## BC-80
## *LEISHMANIA COLOMBIENSIS* AND *LEISHMANIA EQUATORENSIS*: PECULIAR *VIANNIA* SPECIES

Catanho MP, Cupolillo E, Campbell D**, Fernandes O/*
Departamento de Medicina Tropical e Imunologia, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900, Rio de Janeiro, RJ, Brasil *Departamento de Patologia, UERJ, Rio de Janeiro, RJ, Brasil **Department of Microbiology and Immunology, UCLA, USA

The taxonomic criterion used to classify a *Leishmania* species as *Viannia* is the perypilarian pattern of development, with a phase in the hindgut of the sandfly. The use of a sole marker to discriminate this group of parasites can lead to misclassifications. The mini-exon gene non-transcribed spacer has been used as a molecular tool to distinguish different *Leishmania* species. For example, although *L. lainsoni* is taxonomicaly identified as a *Viannia* species, the mini-exon gene non-transcribed spacer revealed that it comprises a very divergent species inside the subgenus *Viannia*, behaving as an independent complex. Recently, we have observed that the variable region of the minicircle molecules of both *L. colombiensis* and *L. equatorensis* are different in length when compared with all the other *Leishmania* species. In order to investigate this discrepancy with another molecular tool, we PCR amplified the mini-exon gene of both *Leishmania* species. The amplified products corresponded to a 400 bp repeat, different from all the other *Viannia* species, which are 250 bp or even from *L. lainsoni* that is 300 bp. The PCR product from *L. colombiensis* was cloned and sequenced. The primary DNA sequenced showed a 89.5% of similarity with *E. schaudinni* and diverged from all the described *Leishmania* mini-exon genes. These results, in association with MLEE, minicircle conserved region sequences, ITS of the rRNA gene analysis and sialidase activity demonstrated that *L. colombiensis* and *L. equatorensis* are very related to *E. schaudinni* and may represent an eventual evolutionary link between the genus *Leishmania* and *Endotrypanum*.

## BC-81
## *LEISHMANIA AMAZONENSIS, L. MAJOR* AND *L. MEXICANA* RDNA PROMOTER MINIMAL FUNCTIONAL DOMAINS PRESENT A HIGH LEVEL OF SEQUENCE SIMILARITY

Stempliuk VA, Franco AR, Uliana SRB, Floeter-Winter LM
Departamento de Parasitologia, Instituto de Ciências Biomédicas, USP, Caixa Postal 66208, 05389-970 São Paulo, SP, Brasil

The rDNA promoter region of *Leishmania amazonensis* has been structurally and functionally characterized (Uliana et al. 1996 *Mol Biochem Parasitol 76*: 245). Unexpectedly, *L. major* and *L. mexicana* transfected with the *L. amazonensis* promoter showed higher levels of CAT expression than the observed in experiments with the homologous system (Stempliuk et al. 1997 *Mem Inst Oswaldo Cruz 92*: 176). To determine the reasons for the differential levels of expression, we characterized the regions mapped as being the promoter minimal functional domains in *L. amazonensis* and in the other species. Two regions, encompassing the putatives UBF plus SL1 binding sites (nucleotides –196 to + 170) and the SL1 binding site alone ( –74 to 170) of the *L. amazonensis, L. major* and *L. mexicana* promoter sequences were obtained by PCR, cloned and sequenced. These sequence showed a high degree of identity. Constructs bearing the promoter regions derived from the three different *Leishmania* species, upstream to the CAT reporter gene were used for functional studies on the cross-species activity of these promoters. Transient transfection experiments showed that the region –74 to 170 contains a functional element, although the levels of expression for this sequence were lower than the one observed for original construct pLaΔ14ASCAT. This observation might be explained by the absence, in that construct, of a fragment of the ETS, in the 3'region, that contains a polypyrimidine track. Other constructs are being made to test the functional role of those domains and their cross-species activity.

Supported by Fapesp and CNPq.

## BC-82
## *LEISHMANIA AMAZONENSIS* IN TAXIDERMIZED RODENTS

Cantarino L, Araújo A, Sabroza P, Ferreira LF, Fernandes O, Pirmez C
Escola Nacional de Saúde Pública, ENSP, Fiocruz *Dept. Medicina Tropical e Dept. Bioquímica e Biologia Molecular, Instituto Oswaldo Cruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, Brasil

Starting in the forties, the National Service of Rural Endemic collected 60,000 rodents in attempt to seek out plague reservoirs. These animals were examined, taxidermized, and kept in archives at the National Historical Museum of the Federal University of Rio de Janeiro (UFRJ). Since many of these animals were collected from areas where leishmaniasis was also present, we decided to investigate if these rodents were parasitized by *Leishmania* through the polymerase chain reaction (PCR). We examined animals from two well-known *Leishmania* endemic

areas: 20 from Baturité, Ceará and 19 from Ilha Grande, Rio de Janeiro. Three skin fragments were collected from each animal. Genomic DNA was isolated by using QIAmp tissue kit (QUIAGEN, California, USA), ethanol precipited and resuspended in TE. Oligonucleotides that amplify the conserved region of minicircle kDNA were used in a hot start PCR. The amplified products were analysed by agarose gel electrophoresis, and dot-blots were hybridized with a preamplified product of *L. panamensis* or *L. amazonensis* as probes. Our results show the presence of a 120bp band in two animals, both from Baturité: one *Oryzomys eliurus* and one *Trichomys apereoides*. These animals were captured in 1953. Hybridization showed that these positive PCR products were *L. amazonensis*. These results raises the possibility to reconstruct the history of leishmaniasis through retrospective studies by using molecular approaches.

## BC-83
## ISOLATION OF SPECIFIC GLYCOLIPID ANTIGENS OF *LEISHMANIA (VIANNIA) BRAZILIENSIS*

Silveira TGV, Straus AH,Takahashi HK
Departamento de Bioquimica, Universidade Federal de São Paulo, Escola Paulista de Medicina, 04023-900 São Paulo, SP, Brasil

In order to identify possible glycolipid antigens of *Leishmania (Viannia) braziliensis*, monoclonal antibodies (MoAbs) were produced. BALB/c mice were immunized with promastigote membranes obtained by $N_2$ cavitation and ultracentrifugation. A hybridoma line was established, and termed SST-1. The monoclonal antibody (IgG3) secreted by SST-1 showed to be directed to the glycolipid fraction of *L. (Viannia) braziliensis* promastigotes. Glycolipids of *L. (V) braziliensis* promastigotes were extracted with mixtures of chloroform/methanol and isopropyl alcohol/hexane/water. The glycolipids were purified by chromatography on Octyl-Sepharose, Silica-Gel 60 and C18 reverse-phase columns and by HPLC using Iatrobeads columns. Reactivity of the MoAb SST-1 with the glycolipids was analyzed by HPTLC immunostaining and solid-phase radioimmunoassay. The glycolipids recognized by SST-1 were present in all *L. (V.) braziliensis* serodemes analyzed. The SST-1 reactivity was abolished after treatment of these glycolipids with sodium m-periodate, indicating that the epitope recognized by SST-1 is present in their carbohydrate moiety. By indirect immunofluorescence and radioimmunoassay no cross- reactivity of SST-1 was observed with promastigote forms of *L. (Leishmania) amazonensis*, *L. (Leishmania) major*, *L. (Leishmania) chagasi*. The structure of *L. (Viannia) braziliensis* glycolipid antigens are under investigation.

Supported by Capes, CNPq, Fapesp and Pronex.

## BC-84
## INVOLVEMENT OF *LEISHMANIA AMAZONENSIS* PROMASTIGOTES SURFACE PROTEINS IN THE RESISTANCE OF PARASITES TO AUTOLYSIS

Menezes EAG, Almeida-Campos FR, Horta MF
Depto. de Bioquímica e Imunologia, Instituto de Ciências Biológicas, Universidade Federal de Minas Gerais, Av. Antônio Carlos 6627, 31270-901 Belo Horizonte, MG, Brasil

We have recently described in *Leishmania amazonensis* extracts a pore-forming protein, named leishporin, that lyses erythrocytes and nucleated cells including its host cell, the macrophage. In the present work we report that leishporin is secreted by promastigotes. However, parasites are not lysed by its own cytolysin. Parasites were incubated for different periods of time (30 min to 5 hr) at 24°C and pH 7.2 and the hemolytic activity of the supernatant was determined. As early as 30 min, the supernatant presents hemolytic activity, indicating that promastigotes secrete leishporin in its active form. However, parasites remain intact and viable in contact with its own cytolysin, even after 5 hours of incubation, as revealed by the vital dye Erythrosin B. To investigate the resistance of parasites to autolysis by leishporin, viable promastigotes were incubated with a cytolytic promastigotes extract (p-ext) at 37°C for 30 min at pH 5.5, the optimal conditions for leishporin full activity. Parasites were stained with Erythrosin B and dead and alive parasites were counted. We observed that parasites are resistant to lysis by p-ext when compared to erythrocytes and nucleated mammalian cells. Parasite resistance increases during its growth *in vitro*, reaching a peak at late logarithmic phase or early stationary phase. To determine whether parasite surface proteins are involved in resistance to lysis by leishporin, viable promastigotes were incubated with proteases (proteinase K, trypsin and chymotrypsin) in different concentrations (6.25, 12.5, 25 and 50 µg/ml) for 2 hr at 34°C. Parasites were then washed, incubated with p-ext for 30 min at 37°C and assayed for viability. We found that proteinase K and trypsin caused a dose-dependent reduction of parasites resistance to its own cytolysin. Chymotrypsin, on the other hand, had no effect on parasite's sensitivity to lysis. These data indicate that trypsin-sensitive and chymotrypsin-resistant promastigotes surface proteins are involved in the resistance of the parasite to autolysis.

Supported by CNPq, Capes, and Fapemig.