly different from ND. CL patients and ND $CD4^+CD25^+$ cells inhibited more efficiently proliferation of ND than CL patients PBMC, but inhibited IFN-γ production of CL patients and ND PBMC stimulated with anti-CD3 similarly. They also suppressed IFN-γ production in CL patients PBMC stimulated with parasite antigens. TNF-α production was suppressed by CL or ND $CD4^+CD25^+$ cells when patients' PBMC were stimulated with parasite antigen, while no suppression occurred in CL and HD PBMC stimulated with anti-CD3. We observed a not significant positive correlation between the expressions of FoxP3, IL-10 and IL-10R and DTH responses. These results suggest that CL patients' Tregs are as effective as ND Tregs in suppressing effector T cell responses, however, CL patients effector cells seem to be less susceptible to suppression. Future experiments will focus on cells isolated from the lesions, where most strongly activated cells are located and Tregs may play major role in controlling tissue damage. Support: FAPESP, CAPES, NIH.

## IM.54 – *LEISHMANIA (VIANNIA) BRAZILIENSIS* ISOLATED FROM CUTANEOUS LESIONS INDUCES EARLY DISEASES IN IFNγ KNOCKOUT MICE THAN ISOLATES OBTAINED FROM MUCOSAL LESIONS.

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*Leishmania (Viannia) braziliensis* is the major parasite that causes tegumentary leishmaniasis in Brazil. The clinical manifestations of the disease caused by this pathogen include the cutaneous and mucosal forms. The control of the parasite is dependent on the activation of macrophages by IFNγ. In that sense, IFNγ knockout are highly susceptible to the infection. To evaluate the virulence of the parasites isolated from patient with cutaneous (CL) or mucosal (ML) leishmaniasis, IFNγ knockout mice were inoculated with doses among 100 to 100.000 amastigotes obtained originally from two CL (JCJ8c and RPL5c) or ML (PPS6m and JBC8m) patient. The isolates were characterized as *L. (V.) braziliensis* by PCR using specie specific primers to glucose-6-phosphate dehydrogenase. The lesion in the infected foot pad of mice and the metastasis in the contra lateral paw were followed weekly by a gauge. Infections with doses of 100.000 parasites induced similar lesions for all isolates, reaching in the fourth week the sizes of 1.9±0.8 mm (JCJ8c); 2.7±1.1 mm (RPL5c); 2.5±1.5 mm (PPS6m) and 2.6±0.5 mm (JBC8m). At the same time, the dose of 100 parasites of the CL isolates induced lesions that reached the size of 1,7±0,7 mm (JCJ8c) and 0.9±0.19 mm (RPL5c), however, the ML isolates were unable to induced any lesion until this time. Metastasis in the non inoculated paw was observed for all isolates, and the CL isolates induced measurable metastasis six weeks after infection in all doses. ML isolates induced measurable metastasis only with the highest doses. These data suggest that ML parasites grow slowly than CL isolates *in vivo*. This behavior can induce a weaker immune response and consequently a better survival of the parasites in the host. Supported by CAPES, CNPq, FUNAPE and FAPEG

## IM.55 – EVALUATION OF TUMOR NECROSIS FACTOR AND INTERLEUKIN 10 PRODUCTION IN WHOLE BLOOD CULTURES OF AMERICAN TEGUMENTARY LEISHMANIASIS PATIENTS

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The role of tumor necrosis factor (TNF) and interleukin 10 (IL-10) in American Tegumentary Leishmaniasis (ATL) is not completely understood, mainly in infections caused by *Leishmania* (*Viannia*) complex. The aim of this study was to evaluate the production of TNF and IL-10 *in vivo*, and *ex vivo* in whole blood cultures of ATL patients. ATL patients (22 cutaneous leishmaniasis, CL; 8 mucosal leishmaniasis, ML) and healthy controls were evaluated. Biopsy fragments were used to determine *Leishmania* subgenus using kDNA-PCR and PCR-RFLP. Whole blood cultures were incubated with *Toll*-like receptor agonists (LPS; $Pam_3Cys$), and *L. (V.) braziliensis* antigen. TNF and IL-10 were measured by using immunoenzymatic assay. West-Central Region contributed with 64.3% of ATL cases and *L.* (*Viannia*) sp was detected in 21 biopsy fragments. TNF levels were higher in serum and in cultures of ATL patients than in controls (serum: 198.5, from 50 to 1,976.0 pg/mL *vs* 114.9, from 50 to 450.7 pg/mL, $n = 25$, $p < 0.05$; LPS: 2,308.0, from 652.7 to 4,262.0 pg/mL *vs* 1,292.0, from 312.4 to 3,954.0 pg/mL, $n = 27$; $p < 0.05$). There was a positive correlation between number of lesions (CL) and TNF concentrations in activated cultures (LPS, $r = 0.62$, $p < 0.01$, $n = 21$). Serum levels of IL-10 did not significantly differ between patients and controls. Whole blood cultures of patients and controls produced similar amounts of IL-10. A positive correlation was detected between TNF and IL-10 in serum of CL patients ($r = 0.54$, $p < 0.05$, $n = 17$), but not in ML patient serum. Our data showed that patients infected with *L.* (*Viannia*) sp parasites produce high levels of TNF and similar levels of IL-10 compared to healthy controls. Data suggest that TNF can be related to severity of CL. Financial support: FAPEG, CNPq, CAPES

## IM.56 – IMPROVED INTRANASAL VACCINE EFFICACY OF LACK-DNA AND LaAg LEISHMANIAL ANTIGENS BY ENCAPSULATION IN CHITOSAN MICROPARTICLES AND COMBINED DELIVERY.

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We have previously shown the effectiveness of intranasal vaccination against cutaneous leishmaniasis in mice using both LACK DNA and free particulate leishmania antigen (LaAg) (Pinto E.F. et al., 2004). Chitosan microparticles (CMP) are biocompatible, cationic and mucoadhesive delivery systems potentially suitable for mucosal delivery of negatively charged antigens. In this work, we proposed to optimize LACK DNA and LaAg efficacy by absorption onto CMPs, and combined delivery. For this, CMPs with mean diameter of 5 um and + 30,0 mV of surface charge were constructed and adsorbed with LACK DNA or LaAg at 1:50 and 2:1 Ag:polymer (w/w), respectively. BALB/c mice were immunized twice by the intranasal route with suboptimal doses of LACK DNA plus LaAg in their free forms or complexed with CMPs and then challenged with fluorescent *L. amazonensis*-GFP promastigotes. Animals immunized with a combination of both antigens complexed with CMPs presented slower lesion growth ($p \leq 0{,}001$) and reduced parasite burden ($p \leq 0{,}001$), as compared to PBS controls. On day 97 of infection, those mice were producing higher levels of IFN-$\gamma$ in the infected footpads as compared to animals receiving free antigens ($p \leq 0{,}001$) or PBS ($p \leq 0{,}001$), whereas local IL-4 production was not significantly affected. These data show that i.n. vaccination with LACK DNA and LaAg adsorbed onto CMPs led to Th-1 type immunity at the infection site, and increased the effectiveness of the combined vaccination. Supported by CNPq.

## IM.57 – BALB/c and C57BL/6 MACROPHAGES UNDERGO APOPTOSIS DURING *IN VITRO* INFECTION OF *LEISHMANIA AMAZONENSIS*, BUT NOT DURING INFECTION OF *L. MAJOR* OR *L. GUYANENSIS*

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Apoptosis of host cells has been widely demonstrated as a key event on the outcome of several infectious diseases, downregulating immune responses against infection. Leishmaniasis is a wide-spectrum illness caused by species of the protozoan parasite *Leishmania*, which replicates within macrophages of the mammalian host. In the murine model, *Leishmania amazonensis* can cause a growing lesion at the site of infection, which can either lead to death of some strains of mice, such as BALB/c, or to the control of infection, as C57BL/6. *Leishmania major* and *L.guyanensis* are also responsible for the cutaneous form of the disease, but have different host susceptibility/resistance patterns in the murine model. Using PI permeabilization assay we have demonstrated previously that there is a viability reduction of BALB/c or C57BL/6 peritoneal macrophage infected *in vitro* with *L.amazonensis* when compared to non-infected cells. Macrophages from both strains infected with *L. amazonensis* presented a typical DNA fragmentation pattern, seen in agarose gels and TUNEL assay, starting within the first hours of infection, indicating that macrophage death occurred through apoptosis. These results were corroborated with the increase of hypoploydic nuclei in macrophage cultures infected with *L.amazonensis* when compared to the non-infected control. In addition, using flow cytometry, we have shown that macrophages infected with *L. amazonensis* also expose phosphatidylserine, as demonstrated by the increased binding of AnnexinV to the host cells. On the other hand, the typical ladder pattern of DNA fragmentation was not observed in BALB/c macrophages infected with *L.major* or *L.guyanensis* Together, our results show that macrophages infected *in vitro* with *L. amazonensis* die through apoptosis. They also suggest that *L.major* or *L.guyanensis* do not induce apoptosis in mice macrophages. It seems therefore that apoptosis of host cells does not correlate with susceptibility or resistance of the host, but rather with strain-specific features. Financial support:FAPEMIG, CNPq

## IM.58 – IMMUGENICITY AND EFFICACY OF *LEISHMANIA AMAZONENSIS* EXTRACELLULAR SERINE PROTEASES FRACTION (LASP-EX) AND OLIGOPEPTIDASE B2 AS ANTILEISHMANIAL VACCINES

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*Leishmania amazonensis is* the main agent of anergic diffuse cutaneous leishmaniasis. Our previous studies demonstrated that contrary to intramuscular (i.m.) immunization with whole *Leishmania amazonensis* antigens (LaAg) that enhances mouse susceptibility to cutaneous leishmaniasis, intranasal (i.n.) vaccination confers protection. Using a single-step aprotinin-agarose chromatography, serine proteases were partially purified from extracellular extracts of *L. amazonensis* promastigotas (LaSP-Ex). Their effectiveness in i.m. and i.n. vaccination was compared using a protocol similar to used with LaAg. BALB/c mice were twice vaccinated by the i.m. or i.n route with 25μg of LaSP-Ex, prior to footpad infection with *L. amazonensis*-GFP. We found that i.m. immunization with LaSP-Ex promoted increased susceptibility to subsequent infection, irrespective of the presence of saponin as adjuvant. When using the i.n. route, LaSP-Ex induced a strong protective immunity, as seen by the significantly smaller lesion sizes and parasite burden at day 125 after infection. These findings indicate that similarly to observed with LaAg, LaSP-Ex displays opposing effects when used by the i.m. or i.n. routes. I.n. vaccination with LaSP-Ex induced TGF-β production, but not IL-10 in cervical lymph nodes. At PID 7, decreased IL-10 and increased TGF-β were produced in lesion-draining popliteal lymph nodes, suggesting the involvement of T regulatory cells both in the mucosa and the periphery. At PID 125, protection was accompanied by elevated IFN-γ and IL-12 in the infected footpads of LaSP-Ex vaccinated mice in relation to non-immunized mice, indicating expansion of Th1 cytokines in infected site. A possible active component of this fraction is oligopeptidase B2 (OPB2), a new serine oligopeptidase described in *Leishmania amazonensis* (de Matos Guedes et al, 2008). I.n. vaccination with recombinant OPB2 induced protection as observed for LaAg and LaSP-Ex. This study proposes that LaSP-Ex and recombinant OPB2 are further explored as a potential defined adjuvant-free mucosal vaccines against cutaneous leishmaniasis.
Supported by FAPERJ and CNPq.

## IM.59 – METACYCLIC OR BLOOD *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES TRIGGERED CHANGES IN NK AND T-CELLS DERIVED CYTOKINES IN SPLEEN

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We previously demonstrated that infection by different infective forms of *T. cruzi* causes different immune responses in peripheral blood. Herein we have further focus in the cytokines produced by NK and T cells in the spleen following infection with metacyclic or blood trypomastigotes of Be-78 strain. Our results demonstrate that in animals infected with metacyclic forms there is a decrease in the percentage of NK cells in 42 days after infection in the spleen. Moreover, regardless of the infecting form there is an increase of $CD8^+$ lymphocytes in the spleen of animals at 28 and 42 days after infection. Regarding the production of cytokines, we observed an earlier production of IFN-$\gamma$ by CD8 cells in mice infected with metacyclic trypomastigotes on day 7 post infection, whereas in animals infected with blood forms have an early production only of TNF-$\alpha$, by both $CD4^+$ and $CD8^+$ T lymphocytes and NK cells. But this increase in IFN-$\gamma$ and in TNF-$\alpha$ returns to baseline levels at 42 days after infection in animals infected with metacyclic forms, however in animals infected with blood forms, levels for these cytokines do not return to baseline remaining elevated until 42 days after infection. For immunomodulatory cytokine IL-10, there is an increase of the levels in animals infected with metacyclic forms in 28 and 42 days after infection, whereas animals infected with blood forms there is a decrease in the production of this cytokine at day 7 after infection by CD4 and NK cells. These results re-emphasize the importance of the inoculum source triggering distinct aspects of the immune response, showing that the infection with blood forms leads to an exacerbation in the production of inflammatory cytokines, with higher levels even after controlling parasitemia. Supported by FAPEMIG (PPM, Redes Toxifar e Bioterismo), CNPq and UFOP.

## IM.60 – IMMUNOHISTOCHEMICAL STUDY OF CELLS INFILTRATING THE MYOCARDIUM OF DOGS EXPERIMENTALLY INFECTED WITH METACYCLIC OR BLOOD TRYPOMASTIGOTE FORMS OF *TRYPANOSOMA CRUZI*

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Similarly to that observed in humans, *Trypanosoma cruzi* experimentally infected dogs showed a mononuclear inflammatory infiltrate during the acute phase of infection. The identification of these cells is an important strategy to understand the immunopathological mechanisms triggered by the parasite during the infection. Therefore, the cells involved in acute experimental infection of dogs by metacyclic (MT) or blood trypomastigotes (BT) of Berenice-78 *T. cruzi* strain were quantified by morphometric analysis in right atrium and interventricular septum. Quantification of cell phenotype showed that $CD4^+$ T lymphocytes are predominant in the inflammatory infiltrate of animals infected with both forms and both tissue sections. On the right atrium the $CD8^+$ T lymphocytes were more present in the infected groups in comparison to the control group. However, $CD14^+$ macrophages cells were prevalent only in animals infected with MT when compared to the control group. In the interventricular septum the $CD8^+$ T lymphocytes and the $CD14^+$ macrophage cells were found in large numbers in the MT group when compared to control and BT group. Neutrophils are present in less number if compared to the other cells analyzed, but were found in greater numbers in the right atrium of both infected groups. The better preserved histopathological picture observed in the animals infected with MT may be directly related to the interaction between $CD8^+$ T lymphocytes and $CD14^+$ macrophages because they are potent producers of interleukin-12, a cytokine that stimulates the production of interferon-gamma by $CD8^+$ T lymphocytes. This cytokine is crucial in the process of infection resistance by *T. cruzi* during the acute phase via stimulation of nitric oxide synthesis by macrophages. The results showed that the MT forms was more efficient and less harmful for the host.
Supported by FAPEMIG (PPM, Redes Toxifar and Bioterismo), CNPq and UFOP.

## IM.61 – QUANTIFICATION OF CARDIAC INFLAMMATION IN BALB/c MICE WITH *TRYPANOSOMA CRUZI* CLONES MIXED INFECTION SUBJECTED TO TREATMENT WITH BENZNIDAZOLE

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Analysis of *Trypanosoma cruzi* reservoirs, vectors and the man has revealed that the presence of mixed infections (MI) is frequent in nature. Moreover, experimental studies demonstrated that in these cases there is not only an overlap of effects of mixing clones, but an interaction between fundamental biological properties. This shows the importance of mixed infections studies and our group has assessed MI with *T. cruzi* clones belonging to genotypes main 19, 20, 39 and 32, which are widely distributed in American continent. Female BALB/c were inoculated intraperitoneally with 5,000 blood trypomastigotes of each clone (Gamba cl1 and OPS21 cl1 – 19 genotype, P209 cl1 and Cuica cl1 - genotype 20, Bug2148 cl1 and SO3 cl5 – 39 genotype, SO3 cl5 - genotype 39, IVV cl4 and MAS cl1 – 32 genotype) combined in pairs and treated with benznidazole, during 20 consecutive days. We evaluated the cardiac inflammatory response and the impact of treatment on MI. Two animals of each combination were necropsied 30-35 dpi, (acute phase-AP) and others two animals between 120-125 dpi (Chronic phase-CP). The results were compared with their respective untreated groups. The hearts samples were fixed in formalin, processed, submitted to microtomy and staining with hematoxylin-eosin for inflammatory process quantification. The inflammatory response was different between the monoclonal and mixed infections in almost all cases, and the mixed infection triggered inflammatory response more intense than expected, mainly in the untreated group. In general, the treatment reduces heart inflammation. Only in monoclonal infections and in acute phase occurred increased inflammation after treatment and this was not correlated with any specific genotype. These data corroborate with previous studies from our group in relation to virulence and drug susceptibility, demonstrating that in mixed infections, the cardiac lesions development is difficult to be predicted given the behavior of individual clones.
Supported by FAPEMIG (PPM, Redes Toxifar and Bioterismo), CNPq, CAPES and UFOP.

## IM.62 – IMPACT OF GALECTIN-1 ON THE EVOLUTION OF ACUTE EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION.

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Galectin-1 (Gal-1) is a β-galactoside-binding protein and participates in several biological processes, including modulation of immune response. In the literature, there are several reports about the potential therapeutic use of Gal-1 for autoimmune processes, degenerative and infectious diseases. However, there are few reports on the involvement of Gal-1 in Chagas disease. Thus, this work was conducted to study the participation of endogenous Gal-1 in acute experimental infection by *T. cruzi*. Galectin-1-deficient mice (KO - Gal-$1^{-/-}$) or wild type (WT - Gal-$1^{+/+}$) mice were used to perform the *in vivo* experiments. The animals were infected with trypomastigotes of *T. cruzi* (strain Y), intraperitoneally. The biological parameters analyzed were parasitemia and survival; histopathology of heart tissue; leukocyte immunophenotyping by flow cytometry; cytokine detection by ELISA. Infected-Gal-$1^{-/-}$ mice showed the lowest levels of parasitemia. Interestingly, all infected-KO mice survived after the infection, whereas the infected-WT mice showed a drastic reduction in survival. The absence of endogenous Gal-1 promoted a reduction on inflammatory cells infiltrate in the cardiac muscle of infected mice. The number of circulating TCD8+ cells in the infected-WT mice were higher, and the sera of infected-WT mice showed higher levels of IFN-γ and IL-12, compared with the infected-KO mice. Taken together, these results suggest that the absence of endogenous Gal-1 promoted immunological profiles that cooperate to the resolution of acute experimental infection by *T. cruzi*. Supported by CAPES.
Keywords: Galectin-1, *Trypanosoma cruzi*, infection, lectin, immunoregulation

## IM.63 – THE ROLE OF Rip2 IN THE SIGNALING PATHWAY DOWNSTREAM OF NOD1 IN RESPONSE TO *Trypanosoma cruzi* INFECTION

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Chaga's disease, caused by *Trypanosoma cruzi*, is a serious parasitic disease in Latin America, affecting approximately 18 million people. It was described that the immune response against *T. cruzi* is initially triggered by pattern recognition receptors (PRRs), such as Toll-like receptors (TLRs). However the participation of non-TLR PRR remains largely obscure. We have recently demonstrated that Nod1, a member of the Nod-like receptors (NLRs) family, accounts for host response and resistance against *T. cruzi* infection in macrophages and in a murine model of Chagas disease. Here, we aimed to investigate the signaling pathway downstream of Nod1 that operates in response to T. cruzi infection. It is well established that Nod1 signals via the Rip2 kinase to trigger mitogen-activated protein kinase (MAPK) and NF-kB activation. Thus, we used macrophages from C57BL/6 (WT), $Nod1^{-/-}$ or $Rip2^{-/-}$ mice to investigate the role of Rip2 for macrophage response against the infection. Macrophages were pre-treated with IFN-$\gamma$ and infected with *T. cruzi*. The number of intracellular amastigotes was analyzed 48 h after infection by Giemsa staining. In addition we evaluated the release of trypomastigotes from cells infected for 3, 4 and 5 days. The, activation of MAPK Erk1/2 and p38 was evaluated by western blot in infected macrophages. We found that as compared to WT, macrophages from $Nod1^{-/-}$ and $Rip2^{-/-}$ contained a higher number of intracellular amastigotes and released higher numbers of trypomastigotes to the tissue culture supernatant. Besides, we found that Erk1/2 and p38 were activated regardless to the presence of Nod1 and Rip2. These results suggest that activation of MAPK is not dependent on Nod1/Rip2 pathway. Nonetheless, Rip2 seems to be at least partially involved in the signaling pathways downstream to Nod1 for restriction of intracellular parasitism by macrophages.
Supported by FAPESP, CNPq, PEW, WHO/TDR and FAEPA.

## IM.64 – PRODUCTION OF MONOCLONAL ANTIBODIES AGAINST *Trypanosoma cruzi* VIRULENCE FACTOR NUCLEOSIDE TRIPHOSPHATE DIHOSPHOHYDROLASE 1 (NTPDase-1)

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*Trypanosoma cruzi* is the etiological agent of Chagas Disease, a tropical neglected disease that remains as a Public Health problem in many countries including Brazil. NTPDase-1 is an ecto-nucleotidase from apyrase/CD39 family that was previously demonstrated as a virulence factor and infectivity facilitator in in vitro and in vivo *T. cruzi* infections. Because of this NTPDase-1 is considered as a good target in rational drug design to Chagas disease chemotherapy. Monoclonal antibodies can be applied as chemotherapeutic agent and used in biological and biochemical assays. The main goal of this work is to produce specific monoclonals against *T. cruzi* NTPDase-1. To do this we expressed the NTPDase-1 in bacterial heterologous system pET21b. The recombinant protein was purified by Ni-NTA affinity chromatography. The purified protein was used to immunize mouse. Specific antibodies were detected by indirect ELISA and Dot-ELISA assays using the recombinant *T. cruzi* NTPDase as antigen. Spleen cells were isolated and fused with tumor cells to produce stable hybridomas. We isolated seven stable hybridomas and 51 isolated clones producing monoclonals. Many of them were not able to recognize *Leishmania* NTPDases. These monoclonal will be analyzed about its ability to inhibit nucleotidase activity of *T. cruzi* NTPDase and the in vitro *T. cruzi* infectivity.
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## IM.65 – NEUROLOGICAL MANIFESTATION IN EXPERIMENTAL *Trypanosoma cruzi* INFECTION: DEPRESSION AND MEMORY

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Since the discovery of the American trypanosomiasis by Carlos Chagas in 1909, it is known that the protozoan parasite *Trypanosoma cruzi* affects the central nervous system (CNS). Neuropathological lesions and parasites inside glial cells of the CNS are detected in humans, mainly, during the acute phase of infection. In the chronic phase, cases of neuropathological alterations are rare, while more frequent and severe in immunocompromised individuals with co-infections (eg, HIV), in transplanted and cancer patients. In Chagas disease, psychiatric disorders, cognitive and behavioural alterations are detected during the chronic phase, probably related by cardiac features and/or by *T. cruzi*-elicited direct or indirect CNS injuries. Experimentally, C3H/He infected mice develop a severe meningoencephalitis during the acute phase that self resolves in the chronic infection, although parasite antigens persist in the CNS. Further, drug-induced immunosuppression results in reactivation of the CNS inflammation. Therefore, we investigated the presence of neuropsychiatric changes, in special, depression and memory impairment during the experimental *T. cruzi* infection. Adopting *T. cruzi*-infected C3H/He mice during the acute phase (30 days post-infection) and chronic phase (90 days post-infection), we performed forced swimming and tail suspension tests to assess depression. Additionally, object recognition test was elected to investigate memory status. Acute and chronically infected mice showed high immobility time when submitted to forced swimming and tail suspension tests. Importantly, when compared of non-infected control mice, the infected C3H/He mice did not show difference in the open field test, specific to assess locomotor alterations. In memory test, chronically infected C3H/He mice showed reduced time of exploration of a novel object, revealing mnemonic deficit. In conclusion, depression and memory deficit are present during experimental infection by *T. cruzi*.
Supported by: IOC-Fiocruz, CAPES, FAPERJ.

## IM.66 – CD8 + T CELL FUNCTION IN THE PATHOGENESIS OF CHRONIC CHAGASIC CARDIOMYOPATHY

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Persistence of *Trypanosoma cruzi* associated with inflammation at target tissue is proposed to be the main immunopathogenic mechanism leading to chronic cardiomyopathy (CCC) in Chagas disease. CD8+ T cells comprehend the majority of inflammatory cells invading the heart tissue in CCC. Although human CD8+ T cells expressing cytokines and cytolytic effector molecules in a segregated manner were detected in different infectious diseases, less is known about the phenotype and function of CD8+ T cell subsets in *T. cruzi* infection. Herein, we approached the role of CD8+ T cell subsets, segregating pro-inflammatory (interferon-gamma-IFN-g+) and cytotoxic (perforin-pfp+) phenotypes, particularly recognizing parasite antigens, in the immunopathogenesis of *T. cruzi*-elicited experimental CCC. *T. cruzi*-infected C57BL/6 mice present parasitemia and cardiac parasitism during the acute infection peaking at 42-45 days post-infection (dpi). Specific IFN-g+ and cytotoxic (CTL) CD8+ T lymphocytes recognizing ASP-2 peptide were first detected at 15 dpi, while high frequency of these subsets were detected at 30 dpi, preceding parasitemia peak, and persisted in high percentage after parasite control (120 dpi). The persistence of high frequencies of peripheral CTL and IFN-g+ CD8+ T cells during the chronic phase paralleled the increase in numbers of these cells in the heart tissue and the increase in CK-MB levels in serum and a disorganized expression of connexin 43, markers of cardiomyocyte lesion, as well as significant electrocardiographic abnormalities. All these cardiac alterations were first detected at 30 dpi paralleling the entrance of these CD8+ T cell subsets in the heart tissue. Furthermore, higher frequency of inflammatory (IFN-g+) than cytotoxic (pfp+) CD8 + T cells is detected in the spleen of infected mice, however CD8+pfp+ present a more prone migratory phenotype with higher frequency of CCR5+LFA-1+ cells. Presently, we are studying the real migration potential and effector function of these CD8+ segregated subsets in *T. cruzi*-elicited cardiomyopathy.
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## IM.67 – TREATMENT WITH THE IMMUNOMODULATORY AGENT PENTOXIFYLLINE DURING THE CHRONIC PHASE OF EXPERIMENTAL CHAGAS DISEASE DECREASES CARDIAC ALTERATIONS

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Chagas disease, caused by *Trypanossoma cruzi* infection, is one of the main causes of death due to heart failure in Latin America. Pentoxifylline (PTX), a methylxanthine derivate, has shown to display immunomodulatory, anti-inflammatory and anti-tumor effects. In the present study we used a C57BL/6 mouse model of chronic Chagas disease to investigate the effects of PTX treatment during the chronic phase, targeting disease progression. PTX treatment did not alter the survival rate, parasitemia and heart parasitism during the chronic *T. cruzi* infection. However, the heart of PTX-treated mice showed a significantly reduced deposition of fibronectin and impaired connexin 43 loss compared with saline-treated mice, resembling non-infected controls. More importantly, PTX treatment of *T. cruzi*-infected animals decreases serum levels of CK-MB, one of the markers of myocardial injury. Furthermore, PTX treatment improves the cardiac function revealed by electrocardiographic alterations. These results demonstrated that treatment with PTX during the chronic phase of *T. cruzi* infection prevents the development of severe chronic cardiomyopathy. Therefore, PTX might be considered a potential adjunct therapy for Chagas disease.
Supported by CNPq

## IM.68 – SPLENECTOMY INCREASES TISSUE PARASITISM IN MURINE *TRYPANOSOMA CRUZI* INFECTION

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The spleen is a secondary lymphoid organ that harbors a variety of cells such as T and B lymphocytes and antigen presenting cells important to immune response development. In this study we evaluated the impact of spleen removal on the cardiac and liver inflammatory response in these animals to *T. cruzi* infection. C57BL/6 mice were infected with Y strain. Animals were submitted to necropsy during the acute phase of infection. Fragments of heart and liver were fixed in 4% paraformaldahyde (pH7.2), dehydrated in alcohol and embedded in paraffin. Sections were stained with Haematoxylin-Eosin (HE) for standard histological procedures. At day 9 after infection, we observed a higher number of amastigotes in hearts of splenectomized mice but there was no difference in the heart inflammatory infiltrates between splenectomized and control group. No difference was observed either at day 16 in heart parasitism. In mice without spleens, liver sections displayed a higher number of amastigotes 9 and 16 days post infection, but presented more inflammatory infiltrates in the liver only at day 9 after infection when compared to the control group. These results demonstrate that most of the parasites were lodged in tissues and not in the bloodstream at day 9 post infection and this could contribute to mice death. In addition, *T. cruzi* infection is more severe in splenectomized mice as a result of impaired immune responses that are essential for protective immunity against the parasite. Supported by UFMG and CNPq.

## IM.69 – REGULATORY EFFECTS OF IL-18 ON CYTOKINE PROFILES AND DEVELOPMENT OF MYOCARDITIS DURING *TRYPANOSOMA CRUZI* INFECTION

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*Trypanosoma cruzi* causes Chagas disease, an important cause of heart disease in South and Central America. Resistance to *T. cruz*i infection is associated with the production of cytokines such as TNF-$\alpha$, IFN-γ and the production of nitric oxide. The imbalance of cytokine production is thought to contribute to the pathogenesis of *T. cruzi* infection in the mouse model. IL-18 plays an important role in the immune system by enhancing T-cell responses, regulating IFN-γ production and promoting the development of Th1 immune responses. Here, we investigated the role of IL-18 in the modulation of cytokine production and development of myocarditis during murine *T. cruzi* infection. C57BL/6 and IL-18$^{-/-}$ mice were infected with trypomastigotes of *T. cruzi* (Colombian or Y strain) and parameters such as parasitemia, immune response (by FACs, Real-Time PCR, ELISA) and histopathology were assessed. Infected IL-18$^{-/-}$ mice displayed significantly higher levels of IL-12 and IFN-γ and reduced levels of IL-10 when compared with infected wildtype and Y strain infected mice. The number of T helper type-1 IFN-γ-producing CD4 and CD8 T-cells was significantly elevated in infected IL-18$^{-/-}$ mice and the percentage of cells expressing CD4$^{+}$CD25$^{+high}$ and FOXP3 in the spleen of infected mice was reduced. We also found reduced expression of iNOS and a reduction in the infiltration of leukocytes in the heart at the early onset of chronic disease in IL-18$^{-/-}$ mice when compared with wildtype counterparts. Taken together, our results indicate that IL-18 production, during experimental infection only with the Colombian but not the Y strain contributes to the balance of IFN-γ, IL-12, TNF-$\alpha$ and IL-10 production and correlates with the modulation in the development of infection-induced myocarditis. #Contributed equally. Supported by CNPq, FAPEMIG, CAPES and Pró-Reitoria de Pesquisa-UFMG (Programa de Auxílio à Pesquisa de Doutores Recém-Contratados).

## IM.70 – 3642 EFFECTS OF ZILEUTON TREATMENT DURING *TRYPANOSOMA CRUZI* EXPERIMENTAL INFECTION

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Chagas' disease is an illness caused by the protozoan *Trypanosoma cruzi* and it is the major cause of heart disease in the endemic areas. The vigor of immune response against *T.cruzi* is directly related with the development of chagas' cardiomyopathy. Lipoxin(LX)$A_4$ production by the activation of the enzyme 5-lypoxigenase(5-LO) down-modulate and promote the resolution of inflammatory processes. The beneficial effects of LXA administration in models of inflammatory pathology, along with the fact that administration of Zileuton leads to the inhibiton of 5-LO activity, has suggested therapeutic promise for specific harnessing of the biological activities of LXA. Herein, we investigated if manipulation of lipoxins production by Zileuton during *T.cruzi* infection could be a potential therapeutic toll. WT mice were infected with *T.cruzi* and treated with Zileuton (30mg/kg) and at different time point after infection the parasitemia, pathology and the expression levels of cytokines were verified in the heart, spleen, liver and serum. Our results showed that treatment with Zileuton results in decreased levels of IFN-$\gamma$ production by splenic CD4 and CD8 T cells when compared with untreated counterparts. In addition, we found reduction of mRNA expression levels for AhR (an nuclear lipoxin receptor), IL-6, IL-10 and IFN-$\gamma$ in the heart of treated mice. Moreover, the treatment with Zileuton results in the lower parasitemia and lower leukocyte infiltration into the heart when compared with untreated mice. In addition, our electrophysiological analyses, using the patch-clamp technique, demonstrated that the treatment with Zileuton during *T.cruzi* infection results in the protection of cardiomyocytes activities, including decreased in the repolarization time. In summary, our results suggest that the treatment with Zileuton could be a "powerful toll" in the therapeutic field to modulate the development of cardiomyopathy in Chagas' disease. Supported by CNPq, FAPEMIG, PRPq-UFMG, and FUNDEP-SANTANDER.

## IM.71 – INFLAMMATORY ANGIOGENESIS INDUCED BY *Trypanosoma cruzi* ANTIGENS IN MURINE SPONGE MODEL

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Chagas heart disease is an inflammatory illness caused by *Trypanosoma cruzi* and responsible for thousand deaths/year around the world. *T. cruzi* antigens (glycoproteins) appear to be the main trigger for the acute and chronic inflammation driving cardiomyocytes toward a progressive damage process with consequent fibrosis and loss of functionality. Angiogenesis, the formation of blood vessels, has emerged as an essential key for repair process in distinct chronic inflammatory process, especially cardiovascular diseases. Here, we analyzed the angiogenic and inflammatory components of the fibrovascular tissue induced by sponge implants in Swiss mice (n=10) and the modulation of these components induced by total antigens from Y strain of *T. cruzi*. Angiogenesis was assessed by hemoglobin content and VEGF by production in the implants and inflammation determined by measuring the levels of myeloperoxidase (MPO), N-acetylglucosaminidase (NAG), chemokines (CCL2 and CCL5) and regulatory/inflammatory cytokines (IL-10, IFN-g and TNF-a). Both parameters were evaluated at days 1, 4, 7 and 14 post-implantation and corroborated by histological evidence. In our preliminary challenges with antigens (100ul from $10^8$ parasites injected inside the sponge) at day 1$^{st}$ post-implant, showed an early accumulation of neutrophils (MPO) at day 4$^{th}$ in contrast with a predominance of macrophages (NAG) population at 7$^{th}$ day post-implant. Surprisingly, even inducing early inflammation, *T. cruzi* antigens inhibited angiogenesis evidenced by decreasing in hemoglobin and VEGF contents as well as by histological sections, when compared with non-challenge animals. Our data suggest that inflammatory angiogenesis is directly affected by *T. cruzi* antigens and it might be a close genetic-related process.
**Financial support**: CNPq, CAPES, ISID, CNPq-TWAS, UFOP.

## IM.72 – 4721 THE ROLE OF SOCS-2 IN THE REGULATION OF IMMUNE RESPONSE DURING *TRYPANOSOMA CRUZI* INFECTION

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Infection with *Trypanosoma cruzi* results in a robust inflammatory reaction in many organs which limits parasite proliferation and results in tissue damage. The regulation of parasite-induced inflammation is multifactorial involving many complex pathways including the lipoxin pathway. Lipoxin (LX) A4 production is mediated by the enzyme 5-lipoxigenase (5-LO) which induces suppressor of cytokine signaling (SOCS)-2 expression which in turn down-regulates pro-inflammatory cytokine production. To investigate the contribution of lipoxins in *T. cruzi* infection we infected wild type (WT), SOCS-2 and 5-LO null mice with the Y strain. When compared with WT mice, the parasitemia was significantly reduced in SOCS-2 and increased in 5-LO null mice. The expression of IFN-γ, TNF-$\alpha$, IL-6, SOCS-1 and SOCS-3 was reduced in the spleen of SOCS-2 infected mice. The expression of IFN-γ and TNF was reduced in the hearts of *T. cruzi*-infected SOCS-2 and 5-LO null mice compared with WT mice. Infection of 5-LO null mice resulted in lower expression of SOCS-2 in the spleen and heart compared with WT mice. In peritoneal macrophages isolated from WT and SOCS-2 null mice pre-incubated with LXA and then infected there was an increased nitric oxide production and trypanocidal activity in infected SOCS-2 null macrophages simultaneously stimulated with IFN-$\gamma$ when compared with WT macrophages. SOCS-2 null macrophages exhibited low trypanocidal activity when incubated without IFN-γ. The inhibition of parasite growth by IFN-γ was significantly blocked when WT cells, but not SOCS-2 null cells, were pre-incubated with LXA. The observations indicate a role for the 5-LO/LXA/SOCS-2 pathway in the regulation of the inflammatory response during experimental *T. cruzi* infection. Supported by CNPq, FAPEMIG and Programa de Auxílio à Pesquisa de Doutores Recém-Contratados-Pró-Reitoria de Pesquisa (UFMG).

## IM.73 – ESSENCIAL ROLE OF CASPASE-1 FOR THE HOST INNATE IMMUNE AGAINST PARASITE *TRYPANOSOMA CRUZI*

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Chagas disease is caused by the protozoan parasite *Trypanosoma cruzi.* Several studies have demonstrated that IL-1β is important in the experimental infection against this parasite. A new family of pattern recognition receptors, Nod-like receptors (NLRs) has been described. Among NLRs, NLRC4 and Nalp3 form an intracellular multimolecular complex with active caspase-1, called inflammasome, which is necessary for cleavage and secretion of the active forms of the IL-1β. In this study we aimed to determine the role of caspase-1 in the innate immune response against *T. cruzi*. First we investigated if *T. cruzi* is able to trigger caspase-1 activation in murine bone marrow-derived macrophages (BMMs). We found that C57BL/6 (WT) and NLRC4$^{-/-}$ BMMs presented robust caspase-1 activation and IL-1β secretion. However, ASC$^{-/-}$ BMMs are not able to activate caspase-1. To study the signaling pathways mechanisms involved in inflammasome activation we investigated the influence of the potassium efflux and oxygen radicals reactive (ROS), which are described to be required for activation of caspase-1 via ASC. Interesting, in the absence of ROS and potassium did not occur activation caspase-1 in WT BMMs. To test the susceptibility of these animals *in vivo*, WT, caspase-1$^{-/-}$, ASC$^{-/-}$ and NLRC4$^{-/-}$ mice were infected with 1000 forms of *T. cruzi* Y strain. Parasites in bloodstream and heart inflammation were measured. Further, the mortality was daily evaluated. Our results show that ASC and Caspase-1-/- mice present higher mortality whereas WT and NLRC4-/- did not die. Beyond the Asc-/- and caspase-1 mice presented diminished inflammation in the heart after 14 days of infection. However 17 days post infection, ASC$^{-/-}$ and caspase-1$^{-/-}$ mice showed increased inflammatory score compared to WT. Together, these results suggest that ASC inflammasome is crucial in the host innate immune response against *T. cruzi* infection.
Financial Support: FAPESP

## IM.74 – 5171 THE ROLE OF ARYL HYDROCARBON RECEPTOR (AHR) IN THE REGULATION OF IMUNE RESPONSE DURING *TRYPANOSOMA CRUZI* EXPERIMENTAL INFECTION

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Chagas' disease is caused by the protozoan *Trypanosoma cruzi* and is a major cause of heart disease in endemic areas. The *T.cruzi* infection is an appropriate and interesting model for study/understanding the balance between effective immune response and immune-pathology. Lipoxin(LX)$A_4$ production by the activation of the enzyme 5-lypoxigenase(5-LO) is important to the regulation of pro-inflammatory cytokine production during *T.cruzi* infection. Therefore, the role of aryl hydrocarbon receptor(AhR), the nuclear receptor for LXA, during Chagas' disease is not know. Herein, we infected WT and AhR$^{-/-}$ mice with *T. cruzi* (Y strain) and parasitemia and imune response were assessed. We found significantly reduced parasitemia in AhR$^{-/-}$ mice when compared with WT. AhR deficiency resulted in the increased levels of IL-12p70 and IFN-γ production during the early and late acute phase of *T. cruzi* infection, as detected by FACs and ELISA. Next, to investigated which was the mainly factor responsible for the dramatically increase of parasite clearance in the AhR$^{-/-}$ mice, we test whether parasite killing was mediated by nitric oxide (NO). For this, peritoneal macrophages were isolated from WT and AhR$^{-/-}$ mice, infected with *T.cruzi* and/or followed by IFN-γ stimulation and evaluated for nitrite production (Griess method) and trypanocidal activity. Our in vitro results demonstrated no diference in the uptake of parasite by AhR$^{-/-}$ and WT cells, however, we found an increased trypanocidal activity by *T. cruzi*-infected AhR$^{-/-}$ macrophages when compared with WT. Nevertheless, no significant difference in NO levels was detected in the supernatants harvested from *T. cruzi*-infected-AhR$^{-/-}$ and -WT macrophages cultures. By contrast, an increased NO production by AhR$^{-/-}$ macrophages was detected when IFN-γ was added in the uninfected cultures when compared with WT counterparts. In summary, our data indicate the role of AhR in the regulation of immune response during *T. cruzi* experimental infection. Supported by CNPq and PRPq-UFMG.

## IM.75 – TREATMENT WITH A PARTIAL CCR1/CCR5 ANTAGONIST (Met-RANTES) ameliorates *Trypanosoma cruzi*-ELICITED CARDIOMYOPATHY: POTENTIAL DELETERIOUS ROLE OF CCR1

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Chagas' heart disease, caused by Trypanosoma cruzi, is mainly characterized by chronic fibrogenic inflammation and loss of heart function. In affected cardiac tissue, the presence of pro-inflammatory cytokines and chemokines might drive leukocyte migration contributing to CCC formation. Chemokines, a group of mediators of the inflammatory process play an essential role in the recruitment and activation of leukocytes in various models of inflammatory diseases. Chemokines type CC (CCL2, CCL3, CCL4 and CCL5) are produced by cardiomyocytes and macrophages in response to T. cruzi infection, preferentially attracting mononuclear cells to inflammation sites. These are the predominant cell types in heart lesions of patients and infected animals. Therefore, CC-chemokines are proposed to crucially define the leukocyte subtypes composing the inflammatory infiltrates in the heart tissue of T. cruzi-infected individuals, being, consequently, involved in the immunopathogenesis of T. cruzi-triggered cardiomyopathy. Independent studies showed that CCR5 and LFA-1 are expressed in PBMCs enabling their migration to the heart. Further, most of the heart invading inflammatory cells are CCR5+ and LFA1+. Importantly, treatment with the selective partial antagonist CCR1/CCR5 during the chronic phase of infection resulted in 20-30% reduction in CD4+ cell numbers as well as IL-10, IL-13 and TNF expression in the cardiac tissue. Furthermore, Met-RANTES treatment led to reduction in parasite load, fibronectin deposition and cardiomyocyte lesion. Mice CCR5-/- infected with T. cruzi had lower survival when compared to C57BL/6 counterparts. Our preliminary data suggest that treatment of CCR5-/- mice with CCR1/CCR5 partial antagonist increases the survival of these animals, indicating a role for CCR1 in pathogenesis during T. cruzi infection. Thus, therapeutic strategies based on the modulation of CCR1/CCR5-mediated cell migration and/or effector function may contribute to cardiac tissue damage limitation during chronic Chagas disease.

## IM.76 – EXPERIMENTAL INFECTION WITH *Plasmodium berghei* COMPROMISES THE THYMUS IN MULTIPLE WAYS

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Maturation of T cell occurs within the thymus and pathologies such as malaria cause thymus atrophy, which alters thymic microenvironment and impairs T cell development in multiple ways. Leptin is an important hormone linked to obesity and immunological activities whose decrease can also lead to thymic atrophy. Cytokines are essential for proper T cell maturation and besides them other factors such as the extracellular matrix and galectins control cell migration into the thymus. Galectin3, for instance, arouses cell de-adhesion smoothing cell migration. Here, we used 8-week old male mice infected with *Plasmodium berghei* and non-infected control mice to analyze serum levels of leptin (by ELISA), expression of thymic cytokines (by real time PCR) and the expression of thymic gal-3 (by IHQ) as well as the role of exogenous gal-3 in thymic-nurse cell complex. Thymuses from infected and control mice were collected, washed and submitted to Real time PCR to detect expression of IFN-γ, TNF-α, IL-17, IL-7, IL-10 and TGF-β; they were also embeded in paraffin for IHQ analysis and enzymatically digested for TNC isolation. Thymic expression of IFN-γ, TNF-α and IL-10 was higher in infected mice (twenty-fold, eight-fold and thirty-fold higher than in control, respectively). Expression of IL-7 and TGF-β was lower in infected mice (three-fold higher than in control for both cytokines). IL-17 expression did not alter in infected mice whereas these mice had lower levels of leptin when compared to control. Gal-3 increased in infected thymic tissue while TNC levels were smaller in infected mice. Further, the treatment of gal-3, ex vivo, altered thymocyte migration. We conclude that mice infected by *Plasmodium berghei* present clear alterations in thymic function arising from different pathways, and compromising the profile of mature peripheral T cells.
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## IM.77 – ANTIBODIES PRODUCTION AND CIRCULATING LEUCOCYTES IN BALB/c MICE REINFECTED WITH RECOMBINANT *TOXOPLASMA GONDII* STRAINS AFTER IMMUNOSUPPRESSION WITH CYCLOPHOSPHAMIDE

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It still is not established if reinfection by *Toxoplasma gondii* is genotype-dependent and no work is available on reinfection of immunosuppressed mice. To verify the interference of differences among genotypes of *T. gondii* strains and immunosuppression with cyclophosphamide (Cy) in reinfection process, groups of BALB/c mice were prime-infected with the non-virulent strains: D8 (recombinant I/III) or ME49 (type II). Mice were challenged 45 days after primary infection with CH3 or EGS strains (recombinant I/III and virulent). Other groups of mice were inoculated following the same protocol, however, they were treated weekly with Cy, beginning 5 days before challenge. To evaluate the kinetic of anti-*T. gondii* antibodies (IgG, IgG1, IgG2a, IgM and IgA) by ELISA, blood was collected from each mouse immediately before challenge and at 30 days after challenge. Thirty days after challenge, the brain from surviving mice was submitted to PCR-RFLP analysis (genetic markers *L363* and *cS10-A6*) to confirm reinfection. Besides, circulating leucocytes of mice was evaluated 45, 55 65 e 75 days after primary infection with D8 or ME49. PCR-RFLP demonstrated that mice prime-infected with ME49 strain were reinfected with CH3 and EGS strains, while mice prime-infected with D8 strain were reinfected only with EGS strain. The immunosuppression increased reinfection and mortality after challenge. Reinfection in non-immunosuppressed mice was associated with increase of IgM and IgA levels after challenge. Otherwise, 30 days after challenge, immunosuppressed mice presented IgM and IgA levels significantly smaller than non-immunosuppressed mice, suggesting that those immunoglobulins participated in the process of protection against reinfection. Cy treatment caused significant leukopenia in prime-infected mice, with reduction of lymphocytes and neutrophils which probably favored reinfection. In conclusion, reinfection is related to genetic differences among *T. gondii* strains and to alteration of immune integrity of the host, which probably favor reinfection.
Supported by CNPq and FAPEMIG

## IM.78 – 2751 QUANTIFICATION OF SPECIFIC AND LOW AVIDITY IgG IN THE DIAGNOSIS OF TOXOPLASMOSIS

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Toxoplasmosis is usually benign and asymptomatic, but affects humans by visual loss or severe diseases, often lethal in fetuses and immunocompromised patients. The transmission is related to acute infection allowing adequate treatment. The avidity of IgG antibodies has been used for diagnosis of acute infection, but conventional indirect avidity tests allow only the proportion of low avidity antibodies. Markers of disease activity, low avidity antibodies (LAA), are highly prevalent in acute infection. We devised an enzyme immunoassay (ELISA), with pH as chaotrope, which allows LAA elution, with subsequent neutralization and direct determination. We tested 150 serum samples from L.Protozoology IMTSP. ELISAs were performed in microtiter plates (Costar ®), adsorbed with 10 µg/mL of *T. gondii* soluble extract antigen and reacted with 1/200 sample dilution. Low avidity immune complexes were dissociated with citrate phosphate buffer at different pHs for 10 minutes, transferred to a new well, neutralized to neutral pH and incubated by 1 hour and 12 hours with the absorbed antigen. Bound IgG was revealed with 1/20.000 of the anti-human IgG conjugate (Sigma ®). Washing with pH 3.5 present similar results to usual 6M urea ($r^2$=0,7348), allowing recovery of the antibodies of low avidity. Neutralization allows reaction to the antigen, with similar results between 1 hour and 12 hours incubation. Antigen specificity of LAA were tested by western blotting against *T. gondii* antigens, compared to total or high avidity antibodies from our samples and also from samples from rabbits experimentally infected with *T. gondii*. LAA showed a clear difference in antigen specificity as compared to whole serum or high avidity antibodies. Direct quantification of low avidity antibodies open a new perspective for future studies both on diagnosis and pathogenesis of toxoplasmosis related to disease severity, especially in congenital infection and in immunocompromised individuals. This work was partially supported by CNPq and LIM49HCFMUSP.

## IM.79 – DETECTION OF TH17 AND T REGULATORY CELLS DURING THE COURSE OF EXPERIMENTAL TOXOPLASMOSIS IN SUSCEPTIBLE AND RESISTANT MICE

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It has been described that peroral infection with *Toxoplasma gondii* in mice leads to the development of an intense intestinal inflammation dependent on Th1 cytokines. C57BL/6 susceptible mice succumb to this exacerbated inflammation, while resistant BALB/c mice are able to control this inflammatory response and develop a chronic disease. Previous studies have implicated IL-17 production in resistance to *T. gondii* and in development of immune-mediated pathology during infection. In parallel the regulatory T cells (Tregs) function is suggested to be corrupted during toxoplasmosis. In this work, we compared the Th17 and Treg responses in susceptible and resistant mice during course of *T. gondii* infection. C57BL/6 and BALB/c mice were orally infected with 40 cysts of ME49 *T. gondii* strain and, 4, 8 and 11 days post-infection the immune response was evaluated. During the course of infection, the number of T $CD4^+$ lymphocytes increased in Lamina Propria and decreased in Peyer´s patches from C57BL/6 mice. In BALB/c mice T $CD4^+$ cell numbers in Lamina Propria were significantly reduced when compared with C57BL/6 mice, but in Peyer´s patches, the number of these cells increased in the $8^{th}$ day. The frequency of Tregs ($CD4^+CD25^+Foxp3^+$) decreased and the Th17 ($CD4^+ROR\gamma^+IL\text{-}17^+$) cells increased during the toxoplasmosis progression in C57BL/6 mice while BALB/c mice sustained increased frequency of Tregs. In addition, the frequency of Th17 cells in BALB/c mice also increased during the course of infection, but it was lower than in C57BL/6 mice. The transfer of $Foxp3^+(GFP^+)$ cells sorted from non-infected mice, but not from infected mice, increased the survival of C57BL/6 infected mice. In addition IL-17 knockout mice were even more susceptible than C57BL/6 mice to oral infection with *T. gondii*. These results suggest that the balance between Tregs and Th17 cells might be essential to resistance of *T. gondii* infection in BALB/c mice.
Supported by: FAPESP and CNPq

## IM.80 – ASSOCIATION BETWEEN IMMUNOGLOBULIN LEVELS AND DIFFERENT TYPES OF SCARRED OCULAR LESIONS DUE TO *TOXOPLASMA GONDII* INFECTION

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*Toxoplasma gondii* infects almost one third of human population worldwide. Congenital and acquired toxoplasmosis may cause acute scarring ocular lesions. It has been shown that the size of scarred lesions is related to the specific anti-*T. gondii* IgA levels in human sera. This study aimed to characterize the anti-*T. gondii* specific humoral immune response in 1714 individuals of three villages, located in Vale do Jequitinhonha, Brazil and to associate the specific antibody levels against *T. gondii* with number and size of lesions. The presence of specific anti-*T.gondii* IgG was measured by the ELISA assay. The number, type, size and location of retinochoroidal lesions where documented by retinographs. The lesions were classified into three types: A, B and C. Lesions of type A and B fulfill the morphological criteria of being caused by *T. gondii*, while type C lesions are considered to be of uncertain etiology. Our findings show a seroprevalence of 43.4% (744/1714) in the population studied. The prevalence increased with age, revealing the cumulative effect of age in toxoplasmosis seropositivity and was gender independent. Lesions were present in 9.95% of individuals studied. We observed that 87% of type A lesions (27/31) and 73% of type B (47/64) were present in seropositive subjects. The chance of being seropositive once the individual has developed a type A lesion is 10.3 higher as compared to individuals without a lesion. In comparison with those without lesions, the chance of being seropositive is of 5.3 for the type B lesions, ($p$<0.0001/99% IC). The levels of anti-*T. gondii* specific IgG and IgG1 did not correlate with the numbers of lesion per individual. The association between lesion size and immunoglobulin levels is still under investigation. In conclusion we found a strong relationship between the seropositivity to *T. gondii* antigens and the presence of ocular lesions type A and B.
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## IM.81 – IgG AND IgM WESTERN BLOT ASSAY FOR DIAGNOSIS OF CONGENITAL TOXOPLASMOSIS

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The aim of this study was to evaluate the use of Western blot (WB) in 215 newborn infants under suspicion of congenital toxoplasmosis. The children were submitted to clinical examinations to assess macular, neurological and hearing signals. The WB results obtained were compared to the persistence of IgG antibodies at the end of 12 months, regarded as the 'gold standard' diagnosis of congenital toxoplasmosis. It was also verified whether there was any association between the WB results and the clinical signals presented by the infants. Of the 215 children, 177 had confirmed congenital toxoplasmosis diagnosis, and 38 did not have the infection. IgG-WB showed sensitivity of 73.5% and specificity of 97.4%. IgM-WB showed sensitivity of 54.8% and specificity of 94.7%.The IgG-WB and IgM-WB combination increased the sensitivity to 86.5%. The IgM-WB positive children were found to have 1.4 times more risk to present active macular lesion than those with negative testing of IgM-WB. This study showed that WB is a useful tool in the confirmation of congenital toxoplasmosis diagnosis, and that the IgM-WB positive results can be an indication of active macular lesion in newborn infants. Financial support was provided by Secretaria do Estado de Saúde de Minas Gerais, Brazil; FAPEMIG (CBB-APQ-00129-09) and CNPq (301110/2009-3).

## IM.82 – ANALYSIS OF ACUTE IMMUNE RESPONSE IN CD1$^{-/-}$ AND WILD TYPE C57BL/6 MICE INFECTED WITH *ENTAMOEBA HISTOLYTICA*

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Recent studies have shown that Natural Killer T (NKT) cells may represent a barrier against hepatic amebic abscess formation at acute phase of infection. The aim of the study was to characterize the immune components involved in the development and control of lesions caused acute infection with *E. histolytica* and its relationship with NKT cell activity. Frequencies of spleen and mesenteric lymph node (MLN) NKT, TCD4$^+$, TCD8$^+$, and B cells were analyzed in CD1-deficient C57BL/6 mice infected with *E. histolytica*. Four CD1 $^{-/-}$ C57BL/6 mice (Eh-CD1$^{-/-}$ group) and four wild type mice (Eh-WT) were inoculated via intracecal with 10.000.000 *E. histolytica* trophozoites of EGG strain. Control groups (CTRL-CD1$^{-/-}$ [N=4] and CTRL-WT [N=4]) were inoculated with sterile culture medium YI-S-32. 48hr after infection were collected spleen, MLN and caecum of the mice. Spleen and MLN cells were isolated for cell culture and analyzed by flow citometry. Caecum was analyzed by histopathology. The frequency of spleen NKT (CD3$^+$panNK$^+$) cells was reduced in CTRL-CD1$^{-/-}$ when compared to CTRL-WT groups (3.86 ± 0.39 to 2.61± 0.18) as expected since CD1 is a restriction element for NKT cells. Activated T cells were also reduced in CD1$^{-/-}$ mice but activated B cells were elevated. Cecal lesions have occurred more frequently and with higher magnitude in the animals of Eh-CD1$^{-/-}$ group. Thus, our results suggest that NKT cells may function as regulatory cells for the inflammatory effects of Eh infection.
**Financial support:** FAPEMIG

## IM.83 – LOCAL IMMUNE RESPONSE TO TROPHOZOITES IN LIVER OF HAMSTERS INFECTED WITH *ENTAMOEBA HISTOLYTICA* AND *ENTAMOEBA DISPAR*

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*Entamoeba histolytica* is a protozoan parasite capable to penetrate and destroy the intestinal mucosa leading to the amoebic dysentery. Since then, trophozoites may reach the liver through blood circulation. Amoebic liver abscesses are the most frequent extraintestinal form of amoebiasis. The mechanisms involved in generating lesions by *E. histolytica* are not still completely understood, as well as the role of immune responses raised against trophozoites. In this study we evaluated the *in situ* binding of antibodies, C3 and C9 complement components on trophozoites, in livers of hamsters infected with *E. histolytica* and *E. dispar*. These parameters were correlated with the extension of the hepatic lesions observed in these animals and with trophofozoites survivor. Hamsters were inoculated intra-hepatically with 100,000 trophozoites of *E. histolytica* or *E. dispar* strain. Different groups of animals were necropsied 12, 24, 48, 72, 144 and 192h after inoculation. Antibodies, C3 and C9 binding to trophozoites were detected by immunohistochemistry. Although, binding of antibodies on trophozoites was searched for both strains, it was weaker in *E. histolytica* ($p<0.05$). Trophozoites of *E. dispar* were also more frequently vacuolated and high labeled cellular debris observed in the lesions. $C3^+$ and $C9^+$ trophozoites debris immunostaining was higher in livers of *E. dispar* than in livers of *E. histolytica*. Overall, these findings indicate that *E. histolytica* strain possesses an enhanced ability to evade the immune responses compared to *E. dispar,* although it also causes experimental hepatic lesions. This might be related to the larger amount and types of cysteine proteinases and expression of CD59-like lectin produced by *E. histolytica* trophozoites. The binding of antibodies and activation of the complement system were not sufficient to impair the progression of the amebian abscess, on the other hand, their action on trophozoites could promote selection of resistant subpopulations.
**Financial support:** FAPEMIG

## IM.84 – CELL MIGRATION INDUZED BY DIFFERENT VACCINE ADJUVANTS IN MICE

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The use of the adjuvant for generation of effective cell-mediated immune response is of fundamental importance in vaccine against visceral leishmaniasis. In the context of innate immunity, inflammatory response deserves special attention, since it is rapidly initiated and independent of antigen, and precedes the onset of antigen-specific response. In these study was evaluated the inflammatory reaction and the activity of the enzymes myeloperoxidase (MPO) and N-acetyl-β-D-glucosaminidase (NAG) in the inoculation local by adjuvants: aluminum hydroxide ($AlOH_3$), saponin (SAP), Bacille Calmette-Guerin (BCG), monophosphoryl lipid A (MPL) and Freund Incomplete Adjuvant (FIA) at times (1, 12, 24, 48, 96 hours and 7, 14 days) in *Swiss* mice. The results demonstrated an increase in the cell recruitment in 1 hour in SAP and BCG groups until 14 days after the inoculum. In MPL group, this increase was observed after 12 hours. In FIA group this increase was visualized in 7 and 14 days. Any alteration in relation to number of cells circulation was observed in the $AlOH_3$ group. In concerning to the MPO activity enzyme, increase levels of this enzyme in BCG and AIF groups during 1 hour when compared with MPL. The increase of MPO was maintained in the BCG group rather than MPL group in 12 hours. Also, we analyzed the activity NAG enzyme and were observed an increase NAG levels in $ALOH_3$ group (1 hour) when compared to the groups (CS, SAP, BCG, MPL and AIF). In 96 hours, an increase of NAG levels occurred in SAP group when compared to MPL. In 14 days, were observed increased NAG levels in AIF group when compared to the CS group. These results will help in the understanding of the mechanism involved in the cell recruitment induced by adjuvants and thus may contribute in future vaccine formulations with the *Leishmania* antigens. Supported by PRONEX - FAPEMIG/CNPq and UFOP.

**IM.85 – N VITRO BINDING AND SURVIVAL ASSAYS OF *LEISHMANIA* PARASITES TO MONOCYTES AND MONOCYTE-DERIVED MACROPHAGES ISOLATED FROM DOGS WITH VISCERAL LEISHMANIASIS AND TREATED WITH MEGLUMINE ANTIMONIATE-CONTAINING NANOMETRIC LIPOSOMES**

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Canine visceral leishmaniasis (CLV), in America, is a zoonotic disease caused by *Leishmania (Leishmania) chagasi* (*syn* = *infantum*). It is endemic in several Latin American countries, where the parasite is transmitted to man and animals by infected blood-sucking sandflies of the genus *Lutzomyia* (Grimaldi et al. 1989). Our group, following the treatment protocol with meglumine antimoniate-containing lipossomes (LMA), has recently reported of both long-term parasite suppression and reduction of infectivity to sand flies in naturally-infected dogs (Ribeiro et al. 2008). The aim of this study was to evaluate the influence of LMA treatment in the in vitro binding and survival assays among amastigotes forms of *L. chagasi* and monocytes and macrophages derived from peripheral blood monocytes of dogs with CVL. Twenty four dogs was obtained from City Hall Zoonosis Department of Ribeirão das Neves (Metropolitan area of Belo Horizonte, MG) and divided in three groups of eight animals. Monocytes were obtained from the peripheral blood and resuspended to 3.0 x $10^6$ cells/mL. Then, these cells were distributed in plates of 24 wells. The binding and the survival assays were carried out during 1 and 48 hours, respectively. The same protocol was applied to monocyte derived macrophages obtained after ten days cultures. The numbers of monocytes and monocyte derived macrophages bound to *Leishmania* promastigotes were significantly increased when C5D serum was used during the interaction with the canine cells. Moreover, parasite survival, characterized by the presence of amastigote forms of *Leishmania* in macrophages, was also significantly increased in the presence of serum ($p < 0.01$) in despite of the treatment with LMA.
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## QT.01 - THE EFFECTS OF CAMPTOTHECIN IN *TRYPANOSOMA CRUZI* PROLIFERATION AND ULTRASTRUCTURE SUGGEST ITS PARTICIPATION IN DNA REPAIR

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*Trypanosoma cruzi* contains an unique mitochondrion with an enlarged portion termed kinetoplast where is harbour the kDNA. The nucleus presents compartments, as the nucleolus, and a condensed chromatin associated with the nuclear envelope. The topological state of the DNA is modulated by topoisomerases, which act by reverting supercoilings of the double stranded DNA during replication, transcription and repair, thus representing an important target in chemotherapy. According to this, camptothecin, a topoisomerase type I inhibitor, has been widley used in cancer research, once it is effective against cell proliferation by DNA damage that leads to DNA repair or apoptosis. Our previous works showed that camptothecin strongly inhibits the proliferation of *T. cruzi* epimastigote and causes a remarkable unpacking of nuclear chromatin. In this study, we compared the proliferation between wild-type cells and protozoa over-expressing the *TcRad51* gene, that is involved in DNA repair, after removal of the drug from the culture medium. Thus, cells were cultivated in medium containing different drug concentrations (1, 5, 10 and 50 µM) and samples were collected after each 24 hours for counting on Neubauer's chamber or for processing to transmission electron microscopy. Our data showed that removal of camptothecin, after treatment with 1µM drug for 24h, restored cell proliferation. With respect to the transfected cells, protozoa over-expressing Rad51 gene presented a higher resistance to camptothecin, since in this case the number of viable protozoa was two fold higher when compared to the wild-type cells. These results suggest that DNA repair mechanisms are activated, with participation of the RAD51 enzyme, after cell treatment with camptothecin. Supported by CNPq.

## QT.02 - TETRAZOLIUM SALTS BASED METHODS AS TOOLS FOR QUANTITATIVE EVALUATION OF ANTI-PARASITE CHEMOTHERAPY.

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Genome projects brought much information about metabolic pathways and enzymes that are good targets for neglected diseases chemotherapy, increasing the need for sensitive and reliable high throughput screening of drugs in parasites. The widely used tetrazolium salt MTT, form insoluble formazan crystals that need to be solubilized. In contrast, the second generation, MTS and XTT are reduced by metabolically active organisms, producing hydrosoluble colored formazans. The water soluble tetrazolium methods require an intermediate electron transfer reagent, 5-methyl-phenazinium methyl sulfate (PMS), that is reduced by agents produced by viable cells, NADH and NADPH. The reduced PMS, transfer its electrons to the tetrazolium salts, producing water soluble reduced formazan, proportional to viable cells number. We improved the detection limit by removing growth culture medium, and compared MTT, XTT and MTS tetrazolium assays using a density curve of *T.cruzi* epimastigotes in buffer saline/glucose 10 mM. The MTS/PMS method was faster and displayed the highest sensitivity, detecting $10^5$ epimastigotes/ml (n=3). To validate the MTS/PMS method for anti-parasite chemotherapy studies, epimastigotes were treated with oligomycin, followed by evaluation of mitochondrial potential with Rho123 (2µg/ml) by flow cytometry. Oligomycin at 10µg/ml affected 72.7% ± 7 (n=2) epimastigotes population, compatible with the MTS/PMS method that displayed 67% ± 7 reduction of viable cells. The $IC_{50}$ for the drug amiodarone in epimastigotes was 10.5 ± 2 (n=2) corroborated the $IC_{50}$ in the literature, 9 µM. The MTS tetrazolium method was also tested in Giardia lamblia and demonstrated a detection limit of $10^5$ trofozoites/ml (n=2). The MTS/PMS tetrazolium method was also used to evaluate the effect of the anti-helminth drug albendazol in *Caenorhabditis elegan*. This is the first report of the MTS/PMS tetrazolium based method for Giardia, helminthes and *T.cruzi*. The MTS/PMS reduction is associated not only with mitochondria function, but also with cytoplasm and with non-mitochondrial membranes.

Financial support: CNPq and FAPERJ.

## QT.03 - EXPLORING PLANTS AND FUNGI FOR DRUG DISCOVERY AND DEVELOPMENT AGAINST CHAGAS DISEASE

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One of the major challenges for drug discovery and development from natural products, especially in the third world, is the lack of standardization, reproducibility and traceability of the produced data. The Program of Technological Development for Health (PDTIS) of the Oswaldo Cruz Foundation (FIOCRUZ) has stimulated building and consolidation of Technological Platforms (TP) in order to improve research and development of health products with quality. The Bioprospecting TP (RPTA 10A) has more than 8,000 plant and fungi crude extracts deposited. All collected plants have a voucher deposited at the Herbarium BHCB and all fungi sample were deposited in the culture collection of UFMG. The extracts were concentrated, dissolved in DMSO at 20mg/ml and as a microtiter film at 10ug/well and kept at -20$^0$C. All procedures are guided by the Standard Operating Procedure of RPT10A. With the aim to detect bioactive extracts against *T.cruzi,* the crude extracts at microtiter plates were *in vitro* assayed at the Chagas Disease TP (RPT11F) using *T. cruzi* (Tulahuen strain) expressing the *Escherichia coli* beta-galactosidase gene (Buckner et al. 1996). Briefly, trypomastigotes were left for 2h to infect L929 fibroblasts seeded in tissue culture micro plates. After 48h, the medium was discarded and replaced by fresh medium and test compounds. After 7 days, chlorophenol red beta-D-galactopyranoside was added to the plates, incubated overnight and the absorbance measured at $\lambda_{570}$ nm. Benznidazole at its $IC_{50}$ (1µg/mL or 3.8µM)) was used as positive control. The results are expressed as percentage of *T.cruzi* growth inhibition. Bioactivity ≥ Benznidazole was observed in 70 extracts (0.8%). The $IC_{50}$ of 20 extracts showed 4 from plant and 2 from fungi with $IC_{50}$ varying from 1.4 to 6.6µg/mL. The remaining 14 extracts were cytotoxic for the L929 fibroblasts.The results found are encouraging to explore plants and fungi as source of anti-*T.cruzi* drugs. Supported by PDTIS/FIOCRUZ, FAPEMIG and CNPq

## QT.04 - INCREASE OF REACTIVE OXYGEN SPECIES BY DESFERRIOXAMINE DURING EXPERIMENTAL CHAGAS' DISEASE

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Oxidative stress is common in inflammatory processes associated with many diseases including Chagas' disease. Work undertaken by our group has demonstrated that within the interaction *T. cruzi*/host in a murine model, changes in iron stores may be a factor favorable to the host. The antioxidant activity of DFO (Desferrioxamine) has already been proven, but until now no work is proposed to evaluate the antioxidant capacity of this iron chelator associated with *T. cruzi* infection. Based on this, this study aims to evaluate the oxidative stress together with components of the antioxidant system in animals treated or not with DFO through the measurement of thiobarbituric acid reactive species (TBARS), protein carbonyl (PC), and serum nitric oxide (NO) and antioxidant defenses through the measurement of superoxide dismutase in serum (SOD) and total glutathione (GT) in liver were determined on 0, 7, 14 and 21 days post-infection (dpi). Swiss mice (n=48) infected or not with the Y strain of *T. cruzi* were divided into four groups: (C) control, (I) infected, (DFO) treated with DFO and (IDFO) infected and treated with DFO. Among the five measurements performed treatment with DFO decreased GT and increased SOD activity in IDFO group. We observed an increase in NO production at 21 dpi and PC at 7 dpi in IDFO group. An increase in TBARS levels at 7 and 21 dpi for the animals of I and IDFO group were observed, respectively. Within the parameters used to evaluate the oxidative status, DFO has a capacity to provide protection while increasing the production of ROS, indicating that the mode of action of the drug involves a positive contribution to the host together with an effect that is not beneficial to the parasite. Supported by FAPEMIG (PPM, Redes Toxifar and Bioterismo), CNPq and UFOP.

## QT.05 - EVALUATION OF TRYPANOCIDAL AND CITOTOXICITY ACTIVITY OF N-ALKYL STERS OF GALLIC ACID IN *T. CRUZI* AND HEPG2 CELLS

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Gallic acid (3,4,5-trihydroxybenzoic acid) is a plant phenol obtained by the hydrolysis of tannins. Esters of gallic acid have different uses, such as, antioxidant additives in foods and cosmetics, besides having antibacterial activity, as a synergistic action with antibiotics, antifungal and antiviral activities. In this study we evaluated the activity of gallic acid and nonyl gallate against the *Trypanosoma cruzi* parasites, and the cytotoxicity of nonyl gallate in HepG2-hepatoma cell line, which is used as a model for studying the human liver. The nonyl gallate was obtained by structural modifications of gallic acid. The substances were tested for trypanocidal effect on *T. cruzi* (Y strain) epimastigote forms by the MTT technique, and calculating the citotoxicity index ($IC_{50}$). Because of the higher activity of nonyl gallate (2.0 µM) when compared with benznidazole (33.6 µM), respectively, both molecules were also evaluated in HepG2 cells. The nonyl gallate citotoxicity in HepG2 cells presented a $IC_{50}$ of 57.67 µM, which was much higher when compared with the $IC_{50}$ in *T. cruzi* (safety index, SI = 28.8). In contrast, gallic acid showed no toxic effects in *T. cruzi* ($IC_{50} \geq$ 300 µM), showing that the esterification on this molecule can produce a very potent trypanocidal effect. These results show that the nonyl gallate would be an interesting molecule for further studies *in vivo* to treat Chagas' disease.
Supported by Capes, FUNDUNESP and FAPESP.

## QT.06 - *TRYPANOSOMA CRUZI* ABC TRANSPORTER: GENE STRUCTURE AND ROLE IN DRUG RESISTANCE

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Benznidazole (BZ) therapeutic failures are widely documented in Chagas disease and have been associated to variations in the drug susceptibility of *Trypanosoma cruzi* strains. DNA microarrays indicated that one ABC transporter gene (*Tc*ABCG) was overexpressed in BZ-resistant strains, as compared to susceptible strains. This was confirmed by real time RT-PCR. *Tc*ABCG shares high similarity with ABCG genes of *Leishmania* and *T. brucei*. The goal of this study was to further characterize the association of *Tc*ABCG with BZ resistance and analyze the structural characteristics of this gene in *T. cruzi* strains. The effect of known ABC inhibitors on the drug sensitivity was investigated. In four analyzed strains 20 µM and 30 µM verapamil decreased the $IC_{50}$ to BZ by 38% and 50%, respectively. On the other hand, cyclosporin-A and fumitremorgin-C had no effect. *T. cruzi* strains will be transfected with the *Tc*ABCG gene to verify if overexpression of this protein increases BZ resistance. Single Nucleotide Polymorphisms (SNPs) in ABC transporter genes were associated with alterations of gene expression and/or functionality. The *Tc*ABCG gene of six strains was cloned and sequenced (six clones of each strain). Esmo and Non-Esmo haplotypes of CL Brener *Tc*ABCG display 41 nucleotide variations, of which 28 are synonymous. *Tc*ABCG of two DTUII strains (one susceptible and one resistant) shows predominance of the Esmo haplotype and 2 synonymous SNPs. In three DTUI BZ-resistant strains *Tc*ABCG exhibits alternation of Esmo and Non-Esmo variations and a total of 17-20 SNPs. Most of the SNPs are identical in the three strains and occupy the same positions. The structure of *Tc*ABCG gene in one DTUV strain revealed at least three different "allele" types. Type I and Type II display, respectively, the Esmo and Non-Esmo haplotypes, whereas Type III displays Esmo stretches followed by Non-Esmo stretches, suggesting intragenic recombination. Support: FAPESP; CAPES; CNPq.

## QT.07 - EVALUATION OF THE THERAUPETIC PERSPECTIVES OF THE *L*-THIAZOLIDINE-4-CARBOXYLIC ACID, A PROLINE ANOLOGUE, ON MICE INFECTION BY *TRYPANOSOMA CRUZI.*

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*Trypanosoma cruzi* is dependent on proline for a variety of processes such as energy metabolism, host cell invasion, differentiation and resistance to osmotic, metabolic and oxidative stress. *L*-thiazolidine-4-carboxylic acid (T4C), a proline structural analogue that inhibits the proline uptake, diminishes the ability of *T. cruzi* to deal with several of the stresses that the parasite naturally undergoes throughout its life cycle. From these results, we hypothesized that T4C interferes with proline-dependent mechanisms of resistance to stress conditions, reducing the virulence and supporting a role for proline in the *in vivo* infection. Experiments conducted to test this hypothesis showed that the parasitemia peak was reduced in 49% in the infected mice that were treated with a unique dose of T4C (100 mg/Kg). In addition, histological analysis of lung, heart, bladder, skeletal muscle, spleen and intestine revealed that only the latter showed a reduction (90,3 %) of the number of amastigotes nests when the animals were treated with 150 mg/kg. These results were confirmed by quantitative PCR. Besides, the toxicity of T4C was also evaluated. T4C was inoculated i.p. (0, 50 or 200 mg/kg) in a single dose, and the body weight of animals were followed up for 40 days. At day 12 after treatment it was observed that mice treated with 200 mg/kg had their weight reduced in 6.3 % ($p<0.05$), and the survival of this group was diminished in 20% with respect to control. When T4C was administrated daily for 10 days (200 mg/Kg), treated animals showed a progressive weight reduction and only 60% of the animals survived the treatment when compared to the controls. The present results suggest that T4C-treatment contributes to reduce the virulence of *T. cruzi* infection, in accordance to our hypothesis, but it was toxic in doses over 150 mg/kg.

Supported by CNPq and FAPESP.

## QT.08 - THE ANTI-*TRYPANOSOMA CRUZI* EFFECT OF DRUGS USED TO TREAT PSYCHIATRIC DISORDERS

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The search for new therapeutic alternatives against *Trypanosoma cruzi*, the etiological agent for Chagas´ disease, is a main issue due to the serious drawbacks of currently used drugs Nifurtymox and Benznidazole (high toxicity, low efficiency and emergence of resistant strains). Several amino acids have several roles in the biology of trypanosomatids, participating in the energetic metabolism, host cell invasion, resistance to different stresses and differentiation. In previous work we demonstrated that *T. cruzi,* metabolizes glutamate and GABA. In this study, we analyzed the effects of interfering with the metabolism of these amino acids by using the GABA analogue 3-(acetylamino)-1-propanesulfinic acid (Acamprosate), a drug currently in use to treat alcohol dependence. The effect of this drug on epimastigotes growth was evaluated by using concentrations of Acamprosate ranging from 0.25 to 150 μM, showing a dose-response effect on epimastigote growth with an $IC_{50}$= 0.9±0.043 μM. We also evaluated the possible interferences of Acamprosate with the ability of the parasite to resist stressing conditions like high temperature, acidic pH, nutrient starvation and oxidative stress. We observed a significant diminution of parasite survival ($p<0.05$) when treatment was combined with nutrient starvation or oxidative stress. Finally, the trypanocid effect of this drug on infected mammalian cells (CHO-$K_1$) was evaluated. It was observed that Acamprosate diminished 65 % the trypomastigote bursting when cells were treated with non-toxic concentrations of this drug (between 0.25 and 150 μM). All these data suggest that Acamprosate may act in the GABA-glutamate metabolism and interferes with mechanisms of resistance to stress that *T. cruzi* naturally faces along its life cycle. Finally, Acamprosate could be an interesting therapeutic drug against *T. cruzi* infection by itself or if combined with others.

Supported by CNPq and FAPESP.

## QT.09 - EVALUATION OF EFFECTS OF AN ANTIPSYCHOTIC DRUG (LEVOMEPROMAZINE) IN *TRYPANOSOMA CRUZI* FORMS

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Chagas' disease, caused by the protozoan *Trypanosoma cruzi*, is a relevant parasitic disease in the Americas. The current chemotherapy relies on Nifurtimox and Benznidazole, which present serious drawbacks (high toxicity, low efficiency and emergence of resistant strains). Trypanothione reductase (TR), an enzyme involved in the resistance to oxidative stress, constitutes an attractive target for chemotherapeutic research in relation to Chagas' and other trypanosomatid-caused diseases since this enzyme is present in the parasite but not in mammalian hosts. In this work, we investigate the effect of phenotiazine (levomepromazine), a drug currently in use to treat schizophrenia, which showed a potent inhibitor activity for (TR). Levomepromazine interfered on epimastigotes growth. Growth curves were performed using drug concentrations ranging from 75 to 1000 μM, showing a dose-response effect on epimastigote growth with an $IC_{50}$= 405±2.43 μM. We also evaluated the possible interferences of levomepromazine with the ability of the parasite to resist stressing conditions like high temperature, acidic pH, nutrient starvation and oxidative stress. We observed a significant diminution of parasite survival ($p<0.05$) when treatment was combined with nutrient starvation or oxidative stress. Also we evaluate the trypanocid effect of this drug on infected mammalian cells (CHO-$K_1$). It was observed that levomepromazine diminished 75% the trypomastigote bursting when cells were treated with non-toxic concentrations of this drug (between 75 and 1.000 μM). All these data suggest that levomepromazine may inhibit the TR function, interfering with essential biological functions of this enzyme, such as mechanisms involved in the resistance to stress that *T. cruzi* naturally faces along its life cycle. The fact that levomepromazine selectively inhibit the parasite infection *in vitro*, with little compromise of the viability of host cells is promising to propose the use of this drug (already applied to the treatment of a psychiatric disorder), to treat Chagas´ disease.
Supported by CNPq and FAPESP.

## QT.10 - 243 2 BIOTHERAPIC 17DH OF *TRYPANOSOMA CRUZI*: EFFECT *VERSUS* AGE

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*Trypanosoma cruzi* infection in mice is well known, making this is a good model for understanding the effect of ultra-diluted medicines. The objective of this work was to evaluate the effect of biotherapic-17DH-*T. cruzi* at different ages of mice infected with the parasite. In a double-blind, controlled, randomized by draw assay, 109 Swiss male mice, 4 or 8 weeks old were divided into groups: control treated with water-alcohol solution-7% (CI-4=34 animals/CI-8=21 animals) and treated with biotherapic-17dH (BIOT-4=33 animals or BIOT-8=21 animals). It were used 1400 trypomastigotes intraperitoneally, *T. cruzi Y*-strain. The biotherapic-17dH was prepared by adding 0.9mL of blood with *T. cruzi* ($10^7$ trypomastigotes/mL) to 9.1mL of distilled water in laminar flow. The following dilutions were made in water-alcohol solution-7%. Microbiological control and biological risk *in vivo* were performed. Treatment: 0.2mL/20 consecutive days/oral route. Parasitological parameters were compared using the program Statistica-7.0. Work approved by the Ethics Committee for Animal Experimentation/UEM. Parasitemia (0.5718) and mortality (0.9136) did not differ between treated and control group in 4 weeks animals. For animals of 8 weeks, the treated group had a higher parasite peak ($p=0.0424$) and total parasitemia ($p<0.005$) than control. The mortality started later in BIOT-8, but was not significantly different from CI-8 ($p=0.8815$). Considering the parasitemia of the 8th day of infection, animals in both group CI-8 and BIOT-8, could be classified as: high, medium and low sensitivity to infection. The difference between BIOT-8 and CI-8 has been identified in animals classified as high and especially medium sensitivity to infection ($p=0.001$ and $p=0.041$). Mortality for each mentioned extract followed the same pattern observed for all BIOT-8 group. Ultra diluted drug has different effect on 4 or 8 weeks old mice and on the group of animals with 8 weeks there is individuality of response to ultra diluted medication.
Supported by PROAP CAPES

## QT.11 - Synergistic effect of Semicarbazones/Thiosemicarbazones and Benznidazole on *Trypanosoma cruzi*

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A specific treatment, with more efficiency and less toxicity for neglected diseases, as Chagas' disease and Leishmaniasis, is the main objective of several studies in this field. It has been demonstrated a synergistic effect when reference drugs and new compounds have been associated and used against those pathologies. In a previous study we evaluated the activity of three thiosemicarbzazones (HTIO, 2MEOTIO, VATIO) and two semicarbazones (OVASEMI, 2MEOSEMI) against *Trypanosoma cruzi* (Y strain) and *Leishmania amazonensis* (LTBOO16 strain) with promising results. Based in those data, in the present work we assayed the activity of those compounds associated to Benznidazole, in several concentrations (from respectives LDs50/24 hours to decreasing dilutions), against *Trypanosoma cruzi* trypromastigote bloodstream forms using a viability cellular assay (MTT) and it was observed a significant **increase in** the activities in 24 hours of incubation. Among those thiosemicarbazones and semicarbazones plus Benznidazole, HTIO, 2MEOTIO and VATIO **showed a LDs50** significantly lower, while for 2MEOSEMI no difference was observed as compared to semicarbazones alone, which is interesting considering that the last one was the most active when assayed alone against *T. cruzi*. Concerning semicarbazone, OVASEMI presented only slight diminishing in the LDs50. Further experiments are been realized with a larger concentration range for the reference drug (Benznidazole) and the drugs test.
Supported by: CNPq/FAPERJ/FIOCRUZ-PDTIS/FIOCRUZ-IOC

## QT.12 - TRYPANOCIDAL ACTIVITY AND SELECTIVITY OF NEW FUNCTIONALISED CYNAMIL-*N*-ACYLIDRAZONES

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Chagas disease, caused by *Trypanosoma cruzi*, is a widely distributed debilitating human illness, representing an important health problem. The available chemotherapy for this disease is unsatisfactory; therefore there is an intense effort to find new drugs (Soeiro et al., 2009). Natural products are a rich potential source of drugs since they contain a countless quantity of molecules with a great variety of structures and pharmacological activities (Hoet et al., 2004). Polyphenolics have been known to exert diverse biological effects and those containing a cinamoyl moiety, like caffeic acid, were described as potential antiparasitic agents (Hamilton et al., 2005). In this context, new $\alpha,\beta$-unsaturated N-acylhydrazones were designed as trypanocidal candidates by the molecular hybridization of caffeic acid with an acylhydrazone derivative, a potent cysteinyl protease inhibitor (Rodrigues et al., 2002) in order to enhance the trypanocidal activity by the incorporation of the acylhydrazone subunit. Here we investigated the *in vitro* effect of $\alpha,\beta$-unsaturated N-acylhydrazones on bloodstream trypomastigotes of *T. cruzi* and the possible toxic effects of the compounds on mammalian host cell (peritoneal macrophages). Six of them showed activity ($IC_{50}$/1d) at concentrations below 180 µM, being the most active **HD24**, with $IC_{50}$ = 59.3 µM. Our results demonstrate the promising activity on the derivatives against *T. cruzi*, especially **HD24**, justifying further others assays to elucidate the mechanism of action of these compounds and the *in vivo* activity. Supported by FAPERJ, CAPES and CNPq

## QT.13 - ALTERATION OF *Trypanosoma cruzi* CELL MEMBRANE INTEGRITY INDUCED BY ELATOL

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, represent an important public health problem. The available drug for treatment of this infection is ineffective during the chronic phase of disease, causing serious side effects. In this context, research groups are focusing their studies on biological effects of compounds extracted from shellfish, fish and plants to find new medicines. Recently we described the antiproliferative effect of elatol, a secondary metabolite extracted from red macroalgae *Laurencia dendroidea*, present in Brazilian coast in trypomastigote forms of *T. cruzi*. Thus, the goal of this study was to evaluate the alterations caused by elatol on cell membrane plasma integrity and mitochondrion function of trypomastigote forms of *T. cruzi* in a way to try to elucidate possible mechanism of elatol action. For this, trypomastigotes were treated with elatol at concentrations 0.25, 0.5, 1.0 and 2.0 µg/mL and evaluated by flow cytometry using rhodamine 123 and propidium iodide (PI). It was observed a positive staining for propidium iodide, indicating a possible alteration of cell membrane integrity and no depolarization of mitochondrial membrane. These results confirm the ultrastructural alterations caused by elatol in trypomastigote forms of *T. cruzi* described in a previous study made by our group. Therefore, it is possible to suppose that the tripanocidal action of elatol maybe involve its effect on the plasma membrane of the parasite leading cell death.
Supported by CAPES, CNPq, FINEP, PRONEX/Fundação Araucária.

## QT.14 - MONITORING OF CURE BY PCR FOR MICE INFECTED WITH *TRYPANOSOMA CRUZI* AND TREATED WITH DIFFERENT BENZNIDAZOLE SCHEDULES

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The polymerase chain reaction (PCR) was used to monitor cure of mice infected with *Trypanosoma cruzi* and submitted to treatment with benznidazole (BZ). For this, 21 mice, male, 28 days were intraperitoneally inoculated with $1x10^4$ blood trypomastigotes (BT)/animal of *T. cruzi* Y strain (DTU TcII). Ten were orally treated with BZ (Rochagan, Roche ®) 100mg/kg/day and 11 constituted the not treated control group (NT). Of the ten treated animals, five received the drug for 20 consecutive days (TBZ-20) and five for 60 days (TBZ-60). From the 4th day of infection (di), parasitemia and cumulative mortality were recorded. In assessing the cure, flesh blood examination (FBE), blood culture (BC) and PCR 30 days after treatment were used. Further blood samples for PCR were collected at different times post-treatment: two and 15 days (TBZ-20 group), and six months (TBZ-60). The mortality rate was 0% for the treated groups and 100% (33 di) for the NT group. Cure rates were 40% (2 / 5) and 100% (5 / 5), respectively for TBZ-20 and TBZ-60 groups. TBZ-20 group was 100% agreement between the results of FBE, BC, and PCR performed with two, 15 and 30 days post-treatment. TBZ-60 group was also 100% agreement between the FBE, BC and PCR performed six months after treatment. However, in all animals TBZ-60, *T. cruzi* DNA was detected in samples collected 30 days after treatment. These results were confirmed with further DNA extractions and repetition of reactions. TBZ-20 animals showed cure rate similar to that of literature and animals TBZ-60 an index higher, which reclassify the Y strain as sensitive to BZ. Explanations for the unusual PCR results (positive 30 days and negative 6 months post-treatment) for the TBZ-60 group are searched while performing serology. Supported by CNPq

## QT.15 - BIOLOGICAL EFFECTS OF A CYSTEIN-RICH SECRETORY PROTEIN ISOLATED FROM THE *CROTALUS VIRIDIS VIRIDIS* SNAKE VENOM ON *TRYPANOSOMA CRUZI* TRYPOMASTIGOTES.

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Snake venoms have been used as pharmacological tools for drug development. Treatment of Chagas' disease, caused by *Trypanosoma cruzi*, is based on drugs that exhibit toxic effects and limited efficacy. Therefore the search for new drugs is a lining research to be exploited. This work shows the purification of a novel protein belonging to cysteine-rich secretory protein (CRiSP) family, isolated from *Crotalus viridis viridis* (Cvv) snake venom, and its effects over *T. cruzi* and murine muscles. The crude venom extract was loaded onto to a reverse phase analytical (C8) column using a high performance liquid chromatographer. A linear gradient of water/acetonitrile with 0.1% trifluoroacetic acid was used. The peak contained the isolated protein (confirmed by SDS-PAGE and mass spectrometry) was collected, lyophilized, ressuspended in distilled water and its protein content measured. The isolated protein exhibited a molecular mass of 24,893.64 Da and the MS/MS-derived sequences are nearly identical to the protein Catrin and related snake venoms CRiSPs. Trypomastigotes obtained from LLC-$MK_2$ cells cultures were incubated with 0.6 to 4.8 μg/ ml of the protein, and the effect on the cells lysis was evaluated by counting with a Neubauer chamber. The treatment caused a significant reduction (36% to 88%) in the parasites living after 24h, with a $LD_{50}$ of 0.93 μg/ ml. The mainly morphological alterations observed by transmission electron microscopy were at the plasma membrane, with blebs exhibiting different shapes and sizes, and in lysosomes related organelles, which were often enlarged. No myotoxicity was observed in isolated murine gastrocnemius muscles treated with 0.93 μg/ ml, where only basal creatine kinase release was detected. This work presents, for the first time, the purification of a protein from Cvv venom belonging to the CRiSP family with trypanocidal and no myotoxical effects, and which could be a promissory compound to Chagas' disease treatment.
Suportted by CAPES, CNPq and FAPERJ.

## QT.16 - ANTITRYPANOSOMAL ACTIVITY OF DIFFERENT SYNTHETIC COMPOUNDS AGAINST EPIMASTIGOTE FORMS OF *Trypanosoma cruzi*

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Chagas disease is present in almost all Latin American territory and represents a serious health problem, by reaching millions of people and lack of effective and appropriate treatment. Benznidazole, the only available drug for the treatment of this infection is not effective in chronic phase, in addition to having high toxicity. The search for new drugs that are able to cure or even prevent the disease progress is necessary and a priority. The purpose of this study was to report the antitrypanosomal activity from synthetic compounds, being 8 nitroketene *N,S*-arylaminoacetals and 8 2,3-disubstituted-quinoxaline derivatives. In 24-well plates, $1x10^6$ parasites were inoculated in LIT medium supplemented with 10% of fetal bovine serum in different concentrations of compounds. After 96 h of incubation at 28℃ the parasites were counted and the gro wth inhibition determined. The results showed that 13 of the 16 substances tested showed activity against epimastigotes at concentrations below 50 μg/mL, and the most effectives were 6-methoxy-3-(methylsulfonyl)-2-phenylquinoxaline, *N*-[1-(methylthio)-2-nitroethenyl]-benzenamine and 4-fluoro-*N*-[1-(methylthio)-2-nitroethenyl]-benzenamine with IC50 of 0,34, 2,40 and 4,50 μg/mL, respectively. However, *in vitro* and *in vivo* studies are necessary to elucidate the mechanism of action of these compounds.
Supported by CAPES, CNPq, FINEP, PRONEX/Fundação Araucária, FAPESP.

## QT.17 - DEPOLARIZATION OF MITOCHONDRIAL MEMBRANE INDUCED BY EUPOMATENOID-5 ON TRYPOMASTIGOTE FORMS OF *Trypanosoma cruzi*

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Chagas disease, an infection caused by the protozoan *Trypanosoma cruzi,* is present in almost all Latin American territories. Since it has been discovered, the pharmacological treatment for this infection is unsatisfactory due to limited efficacy and toxic side effects. Therefore, the study for new pharmacological agents is a priority. Eupomatenoid-5 isolated from leaves of *Piper regnellii* var. pallescens has already been described as a tripanocidal agent, making this compound a new alternative for Chagas disease treatment. Thus, the goal of this study was to investigate a possible mechanism of action of eupomatenoid-5 through the study of membrane plasma integrity and mitochondrion function of trypomastigote forms of *T. cruzi*. Previously, it was found that the $EC_{50}$ of eupomatenoid-5 on trypomastigote forms was 100 µg/mL. Then, the trypomastigotes were pre-treated with eupomatenoid-5 at concentrations of 50 and 100 µg/mL for 2 hours and analyzed by flow cytometry using rhodamine 123 and propidium iodide (PI). The results showed depolarization of mitochondrial membrane in all concentrations tested. However, it had no effect on staining for propidium iodide in the tested concentrations, indicating that the cell membrane integrity was not changed. On this basis, our results show that the trypanocidal action of eupomatenoid-5 may be associated with mitochondrial dysfunction which could lead cell death. Further studies to elucidate better the mechanism of action of eupomatenoid-5 on *T. cruzi* death are been performed.
Supported by CAPES, CNPq, FINEP, PRONEX/Fundação Araucária.

## QT.18 - DESFERRIOXAMINE, AN IRON CHELATOR, DECREASES MORTALITY AND PARASITEMIA IN *Trypanosoma cruzi* INFECTED MICE THROUGH DIRECT ACTION ON PARASITE

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Although DFA is known to reduce the intensity of *T. cruzi* mice infection, the mechanism underlying this effect is still unclear and may involve host and parasite factors. To investigate the impact of DFA on mice disease outcome, on *T. cruzi* biology and on host biomarkers, disease and parasitological studies were performed. The effect of DFA in disease outcome was verified by parasitemia and mortality studies as well as host iron metabolism, blood cells and lymphocyte subsets analysis. To evaluate the activity of DFA directly on parasites we tested culture growth inhibition and performed mobility, membrane integrity and apoptosis assays. DFA treated animals presented lower cumulative mortality rate in long term infection and lower parasitaemia in both short and long term infection. No effect was observed in iron metabolism markers, erythrogram, leukogram, lymphocyte subsets, except for an increase in lymphocyte counts at $7^{\underline{th}}$ d.p.i. DFA inhibited amastigotes and trypomastigotes growth in fibroblast culture, decreased parasite mobility, induced minor parasite apoptosis but did not change viability measured by trypan blue staining. Beneficial DFA effects on mice *T. cruzi* infection may be due to trypanostatic effect, independently of interference on host iron metabolism and with minor effects on lymphocyte subpopulation counts.
Supported by CAPES, FAPEMIG, UFOP, UFMG and CPqRR.

## QT.19 - INDUCTION OF RESISTANCE TO THIOSSEMICARBAZONE AND BENZNIDAZOLE IN *Trypanosoma cruzi* AND ITS ASSOCIATION WITH P-GLYCOPROTEIN

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Chagas disease, caused by the protozoan *Trypanosoma cruzi*, represents an important health problem in Latin America. Specific treatment for this pathology is up to now not efficient and present high toxicity. Furthermore, studies have shown that resistance to benznidazole (Bz) for a variety of *T. cruzi* strains, results in failure of patients treatment. One of the mechanisms related to drug resistance in different pathogenic protozoa is the transport of drugs across the membrane by ATP-binding cassette (ABC) transporters. Recently, a member of the ABC superfamily, P-glycoprotein (Pgp), have been described in *T. cruzi* although its function has not been characterized. In the present study, the effects of (2-methoxy-styryl)-thiosemicarbazone (2-MEOTIO), a synthetic compound presenting activity on trypomastigote and amastigote forms of *T. cruzi* and Bz (reference drug) were investigated on *T. cruzi* epimastigotes (Y strain). The $IC_{50}$ values observed for 2 MEOTIO and Bz were 230 ± 20,2 µM and 182,1 ± 10,7 µM, respectively. Both drugs were then used to induce resistance in *T. cruzi* epimastigotes. After at least 5 passages under drug pressure, It was obtained resistant parasites as demonstrated by the increase of the $ICs_{50}$ for 2 MEOTIO (401,8 ± 33,7 µg/mL) and Bz (474,1 ± 15,7 µM). In order to verify the influence of P-gp, in the mechanism of drug resistance in *T. cruzi* it was analyzed the efflux of Rhodamine 123 (Rho-123) by resistant and wild-type epimastigotes. Parasites were incubated with Rho-123 (a fluorescent probe) in the presence or absence of verapamil (Pgp inhibitor) and the Rho-123 fluorescence was analyzed on a FAC-Scan flow cytometer. It was observed a significant time-dependent reduction of Rho-123 fluorescence in resistant parasites in comparison with wild-type. The results suggest participation of Pgp in *T. cruzi* resistance induced by benznidazole and (2-methoxy-estiryl)-thiosemicarbazone.
Supported by FAPERJ, CAPES and CNPq.

## QT.20 - EFFECT OF EFAVIRENZ AND NEVIRAPINE ON THE PROLIFERATION OF *TRYPANOSOMA CRUZI* EPIMASTIGOTES

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Chagas' disease is a zoonosis caused by the haematic protozoan *Trypanosoma cruzi*. The therapeutic possibilities rely on two drugs, nifurtimox and benznidazole whose efficiency is highest during the acute phase of the disease. In addition, these drugs are highly toxic, with systemic side effects on patients. From the above, it is clearly necessary to validate new drugs that allow more effective treatment. The aim of this work was to evaluate the activity of two antiretroviral drugs, efavirenz and nevirapine, on *T. cruzi* growth. These drugs belong to the first generation of NNRTIs (non-nucleoside reverse transcriptase inhibitors). Used in combination with other antiretroviral drugs, these compounds have become a cornerstone for the treatment of HIV-1 infection. Efavirenz and nevirapine presented $IC_{50}$ values of 45 ± 0.98 µM and 75 ± 1.65 µM for the Hep3B cell line, respectively. For the HeLa cells the values were 39 ± 1.05 µM and 67 ± 0.95 µM, respectively. The effect on *T. cruzi* proliferation was evaluated in the epimastigote form, cultured in media LIT. The efavirenz and nevirapine concentrations are ranged between 1 and 100 µM using rotenone (60 µM) and antymicin (0.5 µM) mixture as positive controls. The data were obtained daily, monitoring the absorbance change to 620 nm in 96-well-plates during 7 days. The dose-response on growth showed for efavirenz and nevirapine, $IC_{50}$ values of 54.13 ± 0.75 µM and 62.86 ± 0.07 µM respectively. When comparing the cell growth in control and treated parasites, statistical differences were found ($p<0.01$). Our *in vitro* results showed that efavirenz and nevirapine affects the cell growth of epimastigotes from *T. cruzi.* Further experiments will be done to confirm its therapeutic potential. Possible synergism between the two drugs and their potential effect on different stages of the life cycle of *T.cruzi* will also be analyzed. Supported by FAPESP, CNPq and USP.

## QT.21 - VITAMIN C EFFECTS IN MICE EXPERIMENTALLY INFECTED WITH *Trypanosoma cruzi* QM2 STRAIN

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Studies on new perspectives for the human treatment of Chagas' disease, concluded that the combined administration of 500mg/day of vitamin C and vitamin E 800UI/dia was able to halt the progression of oxidative stress caused by the disease. However, several studies describe the use of ascorbic acid by *T. cruzi* to protect itself from free radicals produced by immune cells of host. Thus, 60 mice were infected by *T. cruzi* QM2 strain and divided into six groups: G1, G1', G2, G2', G3 and G3', and G1, G2 and G3 for the acute phase and G1', G2 'and G3' to the chronic stage. G1 and G1' received 8.6 x $10^{-4}$ mg vitamin C, G2 and G2', 7.14 x $10^{-3}$ mg of vitamin C, and G3 and G3' were the placebo groups. The study of the acute phase showed statistically significant differences between G1 and the other groups at various count days of the parasitemia evolution, and until the $11^{th}$ day multiplying parasite was slower in G1, but at $22^{th}$ day it has parasitemia greater than G2 and G3, and from $36^{th}$ day stabilizes its parasitemia at higher levels, however there was no significant difference in histopathological analysis. In the chronic phase was not significant difference in histopathological analysis between the groups. So, it was found that even with a significant difference in parasitemia during the acute phase, the administration of two different vitamin C doses was not able to protect mice and likely contain the oxidative stress caused by free radicals formed by metabolism of oxygen and nitrogen, establishing the characteristic lesions of Chagas' disease.
Supported by Fapesp.

## QT.22 - THE INVOLVMENT OF RESERVOSOMES, GOLGI AND AUTOPHAGY IN TRIAZOLIC NAPHTHOQUINONE MECHANISM OF ACTION IN *TRYPANOSOMA CRUZI* EPIMASTIGOTES

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The current chemotherapy for Chagas´ disease caused by the pathogenic protozoan *Trypanosoma cruzi* is based on two nitroderivatives, but its variable efficacy and serious side effects require the development of alternative drugs for the treatment of this disease. In this framework, among several naturally occurring quinones, emerge the naphthoquinones with a broad distribution in the plant kingdom and involved in oxidative processes. In the last decade, our group has been synthesized and screened on bloodstream trypomastigotes, 80 derivatives of quinones, being a novel triazolic naphthoquinone (**TN**) derived from nor-lapachol, the most active compound. Here, we evaluate the ultrastructural effect of this compound in epimastigote forms of *T. cruzi*. Scanning electron microscopy showed important morphological alterations in **TN**-treated epimastigotes (3-9 µM) such as retraction of the posterior region of the body and the presence of multiple flagella. Transmission electron microscopy pointed to a remarkable disorganization in reservosomes morphology, a severe Golgi disruption as well as blebs in the flagellar membrane and the formation of concentric membranar structures in the cytosol in treated parasites. 9 µM **TN** also triggered an extensive autophagic process, being endoplasmic reticulum profiles observed surrounding cytosolic portions and organelles such as reservosomes. Our scanning electron microscopy data showing parasites treated with 3-9 µM with multiple flagella, together with flow cytometry experiments indicated the partial blockage of cytokinesis, leading to the cell cycle arrestment. Our ultrastructural data together with the cell cycle analysis strongly suggests that **TN** blocks cytokinesis and impairs the parasite proliferation. Further electron microscopy assays must be performed to evaluate the targets of this compound in bloodstream trypomastigotes, a clinical relevant form of *T. cruzi*.
Supported by CNPq, FAPERJ and IOC/FIOCRUZ.

## QT.23 - INHIBITING EFFECTS OF GALLIC ACID AND ESTERS DERIVATIVES ON *Trypanosoma cruzi* TRYPANOTHIONE REDUCTASE

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Trypanothione reductase (TR) is a key enzyme in the trypanothione-based redox metabolism of pathogenic trypanosomes. Due its absence in mammals, were glutathione reductase (GR) takes place, TR represents a potential drug target. Since gallate esters were reported to be active against *T. cruzi*, the aim of this work was to assess their potential as inhibitors of *T. cruzi* TR (*Tc*TR). For that, *Tc*TR was cloned and expressed in *E. coli* and the purified active enzyme was evaluated against gallic acid (GA) and its esters as well as against a commercially available *S. cerevisae* GR. The *Tc*TR ORF was PCR amplified from *T. cruzi* Y strain genomic DNA, cloned into pET14b and expressed in *E. coli* BL21(DE3) by IPTG (1mM) induction at 37℃ for 4 hours. After purification of the soluble recombinant protein by affinity columns (Ni-NTA) under non-denaturing conditions, *Tc*TR inhibition assays were performed using different concentrations of gallates, 40mM Hepes (pH 7.5), 1mM EDTA, 150µM NADPH, 1µM trypanothione, 25µM of DTNB and 230ng of purified *Tc*TR. *Tc*TR and GR activities were assessed spectrophotometrically at 412nm and 340nm, respectively, using clomipramine (12.5-100µM) and carmustine (10µM) as inhibition controls. Among six compounds, only GA and decyl gallate were able to inhibit *Tc*TR activity, showing $IC_{50}$ values of 45.6 and 78.9µM, respectively. This GA-induced inhibition was higher than clomipramine control ($IC_{50}$ of 63.6µM), showing a selective *Tc*TR inhibition. Along with an efficient heterologous expression of *Tc*TR, the results reveal that GA is a prominent prototype for the development of new molecules with selective trypanocidal activity. Supported by CNPq, FINEP and UFSC.

## QT.24 - EFFECT OF *APIS MELLIFERA* VENOM ON *TRYPANOSOMA CRUZI* INTRACELLULAR CYCLE

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Chagas' disease chemotherapy is based on drugs that exhibit toxic effects and limited efficacy such as Benznidazole. Therefore, new chemotherapeutic agents from natural sources are a lining research to be exploited. Honeybee (*Apis mellifera*) venom consists of many biologically active compounds and has been reported to exhibit anticancer effects. In the present study we analyzed the effect of *A. mellifera* venom on *T. cruzi* intracellular cycle. Briefly, LLC-$MK_2$ cells were incubated in the presence of 0.1, 0.5, 1 e 2 µg/ ml of *A. mellifera* venom for 5 days. Coverslips were collected daily, fixed and stained with Giemsa to analyze the venom effects on host cells. None of these dose was toxic to them. To analyse the effect on the parasite intracellular cycle, host cells were infected with tissue culture trypomastigotes at a 10:1 parasites: host cell ratio in the absence of the venom. After 24 h interaction, cells were washed and different venom concentrations (0,025- 0,4 µg/ ml) in RPMI medium were added to distinct wells, and then incubated for 24 to 96 h at 37℃. The percentage and number of infected cells, and the number of amastigotes per 100 cells were daily evaluated. The venom's presence during the intracellular development of *T. cruzi* caused a significant reduction in the percent (32 to 76 %) and number (17.5 to 56%) of infected cells, after 24h, reaching 100% and 69% after 96h of incubation with 0.4 µg/ ml, respectively. The parasites number per 100 cells was 59 to 87.9% after 24 h, reaching 68 to 95.1% after 96h. Our data demonstrate that *A. mellifera* venom gains access to the host cells cytoplasm where it was effective against the intracellular forms of *T. cruzi*. Further studies are underway to investigate the venom targets in *T. cruzi* amastigotes forms.
Supported by CNPq, CAPES and FAPERJ.

## QT.25 - ULTRASTRUCTURAL STUDY OF *Trypanosoma cruzi* TREATED WITH POSACONAZOLE PLUS AMIODARONE

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Chagas disease, caused by the protozoan *Trypanosoma cruzi,* represents an important public health problem, with overall prevalence estimated in 10-16 millions of cases. There is no effective treatment for the prevalent chronic form of Chagas' disease. Posaconazole, an ergosterol biosynthesis inhibitor acting at cytochrome P 450-dependent lanosterol C14 demethylase (CYP51), has potent and selective anti-*T. cruzi* activity *in vitro* and *in vivo* and is currently entering clinical trials for the specific treatment of this condition. On the other hand, the antiarrhythmic drug amiodarone has been shown to have also direct activity against *T. cruzi*, disrupting the parasite's $Ca^{2+}$ homeostasis and inhibiting ergosterol biosynthesis at the level of lanosterol synthase (Benaim et al, 2006). Furthermore, it was shown that amiodarone acts synergistically with posaconazole against the clinically relevant form of the parasite, intracellular amastigotes. These results have now been reproduced our laboratory. Epimastigotes and intracellular amastigotes were treated with $IC_{50}$ and $IC_{90}$ of posaconazole and fixed with 2.5% glutaraldehyde. For transmission electron microscopy (TEM), cells were post-fixed in osmium tetroxide, dehydrated in acetone, embedded in Epon and observed by TEM. For field-emission scanning electron microscopy, parasites were dehydrated in ethanol, critical-point dried in $CO_2$, and observed. Epimastigotes treated with posaconazole and observed by field-emission scanning electron microscopy displayed extensive loss of integrity of the plasma membrane, with blebs formation and parasite body retraction. Observations by TEM showed intense changes in organization of microtubules in the flagellum, alterations in the Golgi complex organization and extensive autophagic vacuolization. In addition, analysis of intracellular amastigotes treated with posaconazole by TEM showed alterations in the Golgi complex organization and plasma membrane shedding. Further microscopic assays are being performed to study alterations in the ultrastructure of *T. cruzi* amastigotes and trypomastigotes treated with amiodarone plus posaconazole, to better understand the synergic effects of these drugs. Supported by CNPq, CAPES and FAPERJ.

## QT.26 - ACTIVITY OF AN INHIBITOR OF TRANSFORMING GROWTH FACTOR BETA SIGNALING DURING THE ACUTE PHASE OF *TRYPANOSOMA CRUZI* INFECTION

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Transforming growth factor beta (TGF-β) is a key mediator in the pathogenesis of cardiac remodeling during Chagas` disease. High TGF-β levels are associated with extensive fibrosis in acute and chronically *T. cruzi*-infected humans and mice. We recently reported that a TGF-β type I receptor inhibitor, SB-431542, partially inhibits *T. cruzi* invasion, reduces amastigote numbers per infected cell and inhibits the differentiation into trypomastigotes at the end of the intracellular cycle in cardiomyocytes. Our present aim is to investigate the role of a novel orally active TGF-β type I receptor inhibitor, GW-788388, in attenuating heart damage and cardiac dysfunction, using a *T. cruzi* mouse infection model. In our experimental model of acute infection, male Swiss mice were inoculated with the *T. cruzi* Y strain and evaluated by clinical, parasitological and histopathological investigations up to 30 days post-infection (dpi). One dose of GW-788388 was orally administered at 3 or 13 dpi. Our results show that GW-788388 treatment significantly increased mice survival rates and reduced the number of circulating parasites, as compared to non-treated mice. Moreover, GW-788388 also reduced tissue lesions, showing lower cardiac parasitism and numbers of cardiac inflammatory cells. Interestingly, blocking of TGF-β intracellular signaling at 13 dpi increased the mortality of treated animals, suggesting that TGF-β could have, at this stage of infection, an important regulatory role in the inflammatory process. Thus, we believe that inhibition of the exacerbated biological effects of TGF-β might represent an attractive strategy to combat the severity of this disease. Supported by Fiocruz and CNPq.

## QT.27 - EFFICACY OF BENZIDAZOLE TREATMENT IN CHRONIC CANINE CHAGAS DISEASE: HISTOPATHOLOGY AND FUNCTIONAL ANALYSIS

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The analysis of available information reveals that the efficacy of benznidazole treatment in chronic chagasic infection is doubtful. In this study we evaluated the effect of Bz-treatment on the cardiac alterations using dogs infected with Berenice-78 strain as experimental model. The infected animals were divided in two experimental groups: (i) 12 dogs were Bz-treated at 7.0 mg/kg bid (Q12) for 60 days during the chronic phase; (ii) 12 dogs were maintained as non-treated control. Another 8 animals were maintained as non-infected control group. For cardiomegaly and systolic or diastolic function evaluation the animals were examined by echocardiography in the 1$^{st}$ and 12$^{th}$ month post-treatment. The parameters fractional shortening, Left Atrium (LA) volume, Left-ventricle (LV) ejection fraction, diastolic volume and systolic diameter were measured. After this evaluation a half of animals were euthanized in the same period for histopathological analysis of heart tissue. Bz-treatment led to a reduction of around 20% to 36% of inflammatory cells and intra-fascicular collagen deposition when compared to non-treated animals in the first month post-treatment. Additionally, all animals evaluated showed echocardiographic parameters similar to non-infected animals. Differently, 12 months post-treatment the intensity of cardiac lesions were similar to treated and non-treated animals and significantly larger than those detected in non-infected animals. Also, the echocardiographic parameters, related with cardiomegaly (LV and LA volume, LV systolic diameter) and diastolic function (LA volume), were similar among treated and non-treated animals and significantly higher than those observed in non-infected animals. Interestingly, the Bz-treatment was able to prevent alterations related to cardiac functions (LV ejection and shortening fraction), such this parameters were similar to treated and non-infected animals. Taken together, the results indicate that Bz-treatment performance during the chronic phase of the dogs' infection is efficient in preventing cardiac lesions immediately after the treatment and the systolic cardiac function long-time post-treatment. Financial Support: CNPq, FAPEMIG, UFOP.

## QT.28 - EVALUATION OF THE BENZNIDAZOLE THERAPY EFFICACY OF BLOCKING HOST CELL APOPTOSIS IN DOGS INFECTED BY *Trypanosoma cruzi*

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The identification of the deleterious effects of apoptotic cells and their effect on *T.cruzi* replication provide a new conceptual framework for the pathogenesis of Chagas disease. However, several questions remain unsolved, including the role of apoptosis in cardiac inflammation and the therapeutic efficacy of blocking host cell apoptosis. In the present study, we investigate the effect of the Benznidazole (Bz) treatment in the occurrence of apoptosis in peripheral blood mononuclear cells (PBMC) and in cardiomyocytes of dogs infected with VL10 strain (resistant to Bz-terapy). Infected animals were divided in two experimental groups: (i) 5 dogs were Bz-treated at 7.0 mg/kg bid (Q12) for 60 days; (ii) 5 dogs were maintained as non-treated control. Another 5 animals were maintained as non-infected control group. The animals were treated during the acute phase, and after treatment the blood was collected for PBMC analysis and the animals were euthanized for heart tissue evaluation. The results showed that infected and Bz-treated animals presented a higher proliferative response (proliferative index:1.96±0.63), similar to infected control (1.95±0.24) and significantly higher to no-infected group (0.95±0.24). Differently, the apoptosis index was truly influenced by Bz treatment, while the apoptotic index among treated animals (1.36±0.45) and non-infected (1.26±0.42) was similar and significantly ($p<0.01$) smaller to the one detected in the PBMC of those infected and non-treated animals (2.63±1.1). Differently, cardiomyocytes apoptosis (analyzed by TUNEL method) was not influenced by Bz-treatment, as the apoptotic index showed similar value among Bz-treated (13.13±0.45) and non-treated (12.17±2.04) infected animals, and significantly higher to non-infected animals (9.42±0.57). Additionally, similar intensity of inflammatory infiltrated and fibrosis area were detected among Bz-treated and non-treated animals. Taken together, these results confirm a causal link between apoptosis and heart damage and suggest that influence of the Bz-therapy in cell apoptosis is related with the cell type evaluated, PBMC or cardiac. Supported by: FAPEMIG and CNPq

## QT.29 - EXPERIMENTAL CHEMOTHERAPY WITH COMBINATIONS OF ITRACONAZOLE PLUS BENZNIDAZOLE IN MURINE MODELS OF CHAGAS' DISEASE

Tassiane Assiria Fontes Martins[1], Isabel Mayer de Andrade[1], Livia Figueiredo Diniz[1], Ivo Santana Caldas[1], André Talvani[1], Isabela Ribeiro[2], Maria Terezinha Bahia[1*]

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We report the effects of benznidazole (Bz) acting alone or in combination with Itraconazole (Itz) in murine models of Chagas' disease. The following treatment arms were selected: Itz 100, 75 and 50 mpk (mg/kg/day); Bz 100, 75 and 50 mpk; Bz/Itz combination: 50/50; 75/75 and 100/100 mpk of each of the drugs. Female Swiss mice, 18-20g, 10 animals/group, were infected with $5 \times 10^3$ bloodstream trypomastigotes of Y strain. Treatment was administered orally for 20 consecutive days, beginning at 4 days after inoculation. Parasitological cure was assessed by parasitemia at and up to 60 days after treatment through optimal microscopy and blood PCR. All Bz-treated animals survived for 60 days after treatment. In contrast, only animals that received 100 mpk of Itz survived during same period, while 50% and 60% of animals treated with 50 and 75 mpk of Itz died up to 50 days post-infection. Although there was significant reduction in the level of parasitemia after treatment, parasitological cure was not achieved in all treatment groups. Parasitological cure was documented in 60% (6 of 10) of mice treated with 100mpk of Bz; whilst there was no cure among animals treated with Bz 50 and 75 mpk or Itz 50, 75 and 100mpk. Bz/Itz combination treatment induced parasitological cure in 80% (2 of 10), 70% (7 of 10) and 20% (2 of 10) of mice that received 100, 75 and 50mpk of Bz/Nfx combination therapy, respectively. These results suggest a beneficial effect of the combination, as parasitological cure was observed in a higher proportion of animals treated with the drugs in association than in animals receiving the same dose of each of the drugs in monotherapy. Supported by DNDi, UBS Optimus Foundation, Fapemig, CNPq and UFOP.

## QT.30 - EFFICACY OF BENZNIDAZOL OR NIFURTIMOX AND POSACONAZOLE COMBINATION IN EXPERIMENTAL CHAGAS DISEASE

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As there are limited prospects for the near-term introduction of new compounds for the treatment of Chagas disease, an alternative strategy involves the identification of candidate drugs among those already available on the market that could be used in combination with the aim of increased efficacy, safety and potential shortened duration of treatment regimens. We have investigated the anti-*Trypanosoma cruzi* efficacy of combinations of Posaconazole(Ps) and Benznidazol(Bz) or Nifurtimox(Nfx) in mice infected with the *T. cruzi* Y-strain using a rapid treatment protocol, in which each animal received drug suspension by gavage for 7 days. In the initial phase, the effects of half and one-fourth of the curative dose (CD)of each drug alone on the evolution of the infection in mice were evaluated and compared with animals treated with known CD those for long-term treatment (e.g., 100 mpk of Bz, 50 mpk of Nfx and 20 mpk of Ps). The analysis of parasitemia and mortality rate after treatment showed that all compounds had a dose-dependent trypanocidal effect with significantly lower parasitemia levels and mortality rate with CD of Bz, Nfx and Ps compared with those receiving half and one-fourth of the CD. In a second phase of data analyses, the activities of drugs combinations were compared with results of the CD of each drug alone. The results showed a clear beneficial effect of the Bz/Ps or Nfx/Ps combinations, as animals that received the half and one-fourth doses of these drugs in association presented a significant reduction of mortality and parasitemia levels in comparison to those that received the same dose of each drug alone. These data will be confirmed in experiments with 20-day treatment in acute murine model. Supported by DNDi, UBS Optimus Foundation, Fapemig, CNpq and UFOP.

## QT.31 - COMBINED TREATMENT OF HETEROCYCLIC ANALOGUES AND BENZNIDAZOLE UPON *TRYPANOSOMA CRUZI IN VIVO*

Batista, D.G.J.[1], Batista, M.M.[1], Oliveira, G.M.[1], Britto, C.C.[2], Rodrigues, A.C.M.[2], Stephens, C.E.[3], Boykin, D.W.[4] and Soeiro, M.N.C.[1*]

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Chagas disease caused by *Trypanosoma cruzi* is an important cause of mortality and morbidity in Latin America but no vaccines or safe chemotherapeutic agents are available. Combined therapy is envisioned as an ideal approach since it may enhance efficacy by acting upon different cellular targets, may reduce toxicity and minimize the risk of drug resistance. Thereafter, we investigated the activity of benznidazole (Bz) in combination with the diamidine prodrug DB289 and in combination with the arylimidamide DB766 upon *T. cruzi* infection *in vivo*. The oral treatment of *T.cruzi*-infected mice with DB289 and Benznidazole (Bz) alone reduced the number of circulating parasites compared with untreated mice by about 70% and 90%, respectively. However, the combination of these two compounds decreased the parasitemia by 99% and protected against animal mortality by 100%, but without providing a parasitological cure. When Bz (p.o) was combined with DB766 (via ip route), at least a 99.5% decrease in parasitemia levels was observed. DB766+Bz also provided 100% protection against mice mortality while Bz alone provided about 87% protection. This combined therapy also reduced the tissular lesions induced by *T. cruzi* infection: Bz alone reduced GPT and CK plasma levels by about 12% and 78% compared to untreated mice group, the combination of Bz with DB766 resulted in a reduction of GPT and CK plasma levels of 56% and 91%. Cure assessment through hemocultive and PCR approaches showed that Bz did not provide a parasitological cure, however, DB766 alone or associated with Bz cured ≥13% of surviving animals. Our data support additional studies with other diamidines and arylimidamides alone or in combination with other drugs with the goal of identification of new candidate therapies for the treatment of Chagas disease.
Supported by: FAPERJ-APQ1 and Pensa Rio,CNPq, PAPES V/FIOCRUZ and The Consortium for Parasitic Drug Development (CPDD).

## QT.32 - BIOLOGICAL EFFICACY OF NEW ARYLIMIDAMIDES UPON *Trypanosoma cruzi IN VITRO*

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Chagas disease (CD) is a tropical neglected illness affecting mostly the poorest people living in endemic areas of many Latin American countries and also occurring in Europe and North America, due to immigration. Current treatment is based on benznidazole (BZ) or nifurtimox that are indicated for the therapy of all acute cases and early chronic patients, during infection or reagudization processes after transplantation and immune suppression cases as well as for cases caused by laboratory accidents. The aim of this work is to investigate the in vitrotrypanocidal effects of novel arylimidamides (AIAs - DB667, DB709, DB745B, DB749 and DB946) against *T. cruzi*. All the AIAs tested exhibited significant trypanocidal effects, giving dose and time-dependent activity against bloodstream trypomastigotes and intracellular amastigotes (Y strain), and exhibiting $IC_{50}$ values ranging from 15 nM up to 2.48 µM. DB745 also exerted striking effects upon different parasite stocks, including those naturally resistant to benznidazole such as YuYu and Colombiana strains, displaying higher efficacy than the reference drugs (BZ and gentian violet). Our data clearly demonstrates the trypanocidal effect of the novel AIAs, showing that this class of compounds exhibits the potential to provide new leads compounds for CD therapy.
Supported by: FAPERJ, CNPq , CPDD and PAPES/FIOCRUZ.

## QT.33 - INHIBITORY ACTIVITY AGAINST *Trypanosoma* AND *Leishmania*: A KNOWN DRUG REVISITED.

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Neglected diseases are in its majority tropical infectious pathologies transmitted by insect vectors or by contaminated water and soil. The World Health Organization lists a total of fourteen main tropical neglected diseases among which are leishmaniose, sleeping sickness, malaria, Chagas disease, schistosomiasis and dengue. Regardless of all the knowledge already reached about those infectious diseases, they still present high worldwide morbidity and mortality rates. Moreover, the investment in drug research and development to its treatment is stills low, when compared to other diseases. The development of novel drugs for the Kinetoplastida parasites (*Leishmania, Trypanosoma cruzi* or *Trypanosoma brucei*) is the main focus of several research efforts. We have tested the use of a known human rheumatoid arthritis drug that is believed to interact selectively with selenoproteins. We have shown that this proteins are vital components to organisms belonging to Kinetoplastida order. *In vitro* experiments with this drug have shown a $LD_{50}$ of 75µM and 2.88µM for the trypomastigote and epimastigote forms of *T. cruzi* respectivelly. Moreover, this drug is effective against *Leishmania* and *T. brucei* cells. The *in vivo* experiments, following the World Health Organization protocols, are been conducted to verify the efficacy of this compoind as a treatment for Chagas disease as a less toxic alternative to the current treatment Benznidazol.
Supported by grants from CNPq and FAPESP.

## QT.34 - THE NEOLIGNAN GRANDISIN PRESENTS ACTIVITY AGAINST AMASTIGOTES OF *Leishmania chagasi*

Caldas, J.P.A.[1], Amorim, F.M.[1], Néris, P.L.N.[1], Penha, A.R.S.[1], Barbosa-Filho, J.M.[2], Oliveira, M.R[1*].

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Leishmaniasis treatment relies mainly on pentavalent antimonials, amphotericin B and pentamidine, expensive and highly toxic treatments that require prolonged parenteral administration. Thus, there is an urgent need for developing new drugs to replace those in current use. The plants are valuable sources of new medicinal agents. Lignans are an important group of plant metabolites with several biological activities documented. The objectives of this study were to evaluate the cytotoxicity of the neolignan grandisin on murine macrophages (MØs), and its antiamastigote activity against *Leishmania* (*L.*) *chagasi*. MØs were incubated in the presence or absence of grandisin at 37ºC in a 5% $CO_2$ atmosphere for 24h, and their viability was assessed by MTT reduction and trypan blue exclusion assay. MØs were infected with stationary-phase *L. chagasi* and incubated as aforementioned with different concentrations of grandisin (200 and 400 µg/mL) for 24, 48 and 72h. The cells were dyed, and the amastigotes were counted under light microscopy. Cell supernatants were analyzed for nitric oxide (NO) and tumour necrosis factor (TNF)-α production. Cytotoxicity of grandisin (concentrations up to 800µg/mL) was not detected by MTT reduction assay. Cell viability measured by trypan blue exclusion assay resulted in a $CC_{50}$ of 729.79µg/mL. The treatment of infected MØs with grandisin at 400µg/mL, for 24h, significantly reduced the percentage of infected cells (29.87%). At 48h, infection was reduced by 21.19% (200µg/mL) and 33.67% (400µg/mL). After treatment for 72h, a further reduction of infection was observed at 200 (45.65%) and 400µg/mL (55.65%). The antiamastigote activity of grandisin correlated with NO production at 24 (at 400µg/mL), 48 and 72h (at 200 and 400µg/mL). No correlation was found between NO and TNF-α production. These data demonstrate grandisin presents antiamastigote activity against *L. chagasi*, little cytotoxicity for MØs, and is able to modulate MØs infection by inducing NO production.
Supported by CAPES.

## QT.35 - LEISHMANICIDAL ACTIVITY OF *Momordica charantia* (CUCURBITACEAE)

Vasconcelos, M.F.[1], Marques, M.C.S[2], Garcez, W.S.[2], Garcez, F.R.[2], Canto-Cavalheiro, M.M.[1], Torres-Santos, E.C.[1]*

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In spite of the advances in last decade, such as miltefosine registry and the development of new formulations of amphotericin B, control of leishmaniasis remains in the order of day and the search for new treatment alternatives is an actual priority. Thus, the study of large chemical potential of Brazilian biodiversity can be an interesting approach to the development of new treatment alternatives. This study aimed to evaluate the leishmanicidal activity of *Momordica charantia* (Cucurbitaceae), originally known for its use in cooking and medicine. Specimens of *M. charantia* were collected, air parts were dried, macerated and subjected to extraction with ethanol by dynamic maceration. *Leishmania amazonensis* promastigotes were cultured in the presence of several concentrations of crude extracts and their subsequent partitions up to 100 µg/mL for 72 hours and quantified colorimetrically by MTT assay. Under these conditions, the ethanolic partition was the most active, with $IC_{50}$ of 4.5 µg/mL. This sample was sequentially fractionated and the partition in ethyl acetate (MSØAc) was significantly more active, with $IC_{50}$ of 6.12 µg/mL. The fractionation of this partition led to a fraction rich in triterpenes (F7), with $IC_{50}$ of 2.1 µg/mL. Again, this partition was fractionated and the activity was concentrated in three fractions, with $IC_{50}$ of 1.50 µg/mL, 1.08 µg/mL and 1.16 µg/mL. Five pure substances were obtained from these fractions, all with $IC_{50}$ around 5.0 µg/mL. The activity of MSØAc and F7 fractions was evaluated in intracellular amastigotes. The infectivity index was determined by optical microscopy. F7 and MSØAc showed a significant antiamastigote activity, with $IC_{50}$ of 1.8 µg/mL and 2.3 µg/mL, respectively. The structural identification of the isolated active compounds may lead to a new antileishmanial prototype. Supported by Papes/CNPq.

## QT.36 - EVALUATION OF A COMBINED MEGLUMINE ANTIMONIATE AND TAMOXIFEN THERAPEUTIC SCHEME FOR LEISHMANIASIS

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Tamoxifen is an antiestrogen used for the treatment of breast cancer. Recently, our group has demonstrated that this drug is effective against *Leishmania in vitro*, reduces the parasite burden in *L. amazonensis* and *L. braziliensis*-infected BALB/c mice and in hamsters infected with *L. chagasi*. The aim of this study is to assess the effect of the association of tamoxifen and meglumine antimoniate (Glucantime®), a drug regularly used in the clinical practice as the first choice treatment for cutaneous leishmaniasis. Our assays were performed after infecting the basis of the tail of BALB/c female mice with 1 million *L. amazonensis* stationary-phase parasites. Fifty days post-inoculation, animals were distributed in different groups (n=6-7/group) for the following i.p. treatment protocol for 15 days: 1) saline solution; 2) 20 mg/kg/d tamoxifen; 3) 10 mg/kg/d Glucantime; 4) 20 mg/kg/d Glucantime; 5) 10 mg/kg/d Glucantime plus 20 mg/kg/d tamoxifen and 6) 20 mg/kg/d Glucantime plus 20 mg/kg/d tamoxifen. The ulcerated area and lesion size were measured weekly up to 65 days after the interruption of the treatment. Two weeks after the end of treatment, the response in groups receiving the association of tamoxifen and Glucantime was comparable to groups receiving Glucantime alone. The pattern of response was maintained up to 9 weeks after the end of treatment and was also observed when the ulcer size was evaluated. Experiments testing this association in different dose schemes are being carried out to confirm these initial observations, which indicate that the association of Glucantime and tamoxifen in vivo does not show antagonic or synergic properties. On the other hand these drugs may show an additive effect in the treatment of cutaneous leishmaniasis in this experimental model. Other studies are also being conducted to establish the effectiveness of Amphotericin B-tamoxifen association in *L. amazonensis*-infected mice.
Support: FAPESP, CNPq.

## QT.37 - EVALUATION OF MECHANISM OF ACTION, IN VITRO AND IN VIVO ACTIVITIES OF LQB118, A NEW ANTILEISHMANIAL PROTOTYPE

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Leishmaniasis is one of the most neglected diseases, affecting the poorest segments of populations, resulting in substantial morbidity and mortality in 2 million people worldwide. Nevertheless, there is scant investment for development of new effective and safe drugs. In previous studies, we reported that the naphthopterocarpanquinone LQB118 is a potent *in vitro* antileishmanial agent. Here, we investigated the mechanisms involved in cell death of *L. amazonensis* promastigotes treated with LQB118 and the ability of this compound in controlling lesions in murine model. Morphological alterations induced by LQB118 in promastigotes were evaluated by TEM and SEM. To evaluate the biochemical pathways involved in parasite death, loss of mitochondrial membrane potential (rhodamine, JC1 and mitocapture assays), ROS production ($H_2$DCFDA), lipid peroxidation (TBARS), phosphatidylserine exposure (annexin V) and DNA fragmentation (TUNNEL) were analyzed. The concentration of LQB118 used for in vitro assays ranged between 1.25 and 10.0 µM. In vivo studies were performed in *L. amazonensis* infected mice (license LW07/2010). Our SEM and TEM results showed that LQB118 induced drastic morphological changes, as mitochondrial damage (increase of electron-density, swelling and cristae disorganization), nuclear changes (chromatin condensation and disorganization of the nuclear envelope) and rarefaction of the cytoplasm. Incubation of promastigotes with LQB118 induced dose-dependent generation of reactive oxygen species, lipid peroxidation and loss of mitochondrial membrane potential, which were accompanied by phosphatidylserine exposition and DNA fragmentation. LQB118 was effective in controlling lesions in leishmaniasis murine model by all tested routes of administration (subcutaneous, intraperitoneal and oral), with activity similar to the reference drugs Pentostam and Glucantime. In the present study, we demonstrated that LQB118 exerts its leishmanicidal effect by interfering in mitochondrial activity and triggering several events which suggest programmed cell death apoptosis-like. Altogether, our results indicate that LQB118 is a promising prototype for the treatment of leishmaniasis. Supported by FAPERJ, CAPES, PAPES/CNPq.

## QT.38 - CYTOTOXICITY AND ANTILEISHMANIAL ACTIVITY OF THE NEOLIGNANS BURCHELLIN AND GRANDISIN

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Leishmaniasis chemotherapy, for over 60 years, relies on pentavalent antimonials, amphotericin B and pentamidine, which are toxic and prone to drug resistance. New drugs for the treatment of leishmaniasis are necessary, and the plant kingdom is a valuable source of potential medicinal agents, as the lignans. The objectives of this study were to evaluate the antipromastigote activity of the neolignans burchellin and grandisin against *L. chagasi*, to evaluate farnesyl pyrophosphate synthase of *L. chagasi* (*Lc*FPPS) as a possible molecular target of the neolignans and to investigate their cytotoxicity on MDCK cells. Logarithmic phase *L. chagasi* promastigotes were incubated in Schneider medium ($1x10^6$ cells/mL) with burchellin and grandisin (0-100µg/mL) for 72h at 25ºC. The cultures were diluted and quantified in a neubauer chamber under light microscopy. The crystallized structure of *Lc*FPPS is unavailable, so homology modeling using PDB 1YHL of *Trypanosoma cruzi* FPPS was carried out to obtain a model for molecular docking calculations, which was done using the CCDC Gold Suite v.4.0 with the ChemScore function. MDCK cells cultured in RPMI medium were seeded into 96-well plates ($1x10^5$ cells/mL), treated with the neolignans (0-400µg/mL) and submitted to lactate dehydrogenase (LDH) assay. Burchellin and grandisin inhibited *L. chagasi* growth with an $IC_{50}$ of 16.54 and 7.17µg/mL, respectively. In molecular docking calculations, burchellin scored 21.45, by establishing 1 hydrogen bond and 3 coordinations with the active site of *Lc*FPPS, and grandisin scored 6.76 by establishing 4 hydrogen bonds with it, suggesting this enzyme as a possible molecular target of burchellin. No increment of LDH activity was detected on MDCK cells, implying neither burchellin nor grandisin exerts citotoxicity by altering cell membrane integrity in the concentrations tested (400µg/mL). The data demonstrate burchellin and grandisin present a specific cytotoxicity for *L. chagasi*, with *Lc*FPPS as a possible molecular target of burchellin. SUPPORTED by CAPES.

## QT.39 - CHITOSAN COMPLEXES PRESENT ANTILEISHMANIAL ACTIVITY IN EXPERIMENTAL MODELS *IN VITRO* AND *IN VIVO*

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The drugs commonly used for the treatment of leishmaniasis are administered via parenteral route and present high toxicity for humans. Taking into account that the search for more effective treatments for leishmaniasis is necessary, the objectives of this study were to evaluate the antileishmanial activity of chitosan complexes in experimental models *in vivo* and *in vitro*, as well as to evaluate the cytotoxicity of the complexes on mammalian cells. Logarithmic-phase *Leishmania (L.*) *chagasi* promastigotes were incubated in Schneider medium ($1x10^6$ cells/mL) with chitosan for 72h at 25ºC. The cultures were diluted and quantified in a neubauer chamber under light microscopy. Murine macrophages (MØs) were infected with stationary-phase *L. chagasi* and incubated with chitosan at 37ºC in a 5% $CO_2$ atmosphere for 72h. The cells were dyed, and the amastigotes were counted under light microscopy. MØs and Madin-Darbin Canine Kidney (MDCK) cells cultured in RPMI medium were seeded into 96-well plates ($1x10^5$ cells/mL), treated with chitosan and submitted to lactate dehydrogenase (LDH) assay. The left hind footpad of Swiss mice was subcutaneously infected with stationary-phase *L. amazonensis* ($1x10^7$ cells/mL). The mice were orally treated with 50mg/kg of chitosan for 8 weeks, after which lesion size and parasite load was analyzed. Chitosan presented antipromastigote activity against *L. chagasi*, with an $IC_{50}$ of 88.7µg/mL, and at 50µg/mL reduced the percentage of *L. chagasi*-infected MØs (30%). Additionally, this carbohydrate did not demonstrate cytotoxicity on MØs and MDCK cells at concentrations up to 400µg/mL for 24h. In Swiss mice infected on the footpad with *L. amazonensis*, treatment with 50µg/kg of chitosan reduced lesion size and parasite load in both the footpad and the popliteal lymph nodes. It can be concluded that chitosan complexes present a potentiality in the therapeutic of leishmaniasis, characterized by antileishmanial activity *in vitro* and *in vivo*.
Supported by CAPES.

## QT.40 - RESVERATROL SYNERGIZES WITH AMPHOTERICIN B TO INHIBIT *Leishmania amazonensis*

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Leishmaniasis is a disfiguring and potentially fatal disease caused by parasites of the genus *Leishmania*, which affects around 350 million people worldwide. Leishmaniasis treatment relies mainly on antimonials and amphotericin B that present high toxicity, elevated cost and parasite resistance. Recently, miltefosine was approved for visceral leishmaniasis treatment, but teratogenicy limits its use. All these facts stimulate the search for new anti-leishmanial agents, and natural products constitute an important source of such compounds. Besides, combination therapy for leishmaniasis treatment has been advocated as a way to reduce treatment duration and cost, and limit the emergence of drug resistance. The aim of this study is to validate the anti-leishmanial activity of the natural product resveratrol, alone, or in combination with amphotericin B. Resveratrol, a polyphenolic compound present in wine and grapes has biological activities, such as anti-inflammatory, anticancer and antioxidant. Our previous results have shown that resveratrol presented an anti-*L.amazonensis* activity with an $IC_{50}$ of 27µM for promastigotes and of 42µM for amastigotes. Morphological analysis by optical microscopy demonstrated that resveratrol treated promastigotes (100µM for 48h) presented an irregular number of nucleus and flagella. Corroborating these finds, alteration in the cell cycle was also observed in these parasites analyzed by flow cytometry after propidium iodide stained. Here, by isobolografic analysis, we describe that the combination of resveratrol (R) with amphotericin B (A), showed a synergistic effect for promastigotes (13R + 0.01µM A) as well as for amastigotes 13R + 0.0002µM A) of *L.amazonensis*. Treatment of peritoneal murine macrophages with 600µM of resveratrol was not toxic (75% of viable cells, detected by the XTT assay). Our results confirmed the anti-*Leishmania amazonensis* effect of resveratrol and add its synergic association with amphotericin B, pointing them as possible substances for further studies of drugs combination therapy *in vivo*.
Supported by FAPERJ, CNPq.

## QT.41 - EVALUATION OF THE LEISHMANICIDAL POTENTIAL OF QUINOLINE DERIVATIVES ON *LEISHMANIA MAJOR*

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Leishmaniasis is one of the most important neglected tropical diseases caused by parasites of the genus *Leishmania*. The current chemotherapy for leishmaniasis is limited, because the drugs are toxic and most to be used parenterally for prolonged period. Several drugs based on the quinoline structure have improved the therapy of protozoa diseases. In order to find new drugs against leishmaniasis, we evaluate the *in vitro* antileishmanial activity of the quinoline derivatives. The compounds Methyl 3β-(*N*-[(7-chloroquinolin-4-yl)amino]propylamynomethyl)-1*H*-1,2,3-triazol-1-yl)]7α-12α-dihydroxy-5β-cholane-24-oate **(1)**, Methyl 3β-(*N*-[(7-chloroquinolin-4-yl)amino]buthylaminomethyl)-1*H*-1,2,3-triazol-1-yl)]7α-12α-diidroxi-5β-cholane-24-oate **(2),** 6-chloro-*N*-(4-(di(prop-2-ynyl)amino)butyl)naphtalen-1-amine **(3)**, Platinum(II) Complex from *N*-(2-(di(prop-2-ynyl)amino)ethyl)-7-chloroquinolin-4-amine **(4)** and 6-chloro-*N*-(2-(prop-2-ynylamino)ethyl)naphtalen-1-amine **(5)** were tested against promastigote and amastigote forms of *L major.* The viability of promastigote forms and mammalian cells were determined by tetrazolium-dye (MTT) colorimetric method. The results in promastigotes were expressed as the concentration inhibiting parasite growth by 50 percent ($IC_{50}$) after 72 h of incubation period. For anti-amastigote activity, peritoneal macrophages were infected with promastigotes of *L.major* and treated with the compounds for 72 h. The survival index of amastigote proliferation was obtained multiplying the percentage of infected macrophages by the mean number of amastigote forms per infected cell. All compounds showed activity against promastigote forms of *L. major* ($IC_{50}$ of 32.10 µM, 25.60 µM, 1.80 µM, 19.70 µM and 20.60 µM for **1**, **2**, **3**, **4** and **5** compounds, respectively). Only compounds **3** and **4** showed a significant activity against intracellular amastigotes, with an inhibition of survival index of 78% and 95% at 100 µM respectively, compared to control. The compounds were not toxic to macrophages at the highest inhibitor concentration tested. The present results stimulate further investigations of this class of compounds for the rational design of new chemotherapy agents for leishmaniasis.
Supported by UFJF, CNPq and FAPEMIG.

## QT.42 - EFFECTS OF METHANOLIC EXTRACTS ON SEVERAL *LEISHMANIA* SPECIES

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Leishmaniasis represents an important public health problem with significant rates of morbidity and mortality. The chemotherapeutic agents used for its treatment exhibit high toxicity and drug resistance are frequently encountered. Plants extracts or plant-derived compounds represent an important alternative source of new antileishmanial agents. Therefore, was analyzed the activity of methanolic extracts against *L. amazonensis*, *L. chagasi* and *L. major*, as well as cytotoxic effects on mammalian cells. Methanolic extracts were obtained from of *Achillea millefolium*, *Casearia cf. silvestris*, *Piptocarpha cf. macropoda*, *Casearia sylvestris*, *Vernonanthura divaricata* and *Samanea tubulosa.* Anti-promastigote assay and cytotoxicity test were checked using the tetrazolium-dye (MTT) colorimetric method. The results of anti-promastigote activity are expressed as the concentration inhibiting parasite growth by 50 percent ($IC_{50}$) after 72h of incubation period. Among the extracts analyzed, *C. cf. silvestris* was active against *L. amazonensis*, *L. major* and *L. chagasi*, displaying $IC_{50}$ values of 3.3 µg/mL, 6.5 µg/mL and 6.5 µg/mL, respectively. *P. cf. macropoda* displayed activity against *L. amazonensis* and *L. major* ($IC_{50}$ of 6,9 and 7,0 µg/mL, respectively). *C. sylvestris*, *V. divaricata*, *A. millefolium* and *S. tubulosa* showed activity against only one *Leishmania* species (*L. amazonensis* with $IC_{50}$ of 38.1 µg/mL; *L. amazonensis* with $IC_{50}$ of 46.2, *L. major* with $IC_{50}$ of 29.6 µg/mL and *L. chagasi* with $IC_{50}$ of 12.4 µg/mL, respectively). *A. millefolium*, *S. tubulosa*, *C. sylvestris* and *V. divaricata* were not toxic to macrophages at the highest inhibitor concentration tested (111 µg/mL). *C. cf. silvestris* and *P. cf. macropoda* showed moderate cytotoxicity against mammalian cells, indicating poor selectivity. These results encourag us to continue the experiments in order to isolate and identify the bioactive compounds.
Supported by UFJF, CNPq and FAPEMIG.

## QT.43 - STUDY OF THE POTENTIAL RESISTANCE OF *Leishmania amazonensis* TO A THIOSEMICARBAZONE DERIVATIVE.

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The chemotherapy of leishmaniasis is based until now in drugs which are not totally efficient and present severe side effects and in some case are able to induce resistance to treatment. This resistance could be related to the volume of the drug (dose and frequency and the time of administration, among other factors. The mechanism of resistance have been associated to the increase expression of a transmembrane protein (Pgp), that act as a efflux pump for a wide spectrum of drugs and depends on energy (from ATP) and must be phosphorylated to be active. As part of our research program on chemotherapy against diseases caused by trypanosomatids we have been studied several thiosemicarbazones and semicarbazones derivatives, which have a medical interest because of their capacity of inhibit the growth of several pathogens. Studies concerning its biological activity show that these compounds are active against trypanosomatids, such as *T. cruzi, T. brucei* and *Leishmania sp.*. In the present work, it was used a thisemicarbazone [(3-methoxy-4-hydroxy-stiryl)-thisemicarbazone], that showed to be very active against *Leishmania amazonensis* promastigotes and Pentamidine as a reference drug. Parasites were grown in Schneider's medium, pH7.2, temperature of $26^0$C and resistance was induced in the presence of the compound for several passages in culture. During this process, it was evaluated the potential acquired resistance by new screening in each passage (new $LD_{50}$), besides the assay of infectivity of the parasites through complement lyses test (to detected metacyclic forms) and *in vitro* infection. The results showed that a significant increase in the $LD_{50}$ was observed at passage number 10 and the parasites were able to maintain its infectivity, even after several passages in culture.
Supported by: CNPq/FAPERJ/CAPES/FIOCRUZ-PDTIS/FIOCRUZ-IOC

## QT.44 - INHIBITION OF *Leishmania (Leishmania) amazonensis* ARGINASE BY CONSTITUENTS OF PLANT *Cecropia pachystachya*

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Arginase is a manganese metalloenzyme that catalyzes the hydrolysis of L-arginine to L-ornithine and urea. In *Leishmania*, the arginase is responsible for the production of ornithine, a precursor of polyamines required for the proliferation of the parasite. The plant *Cecropia pachystachya* is used in folk medicine to treat asthma and hypertension. The extracts of *C. pachystachya* can inhibit rat liver arginase and recombinant arginase from *L. (L.) amazonensis*. Previous characterization of the aqueous extract of leaves of *C. pachystachya* showed the presence of chlorogenic acid, (+)-catechin, (-)-epicatechin, isoquercitrin and isovitexin. These five molecules were tested against recombinant arginase from *Leishmania*. The inhibition assays were performed in 50 mM of substrate L-arginine pH 9.6 containing 20 µM of either inhibitor. Between the five inhibitors studied molecules, the highest percentage of inhibition was observed for chlorogenic acid and (-)-epicatechin, which showed 67% and 66% inhibition, respectively. The simulation with the program Dock 6.0 was performed for these five molecules and showed an interaction with the active site of arginase of *Leishmania* that differs from that obtained for the interaction of rat liver arginase. Based on our results, these molecules could be used as prototypes for the molecular development of a new drug against leishmaniasis.
Supported by FAPESP and CNPq.

## QT.45 - ANTILEISHMANIAL ACTIVITY OF THIOPURINE DERIVATIVES CONTAINING TRIAZOLE AND STEROID

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The term leishmaniasis refers to a wide variety of clinical syndromes caused by the *Leishmania* species. The primary chemotherapy of leishmaniasis has been based on the use of pentavalent antimonial drugs. Other medications, such as pentamidine, amphotericin B and paromycin are used as secondary options in resistant cases, despite their high toxicity to the host. However, these drugs are not orally active, requiring long-term parenteral administration and displaying serious side effects. Protozoan parasites are unable to synthesize purines *de novo* and this fact represents potential alternatives for drug design in the treatment of parasitic disease. We reported herein the *in vitro* antileishmanial activity and cytotoxicity on mammalian cells of 6-thiopurine derivatives containing 1,2,3-triazole and steroid substituents. All the compounds were assayed against promastigote forms of *L. amazonensis*, *L. major* and *L. braziliensis*. Antileishmanial activity and cytotoxicity on macrophages were determined using the tetrazolium-dye (MTT) colorimetric method. The results in promastigotes were expressed as the concentrations inhibiting parasite growth by 50 percent ($IC_{50}$) after a three days' incubation period. Among the five compounds tested only one, the 6-thiopurine/bile acid conjugates (6-(3'-colic esther)thiopurine), showed activity against promastigotes of *Leishmania* species. Interestingly, despite the biochemical differences existing between the parasite species, this compound showed activity against the three species of *Leishmania* tested ($IC_{50}$ of 22.8, 13.9 and 17.3 µM for *L. amazonensis, L. braziliensis* and *L. major*, respectively). None of compounds showed cytotoxicity against mammalian cells. These results confirm the antileishmanial activity of thiopurine derivatives and lead to new perspectives about the chemotherapy of this disease.

Supported by FAPEMIG, CAPES, UFJF and CNPq.

## QT.46 - EVALUATION OF THE ANTILEISHMANIAL ACTIVITY OF LIPOPHILIC AROMATIC AMINOALCOHOLS

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*Leishmania* and others protozoa belonging to the trypanosomatid family have distinct polyamine metabolisms compared to mammalian cells, opening the possibility of identifying new targets for antileishmanial drug development. Our purpose is to explore the leishmanicidal effect of thirteen aminoalcohols prepared by the reaction of aromatic halides and aromatic glycidyl ethers with aminoalcohols, aliphatic amides and aliphatic amines. These type of compounds, bearing a covalent bonded aliphatic chain attached to an aminoalcohol fragment, could interact with membrane lipids and be transported into the cytoplasm where they can possibly interfere with the lipid or polyamine transport or metabolism of the parasite. All compounds were assayed against promastigotes of *L. amazonensis, L. major* and *L. chagasi.* The viability of the promastigote forms was determined by the tetrazolium-dye (MTT) colorimetric method. The results are expressed as the concentration inhibiting the parasite growth by 50% ($IC_{50}$) after a 3-day incubation period with the compounds tested. Among the compounds assayed, five compounds displayed a good activity against *L. amazonensis*, seven compounds were active against *L. major,* and only two were active against *L. chagasi.* The results point to the importance of lipophilicity for antileishmanial activity: the two most active compounds were *N*-decyl aminoalcohol and N-dodecanoyl ethylenediamine ($IC_{50}$ of 0.7 µM and 5.2 µM for *L. major*, respectively). None of the less lipophilic compounds was active. These results confirm the antileishmanial activity of these lipophilic aromatic aminoalcohols and further studies will be done in amastigote forms model.

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## QT.47 - IMMUNOCHEMOTHERAPY IN BALB / C MICE INFECTED WITH *Leishmania (L.) amazonensis*

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The aim of the present study was to evaluate the efficacy of the treatment of BALB/c mice infected with *L. (L.) amazonensis* with a palladacycle complex, [Pd(N,N-dimethyl-1-phenethylamine-1,2-ethanebis(diphenylphosphine), DPPE 1.2, associated to the immunization with a recombinant cysteine proteinase from *Leishmania (Leishmania) chagasi*, rLdccys1. Fifteen days after infection with *L. (L.) amazonensis*, BALB/c mice received three doses of 50 µg rLdccys1 plus *Propionibacterium acnes* as adjuvant by subcutaneous route with a 7 days interval. Concomitantly, the animals received 120 µg DPPE 1.2 for 30 days. During the treatment the animal infection was evaluated by measuring the diameter of foot lesions and 10 days after end of the treatment the animals were sacrificed and the parasite burden was also evaluated by the limiting dilution method. A reduction of 99.6%, 99.1% and 97.6% was observed in animals treated with *P. acnes* + DPPE1.2, *P. acnes* + rLdccys1 + DPPE1.2 and DPPE 1.2 alone, respectively, compared to controls that received PBS. T $CD4^+$ and T $CD8^+$ lymphocytes also were analysed in popliteal lymph nodes by FACS during and in the end of the immunochemotherapy. After the second immunization there was a significant increase of both T cell populations in animals that received either DPPE 1.2, DPPE 1.2 plus *P. acnes* or DPPE 1.2 plus *P. acnes* +rLdccys1, indicating that the leishmanicidal activity of this palladacycle complex could be involved with activation of cellular immune responses. The effect of DPPE 1.2 on the activity of *L. (L.) amazonensis* cysteine proteinase was also studied and results demonstrated that the drug inhibited 75% of the cathepsin B activity of *L. (L.) amazonensis* amastigotes. Taken together, these results opened perspectives to evaluate the role of cellular immunity activation and inhibition of *Leishmania* cysteine proteinase activity in the leishmanicidal effect of DPPE 1.2.
Supported by FAPESP and CNPq.

## QT.48 - EVALUATION OF THE LEISHMANICIAL ACTIVITY OF A HYPERVALENT ORGANOTELLURIUM COMPOUND AGAINST *Leishmania (Leishmania) chagasi*

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Organotellurium compounds display several biological activities, such as antioxidant properties, antihelmintic and antibacterial activity. More recently, organotelluranes have been studied as irreversible cysteine proteinase inhibitors. Previous experiments performed in our laboratory showed the *in vitro* leishmanicidal effect of the organotellurane RF07 on *L. (L.) chagasi*, the etiological agent of American visceral leishmaniasis, encouraging us to test the *in vivo* leishmanicidal activity of RF07. Treatment of Golden hamsters infected with *L. (L.) chagasi* with RF07 at 300 µM resulted in a decrease of 75% of the spleen parasite burden. The possible inhibition of RF07 on the activity of *L. (L.) chagasi* cysteine proteinase was also analysed by a spectrofluorometry assay performed in the absence and presence of DTT, an agent that mimics the reducing environment found within the macrophage parasitophorous vacuoles. In the absence of DTT, RF07 showed a significant inhibition of proteolytic activity on substrates used for detection of all cathepsin-like cysteine proteinases (L, B, K, V and S), as well as for those specific for cathepsin B and cathepsins K, V and S. On the other hand, in the presence of DTT, RF07 significantly reduced the hydrolysis of the substrate used by all cathepsin-like cysteine proteinases, whereas it did not inhibit the enzyme activity on substrates specific for cathepsins B, K, V and S. These results indicated that RF07 inhibited the cathepsin L of *L. (L.) chagasi*. There is a body of evidence that shows *Leishmania* cathepsins B and L as virulence factors implicated in the amastigote survival within the parasitophorous vacuoles of the vertebrate hosts. Thus, our data showed the efficacy of RF07 for killing *L. (L.) chagasi* and suggest that its leishmanicidal mechanism could be through the inhibition of cathepsin L of *L. (L.) chagasi* amastigotes, opening perspectives to explore this hyphotesis in our model. Supported by FAPESP and CNPq.

## QT.49 - HIGH TARGETING OF ANTIMONY TO THE LIVER OF DOGS WITH VISCERAL LEISHMANIASIS FROM MEDIUM SIZED (400 NM) LIPOSOMES

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In the 1970s, a major advance occurred when it was found that liposome-encapsulated antimonial drugs were hundreds of times more effective than non-encapsulated drug for the treatment of visceral leishmaniasis (VL). Recent observation that reduction of liposome diameter from 1200 nm to 400 nm improved the targeting of antimony (Sb) to bone marrow of infected dogs suggested the small sized liposomes are more effective in promoting parasitic reduction. In this work, we investigated the influence of reduction in mean vesicle diameter from 400 to 200 nm on the pharmacokinetics of liposome-encapsulated Sb in dogs with VL. Two liposome formulations differing in their mean size were prepared. Formulation 1 (LP1) was obtained by the dehydration-rehydration, in the presence of cryoprotectant sucrose (diameter 410±75 nm, encapsulation 40±4%). Formulation 2 (LP2) was obtained by further extrusion of the liposomes through 200 nm-pore membrane (diameter 175±25 nm, encapsulation 34±3%). The formulations were applied as intravenous bolus injection at 4.2 mg Sb/Kg body weight (LP1) or 6.5 mg Sb/kg (LP2), plasma pharmacokinetics were evaluated and Sb were determined in liver, spleen and bone marrow after 24 h. LP1 exhibited a significantly shorter plasma half-life of Sb than LP2 (27±8 h vs. 127±24 h). Surprisingly, even though a higher dose of Sb (LP2), a lower level of Sb was found in the liver, and similar levels were found in the bone marrow and spleen. Our data suggests that saturation of the mononuclear phagocyte system (MPS) took place with LP2, because of higher lipid dose and total vesicle surface area, resulting in a reduced liver capture efficiency and slower plasma elimination of Sb. In conclusion, our data indicates that medium sized liposomes (400 nm) are more effective in the targeting of Sb to the infected sites of dogs with VL than small sized liposomes (200 nm).
Supported by CNPq and Fapemig.

## QT.50 - DETERMINATION OF *IN VITRO* ANTILEISHMANIAL ACTIVITY OF COPAIBA OIL FROM *Copaifera lucens*

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Leishmaniasis is a group of infectious diseases caused by different protozoa species of the genus *Leishmania*. Generally, the chosen drugs for these diseases are the pentavalent antimoninals, but this drug has high toxicity. Due to this context there is a great need to search new drugs for leishmaniasis treatment. This present study had the purpose to evaluate *in vitro* copaiba oil obtained from *Copaifera lucens* against *Leishmania amazonensis*. Materials and methods: It was evaluated the antiproliferative activity of copaiba oil against promastigotes and axenic amastigotes. The cytotoxic effect of copaiba oil against macrophages J774G8 cells was determined using the colorimetric sulforhodamine-B method. Results: Copaiba oil had significant activity both on promatigote and axenic amastigote forms with $IC_{50}$ values of 22.0 µg/mL and 4.0 µg/mL respectively. The cytotoxicity assay showed that copaiba oil obtained from *C. lucens* has low toxicity against macrophages J774G8 cells with $CC_{50}$ values of 40.0 µg/mL. Conclusion: The effect of copaiba oil obtained from *C. lucens* showed significant activity against *L. amazonensis* parasite.
Suported by: CNPq, FINEP, CAPES, and PRONEX/Fundação Araucária

## QT.51 - 5-HYDROXY-2-HYDROXYMETHYL-γ-PYRONE (HMP), OBTAINED FROM *ASPERGILLUS* FUNGI HAS ANTILEISHMANIAL ACTIVITY *IN VIVO*

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Parasites of genus *Leishmania* are transmitted by the sandflies and infect cells of the mononuclear phagocyte lineage of their vertebrate hosts. The chemotherapy is one of the most effective treatments for this disease. Although a number of antileishmanial drugs are available, these drugs are in general toxic, expensive and require long-term treatment. New drugs isolated from plants and microorganisms have shown leishmanicidal action. The 5-hydroxy-2-hydroxymethyl-γ-pyrone (HMP), produced by some species of *Aspergillus* fungi, has bacteriostatic activity and it effectively inhibits the formation of L-DOPA (3,4-dihydroxy-L-phenylalanine) from tyrosine in the process of melanin biosynthesis. Previous studies of our group showed that HMP was straightly involved with antileishmanial activity *in vitro* and could be useful as selective source for the new antileishmanial agent. However, *in vivo* antileishmanial activity of HMP and its effects are unknown. HMP ointment treatment was initiated after 5 weeks of infection. Control and vehicle groups were also done. Tissue samples were collected and analyzed for histopathological, collagen stain and transmission electron microscopy (MET) techniques. Topical treatment with HMP-ointment decreased the parasite burden observed at histopathological and MET analysis when compared with control group. Healing process observed, suppressing ulcer dissemination. In addition, many collagen fibers were disposal in infection site of HMP-treated animals and absence or few cellular infiltrated were observed. These results demonstrated that HMP effectively inhibits the growth of parasites in lesion sites and does not have cytotoxic effects on the host cells *in vitro*. Thus, HMP may have a great potential as antileishmanial agent. Supported by CAPES, CNPq/UFPa, CNPq/MCT/CT-INFRA/CT-PETRO (Processo nº 620179/2008), MCT/CNPq/FNDCT/CAPES/FAPERJ.

## QT.52 - DEVELOPING NEW TREATMENTS FOR LEISHMANIASIS

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Leishmaniasis is a worldwide problem affecting 12 million people, with 2 million new cases being reported each year, of which 1.5 million cases are of the cutaneous form of the disease. Drugs used currently for leishmaniasis treatment are administered parenterally, are associated with various side effects and do not result in sterile cure. More importantly, some strains of the parasite have started to develop resistance against treatment. Anti-cancer agents are emerging as an alternative treatment against parasites. Our lab previously tested several anticancer compounds against *Leishmania* and observed that one such drug, tamoxifen, when administered by the intraperitoneal route, exhibits antileishmanial activity *in vivo*. Our project is focused on developing and testing topical tamoxifen formulations to be used as adjuvants or sole agents in the treatment of cutaneous leishmaniasis. The infection of BALB/c mice with *L. amazonensis* at the basis of the tail was chosen as the experimental model. Topical administration of tamoxifen as an ethanolic solution for 2 weeks, starting 30 days after infection resulted in decrease of lesion size. Nanoemulsions (NE) containing tamoxifen were developed and characterized for size and entrapment efficiency (EE). High EE (101 ± 0,4 %) and low particle size (191,5 ± 2 nm) were obtained using a hot homogenization method. Sepigel was used to gel the tamoxifen-loaded NE to facilitate the topical application. The activity of these nanoemulsions, containing various concentrations of tamoxifen, is being evaluated in comparison with the ethanolic formulation. Based on the previously observed drug's activity against the parasite and on reports of tamoxifen as a modulator of wound healing, we expect these topical formulations to contribute to the resolution of leishmaniasis ulcers. Funding: PNPD/CAPES 02847/09-4; FAPESP; CNPq.

## QT.53 - ANTILEISHMANIAL ACTIVITY OF IMIDAZOLIDINE DERIVATES ON PROMASTIGOTE E AMASTIGOTE FORMS OF *LEISHMANIA* SPECIES

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Leishmaniasis is caused by parasites of the genus *Leishmania*, which causes illness ranging from skin lesions to systemic infections. Treatment relies on pentavalent antimonials, which have very toxic effects. So, the need to new efficient and safe drugs is urgent. Imidazolidines have shown biological properties, including leishmanicidal activity. In this work, some imidazolidine derivatives were assayed against promastigote and amastigote forms of *Leishmania*. Promastigote forms *of L. amazonensis* and *L. major* were used and the parasite viability was checked by MTT method. The results in the promastigotes were expressed in concentrations inhibiting parasite growth by 50 percent ($IC_{50}$) after three days incubation. For anti-amastigote activity, peritoneal macrophages infected with promastigotes of *L. amazonensis* were used. The antiparasitic effect of the compounds was evaluated by counting the intracellular amastigotes after 72 hours of treatment. The compounds showed a strong activity against *Leishmania* without cytotoxicity for macrophages. Among the compounds assayed, the ethylenediamine derivative 1,2-Bis(p-methoxybenzyl)ethylenodiamine (**4**), and the compounds 1,3-Bis(p-methoxybenzyl)imidazolidines (**5**), 2-(phenyl)-1,3-Bis(p-methoxybenzyl)imidazolidines (**6**), 2-(4'-metoxiphenyl)-1,3-Bis(p-methoxybenzyl)imidazolidines (**7**) and 2-(2'-hydroxyphenyl)-1,3-Bis(p-methoxybenzyl)imidazolidines (**11**) showed activity against *L. amazonensis* and *L. major* promastigotes ($IC_{50}$ values of 1.86 µg / mL and 1.77 µg / mL for the compound **4**, 4.66 µg / mL and 2.42 µg/mL for the compound **5**, 13.57 µg/mL and 4.05 µg/mL for the compound **6**, 9.04 µg/mL and 2.97 µg/mL for the compound **7,** and 12.47 µg/mL and 6.73 µg/mL for the compound **11**, respectively). The compounds **4** and **5** showed the best activity on intracellular amastigotes of *L. amazonensis*, with an $IC_{50}$ value of 2.0 µg/mL and 9.4 µg/mL, respectively. The leishmanicidal activity can be related with inhibition of polyamine synthesis and cellular penetration across biological membrane. These results suggest that these compounds have promising antileishmanial potential and may contribute to the development of new leishmanicidal agents. Supported by FAPEMIG, UFJF and CNPq.

## QT.54 - IN VITRO ACTIVITY OF AQUEOUS EXTRACT OBTAINED FROM ROOTS OF *PHYSALIS ANGULATA* ON *LEISHMANIA (L.) AMAZONENSIS* PROMASTIGOTES AND HOST CELL

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The leishmaniasis is an infectious disease caused by various species of the protozoan parasites in the genus *Leishmania*. The drugs of choice for the treatment of this disease are the pentavalent antimonials, which show high toxicity. Natural products from plants represent an important source of new antileishmanial compounds. Thus, we consider interesting to analyze an aqueous extract from roots of *Physalis angulata,* an annual herb widely used in popular medicine, against promastigotes of *L. amazonensis*. In the present study we showed that extract inhibited 83.5 % and 100% the promastigotes growth in the concentrations of 50 and 100 µg/ mL, respectively. In addition, ultrastructural analysis showed significant morphological changes in promastigotes. On treated promastigotes with 50 µg/mL of the extract were observed some vacuoles in flagellar pocket membrane and alterations in flagellar membrane. Treated promastigotes in the concentration of 100 µg/ mL showed morphological alterations such as myelin-like figures into the flagellar pocket, duplication of kinetoplast DNA, some vesicles inside the flagellar pocket and alterations on shape and swelling of kinetoplast. The tetrazolium-dye (MTT) colorimetric method and Mitochondrial Membrane Potential Detection Kit (JC-1) showed that this compound presented no cytotoxic effects against mammalian cells. These results demonstrated that aqueous extract of *Physalis angulata* effectively inhibits the growth of parasites and does not have cytotoxic effects on the host cells. Thus, this study revealed that extract from *Physalis angulata* has antileishmanial properties. Supported by PIBIC/CNPq/UFPA; MCT/CNPq/ICT (Grant number 16/2008).

## QT.55 - LEISHMANICIDAL ACTIVITY OF MARINE ALKALOID ANALOGUES

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Leishmaniasis is a zoonotic disease that is considered endemic in 88 tropical countries, which is caused by protozoa of the genus *Leishmania*. The World Health Organization estimates that approximately 12 million people are infected and the annual incidence of the disease is approximately 1.5 to 2 million. Currently the treatment of this disease is based on chemotherapy with antimonial derivatives, but these compounds show severe toxicity to patients and require intravenous administration, and even the appearance of cases of drug resistance. Thus, the necessity of discovering new compounds that present high leishmanicidal activity and low toxicity is clear. Results describing the anti-parasitic potential of compounds derived from marine natural products, led us to examine the leishmanicial activity and cytotoxicity on mammalian cells of some synthetic marine alkaloid 3-alkylpyridinium analogues. All the compounds were assayed against promastigote forms of *L. amazonensis* and *L. braziliensis*. Antileishmanial activity and cytotoxicity on macrophages were determined using the tetrazoliumdye (MTT) colorimetric method. The results in promastigotes were expressed as the concentrations inhibiting parasite growth by 50 percent ($IC_{50}$) after a three days' incubation period. In general, the alkaloid 3-alkylpyridinium analogues tested were more active against promastigotes of *L. amazonensis.* Among ten compounds tested, four compounds, 3-(3-(9-azidononacyloxy)propyl)pyridine (**6**) and its corresponding *N*-benzyl salt (**7**), *N*-benzyl salt of methyl *N*-[1,1-dimethylethoxy)carbonyl]-*N*-[9-[3-(3-pyridinyl)propoxy]nonyl]-alaninate (**8**) and the 3-pyridinepropanol Zincke´s salt (**9**), showed significant activity against promastigotes of this *Leishmania* species ($IC_{50}$ of 23.92 µM, 2.88 µM, 1.09 µM and 14.95 µM, respectively). The compounds showed moderate toxicity in mammalian cells. These results confirm the antileishmanial activity of these synthetic marine alkaloid analogues and further studies will be done in amastigote forms model. Supported by FAPEMIG, CNPq and UFJF.

## QT.56 - USE OF PLGA MICROPARTICLES FOR SINGLE-DOSE TREATMENT OF CUTANEOUS LEISHMANIASIS

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The conventional treatment of cutaneous leishmaniasis is based on multiple parenteral injections with antimonial or anfotericin B drugs. In this study, we propose to develop a localized single-dose treatment for this disease using poly-(lactide-co-glycolide) PLGA microparticles (mps) loaded with anfotericin B. 50:50 PLGA mps were prepared with 10 % (w/w) anfotericin B by the multiple emulsion method, followed by solvent evaporation, aqueous washing and dried. Morphological and physical parameters were recorded. Mice were infected in the ear with fluorescent *Leishmania amazonensis* promastigotes*,* and on day 26 they received a subcutaneous injection with 50 ug of anfotericin B: i) in the free form; ii) in PLGA mps; iii) in liposomal formulation (Ambisome®). Controls received empty mps or 10 ul of saline alone. The lesion sizes and parasite loads were measured with a dial calliper and fluorimetry, respectively. Toxicological parameters (AST, ALT and creatinin) were measured in the serum using commercial kits.The drug encapsulation ratio was 89.7% as measured by HPLC. Drug loading did not affect the zeta potential (~ -11 mV) and the size (~ 5.5 µm) of the microparticles. In vivo, despite the transient effect of Ambisome®, only the PLGA formulation controlled the lesion growth throughout infection. Free drug or empty microparticles were not effective. On day 70 of infection, the parasite loads were significantly smaller in the animals treated with drug-loaded PLGA mps but not with free drug. No changes in AST, ALT and creatinin were observed at the completion of the experiment.Loading of anfotericin B in PLGA microparticles may promote a sustained drug release in the lesion site leading to a durable and safe therapeutic effect. These findings support this new approach for single-dose localized treatment of cutaneous leishmaniasis.

## QT.57 - LEISHMANICIDAL ACTIVITY OF ESSENTIAL OILS FROM *Myrcia splendens* AND *Protium hebetatum*

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Leishmaniasis is a widespread tropical disease, with high prevalence around the world. According to WHO, about 4000 new cases are reported every year. The current treatments rely mainly on antimonials and amphotericin B that are unsatisfactory due to their toxic side effects, high costs, and increasing problems with drug resistance. Different approaches have been used to identify novel chemotherapics against Leishmania sp. parasites, and one strategy has been the analysis of naturally occurring plant-derived compounds. In this study we analyzed the effects of two essential oils, extracted from *Myrcia splendens*, identified on the experiments as oil 6 (O6) and *Protium hebetatum* identified as oil 8 (O8) on *Leishmania amazonensis* in vitro. Antileishmanial activity of O6 and O8 were evaluated in vitro on promastigotes culture as well as on amastigotes-infected macrophages. Besides that, we analysed citotoxicity of them for peritoneal macrophages's in vitro through XTT assay. Our results showed that such as O6 as O8 inhibited promastigotes growth in vitro in a dose-dependent manner. It was observed around 31,18%; 42,83 and 50% of promastigotes growth inhibition for 0,1µg/mL, 1µg/ml and 10µg/mL of O6, respectively and 27,25%; 47,67% and 57,44% for 0,1µg/mL, 1µg/ml and 10µg/mL of O8. Moreover, both essential oils (O6 and O8) were able to reduce amastigotes survival inside macrophages in a dose-dependent manner. At 10µg/mL O6 inhibited 42% of amastigotes survival, while O8 10µg/mL inhibited around 52%. At 10µg/mL, neither O6 nor O8 were cytotoxic to peritoneal macrophages as evaluated by the XTT test, after 24 hour of treatment. Furhther experiments will be performed in order to identify possible mechanisms of action of them. Anyway, our results were correlated with the essential oils chemical compositions and point both essential oils as efficient compounds against *L. amazonensis* and provide new perspectives for novel compounds for leishmaniasis treatment.
Supported by: FAPERJ, CNPq

## QT.58 - ANTILEISHMANIAL ACTIVITY OF THIOSEMICARBAZONE DERIVATIVE FROM LIMONENE COMPLEXED WITH COPPER

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Leishmaniasis is a neglected tropical parasitic disease resulting from infection of macrophages by intracellular parasites of genus *Leishmania sp*., which represents an important public health problem. The treatment for leishmaniasis depends on a limited number of drugs, and the basic treatment consists on the administration of the pentavalent antimonials derivatives. However, the use of this drug has been showed serious toxic effects. This way is very important to the development of efficient and safe new drugs for leishmaniasis treatment. Here we investigated the antileishmanial activity of the compound [Bis [N- 4 - [ *R*-1-metil-4-(1-metiletenil)-cicloexeno]-*o*-clorobenzaldeidotiossemicarbazonato)] derivative from limonene complexed with copper, denominated as TSZ against the protozoan *Leishmania amazonensis*. Effects of TSZ on parasites were evaluated on axenic and intracellular amastigote forms, and its cytotoxicity to $J774_{G8}$ murine macrophages. We also used scanning electron microscopy (SEM) to evaluate the effect of TSZ on the morphology of promastigotes, and flow cytometry using Rhodamine 123 as a fluorescent marker to evaluate mitochondrial membrane potential. TSZ showed activity against *L. amazonensis*, with $IC_{50}$ values of 7.25 ± 2.22 µg/ml and 8.15 ± 0.21 µg/ml for axenic and intracellular amastigote forms, respectively. TSZ showed cytotoxicity against machophages $J774_{G8}$ at $CC_{50}$ of 17.6 ± 4.21 µg/ml. The cytotoxicity of the compound to $J774_{G8}$ macrophages and its activity against the protozoa were compared using the selectivity index (SI) ratio ($CC_{50}$ for $J774_{G8}$ macrophages/$IC_{50}$ for protozoa). When observed by SEM, TSZ caused alterations dose-depedent in the shape and size of the parasites, included cellular disintegration. By flow cytometry was observed that Rhodamine 123 showed decreased fluorescence in parasites treated with TSZ, indicating a decreased in the mitochondrial membrane potential. These results could be followed up by *in vivo* testing. Acknowledgements: This study was supported by grants from CNPq, FINEP, CAPES and Fundação Araucária.

## QT.59 - STUDIES OF NOVEL 1,2,3 TRYAZOLE DERIVATIVES AGAINST *LEISHMANIA AMAZONENSIS*

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Leishmaniasis refers to various clinical syndromes caused by intracellular parasites from the genus Leishmania. This disease is an increasing public health problem in Brazil. Most of the antileishmanial drugs currently in use for treatment from the long time established antimonials to the recently introduced miltefosine have side effects or parasite resistance.The need of development of new drugs is necessary. The *in vitro* antileishmanial activity of a series the benzyl piridyl, furanyl ou tiofenyl-1-(phenylamine)-5-methyl-1*H*-1, 2,3-tryazol-4-carbohydrazide sustituted are evaluated against *Leishmania amazonensis* ((MHOM/BR/77LTB 0016 strain) promastigotes and intracellular amastigotes.forms. Promastigotes were evaluated by. counting the parasites in Neubauer´s chamber. Intracellular amastigotes activities were measured by microscopical counting of percentage of amastigote/macrophage.Most of nitro furanyl tryazole derivative exhibited good activity ($IC_{50}$=0.2-2.µM) against the promastigotes forms.In addition, the results showed that these furanyl tryazole derivatives were less active for amastigote ($IC_{50}$=15- 360µM) than for promastigotes. The references antileishmanial agents pentamidine showed active for both amastigotes and promastigotes forms ($IC_{50}$<2.0 µM). It can be suggested that others factors would be associated of this specific 1,2,3 tryazole structure. Further experiments are being carried out in order to define a mechanism of action besides a chemical structure and biological activity.
Supported by CNPq/PDTIS/PAPES/IOC-FIOCRUZ./ UFF

## QT.60 - ANTILEISHMANIAL ACTIVITY OF AERIAL PARTS FROM *Porophyllum ruderale* (Jacq.) *Cass*.

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Leishmaniasis is a group of parasitic diseases caused by different species of *Leishmania* which affects around 2 million people *per annum*. The available therapy still causes serious side effects. On northwest of State of Paraná, Brazil, water and alcohol-water extracts of *Porophyllum ruderale* (Jacq.) *Cass.* have been used popularly as treatment over lesions caused by *Leishmania* sp.. This study includes the extraction process, bioassay-guided fractionation by liquid chromatography method and the *in vitro* antileishmanial activity. The dichloromethane extract, fractions and subfractions of aerial parts from *P. ruderale* (Jacq.) *Cass.* were evaluated against promastigotes of *Leishmania amazonensis* and cytotoxicity against murine macrophages J774G8. Antileishmanial assay were performed in 24-well microplates and $IC_{50}$ (50% growth inhibitory activity) values was determined by direct count in a Neubauer chamber. The viability of the macrophages was determined by MTT method. Cytotoxic concentration of 50% viable cells ($CC_{50}$) was calculated by linear regression analysis. Dichloromethane extract, fraction 6 and subfractions 6.16 and 6.16.12 were the most actives against promastigotes of *L. amazonensis* with $IC_{50}$ values of 57±13.11, 19,50±6,36, 13.5±0.71 and 3.05±0.64 µg/mL, respectively. Cytotoxic concentrations ($CC_{50}$) for dichloromethane extract, fraction 6 and subfractions 6.16 and 6.16.12 were, respectively, 215±21.21, 39.5±7.78, 70±7.07 and 38±4.24 µg/mL. These results suggest that *P. ruderale* (Jacq.) *Cass.* has interesting antileishmanial activity and low cytotoxic effect against murine macrophages J774G8. Studies have been done to understand the mechanism of action of this plant and extracts.
Supported by UNIOESTE, CNPq, CAPES, FINEP and PRONEX/Fundação Araucária

## QT.61 - EFFICACY OF CHALCONE-CONTAINING ELASTIC LIPOSOMES FOR TOPICAL TREATMENT OF CUTANEOUS LEISHMANIASIS

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Previously, we reported that the introduction of a $NO_2$ group into the molecule of a natural chalcone (CH8) improved its efficacy when tested subcutaneously in *Leishmania amazonensis*-infected BALB/c mice, and rendered the drug more effective than Pentostam. Aiming at developing a CH8 formulation for topical application, the CH8 chalcone was encapsulated in conventional liposomes (CL) and in polyoxyethylene glycol (PEG)-functionalized liposomes (PL) for greater cutaneous permeation. BALB/c mice were infected in the ear with promastigotes of GFP-transfected *Leishmania amazonensis*. After 12 days, they were topically treated twice a day, during 30 days with the following formulations of CH8: a) CL, 6.6 μg CH8/ dose; b) PL 6.6 μg CH8/ dose; or lanete cream, 200μg CH8/dose. Controls received empty liposomes or lanete cream. Alternatively, intralesional treatments were carried out twice a week for the same period of time with CH8 in liposomal formulations or in PBS at 3.3 μg CH8/dose. Measurement of lesion size growth showed that on day 42 post infection, encapsulation in CL rendered the topically applied CH8 more effective than when presented in lanete cream in a dose 30-fold higher, comparable to intralesional CH8. The parasite burden in animals given topical CH8 in CL was 90% smaller than in untreated controls. Interestingly, all treatments with CH8 in CL were more effective than CH8 in PL. Permeation through polycarbonate membranes demonstrated the superior elasticity of the CH8 in CL in relation to PL, and this was confirmed in isolated pig skin by tape-stripping and Franz cell HPLC techniques. These results show that intrinsic physical-chemical properties of the antileishmanial CH8 confer enhanced elasticity and skin permeability to CL, simplifying the liposomal preparation process, and increasing topical drug efficacy against cutaneous leishmaniasis. Supported by FAPERJ and CNPQ.

## QT.62 - EFFICACY OF MILTEFOSINE IN THE TREATMENT OF MURINE MODEL OF CUTANEOUS LEISHMANIASIS BY *Leishmania amazonensis*

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Leishmaniasis is one of the most important neglected tropical disease caused by parasites of the *Leishmania* genus. In Brazil, *Leishmania amazonensis* is responsible for cutaneous and diffuse cutaneous leishmaniasis. The current chemotherapy for the treatment of leishmaniasis is based on Pentavalent Antimonials, Amphotericin B and Pentamidine. Recently, Miltefosine has been implemented as the first oral treatment for visceral leishmaniasis in India. However, when Miltefosine was tested in some species of the New World, the results were very unsatisfactory. Then, we decided to study the efficacy of Miltefosine in the treatment of BALB/c murine models infected with *Leishmania amazonensis*. BALB/c mice were inoculated with infective promastigotes of *L. amazonensis* through subcutaneous injections at the base of the tail. After the development of lesions, mice were divided into ten groups: control and vehicle groups, Miltefosine-treated groups (2,5; 5; 10; 20; 30; 40; 50 mg/kg/day), and Glucantime® group (50 mg/kg/day). Miltefosine was administrated by oral gavage, while Glucantime by intraperitoneal route, both treatments for 21 days. The efficacy of Miltefosine was evaluated by measuring the size of the lesions, and the presence of parasite in lesions stained with Giemsa. The result obtained suggested a dose-dependent response, where the size of the lesions decreases significantly with the increase of the Miltefosine doses. In a few days after the last doses, it was observed a gradual increase in the lesions size in mice treated with 5 and 10 mg/kg/day, while in the other groups, only one mouse developed a new lesion after three months of the end of treatment. Evaluation of the lesions stained with Giemsa confirmed a significant reduction of the parasite burden in the Miltefosine-treated groups. Thus, this study shows that miltefosine is effective against experimental cutaneous leishmaniasis caused by *L. amazonensis* in mice and suggests that further studies should be carried out in patients. Supported by CNPq, FAPERJ, and CAPES.

## QT.63 - EVALUATION OF LEISHMANICIDAL ACTIVITY OF *PENICILLIUM WAKSMANII* PRODUCTS

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Leishmaniasis is a neglected disease prioritized by WHO Program of Tropical Diseases (TDR). In the face of this public health problem, treatment and control alternatives are necessary, with development of more efficient and less toxic new drugs. In this study, we demonstrated the leishmanicidal activity of products from the *Penicillium waksmanii* fungus on *Leishmania amazonensis* promastigotes. Tests were performed incubating *L. amazonensis* promastigotes for 72 hours in the presence of 0-100 µg/mL of extracts of several polarities from culture supernatants of *Penicillium waksmanii*. The activity was colorimetrically evaluated by MTT (Thiazolyl Blue Tetrazolium Bromide) assay. ICs50 were calculated through logarithmic regression analysis. We observed that some extracts from supernatants of *Penicillium waksmanii* presented interesting antipromastigote activity, with emphasis on the extracts PW2 and PW7, both with IC50 around 25 µg/mL. These extracts will be purified and further analyzed on intracellular amastigotes to confirm their potential as new antileishmanial drugs. Supported by PAPES/PIBIC/CNPq.

## QT.64 - COMBINED FUROSEMIDE AND PENTOSTAM THERAPY AGAINST CUTANEOUS LEISHMANIASIS

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Intramuscularly administered pentavalent antimonials such as Glucantime® and Pentostam® are the first line therapy against cutaneous leishmaniasisdespite their toxicity and drug-resistance potential. In previous studies, we showed that specific inhibition of Na+-ATPase of *L. amazonensis* promastigotes by furosemide led to parasite killing (De Almeida-Amaral, E.E *et al*., 2008). After determining that the antileishmanial activity of furosemide is extended to the intracellular parasite forms, in this work we proposed to investigate whether furosemide is active in vivo in infected mice, and whether the drug acts synergistically with Pentostam aiming at reducing the antimonial toxic dose. BALB/c mice were infected in the ear with *L. amazonensis* promastigotes-GFP. After 7 days of infection, the animals were treated intraperitoneally with a total of 14 daily doses of 50 mg/kg of furosemide, 20 mg/Kg of Pentostam®, or a combination of furosemide plus Pentostam, in the same dose. Controls received PBS. The lesion sizes were measured throughout the infection with a dial calliper. In the end of the experiment, the animals were parasites were quantified both by fluorimetryand by Limiting Dilution Assay. The results indicated that furosemide is effective in vivo against *L. amazonensis* and that the combination therapy with Pentostam® further decreased lesion growth. Supported by CNPq.

De Almeida-Amaral, E.E; Caruso-Neves, C; Pires, V.M.P.; Meyer-Fernandes, J.R. (2008) Leishmania amazonensis: characterization of an ouabain-insensitive Na+-ATPase activity. *Experimental Parasitology,* 118(2):165-171.

## QT.65 - EFFECTS OF TOMATIDINE ON THE STEROLS METABOLISM OF *LEISHMANIA AMAZONENSIS* PROMASTIGOTES

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The leishmaniasis are clinically different infectious diseases caused by flagellated protozoan of the genus *Leishmania.* It is known to affect ~12 million people worldwide with approximately 2 million new infections per year. Some plants produce substances for their own defense against pathogens and predators. In *Lycopersicon* species, such as tomato *L. esculentum*, the main antimicrobial compound is the steroidal glycoalkaloid α-tomatine. The loss of saccharide side chain of tomatine produces the aglycone tomatidine. In the present study we shown that tomatidine inhibits the growth and promotes alterations in the ultrastructure of *Leishmania amazonensis*. Through transmission electron microscopy was shown that cells treated with tomatidine presented remarkable lesions, such as alterations in the mitochondrial structure and vacuolization. It was also observed a reduction in oxygen consumption and membrane potential in promastigotes treated. Cells exposed to tomatidine for 48 h presented a complete depletion in the level of endogenous 24-alkylated sterols, such as ergosta 5,7,22-trien-3β-ol (ergosterol) and ergosta 7,22-dien-3 β-ol. However, the treated cells accumulated 24-desalkyl sterols (4-methylcholesta-8,24-dien-3 β-ol and cholesta-8,24-dien-3 β-ol). These results are consistent with an inhibition of 24-sterol methyltranferase (24-SMT), an enzyme that methenylates the steroid at the 24 position during ergosterol and others 24-alkylated sterol biosynthesis. Currently, there is no medication that is both perfectly safe and completely efficacious against leishmaniasis. Tomatidine shows great potential as an antileishmanial agent and should be considered as new promissory drugs against leishmaniasis.
Supported by Capes, CNPq and Faperj.

## QT.66 - EFFECT OF *Kalanchoe pinnata* AND ITS FLAVONOID QUERCETIN AGAINST *Leishmania braziliensis in vitro* AND *in vivo*

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Previously, we showed therapeutic effect of the aqueos extract of *Kalanchoe pinnata* and its flanoid quercetin in mice infected with *Leishmania amazonensis*. In the present study, we investigate the effect of *K. pinnata* and quercetin on *L. braziliensis,* the most important dermatropic *Leishmania* species on Brazil. *In vitro* promastigotes *L. brazileinsis* (2,5 x $10^5$/ml) were cultivated with 0-100µg/ml of quercetin for 96h/28℃. Parasites were counted daily in a Neubauer chamber. Monolayers of peritoneal murine macrophages infected with *L. brazilieinsis* (at a ratio of 5 parasites/macrophage) for 4h at 37℃/5%$CO_2$ were incubated with *K. pinnata* aqueous extract or 0-100µg/ml of quercetin for 96h/28℃ for 48h/37℃/5%$CO_2$. Hamster were infected with $10^7$ promastigotes of *L. braziliensis* on the footpad. The experimental groups were treated after seven days of infection during eight weeks with 2mg of quercetin or 40mg of aqueous extract of *K. pinnata* by oral route for five time a week. Controls were non treated or treated animals with 8mg of glucantime five time a week by intraperitoneal injections. The lesion size was measures with dial caliper. Delayed-type hypersensitivity (DTH) against total antigen of *L. braziliensis* was evaluated in the ninth week of the infection. *K. pinnata* extract and quercetin not showed effect inhibitory against promastigote form, but inhibited intracelluar amastigote a dose-dependent fashion. *K pinnata* extract and quercetin inhibited 50% and 70% of intracelluar amastigote at 500µg/ml and 100µg/ml, respectively. *In vivo* quercetin was more active in controlling the growth of the lesion than the *K.pinnata* extract. The DTH was increased in all treated animals.These data demonstrate that the extract of *K. pinnata* and quercetin are active against *L. braziliensis* and may be important for antileishmanial drug development. Supported by Faperj.

## QT.67 - THE ANTI-LEISHMANIAL PTEROCARPANOQUINONE LQB118 TRIGGERS INDUCTION OF APOPTOSIS IN *Leishmania braziliensis*

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We previouly demonstrated therapeutical effect of LQB118 by intralesional and oral route on hamster infected with *Leishmania braziliensis*. The aim of this study was to evaluate the anti-leishmanial activity in *L. brazileinsis*. Promastigote forms were cultivated with LQB 118 at concentrations of 0-20 µM for 96h/28℃. Parasites were counted daily in a Neubauer chamber. Monolayers of peritoneal murine macrophages infected with *L. brazileinsis* (at a ratio of 5 parasites/macrophage) for 4h at 37℃/5%CO2 were incubated with the LQB118 at 0-20 µM for 48h/37℃/5%$CO_2$. The supernatants was collected and nitric oxide was determined by Griess reagent. After staining with Giemsa, the number of intracellular amastigotes was counted under a microscope. To assess whether the anti-leishmanial effect was due to apoptosis, promastigotes were treated with LQB118 at 20µM for 24-48h/28℃ and then incubated with double staining for annexin V-FITC and propidium iodide. *In situ* detection of DNA fragmentation following treatment of promastigote or intracellular amastigotes with LQB118 for 48h at 20µM was performed using the TUNEL Kit and analysed by fluorescence microscopy. The LQB118 showed a dose-dependent inhibitory effect in both promastigote and amastigote. On promastigote inhibited 100%, 78% and 30%, respectively, at 20, 10 and 5µM. Already on intracellular amastigotes the inhibitory effect was 80%, 60% and 10% at 20, 10 and 5µM, respectively. This effect not was acomppanied by increased of nitric oxide. LQB118 induces phosphatiylserine externalization in *L. braziliensis* promastigotes. The percentage of annexin V-FITC-positive cells increased to 9,06% at 24h and 21,78% at 48h. The negative control (untreated promastigotes) at 24 and 48h was 3.68 and 5.22%, respectively. LQB118 induced increase fluorescence in both promastigote and intracelular amastigote as compared to controls. These results demonstrate that the antileishmanial activity of LQB118 in promastigote and amastigote of *L. braziliensis* was mediated via apoptosis. Supported by FAPERJ.

## QT.68 - *TOXOPLASMA GONDII* CELL CYCLE INHIBITION BY A NEW PTEROCARPANQUINONA

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The protozoan parasite *Toxoplasma gondii* is capable of infect almost all warm-blooded and nucleated cells, being responsible for toxoplasmosis disease in humans. In this work, a new pterocarpanquinona, which has recently shown antitumoral activity, is tested in RH strain *T. gondii* crucial cell cycle stages. Invasion assay was performed infecting monolayers of LLC-MK2 cells in the presence of 2,5 or 5µM of the compound. After interaction time, samples were fixed and processed to optical microscopy. The same concentratios were tested during infection development, with samples fixation at 24 and 48 hours post-infection and processed to optical and electron microscopy. On the other hand, to evaluate egress step, calcium ionophore A238187 was used to trigger the evasion of *T. gondii* from host cells, an event of *T. gondii* life cycle that still poorly understood. While invasion and egress were inhibited under the new pterocarpanquinona treatment, by 49 and 33% respectively, no significant changes were observed when the compound was added after interaction, as verified by quantitative and structural assay of *T. gondii* infection development. Supported by CAPES and FAPERJ.

## QT.69 - EFFECT OF ITRACONAZOLE AGAINST TISSUE CYSTS OF *TOXOPLASMA GONDII IN VITRO AND IN VIVO*

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*Toxoplasma gondii* is an important opportunistic pathogen affecting immunocompromised patients with AIDS and other syndromes. The toxoplasmic encephalitis in those patients is responsible for great morbidity and mortality, this clinical presentation of the disease is commonly related to the reactivation of tissue cysts due to a former chronic infection. Although there are very effective drugs against the acute phase of toxoplasmosis, the chronic stage is untreatable and once an individual is infected it remains for the rest of its life. Thus the discovery of potential drugs that affect tissue cysts is very important. Previous studies of our group demonstrated the high susceptibility of *T. gondii* tachyzoites to itraconazole (ITZ) (Martins-Duarte, et. al. 2008). In this work we present data of the effect of ITZ against cell culture infected with encysted bradyzoites analyzed by transmission electron microscopy and the effect of the treatment in chronically infected mice. Monolayers of LLC-$MK_2$ were infected with low burden of parasites and after 2 weeks of infection were incubated with ITZ 2µM for 48h. Transmission electron microscopy analysis of encysted bradyzoites after treatment with ITZ showed many remarkable effects, as the presence of autophagic bodies observed in many bradyzoites. The presence of lysed parasites was also observed inside cysts after the treatment. Preliminary results have demonstrated that this drug might also be effective against chronic toxoplasmosis in murine models as the treatment with ITZ 10mg/kg reduced the numbers of cysts compared with the control group. Altogether, the results obtained up to now suggest that ITZ may have a direct effect against encysted bradyzoites of *T. gondii* and can be considered a potential drug against chronic phase of toxoplasmosis.
This work was supported by CNPq and FAPERJ.

## QT.70 - TREATMENT OF *Toxoplasma gondii* INTERACTIONS WITH LLCMK2 USING A NEW PTEROCARPANQUINONE

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*Toxoplasma gondii*, the agent of Toxoplasmosis, is an obligate intracellular protozoan able to infect a wide range of vertebrate cells including nonprofessional and professional phagocytes. Therefore, the drugs to control this parasite must have intracellular activity. The most usual therapy for Toxoplasmosis is the combination of sulfadiazine and pyrimethamine, although this treatment is associated with adverse reactions. Because of this, the development of new drugs is necessary. In previous studies, naphthoquinone derivatives showed different biological activities as anticancer, as agents capable of acting in groups of DNA, avoiding that it will double the cancer cells. These derivatives also display antiparasitic activity against *Plasmodium falciparum* and *Leishmania amazonsis*. The derivative that had its activity tested in this work was a synthetic structure analog of naphthoquinone. This work show the outcomes of the citotoxicity test with this derivative during *T. gondii* interaction with nonprofessional phagocytes. For this, LLCMK2 were cultured with RPMi 1640 supplemented 10% Fetal Bovine Serum. Before interactions, cells were cultured in 24 well plates. The interaction was realized in the presence or absence of pterocarpanquinone. The compound was able to inhibit intracellular parasite prolifertation with an IC50 of 2,5 µM. Scanning and Transmission Electron Microscopy analysis showed that the concentrations wich damage the parasite did not afect the host cells. Alterations included damage of parasite membranes. The derivative was also capable of to decrease the infection index during interaction with LLCMK2. These results suggest that naphthoquinones are compounds potentially important for the killing of *Toxoplasma gondii*. Supported by: CNPq, FAPERJ and PRONEX.

## QT.71 - EFFECT OF A FUCOSYLATED CHONDROINTIN SULFATE ON CYTHOADHESION OF *Plasmodium falciparum*-INFECTED ERYTROCYTES TO ENDOTHELIAL CELLS

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Severe malaria is characterized by the sequestration of *Plasmodium falciparum*–infected erythrocytes (IEs) in the microvasculature of vital organs. Adhesion of IEs to endothelial cells has a key role in the pathogenesis of life-threatening malaria and could be targeted by anti-adhesion therapy. Therefore, sulfated polysaccharides, such as heparin, chondroitin-4-sulfate (CSA), dextran sulfate, have been tested to prevent severe malaria due to their ability in inhibiting cytoadherence of IEs to host receptors to different extents. However, their uses have been discouraged; since heparin treatment leads to serious side effects, such as intracranial bleedings; and CSA and dextran sulfate, which are extracted from mammals, present a potential risk of carrying harmful contaminants to humans. Indeed, although several compounds have been tested in order to prevent malaria severe forms, none has demonstrated unequivocal evidence in the amelioration of severe malaria outcomes in clinical trials. Here, we showed that fucosylated chondroitin sulfate (FucCS), a highly sulfated polysaccharide isolated from sea cucumber, *Ludwigothurea grisea,* composed of a chondroitin sulfate backbone substituted at the 3-position of the β-D-glucuronic acid residues with 2,4-disulfated α-L-fucopyranosyl branches, is a potent inhibitor of the IEs cytoadhesion to human lung endothelial cells (HLEC) and blocks *P. falciparum* merozoites re-invasion. Inhibition seems to be nonspecific of parasite adhesive phenotype and occurs in a concentration-dependent-manner. Interesting, removal of the sulfated fucose branches on the FucCS practically abolished the inhibitory effect, suggesting a pivotal role played by this compound electrical charge. Furthermore, treatment with FucCS at 1 mg/kg/animal/day showed to improve survival of C57BL/6 mice infected with *Plasmodium berghei* ANKA, an experimental model for cerebral malaria and characterized by a potent inflammatory process. Of note, treated mice did not exhibit visible side effects during therapy. Thus, we propose FucCS as a promising candidate for adjunct therapy to prevent severe malaria outcomes. Supported by FAPESP and Instituto Nacional de Tecnologia em Vacinas (CNPq-INCTV) and CNPq - Doenças Negligenciadas.

## QT.72 - ANTIMALARIAL ACTIVITY OF PRIMAQUINE DERIVATIVES AGAINST *PLASMODIUM FALCIPARUM IN VITRO*

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Adequate therapeutic control of human malaria infections with *Plasmodium vivax* includes the use of the anti-relapsing drug primaquine (8-amino-quinoline) together with chloroquine. Primaquine leads to radical cure, causing complete elimination of the exoerythrocytic liver stages, the hypnozoites, responsible for such relapses. Primaquine however is toxic, causing multiple side effects, thus needs to be replaced. In this work, a series of primaquine derivatives thiazolidinones were obtained by chemistry synthesis using the primaquine as precursor. These thiazolidinones were synthesized by one-pot reaction of primaquine, arenealdehydes and mercaptoacetic acid after purification by column chromatography using silica gel and hexane ethyl acetate as solvent. The activity of the compounds was first evaluated against blood forms of *P. falciparum* (cloroquine-resistant clone – W2), maintained in continuous cultures. Two methods were used to evaluate parasite survival: i) incorporation of tritiated hypoxanthine by the live parasites; ii) enzyme-linked immunosorbent assays (ELISA) which provide very sensitive in quantifying the histidine-rich protein 2 (HRP2) produced during parasite development. From the 13 new compounds tested, six were active ($IC_{50}$ <3ug/ml) in both tests; the others were inactive ($IC_{50}$ $\geq$50ug/ml. The cytotoxicity of the active compounds was evaluated against hepatoma cells (HepG2) in cultures, through MTT. All compounds had low toxicity, thus demonstrating high selectivity index (SI=50 to 200), therefore are potential new antimalarials. Primaquine, less active against the blood forms, was more toxic then all derivatives in vitro (SI= <7ug/ml). These compounds are now tested to evaluate whether they will inhibit sporogony of *P. gallinaceum* in *Aedes fluviatilis*. In this model, primaquine inhibits 100% of the sporogony in mosquitos fed in infected chicken treated once with 15mg/ml (Carvalho et al. 1992). Those new antimalarial aiming to replace primaquine, if identified may be useful, especially because these compounds are also active against *P. falciparum*. Supported by CNPq and FAPEMIG.

## QT.73 - ANTIMALARIAL ACTIVITY OF BIOPRODUCTS FROM *Aspidosperma* sp PLANTS TESTED IN BLOOD CULTURES OF *Plasmodium falciparum*

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Antimalarials currently used as first-line treatments of human malaria, quinine and artesunate, are originated from medicinal plants, *Cinchona* sp and *Artemisia annua*, respectively. Over 1,000 plant species are used in folk medicine against fever in the world, reinforcing the importance of searching new chemotherapeutic agents from ethnopharmacology. Our malaria group has tested about 60 different species of medicinal plants used in the Amazon and other regions, and described antimalarial activity in 50% of them (Krettli *et al.,*Current Drug Targets*,* 2009*).* Now, we tested crude extracts and fractions of *Aspidosperma* sp plants collected in Alagoas state which was characterized phytochemically by one of us (AEGS). This gender is popularly used in Brazil to treat several diseases including malaria, and it is rich in indole alkaloids. Three species were tested, referred here as APM, APT and APP, to preserve future registration as phytotherapics or patenting. The extracts were obtained from plants stems, leaves, roots, wood barks and tested against blood stages of *Plasmodium falciparum* maintained in continuous cultures, following standard protocols. The parasites growth inhibition rates were evaluated by light microscopy or through incorporation of tritiated hypoxanthine, after 42h of parasite incubation with sample and controls (standard antimalarial). Most crude extracts of *Aspidosperma* sp were active at low dose ($IC_{50}$ ≤ 11μg/ml); APM leaves and APT stem barks were partially active ($IC_{50}$ 22 and 19μg/ml). The APP wood bark extract, the most active ($IC_{50}$ 4μg/ml), was further fractioned in ethyl acetate and aqueous fractions, both being active at low concentrations ($IC_{50}$ 3μg/ml and 7μg/ml). Cytotoxicity tests against HepG2 cells showed that all extracts had low toxicicity, thus high selectivity index (SI = 45 to 294μg/ml). These plants provided potential bioproducts for development of new antimalarials, to be further characterized and tested against *P. berghei* parasites in mice.

Supported by FAPEMIG, CNPQ (Project 575746/2008-4).

## QT.74 - ANTIMALARIAL ACTIVITY OF *Kielmeyera variabilis* AGAINST *Plasmodium falciparum* BLOOD PARASITES *IN VITRO*

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About 80% of the populations in developing countries use medicinal plants to treat diseases. The interest in alternative therapies with natural products has been growing. The Brazilian flora has huge pharmacological potential for the discovery of new drugs due to its vast biodiversity. Thus, the screening of compounds obtained from plants through ethnopharmacology is an important strategy aiming new pharmaceutical compounds. *Kielmeyera variabilis* (Clusiaceae) known as “malva-do-campo”, is traditionally used as folk medicine in Brazil to treat several diseases (Alves, TMA. *et al*., 2000, Memorias IOC.). Previous phytochemical studies showed xanthones as the major plant constituent. Such substances are considered as antifungal, antitumoral, antibacterial, tuberculostatic and anti-inflammatory (Pinheiro, L. *et al*., 2003. Memorias IOC.). We now tested 25 plant samples (extracts, fractions, purified substances) from *K. variabilis* against blood forms of *P. falciparum* and used distinct methods for evaluation of antimalarial activity: (a) incorporation of tritiated hypoxanthine, semi-automated; (b) traditional test determining parasitemia through optical microscopy; (c) colorimetric assays with monoclonal antibodies anti-histidine rich protein (HRP-2). Among the extracts of *K. variabilis* tested, ethanolic extract of leaves (EFK) was active ($IC_{50}$ ≤ 10μg/ml); the ethanolic branch extract (EGK) was partially active ($IC_{50}$ ~ 25μg/ml). The active fractions derived from EFK and EGK were EAFK, EHGK and EAGK ($IC_{50}$ 5 to 8μg/ml), whereas other five fractions were inactive ($IC_{50}$ > 25μg/ml). Some subfractions (F1 and L2) and a pure substance (P6→ kielcorin) obtained from the ethyl acetate branch fraction (EAGK) were active, in a preliminary experiment; the subfraction EP5.6 and a pure substance (P3, 5-hydroxy-1,3-dimethoxyxanthone) were partially active. The active fractions will be prepared in larger amounts to be evaluated in mice infected with *P. berghei*. The plant cytotoxicity tests against HepG2 cells in vitro are now undertaken.

Supported by FAPEMIG, CNPQ (PRONEX project 555675/2009-2), PAPES V-FIOCRUZ.

## QT.75 - EVALUATION OF NATURAL PRODUCTS USED TO PREVENT MALARIA IN THE AMAZON REGION IN *PLASMODIUM FALCIPARUM* CULTURE

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Over recent years the incidence of malaria has soared to approximately 500 million clinical cases per year. Chloroquine was the first choice antimalarial drug for more than three decades until the emergence of chloroquine resistant *Plasmodium falciparum* strains rendered its application ineffective in many parts of the world. Furthermore, the main drugs developed for malaria and used up to now, quina alkaloids derived drugs and artemisinin were discovered based on traditional use and ethnopharmacological data. In this context, new efforts to search for novel drugs for treating malaria based on popular knowledge are very important. In our studies were evaluated the *in vitro* susceptibility of *P. falciparum* (chloroquine–sensitive and chloroquine-resistant strains) to twenty nine fractions/extracts from species of Euphorbiaceae, Clusiaceae and Rhamnaceae families. The antimalarial activity was assessed by microtiter plate based on SYBR-Green-I assay. The study of cytotoxicity of these fractions/extracts was performed against HEPG2 cell line using the MTT assay. About 30% of the all tested fractions/extracts showed $IC_{50/48h} \leq 2.0\mu g/mL$ with acceptable selectivity index suggesting that they were more specific in their action against the malaria parasite. Based on these results, we can conclude that these extracts/fractions may be interesting as leads for the development of new antimalarials agents.

Supported by CAPES-GRICES, FAPEAM (PIPT project), IFS (project number AF/18880) and OPCW.

## QT.76 - INHIBITORS OF *PLASMODIUM FALCIPARUM* LACTATE DEHYDROGENASE AS NEW ANTIMALARIAL DRUG CANDIDATES

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Chloroquine, a drug widely used to treat malaria, is believed to interact with *Plasmodium falciparum* lactate dehydrogenase (*Pf*LDH) – an essential enzyme for parasite survival - as part of the mechanism of drug activity. Results from the literature suggest that the amino acid sequence of *Pf*LDH has residues at the active site that are unique to *Pf*LDH, as compared to the human LDH. Therefore, *Pf*LDH is an attractive target for development of new antimalarials. In our present work the docking energies for inhibition of *Pf*LDH were calculated and compared to drugs described in the literature as useful to treat other protozoan infections (unpublished data). Among 50 drugs studied, six generated stable energy conformations at positions coincident with the binding site of NADH cofactor of *Pf*LDH. Next, we tested whether some of these compounds, i.e. Atorvastatin, Itraconazole and Posaconazole, were able to cause *Pf*LDH inhibition acting as potential antimalarials. *P. falciparum* chloroquine-resistant parasites (W2 clone), maintained in continuous erythrocytes cultures, were incubated with various concentrations of those drugs and controls, following standard protocols previously described, with little modification (Krettli, 2009, Expert Opinion Drug Discovery). The inhibition of parasites growth was evaluated through parasite survival curves and $IC_{50}$ values (50% growth inhibition). The ELISA DELI (*double-site enzyme-linked immunodetection LDH assay*) was used to quantify *Pf*LDH, in parallel with ELISA HRP2 (*histidine rich protein II*) tests. Atorvastatin, Itraconazole and Posaconazole were active ($IC_{50}$= 5.9μg/ml, 6.7μg/ml and 1.8μg/ml, respectively) in DELI test; and ($IC_{50}$= 7,7μg/ml, 6.5μg/ml and 3,7μg/ml, respectively) in HRP2 test. Posaconazole was somewhat more active. Itraconazole was also tested in *P. berghei* infected mice (four doses, oral or subcutaneously) being inactive. The reasons for the discrepancy between *in vitro* and *in vivo* tests are being investigated as well as interactions between these drugs and other antimalarials. Supported by CNPq, FAPEMIG and PAPES V-FIOCRUZ.

## QT.77 - EVALUATION *IN VIVO* AND *IN VITRO* ANTIMALARIAL ACTIVITY OF CRUDE EXTRACT FROM *Caesalpinia pluviosa* AND ITS DERIVATES

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Malaria is a disease caused by *Plasmodium spp* protozoan that is transmitted by *Anopheles sp*p vector insect. Present in tropical and subtropical areas, this illness is responsible for 1-2 millions deaths and among 300 to 500 million cases reported annually. Quinine and artemisinin are the mainly antimalarials drugs and both are compounds obtained from plants, thus demonstrating the importance of discovering new plant based compounds. However, the increasing resistance of parasites to conventional antimalarial has been observed worldwide. In this sense, *Caesalpinia pluviosa* tree (Sibipiruna) crude extract was purified and separated in seven fractions. All fractions were tested *in vitro* against *P. falciparum*-infected erythrocytes strains, resistant or sensitive to chloroquine, and their toxicity in MCF-7 cells was also determined. The fraction obtained from ethanol 100% solvent exhibited the highest antimalarial activity, and then it was chosen for in vivo tests in *P. chabaudi*-infected mice. When administrated at a concentration of 50 mg/kg/day/animal, this fraction was capable of reducing mice parasitemia up to 91%. Doses superior of 75 mg/kg/day/animal were toxicity to infected animals. Mass spectrometry (ESI-MS/MS) analysis suggested the presence of quercetin on active fraction. Thus, opening perspectives to isolate and/or synthesize this compound and evaluate its specific antimalarial activity.
Supported by FAPESP, Instituto Nacional de Tecnologia em Vacinas (CNPq-INCTV) and CNPq-Doenças Negligenciadas.

## QT.78 - EFFECT OF CARBON NANOTUBES, FREE OR ASSOCIATED (FUNCTIONALIZED) TO ANTIMALARIALS, AGAINST *P. falciparum*-INFECTED ERYTHROCYTES

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Globally malaria endangers 2.4 billion people, especially those exposed to infection by *Plasmodium falciparum* and *P. vivax*, which accounted for approximately 100-300 and 70-80 million of annual cases respectively. The strategies being used to combat malaria in the world have not been very effective mainly due to the emergence of parasites strains resistant to conventionally used drugs such as chloroquine. Given the impossibility of malaria control with the current strategies, new formulations capable of fighting parasite infection need to be expanded and their mechanism of action evaluated. In this sense, the use of nanoparticles (NP), structures smaller than 100 nm, are a promising alternative for drug-delivery, in which drug supply is directly delivery to target cells, then reducing dose and side effects. Also, due to its small size NP can overcome anatomical barriers, such as the blood-brain barrier or skin. In fact, carbon nanoparticles or carbon nanotubes (CNT) free or associated with drugs or proteins are able to interact with different molecules or antigens. CNT can, via endocytosis, enter into the cells, therefore modifying development and cell function specifically. Based on these factors, we intend to increase efficiency and reduce the toxicity of conventional antimalarial compounds, such as chloroquine, or experimental, such as violacein, through association (functionalization) to carbon nanotubes (CNT). Preliminary data revealed a synergistic effect of carbon nanotubes functionalized with violacein against *P. falciparum*-infected erythrocytes. The functionalization of carbon nanotubes with violacein increased significantly the effect of this compound. In contrast, when these free structures were added concomitantly and separately the effect does not be observed; thus indicating that functionalization of CNT with violacein generates a third structure with a potent antimalarial activity. Collectively, these data open promising perspectives to use CNT to improve antimalarial activity and, in turn, reducing side effects of experimental and conventional drugs.
Supported by FAPESP, Instituto Nacional de Tecnologia em Vacinas (CNPq-INCTV) and CNPq-Doenças Negligenciadas.

## QT.79 - EFFECTS OF AMINOQUINOLINE COMPOUNDS ON *PLASMODIUM BERGHEI*

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**Introduction:** Malaria consists in one of the world's biggest public health problems nowadays, causing over 500 million clinical cases and 1–3 million deaths per year. Although chemotherapy has been the pillar of malaria control, resistance to both chloroquine and alternative drugs has been reported. Thus, there is urgent need for research and development of new antimalarial agents. Quinoline-containing antimalarials have long been used to combat this disease. Their synthesis is easy, cheap and they compose a very versatile group of compounds considering effectiveness. Their action is based on interfering with the parasite heme detoxification process, what is critical for its survival. **Material and methods:** In order to evaluate antimalarial activity, three aminoquinoline derivates were obtained by means of organic synthesis and tested *in vivo* in a murine model using the 4-day suppressive test at 25mg/Kg each. Giemsa stained blood smears were made on days 5, 7 and 9 after inoculation. The compounds are named as follows: N-(2-(di(prop-2-inyl)amine)ethyl)-7-chloroquinolin-4-amine **(1)**, N-(3-(di(prop-2-inyl)amine)propyl)-7-chloroquinolin-4-amine **(2)**, 7-chloro-N-(4-(di(prop-2-inyl)amine)butyl)quinolin-4-amine **(3)**. **Results**: The results are expressed as the inhibition of parasite multiplication percentage. On day 5, it was 64, 68 and 65% for **(1)**, **(2)** and **(3)**, respectively. On day 7: 52, 59 and 13% for **(1)**, **(2)** and **(3)**, respectively. On day 9: 73, 83 and 60% for **(1)**, **(2)** and **(3)**, respectively. Control treated group (chloroquine) exhibited no parasitemia on those days. **Conclusion**: These compounds represent potential sources for new antimalarial agents and may therefore be objects of further research.
Supported by UFJF, FAPEMIG and CNPq.

## QT.80 - EVALUATION OF ANTIMALARIAL ACTIVITY OF DERIVATIVES COMPOUNDS OF CHLOROQUINE.

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Since 1960, several studies have shown the high resistance of *Plasmodium falciparum* to chloroquine and other aminoquinolines used as traditional antimalarials, making the search for new drugs urgent. The aim of this study was to evaluate the *in vitro* antimalarial activity of chloroquine derivatives, a drug of low cost, easy synthesis and low toxicity. Seven chloroquine derivatives compounds (DETA and DMA derivatives) were tested against *P. falciparum* blood forms maintained in continuous cultures, following standard protocols. Enzymatic immunoassay with monoclonal anti-HRP2 (histidine rich protein II) which quantifies a histidine and alanine rich protein was used to evaluate parasite growth. The toxicity of the derivatives was evaluated using the MTT colorimetric assay in two cell lines, HepG2 and BGM. All substances tested were very effective, with $IC_{50}$ values (drug concentration that inhibits 50% of parasites growth) varying from 0,125 ug/ml to 1,66 ug/ml, for DETA and DMA derivatives, respectively. They displayed low toxicity thus, especially the DETA derivatives with the highest selective indices (SI= 189 to 7300). The DMA derivates were less active (SI from 11 to 100). Chloroquine tested in parallel displayed SI=555, thus being less active then DETA derivatives. The DETA molecules seem represent promising alternatives for the treatment of chloroquine-resistant malaria but further studies about the efficacy in vivo need to be undertaken before their use against human malaria. Experiments are in progress in mice as well as *in vitro* test using Hypoxantine test.
Financial Support: CNPq and FAPEMIG.

## QT.81 - EVALUATION OF ANTILEISHMANIAL ACTIVITY OF NIRANTHIN, A COMPOUND ISOLATED FROM *Phyllanthus amarus*

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Infections by protozoan of the genus *Leishmania* are the major world wide health problem, with high endemicity in developing countries. The drugs of choice for the treatment of leishmaniasis are the pentavalent antimonials, which exert renal and cardiac toxicity. Thus, there is a strong need for safer and more effective treatments against leishmaniasis. Genus *Phyllanthus* has been investigated to determine the constituents with pharmacological activities that frequently are attributed to lignans, glycosides, flavonoids, alkaloids, ellagitannins and phenylpropanoids. Pharmacological evaluation showed that this plant possesses anti-inflammatory, antimicrobial, antimutagenic, anticarcinogenic, and antiviral activity. The present study was designated to evaluate the leishmanicidal activity of a lignan (Niranthin) obtained from *Phyllanthus amarus.* Proliferative forms of *Leishmania amazonensis* were treated with several concentrations of the lignans and the parasite growth was determined by counting. The cytotoxic activity macrophage lines was evaluated by sulphorodamine B technique and morphological and ultrastructural alteration was analyzed by electronic microscopy. The niranthin presented a good activity against promastigote, axenic amastigote and intracellular amastigote forms with $IC_{50}$ of 8.5, 2.7 and 8 µg/mL, respectively. The toxicity for cells and the activity against the parasites were compared by using the selectivity index (SI) ratio ($CC_{50}$ for cells/$IC_{50}$ for parasite). Nirantin showed to be more toxic to parasites than to mammalian cells with SI of 46.7 for *L. amazonensi,* moreover niranthin has dose-dependent effect decreasing the infection in macrophages of 81.5% in control for 31.5% in treated cells with 40 µg/mL. In addition, niranthin showed important morphological and ultrastructural alterations, such as swelling of the body, intense exocytic activity in the region of the flagellar pocket, myelin-like figures, and vacuoles in the cytoplasm as compared to control cells. The nirantin showed good activity against the parasite and represents an exciting advance in the search for new antileishmanial agents. **Acknowledgements:** This study was supported through grants from CNPq, FINEP, CAPES, PRONEX/Fundação Araucaria, and FAPESP.

## QT.82 - SYNTHESIS AND ANTILEISHMANIAL ACTIVITY OF NOVEL 8-HALOGENS QUINOLINES DERIVATIVES

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Leishmaniasis is a protozoan disease that affects about 12 million people in the world, particularly in subtropical and tropical regions, causing a serious public health problem. Current chemotherapy of leishmaniasis is unsatisfactory. Efficacious and safe new drugs are needed. In the present work, the antileishmanial efficacy of novel 4-(phenylamino)-3-cyano or 3-(dihydro-1H-imidazol) 8-chloroquinolines and 8-fluoroquinolines was determined against *Leishmania amazonensis*. The quinoline rings structure is already established as a template for antiparasitic drugs; this is exemplified by the drug Sitamaquine (8-aminoquinoline derivative), which is currently undergoing clinical trials for its effectiveness in treating visceral leishmaniasis. The quinolines compounds were obtained, in good yields and all the substances were fully characterized by usual methods (IR, $^{1}H$, $^{13}C$ NMR). The antileishmanial efficacy of eight ciano-8-halogens (Cl,F)-quinolines and derivatives was determined *in vitro* against *L. amazonensis* promastigotes. Parasites were cultured with and without the drugs in Schneider´s medium at 25°C, using Pentamidine as the standard drug. After 24 hours incubation, parasite viability was determined using the MTT( tetrazolium blue) assay. The results showed that all quinoline derivatives assayed were very potent in inhbiting promastigotes forms of *L. amazonensis.* This study reinforces that the quinoline ring structure are potential antileishmanial lead compounds for the design and synthesis of similar heterocycle derivatives.
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## QT.83 - ANTIMALARIAL TESTS WITH HERBAL MEDICINES COMERCIALLY AVAILABLE SPECIALLY THOSE CONTAINING FLAVONOIDS

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The malaria control with specific drug treatment remains a major strategy to reduce morbidity and mortality caused by the disease. However, it is limited by *Plasmodium falciparum* multi-drug resistant parasites, a barrier to disease control. The discovery and development of new antimalarials, an urgent need at present, is complex and require large investments. We focus our research on the study of marketed herbal drugs, especially those containing flavonoid compounds, since they are related to the antiplasmodial activity of plants like *Bidens pilosa* (Andrade-Neto, Brandão *et al*., 2004). Accuvit, Daflon, Ginkgo, Soyfit and some standard flavonoids commercially available (Hesperidin, Genistein, Quercetin) were tested against blood forms of *P. falciparum* (clone W2, chloroquine-resistant) in parallel with chloroquine, a reference antimalarial. To quantify parasite growth, the enzyme linked immuno sorbet asay (ELISA) was performed using monoclonal antibodies to HRP2 (*histidine rich protein II*). *Pf*HRP2 is present in several cellular compartments and expressed by both knob-positive and knob-negative in infected erythrocytes. Through curves of parasite growth inhibition and $IC_{50}$ values, we observed activity of Accuvit, Soyfit and Quercetin ($IC_{50}$= 4,9μg/ml, 11,7μg/ml and 17,2μg/ml, respectively). Surprisingly, Gingko, Daflon, Hesperidin and Genistein, referenced flavonoids, were inactive ($IC_{50}$ > 30μg/ml). The activity of Accuvit and Soyfit was also tested in mice infected with *P. berghei,* the results are under analysis. Since Accuvit and Soyfit are active *in vitro* against the human malaria parasite, they might be useful for human malaria treatment in association with other antimalarials. We concluded that the activity of the tested medicines is not related exclusively to flavonoids, thus, possible synergisms between flavonoids and other compounds present in the medicines tested, the case of the multivitamins in Accuvit, need to be clarified. Supported by CNPq, FAPEMIG and PAPES V-FIOCRUZ.

## QT.84 - EVALUATION OF ACTIVITY AGAINST *Phytomonas serpens* EXTRACTED CRUDE EXTRACTS OF THREE SPECIES OF *Piper, Piperaceae*

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Protozoa of the genus *Phytomonas* are trypanosomatids parasites of several species of plants known for their important pathogenicity. Studies show that some *Phytomonas* are capable of causing lethal diseases, while others cause less damage to the plant. The aim of this study was to evaluate the antiparasitic activity of crude extract of *Piper aduncum, P. crassinervium* and *P. hispidum* against *Phytomonas serpens*. The extracts (aqueous and chloroformic) were obtained by macerating of dried leaves of three species of *Piper* and then was evaluated the activity of these extracts against promastigotas forms of *P. serpens* using microdilution plate. The promastigotes were treated with different concentrations of extracts (10 to 1000 μg/mL) in Warren medium supplemented with 10% fetal calf serum, incubated at 28℃. The Growth was determined by counting the parasites with a hemocytometer chamber every 24 h for 7 days and was calculated the 50% inhibitory concentration ($IC_{50}$). To evaluate the toxicity of extracts on mammalian cells was used $LLCMK_2$. Cytotoxicity assay was performed by sulforhodamine B technique and then calculated $CC_{50}$ (concentration that lyses 50% of cells). The results demonstrated that the crude extract of *P. aduncum, P. crassinervium* and *P. hispidum* have strong effect over the proliferation of *P. serpens*, with $IC_{50}$ of 45μg/mL, 16.5 μg/mL and 22 μg/mL to chloroformic extract, respectively. The aqueous extract showed low antiproliferative activity against the protozoan with $IC_{50}$ of 585 μg/mL, 557 μg/mL and 535 μg/mL, respectively. For cytotoxicity the chloroformic extract showed a moderate toxicity with $CC_{50}$ of 39 μg/mL, 210 μg/mL and 87.5 μg/mL, whereas the aqueous extract showed a low toxicity of $CC_{50}$ above 1000 μg/mL for both species of *Piper*. The results demonstrate substantial activity and selectivity of *Piper* species analyzed against the *P. serpens* although future studies should be continued. **Acknowledgements:** This study was supported through grants from CNPq, Fundação Araucária.

## QT.85 - BIOGUIDED FRACTIONATION OF TRYPANOCIDAL ASTERACEAE PLANT EXTRACT

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Chagas' disease is endemic in 21 Latin American countries, killing every year, more people in the region than any other parasitic disease, including malaria (DNDi, 2010). The disease manifests in two clinical forms, acute and chronic and for both it is necessary the development of better treatment options. Plant extracts were evaluated in two technological platforms of FIOCRUZ, Bioprospecting (RTP10A) and Chagas Disease (RPT11F), in order to identify new sources of compounds for the treatment of Chagas' disease. The active extract EX6464, from one species of the family Asteraceae, was selected for bioassay-guided fractionation. The ethanol extract of the leaves was obtained by maceration and dried under reduced pressure. An aliquot of 100 µg was fractionated into HPLC using Shim-pack ODS column (4.6 X 250 mm), flow of 1 mL/min, gradient of acetonitrile/water with 0,01% TFA and UV detection at 210 nm. The column effluent was collected in 96-well plate, dried and submitted to the test with the amastigotes of *T. cruzi* (Tulahuen strain expressing beta-galactosidase) in L929 cell line. The infection was made with 10 trypomastigote/cell/2 hours. Two days after the infection, samples were added and incubated for 96 h, when percentage of reduction was calculated. The controls used are DMSO 1% and benzonidazole 1 µg/mL. Activity (85% inhibition) was concentrated in a well that corresponds to the peak with retention time of 2.54 min. In this time of elution water content is high, indicating that polar compounds were responsible for the observed activity. Phytochemical analysis indicates that flavonoids and saponins are present in this fraction. New fractionation in a Sepbox apparatus (multidimensional chromatography) will be performed to obtain the purified active(s) compound(s).
Supported by PDTIS/FIOCRUZ, CNPq and FAPEMIG.

## VE.01 - BIOCHEMICAL AND ULTRASTRUCTURAL STUDY OF THE INTERACTION PROCESS OF *RHODNIUS PROLIXUS* WITH *TRYPANOSOMA RANGELI*

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*Trypanosoma rangeli* is the second frequently found species of trypanosomes infecting humans in countries of Latin America. Although pathogenic to the vector, to date there is no evidence of pathogenicity of this protozoan to humans. Several molecules have been identified at the surface of these cells. Surface molecules that cleave sialic acid and carbohydrate mapped with the use of lectins are well described for trypanosomatid. However, little is known about the involvement of the release of these molecules by the parasite in the process of interaction with the vector. Data obtained *in vivo* and observed under transmission electron microscope showed that during infection by *T. rangeli* the perimicrovillar membranes exposed on the surface of epithelial cells of the posterior midgut of *Rhodnius prolixus* undergo agglutination. As a result of this assemblage process, the extracellular membranes form a network in some areas of the intestine leaving others unprotected. We believe that these unprotected areas of the intestine epithelium facilitates the penetration of the parasite. The formed network, because of its perfect organization, suggests that a lectin-like molecule may be released by the parasite. Biochemical analysis of conditioned culture medium after epimastigote growth may identify proteins released by *T. rangeli* that induces this perimicrovillar membrane network.
Supported by: UENF, FAPERJ, CNPq / INCT, CAPES.

## VE.02 - *IN VIVO* ANALYSIS OF *Trypanosoma cruzi* AND *Trypanosoma rangeli* DISTRIBUTION OVER TIME IN THE MIDGUT OF *Rhodnius prolixus*

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During the transmission of trypanosome from mammal to vector, midgut is the first place where parasites remain. In this microenvironment, trypomastigote forms differentiate to epimastigotes, the replicative forms in the insect. Although many studies have been developed about *R. prolixus*-trypanosome interaction, the establishment of the infection in the triatomine gut is poorly understood yet. In this study, the distribution of *T. cruzi* (CL strain) and *T. rangeli* (CHOACHI strain) in the *R. prolixus* midgut was evaluated over a period of five days. Fifth instar nymphs were divided into eight groups of five to ten insects. Four groups were infected with *T. cruzi* and four with *T. rangeli*. For this, insects were allowed to feed for one minute in an artificial feeder containing citrated rabbit blood, previously inactivated, with $1x10^7$ parasites/mL. Insects were weighted before and immediately after feeding in order to estimate the number of ingested parasites. Bugs were dissected on 0.5, 24, 48 and 120 hours post-infection and parasite concentrations inside midgut were determined with a Neubauer chamber. For *T. cruzi*, the percentages of recovered parasites were 38, 29, 18 and 0.7 on 0.5, 24, 48 and 120 hours, respectively. For *T. rangeli*, those percentages were 34, 75, 62 and 15. The concentration of *T. cruzi* epimastigotes in the insect midgut diminished over time. For *T. rangeli*, epimastigotes concentrations increased 24h after infection, decreasing only subsequent to this period. In addition, the concentrations of *T. rangeli* inside *R. prolixus* midgut remained higher than those of *T. cruzi* during the period of evaluation. These results suggest different mechanisms of establishment for *T. cruzi* and *T. rangeli* that could have implications on the parasite growth in co-infections. In the next step we will evaluate molecular aspects of those host-parasite interactions in single and mixed infections to confirm such hypothesis. Supported by FAPEMIG and INCT-EM.

## VE.03 - TEMPERATURE AFFECTS THE INFECTION OF *Rhodnius prolixus* BY *Trypanosoma cruzi*

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Environmental temperature and behavioural thermoregulation can determine the course of an infection in several parasite-insect interactions, affecting both parasite virulence and host development. In this study, the effects of temperature on moulting time and mortality of *Rhodnius prolixus* infected by *Trypanosoma cruzi* were evaluated. For infection, second instar nymphs were fed on blood containing cultured epimastigotes of *T. cruzi* CL strain (1 x $10^7$ par/ml). Control insects were fed only on blood. Immediately after the infective meal, insects were transferred to Petri dishes containing filter paper as a substrate and the plates were maintained in temperature-controlled boxes until moulting. After this, insects remained in boxes until completing 90 days of fasting. Temperatures tested were: 21, 24, 27 and 31 °C. The period required to reach third instar varied with temperature and infection status, such that higher temperatures decreased times to moult. Interesting, the infection by *T. cruzi* decreased moulting time in insects exposed to 21, 24 and 27 °C. The infection associated with higher temperatures (27 and 31°C) increased mortality rates, reaching 21 and 96% in infected insects exposed to 27 and 31 °C, respectively (control insects showed 6 and 73% for 27 and 31°C). Moulting rates were not affected by infection or temperature. Results show that the course of infection by *T. cruzi* is affected by environmental temperature, with implications on ecology and evolution of this interaction.
Supported by: CNPq, Fapemig, CPqRR.

## VE.04 - *TRYPANOSOMA RANGELI*: ECTO- PHOSPHOTYROSINE PHOSPHATASE ACTIVITY IS INVOLVED TO *RHODNIUS PROLIXUS* SALIVARY GLAND INTERACTION

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Membrane-bound ecto-phosphatases have been reported as pathogenic factors in various infectious microorganisms and have been characterized in several members of the Trypanosomatidae family. However, the physiological role of these ecto-phosphatases in trypanosomatids had not been well established yet, although they are supposed to be involved in nutrition, protection, virulence and cellular differentiation. In this context we investigated the role of ecto-phosphatase activity of *T. rangeli* during interaction between these parasites and *R. prolixus* salivary glands. In brief, salivary glands of *R. prolixus* were incubated in the presence of long epimastigote form of *T. rangeli* ($10^6$ parasites in 200 μL). Adhesion assays showed a mean of 2000 ± 380 protozoa /pair salivary glands. We examined the capacity of different *T. rangeli* phosphatases inhibitors to modify the protozoa–salivary gland interaction. Addition of sodium orthovanadate, molybdate and $ZnCl_2$ (PTPase inhibitors) significantly inhibited *T. rangeli* adhesion. However, the addition levamisole and sodium fluoride, that not inhibited PTPase activity, did not affect adhesion to salivary glands. To confirm the involvement of PTPase in the binding of protozoa to the salivary gland we add substrates for this enzyme, *p*-nitrophenyl phosphate and phosphotyrosine on *T. rangeli* adhesion. These PTPases substrates inhibited *T. rangeli* adhesion to salivary glands, inhibition that was not observed with others phosphatases substrates, phosphoserine, phosphothreonine and β-glycerophosphate. These results suggest that an ecto-phosphotyrosine phosphatase activity could be involved in interaction *T. rangeli/* salivary gland.
Supported by CNPq, CAPES and Faperj.

## VE.05 - ULTRASTRUCTURAL STUDY OF THE PRODUCTION OF MUCUS AND RECTAL LINING OF *RHODNIUS PROLIXUS*

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The triatomine *Rhodnius prolixus* has been an excellent biological model to study the development of *Trypanosoma cruzi*, the etiologic agent of Chagas disease. In an attempt to block the development of this parasite in the vector, several drugs have been used, among them, azadirachtin has proved to be effective in blocking infection and its use is associated with cellular changes in the intestinal tract. For the evaluation of mucus secretion by intestinal epithelial cells and deposition of polysaccharides in the wax layer of the rectum, ruthenium red was used. The material was processed for routine transmission electron microscopy. Thin sections were observed by bright field microscopy and transmission electron microscopy. Preliminary data obtained by bright field microscopy showed the presence of large amounts of mucus at the stomach of insect control processed with ruthenium red. In insects treated with azadirachtin mucus was not observed. Insects treated with ecdysone and those receiving azadirachtin and ecdysone showed mucus in the lumen similar to insect control. . In the insect control, ultrathin sections stained with toluidine blue, revealed projections lining the lumen of the organ. These projections presented a colored outer line, a pale median line and a colored inner portion with dark lines elongated fibers. Insects treated with azadirachtin presented an increase in the pale median line. However, when administered azadirachtin/ecdysone, the morphological changes caused in wax were not prevented and suggests that different perimicrovillar membranes of the rectum wax can not be regenerated.
Supported by: UENF, FAPERJ, CNPq/INCT, CAPES

## VE.06 - THE INFECTION BY *Trypanosoma rangeli* CHANGES THE SHELTER USE BEHAVIOUR OF *Rhodnius prolixus*

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Triatomines display a daily rhythm of activity with two peaks, a first one corresponding to the search of food at dusk, while the other precedes dawn, and is directed to the search of refuges. We evaluated whether this behaviour is altered in *R.prolixus* after infection by *T.rangeli*. Fourth instar nymphs were infected intracoelomatically with 100 epimastigotes (CHOACHI strain) in PBS, or with PBS only (control). Fifty fifth instar nymphs starved for 30 days were used for each assay (n=4). Assays were conducted in square glass arenas (40x40x20cm) presenting one central refuge of 10 $cm^2$ with two accesses. Assays were done at 24+2°C and a 12:12 DL. The groups of insects were released in the arenas and after 3 days (time allowed for insect acclimatization) any bugs remaining outside shelters were removed. Afterwards, a mouse kept in a container was placed inside the arena to present chemical and vibratory stimuli signaling the presence of a host. Nevertheless, this did not allow the insects to feed. The hosts were presented two hours before starting the scotophase and kept there for 16 hours. The percentage of insects that entered shelters after acclimatization was 67 and 85% for infected and control groups, respectively. Insects from both groups showed an intense activity in the presence of the hosts, leaving and entering the shelters throughout the assays. At the end of these observations, 40% of the infected insects remained outside the shelters, while only 13% of the insects from control group were found in the open arena. These results show that the infection by *T.rangeli* alters the characteristic refuge behaviour of these triatomines, inducing them to become exposed. This may increase triatomine predation by vertebrates and, in the case of mammals, maintain higher parasite transmission rates by predation in nature. Supported by CNPq, Fapemig, CPqRR, FIOCRUZ.

## VE.07 - *Triatoma infestans* SALIVA AS AN ENHANCER OF *T.cruzi* INFECTION.

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*Triatoma infestans (T. infestans),* is a blood-sucking bug from subfamily Triatominea. It is widespread in the Southern Cone countries of South America and it is a vector of Chagas' disease. *Trypanosoma cruzi*, the etiological agent of Chagas' disease, is transmitted by *T. infestans* and while in the triatomine midgut the parasite differentiates from a non-infective epimastigote stage into the pathogenic trypomastigote metacyclic form. An adult *Triatoma* usually ingests two to three times its own weight of blood in a single meal. Blood-sucking insects possess a variety of anti-hemostatic factors in their salivary glands which maintain blood fluidity during feeding. In this work we shows the influence of *T. infestans* saliva in the *T. cruzi* blood parasitemia *in vivo*.The BALB/c mice were separated in two groups of ten animals,the first group received a subcutaneous injection of sterile PBS and the second group received a subcutaneous injection of *T. infestans* saliva. After 5 minutes both groups received a subcutaneous injection of *T. cruzi* (clone Dm28c) with $5\times10^5$ in 100 µl of saline. Blood parasitemia was measured once a week after the seventh day post infection during the following four weeks. The blood was obtained from a small cut at the end of the tail and diluted fivefold in red blood cell lysis buffer and parasite count was measured in a Neubauer chamber. Our results show that the presence of *T. infestans* saliva increase the infection with *T. cruzi* in BALB/c mice. The effect of bug saliva on *T. cruzi* transmission is under study in our lab and we are searching for the eventual role of biomolecules presents in saliva that may enhance parasite transmission. Supported by CNPq, Faperj, IFS

## VE.08 - HEALTH AND ECOLOGICAL ASPECTS OF CHAGAS DISEASE IN THE DISTRICT BNH OF THE CITY OF BARRA DO GARÇAS, MATO GROSSO

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The presence of vectors triatomines *Triatoma williami* homes BNH district in the municipality of Barra do Garças situated near the Serra Azul, has motivated the application of a questionnaire as part of an exploratory research for better understanding of ecological and health aspects of Chagas disease among the inhabitants of the district, thereby identifying basic knowledge on trypanosomiasis and its vectors. Evaluated-some aspects of Chagas disease in urban population of the district BNH, aiming to address the level of knowledge of their residents on trypanosomiasis and characterization of its vectors, in the municipality of Barra do Garças-MT. All the interviews were carried out after accepted consent free. The data contained in the questionnaire were analyzed, used-if the program ArcView GIS version 3.2 in the construction of maps, while data global positioning were measured with GPS Garmin 60 CSx The analyzes obtained show a possible potential of recrudescence of Chagas disease, even with the certificate of interruption of transmission vectorial and, also a potential vector sinantropic *T. williami*. Front of the actual health in Brazil, the scientific community must propose to public bodies of control epidemiological/sanitary greater attention to the combat and control of Chagas disease The process of occupation and disordered the destruction of natural habitat been contributing to drastic environmental changes, increasing the invasion of wild species, besides the existence of diversity of species of triatomines with potential of transmission. There is an enormous challenge to prevent a recrudescence of the transmission of Chagas disease, since one of vectors has presented high degree of synanthropy. Supported by CAPES.

## VE.09 - MORPHOMETRIC STUDY OF INTRAPOPULATION VARIABILITY OF *TRIATOMA MATOGROSSENSIS* (HEMIPTERA, REDUVIIDAE)

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The insects of the Triatominae subfamily stand out for their importance as vectors of *Trypanosoma cruzi*, the etiologic agent of Chagas disease. The triatomines are distributed throughout the Neotropical regions and the species *Panstrongylus, Rhodnius* and *Triatoma* genus are epidemiologically more important. The genus *Triatoma* is the most numerous and it is subdivided into specific complexes according to morphological similarities and geographic distribution of its species. T. matogrossensis is found in the Central-West region of Brazil and belongs to the oliverai subcomplex, together with *T. baratai, T. guazu, T. jurberg, T. vandae* and *T. williami*. The aim of this study was to determine the *T. matogrossensis* female intrapopulation morphometric variability. For this, we used specimens kept since 02/09/1993 colony in the Insetário de Triatominae, of FCF - UNESP / Araraquara. The original colony was divided according to differences in the size of insects, visually perceptible. For each colony formed, 15 specimens were analysed for parameters of the head (eye diameter, distance ante-ocular and post-ocular and inter-ocular, length of each of the three rostral segments, and head length) and body (body length and length and width of the abdomen and abdomen). According to the results, using unpaired T test, morphometric differences were highly significant for the length of the body, abdomen, head; very significant for length and width of the abdomen, abdomen width, eye diameter and head length; significant for distances ante-ocular and inter-ocular and the second segment; and no significant for post-ocular distance and the length of the first and third rostral segments. We conclude that the parameters used showed significant differences, and there is a real variation in size among the specimens studied, even between individuals of the same species and origin, which justifies the continuity of the observations by other techniques. Supported by: CNPq and Fapesp (Proc. 2010/50355-1)

## VE.10 - PHYLOGENETIC RELATIONSHIP *TRIATOMA* SPECIES (HEMIPTERA, REDUVIIDADE) FROM CENTRAL WEST REGION OF BRAZIL BASED ON CYTOCHROME B GENE SEQUENCE

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The triatomines are vectors of the etiologic agent of Chagas' disease, *Trypanosoma cruzi*. The *Triatoma* genus is the largest and was divided in specific complexes according to morphological similarities and geographical distribution. The seven species studied can be found in the Central West region of Brazil, of which five belong to the oliverai subcomplex (T. *baratai, T. guazu, T. matogrossensis, T. vandae* and *T. williami*) and the other two species, *T. costalimai* and *T. sordida* belong respectively to *T. infestans* and *T. sordida* complexes. The aim of this study was to determine the phylogenetic position of these two species, by comparing the cytochrome b (Cytb) gene fragment sequences of the mitochondrial DNA. The specimens evaluated came from colonies maintained at the Insectary of Triatominae, Faculdade de Ciências Farmacêuticas / UNESP - Araraquara. After extraction of genomic DNA and amplification of Cytb gene fragment, it was sequenced in an automatic DNA sequencer, model ABI 377. The sequences obtained and other sequences (of the same fragment) already available in GenBank were aligned using the Clustal W program, of BioEdit, and the phylogenetic inferences were conducted using the analysis of distance with the MEGA 3.1 program. Sequences of the species *T. sherlocki, T. infestans* and *T. brasiliensis* were included in the analysis to support the phylogeny. The species were distributed in two clades: the first compounded by *T. costalimai, T. sordida, T. matogrossensis* and *T. williami*; and the second compounded by *T. vandae, T. baratai* and *T. guazu*. The phylogenetic analysis using the Cytb sequence show the division of the oliverai subcomplex in two groups and *T. baratai* and *T. vandae* species were included together in one of them. This fragment showed a high degree of polymorphism and homoplasy along the analyzed sequences.
Supported by CNPq and Fapesp

## VE.11 - CHARACTERIZATION OF THE HEMAGGLUTINANT ACTIVITY EXPRESSED IN THE ANTERIOR MIDGUT OF *TRIATOMA INFESTANS* (HEMIPTERA, REDUVIIDAE)

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Hemagglutinins have been described in several hematophagous arthropods. Most of them are lectins with binding specificity for saccharides or lipopolysaccharides. In triatomines, hemagglutination activity was described for different tissues of *Triatoma infestans* and *Rhodnius prolixus*, including haemolymph, salivary glands, anterior and posterior midguts. Each tissue possesses distinct molecules that differ in their binding capacity and their biological role is not known yet. In this study we evaluated the hemagglutinant activity present in the anterior midgut of third instar nymphs of *T. infestans* in different days after molt and partially characterized the hemagglutinin molecule. The insect age after molt influenced significantly the hemagglutinant activity, which is low or absent until approximately 5 days after molt, increases from day 5 to 8 and remains at higher levels from day 7 to more than 25 days. The molecule is thermo-stable and the treatment at 98 °C for 5 minutes doesn't lead to the loss of activity. Hemagglutination was inhibited when midgut extracts were incubated with a nonspecific protease (proteinase K), suggesting that the molecule has a proteic origin. When the midgut extracts were ultra-filtered with 100 kDa cut off membranes, the molecule with hemagglutination activity was present at the portion with molecular weight higher than 100 kDa. New experiments are underway with the aim of identifying the sequence of the molecule, its expression profile and its biological role.
Supported by: FAPEMIG, CAPES and CNPq.

## VE.12 - INFLUENCE OF THE INTESTINAL ANTICOAGULANT IN THE FEEDING PERFORMANCE OF THE TRIATOMINE BUGS (HEMIPTERA; REDUVIIDAE)

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Triatomines are hematophagous insects in all life stages. They are vectors of *Trypanosoma cruzi*, the causative agent of Chagas disease. The vectorial capacity of these insects is directly related to their feeding efficiency, which varies greatly among species due to factors related to the physiology of the host and characteristics of the insect feeding apparatus. Only recently, factors found in the gut environment were reported as influencing the feeding process, as hemagglutination and blood clotting. In this work, we investigated the level of anticoagulant activity achieved by the intestinal contents of three species of triatomines - *Triatoma infestans, Triatoma brasiliensis* and *Rhodnius prolixus* - and correlated the anticoagulant activity of each species with their feeding efficiency on live hosts. For all studied species, the anticoagulant activity was significantly higher in the anterior midgut (crop) contents than in saliva. Among the species, *T. brasiliensis* had the lowest crop anticoagulant activity, the lowest concentration of thrombin inhibitor, and is also the specie that was verified higher difficulty in the feeding process. To confirm the findings that the anticoagulant activity magnitude interferes with the blood pumping into the crop, we knocked down by RNAi the expression of brasiliensin, the intestinal thrombin inhibitor from *T. brasiliensis.* The brasiliensin knockdown nymphs had lower capacity to maintain the cibarial pump contractions frequency throughout the feeding process even in favorable conditions (feeding on a large diameter vessel), and lower blood ingestion rate (mg/min), when compared to control nymphs. However, the difficulty during feeding was reversed in brasiliensin knockdown nymphs fed on mice treated with heparin (a potent systemic anticoagulant), that behaved similarly to the control nymphs. Thus, the intestinal anticoagulant activity is directly related to the blood-pumping frequency modulation, which affects the feeding performance of triatomine bugs.
Supported by: FAPEMIG, CAPES and CNPq.

## VE.13 - THE KNOCKDOWN OF CATALASE AND DUAL OXIDASE INTERFERES WITH OVIPOSITION AND ECLOSION RATES IN *RHODNIUS PROLIXUS*

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The regulation of the generation and elimination of reactive oxygen species (ROS) is key to aerobic life. Here, in order to evaluate the role of dual oxidase (Duox, an $H_2O_2$ producer) and catalase (Cat, an $H_2O_2$ scavenging enzyme) in *Rhodnius prolixus*, we used RNAi to knockdown expression of Duox and Cat genes by injection of gene-specific double-stranded (ds) RNAs into the haemocele of female insects. qPCR showed inhibition of the expression of both genes after the blood meal, reaching 99,5 and 99,7% for Cat and Duox, respectively. In the insects injected with Cat dsRNA, the oviposition and eclosion rates were, respectively, 50 and 75% smaller in comparison with control insects. In the insects injected with Duox dsRNA the oviposition rate was not altered. However, eclosion of first instar was dramatically reduced in these insects, from 55% to 100 % of the control insects. Together, these results show that control of redox metabolism is essential both to oogenesis and embryogenesis.
Supported by CNPq, CAPES, HHMI and FAPERJ.

## VE.14 – Insight into the salivary transcriptome and proteome of *Dipetalogaster maxima*

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*Dipetalogaster maxima* is a blood sucking hemiptera that inhabits sylvatic areas in Mexico. It usually takes his blood meal from lizards but the human population growth resulted in bugs invading suburban areas, feeding also in humans and domestic animals. Hematophagous insects' salivary glands produce potent pharmacological compounds that counteract host hemostasis, including anti-clotting, anti-platelet, and vasodilatory molecules. To obtain a further insight into the salivary biochemical and pharmacological complexity of this insect, a cDNA library from its salivary glands was randomly sequenced. Also, salivary proteins were submitted to one and two dimensional gel (2D-gel) electrophoresis followed by MS analysis. We present the analysis of a set of 2,728 (SG) cDNA sequences, 1,375 of which coded for proteins of a putative secretory nature. Most salivary proteins were described as lipocalins, corresponding to 93% of the transcripts coding for putative secreted proteins. Lipocalins are a large and heterogenous group of proteins that play various roles, mainly as carriers of small ligands in vertebrates and invertebrates. A great array of salivary gland proteins belonging to the lipocalin family has generated a large number of different molecules having anti-hemostatic functions while maintaining the fundamental structure of the protein fold. Lipocalins were found in the saliva of other blood-sucking triatominae bugs as *Rhodnius prolixus*, *Triatoma brasiliensis*, *Triatoma infestans*, and also in tick saliva.
Supported by: CNPq, Finep, FAP-DF, NIH

## VE.15 - INTERACTION OF *PHYTOMONAS SERPENS* WITH THE PHYTOPHAGOUS INSECT *ONCOPELTUS FASCIATUS* (MILKWEED BUG)

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The hemipteran insect *Oncopeltus fasciatus* (milkweed bug) is found in several U.S. states, Mexico and Brazil. Due to its high reproductive activity in the laboratory, short life cycle and size large enough to be easily handled, this insect is used as a model for various studies, including host-parasite interactions, both in natural and experimental conditions. *O. fasciatus*is the natural host of a number of species of the genera *Phytomonas, Crithidia* and *Leptomonas*. Our laboratory colony of *O. fasciatus* is naturally infected with *Leptomonas wallacei*. We have obtained protozoan-free *O. fasciatus* by treating the eggs with sodium hypochlorite. After eclosion, the insects were fed with peeled sunflower seed and mineral water. The absence of trypanosomatids in the gut was confirmed by both scanning electron microscopy and PCR, using primers specific for *L. wallacei*. A colony of *O. fasciatus* free of trypanosomatids has been kept apart from the original colony. Plant trypanosomatids *Phytomonas* have recently attracted attention due to their role as agricultural parasites of both plants and insects. Little is known of the life cycle of *Phytomonas* species in the insect hosts, despite its paramount importance for the transmission of these flagellates to their plant hosts. While *Leptomonas* spp colonize only the digestive tract of their hosts, *Phytomonas* spp cross the intestinal epithelium, reach the hemolymph and infect the salivary glands. Trypanosomatid-free insects were used for assays of interaction with *P. serpens*. Either these parasites or vehicle were injected with a microsyringe into the thorax of adult *O. fasciatus* by puncturing the articulation of a prothoracic coax. At different times from 6 to 72 h after the injections, the insects were lightly anesthetized on ice; the hemolymph was collected from 8 to 10 insects per treatment group, by cutting off metathoracic legs and gently pressing the abdomen. Large numbers of parasites were observed in Giemsa-stained smears from the hemolymph, and interaction of *P. serpens* with hemocytes was examined by both light and transmission electron microscopy. The insects were carefully dissected and their intact salivary glands extracted, fixed and examined by means of scanning electron microscopy. Parasites were found attached to a dense extracellular layer, the basal lamina, closely associated through both the flagellum and the cell body of *P. serpens*. On the other hand, invasion of the basal lamina occurred only via the protozoan cell body. Parasites were also found under the basal lamina, attached to the outer surface of the salivary gland, as well as entering the lumen of the gland. *P. serpens* promastigotes were also allowed to interact with extracted salivary glands from *O. fasciatus*. The basal lamina of the salivary glands showed holes suggestive of parasite penetration. SDS-PAGE-gelatin gels prepared with supernatants of the medium of interaction showed that both the parasites and the salivary glands produce proteinases. The parasites release a 63 kDa enzyme consistent with a metallo-proteinase; proteins released from the salivary glands also showed a proteinase profile consistent with a metallo-proteinase (15 kDa).

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## VE.16 - PHLEBOTOMINE FAUNA (DÍPTERA: PSYCHODIDAE) DISTRIBUTION ACROSS AN URBAN-RURAL GRADIENT OF A VISCERAL LEISHMANIASIS ENDEMIC AREA IN THE MUNICIPALITY OF BARCARENA, PARA, BRAZIL.

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American visceral leishmaniasis (AVL) is a zoonosis caused by a protozoan of specie *Leishmania (Leishmania) infantum chagasi* and it is transmitted by phlebotomines sand flies of genus *Lutzomyia*. The disease was typically rural, but it has become urbanized in consequence of drastic environmental alterations caused by human action. Nowadays AVL has been verified in many Brazilian cities including Santarem (PA), Fortaleza (CE), Rio de Janeiro (RJ) and Belo Horizonte (MG). The aim of this study was to verify the urbanization of *Lutzomyia (Lutzomyia) longipalpis,* vector of AVL in the municipality of Barcarena-PA, Brazil. Systematic captures of phlebotomines were performed using CDC light traps. The captures were carried out in areas of forest, edge of forest, intermediate area and urban area, from 2007 to 2009. A total of 5,089 specimens belonging to eleven species were collected, with predominance of *L. (L.) longipalpis* (95.15%), *Lutzomyia (Sciopemyia) sordellii* (2.06%) and *Lutzomyia (Nyssomyia) flaviscutellata* (1.76%). The highest population densities (88.25%) were from edge of forest belonging to a locality occupied about twelve years. However, the visceral leishmaniasis vector was not captured in urban area, suggesting it has not been urbanized in Barcarena yet. Another two species, *Lutzomyia* (*Nyssomyia*) *flaviscutellata* and *Lutzomyia (Psychodopigus) paraensis* related with cutaneous leishmaniasis transmissions were captured in the forest and edge of forest. More than seventy percent of all *L. (L.) longipalpis* specimens captured were males, whereas to another species, the number of females was more frequent than males. For the diagnosis of infection rate, the microscope analysis was performed but all analyzed samples were negative. These results suggest low rate infection in these areas. The presence of phlebotomine specimens captured in edge of forest reinforces the necessity of entomological monitoring in Barcarena municipality. Supported by CNPq and UFPA.

## VE.17 - CASPAR AND TGF-BETA ARE POTENTIALLY INVOLVED IN *LUTZOMYIA LONGIPALPIS*-PATHOGEN INTERACTION

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*Lutzomyia longipalpis* is the main vector of visceral leishmaniasis in Brazil. In order to identify important events in vector-pathogen interaction we have sequenced ESTs of cDNA libraries from *L. longipalpis* gut RNAs obtained after blood feeding, and after infection with *Leishmania*. Among the sequences we have identified a cDNA coding for a TGF-beta closely related to the Activin subfamily, which regulates immune response and wound repair among other functions. The transcriptional profile of this gene was studied by semi-quantitative RT-PCR using RNA extracted at different times after blood feeding or artificial infection with *Leishmania infantum chagasi*. TGF-beta transcription increased 72h after infection when compared to control uninfected samples. Interestingly, this is the time when the perithrophic matrix degrades and the parasites attach to the vector midgut. Western blot experiments are being performed to investigate the kinetics of protein production. We are also interested in investigating the involvement of the different innate immune pathways in the response to pathogens. We identified a sequence similar to Caspar, a repressor molecule of the IMD pathway. The transcriptional profile of Caspar was determined by semi-quantitative RT-PCR using RNA extracted at different times after blood feeding or artificial infection with *Leishmania mexicana.* Caspar transcription decreased after 72h infection when compared to blood fed samples. RNAi experiments were performed to access the gene function. Females were microinjected with Caspar dsRNA and 72h later were infected with *L. mexicana*. Caspar knocked-down insects showed reduction of parasite count in the midgut when compared to control groups. This phenotype indicates that the non-repressed IMD pathway is capable of reducing *Leishmania* survival in the insect midgut. This is the first report of an immune related gene in sand flies affecting *Leishmania* survival. Supported by: CAPES, CNPq, FAPERJ, Fiocruz.

## VE.18 - PHYLOGENETIC RELATIONSHIPS AMONG FOUR SPECIES OF RUBROVARIA SUBCOMPLEX USING DNA SEQUENCE OF THE MITOCHONDRIAL CYTOCHROME B GENE

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Currently 140 species of Triatominae are recognized. In recent studies, the genus *Triatoma* was grouped into eight complexes and subcomplexes. *Triatoma rubrovaria*, *T. circummaculata*, *T. klugi* and *T. carcavalloi* species are found in similar ecotopes in the Rio Grande do Sul State, Brazil, and they are grouped into one subcomplex (rubrovaria subcomplex), based on morphological characteristics. Aiming to verify the relationship of genetic similarity between those species, the cytochrome B (cytB) fragments were sequenced in this four species. The consensus sequences were evaluated for phylogenetic relationship using the distance methods by the Neighbor Joining algorithm, under the Kimura 2-parameter model implemented in MEGA 4.0 software. The support for the groups was evaluated using distance bootstrap analyses with 500 replicates. The phylogenetic tree was rooted using the cytB sequence of *T. dimidiata*. The phylogenetic analyses using the cytB sequences showed a close relationship between *T. klugi* and *T. carcavalloi* and between *T. rubrovaria* and *T. circummaculata*, agreeing with the morphological data and the current rating.
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## VE.19 - EVALUATION OF *AEDES AEGYPTI* COLONIZATION BY *BLASTOCRITHIDIA CULICIS*

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In a previous work, we demonstrated that *Blastocrithidia culicis,* an endosymbiotic-harbor monoxenic trypanosomatid, is capable of colonizing the mosquito's digestive tract and crossing the intestinal epithelia, reaching the haemocoel of artificially fed *Aedes aegypti*. In order to further characterize this interaction we analyzed midgut and salivary gland proteins that are able to recognize *B. culicis.* With this purpose, mosquito midgut and salivary gland proteins were analyzed by blotting assays using *B. culicis*-biotinylated-epimastigotes as a ligand. In salivary glands we observed five proteins that recognize *B. culicis,* of which two were identified by Edman degradation as apyrase and aegyptin. In the midgut, biotinylated-epimastigotes bound to seven proteins with molecular mass ranging from 10 -50 KDa. All these mosquito midgut proteins are glycoproteins, as they were labeled by different lectins. We also demonstrated that radiolabeled-protozoa inoculated into the mosquito thorax bound to midgut, salivary glands and ovaries. Here, we analyzed the *B. culicis* - *A. aegypti* colonization after feeding mosquitoes with epimastigotes, and following the presence of the protozoa in different organs during 48 days using polymerase chain reaction (PCR). *B. culicis* was detected in midgut, hindgut, abdomen, crop, Malpighian tubules and ovaries early after feeding. Protozoa were detected in salivary glands 28 days post-feeding. These data confirmed our previous assay using radiolabeled-epimastigotes. Taken together these results evidence aspects of *B. culicis* life cycle in *A. aegypti*.
Support: CAPES, CNPq and FAPERJ.

## VE.20 - INITIAL CHARACTERIZATION OF *CULEX QUINQUEFASCIATUS* VITELLOGENIN RECEPTOR

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As occurs with haematophagous insects, *Culex quinquefasciatus* vitellogenesis is triggered by a blood meal. Besides the synthesis of yolk proteins by the fat body, the process is characterized by the development of the endocytic complex at the apical surface of the oocyte, which will allow the uptake of the large amounts of nutrients needed for embryonic development. The endocytic complex is composed of microvilli, specific receptors, mainly vitellogenin receptor (VgR), clathrin-coated vesicles and endosomes. The *Cx. Quinquefasciatus* vitellogenin receptor (CxVgR) cDNA was sequenced using primers based on the *Aedes aegypti* VgR (L77800), since at this time the molecular database of *Cx*. *pipiens quinquefasciatus* was still incomplete. The sequence of the PCR products presented high similarity to the *Ae. aegypti* (L77800) and *Cx. pipiens quinquefasciatus* (CPIJ020278) sequences and the amino acidic deduced sequence showed typical characteristics of the family of receptor low density lipoprotein receptor (LDLR). This family contains five distinct domains: a ligand-binding domain; epidermal growth factor (EGF)-like repeats; repeats containing a YWTD motif; a transmembrane domain anchoring the receptor to the plasma membrane and a cytoplasmic domain. RT-PCR showed that the transcript is present, only in the ovary samples, already in the first day after emergence (AE) and increases progressively until the fifth day AE. During the gonotrophic cycle, the gene increases until the 48 h post blood meal. These results were confirmed by real-time PCR and correlate with females engorgement behaviour and with their fecundity and fertility.
This work was supported by FAPESP.

## VE.21 - CHARACTERIZATION OF *CULEX QUINQUEFASCIATUS* HEXAMERINS

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Insect fat body is a multifunctional organ involved in storage and intermediary metabolism of lipids, carbohydrates and proteins. The analysis of fat body extracts of juvenile and adult stages of *Culex quinquefasciatus* by one-dimension SDS-PAGE revealed the presence of several major proteins with molecular masses between 74 and 91 kDa in the last instar of both larval and pupal stage. The major proteins of the pupae fat body extract were submitted to an *in gel* reduction, alkylation, and trypsinization and resulting peptides were analyzed by mass spectrometry, revealing that these proteins belong to different groups of insect hexamerins. Insect hexamerins are storage proteins with a native molecular mass of around 500 kDa, consisting of a random association of quantities and types of immunologically related homologous subunits (approximately 70-85 kDa each). These proteins, which are synthesized and secreted into the haemolymph during the last larval stage, are incorporated into granules of the fat body cells in the pre-pupal stage. In some genera, hexamerins can be used during the non-feeding phases or during metamorphosis. Moreover, hexamerins act as an amino acid reserve for protein production, mainly during cuticle development of pupae and adults. We are currently analyzing the gene expression profile of *Cx. quinquefasciatus* hexamerins to determine the sites of synthesis and storage.
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## VE.22 - ANALYSIS OF THE PROMOTER REGION FROM A *LUTZOMYIA LONGIPALPIS* CHITINASE GENE

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Leishmaniasis are diseases with high epidemiological impact that affect millions of people around the world. Chitinases are important enzymes in insect biology and, since the 1990s, their involvement in the parasite – vector interaction process is being studied. In *Lutzomyia longipalpis*, the main vector for visceral leishmaniasis in America, the chitinolytic activity in the gut of blood fed females seems to be fundamental for modulation of the peritrophic matrix (PM) thickness and may influence the infection of the vector by the parasite. A *L. longipalpis* chitinase cDNA (Llchit) potentially involved in PM degradation was isolated from blood fed females and characterized. Then, a genomic clone, containing the gene coding for this cDNA, was isolated from a genomic library and sequenced, revealing the presence of 4 introns. The 5`flanking region (FR) of the chitinase gene, present in the genomic clone, was also sequenced and submitted to analysis *in silico*, revealing the presence of a possible promoter region. In order to obtain larger flanking regions, genomic DNA of *L. longipalpis* was subjected to reverse PCR and the products were cloned and sequenced. As a result, we obtained a total of 1270bp of the FR5`. Subsequent bioinformatics analysis identified regions responsive to transcription factors E74 and Kr, initially identified in *Drosophila melanogaster*. E74 regulates mosquito genes after a blood meal and also participates in the chitin synthesis pathway. We also cloned the FR5` in pGL3 basic vector (Promega), to investigate its ability to induce the expression of luciferase in *L. longipalpis* cultured cells (LL5), since we showed previously that these cells express Llchit RNA.
Suported by CNPq, FAPERJ, PDTIS.

# A

## B



## C

## D

## E



## F

## G

## H



## I



## J



## K



## L

## M

## N



## O



## P

## Q



## R

## S

## T

## U



## V

## W



## X



## Y



## Z