MINI-CONFERENCES

XXX ANNUAL MEETING ON BASIC RESEARCH IN CHAGAS DISEASE - XIX MEETING OF THE BRAZILIAN SOCIETY OF PROTOZOOLOGY - HOTEL GLÓRIA, CAXAMBU, MG, BRASIL - 10-12 NOVEMBER 2003. **Rev. Inst. Med. trop. S. Paulo, 45**(Suppl. 13), November, 2003.

MCCB1 - GENETIC HYBRIDISATION IN *TRYPANOSOMA CRUZI*.

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The species *T. cruzi* is divided into 2 divisions (I and II) based on isoenzyme phenotypes, DNA profiles, ribosomal and mini-exon DNA sequence polymorphisms, and microsatellite analysis. Additionally, *T. cruzi* II, which predominates where Chagas disease is more severe, can be divided into up to 5 sublineages (IIa-e). The presence of genetic exchange in *T. cruzi*, and in *Leishmania,* is much debated. Recent phylogenetic and karyotype evidence suggests that *T. cruzi* IId and IIe may have an ancient hybrid origin. However, population genetics has emphasised clonal propagation and the lack of genetic exchange in *T. cruzi* when natural isolates, from dispersed geographical localities, were tested for random mendelian genetic exchange.

As described in detail in Gaunt *et al*. (2003), a mechanisim is observed in *T. cruzi* that is distinct from mendelian inheritance. A pair of putative parental biological clones of *T. cruzi,* transfected to carry different drug resistance markers (hygromycin; neomycin), were passaged singly or together through the various life cycle stages. Six, double drug resistant, biological clones, were recovered from the mammalian stage of the life cycle. DNA amplification showed that the 6 clones contained both the *hyg* and *neo* genes. Isoenzyme analysis and karyotype analysis showed that the clones shared parental characters and were at least in part hybrids. Random amplification of polymorphic DNA (RAPD) revealed characteristic sharing of bands between progeny clones and parents; controls with non-transformed parents showed that shared bands were not attributable to the episomes. For a series of informative microsatellite loci progeny clones showed all parental alleles, whereas for others absence of alleles indicated allele loss. Sequence analysis of nuclear genes indicated fusion, recombination and allele loss. Uniparental inheritance of mitochondrial maxicircle genotype was observed.

There are strong parallels between the experimental hybrids and the genotypes among natural populations of *T. cruzi,* including the presence of polyploid genotypes. Phylogenetic analyses gave unequivocal evidence of genetic recombination among natural populations and infers that the mechanism spans far greater genetic distances than mendelian inheritance.

Aneuploidy via hybridisation explains the wide range in DNA content of *T. cruzi*, and reconciles the paradox between apparent clonal propagation yet occurrence of recombination. Most importantly, aneuploidy via nuclear hybridisation may lead to dramatic speciation events; it is also likely to be present in *Leishmania*.

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Gaunt G. W, Yeo M. , Frame I. A. , Stothard J. R. , Carrasco H. J. , Taylor M. C., Mena S. S., Veazey P., Miles G. A. J. , Acosta N., Arias A. R. de, Miles M. A. (2003) Mechanism of genetic exchange in American trypanosomes. *Nature*; 421: 936-39.

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MCCB2 - *N*-MYRISTOYLATION AS A TARGET FOR INTERVENTION IN KINETOPLASTID PARASITIC INFECTIONS

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Myristoyl CoA: protein *N*-myristoyltransferase (NMT) catalyses the transfer of the fatty acid, myristate, to the *N*-terminal glycine of eukaryotic cellular and viral proteins and is essential for the viability of *Candida albicans*, *Cryptococcus neoformans* and *Saccharomyces cerevisiae*. Selective and specific inhibition of NMT activity in a range of pathogenic fungal species has confirmed a potential role for this enzyme as a drug target in these organisms. To test whether NMT might also be a useful target in kinetoplastid parasites, we are investigating NMT function in *Leishmania* species and *Trypanosoma brucei*.

Myristic acid analogues, non-specific inhibitors of NMT, are toxic to *Leishmania* promastigotes and bloodstream *T. brucei*, inhibiting the *N*-myristoylation of cellular proteins. The single copy *L. major NMT* gene is constitutively-expressed during the parasite life cycle and encodes a 48 kDa protein that is well-conserved in regions essential for myristoyl CoA and peptide substrate binding in *S. cerevisiae*. Gene deletion experiments, targeting the removal of both NMT alleles by homologous recombination coupled with functional complementation by episomal expression, have demonstrated that NMT is essential for viability in *Leishmania.* Similarly, NMT over-expression causes gross changes in *Leishmania* morphology, decreased growth rate and increased cell death. In *T. brucei*, RNA interference assays have confirmed that NMT is also essential in this species.

Soluble active recombinant *L. major* and *T. brucei* NMTs have been produced in *E. coli* and are being used in kinetic analyses to determine the K_m values for the myristoyl CoA and peptide substrates and the inhibitory effects of lead compounds. We have already shown that peptidomimetic NMT inhibitors active against fungal pathogens are highly toxic to bloodstream *T. brucei.* Comparative sequence alignment and homology modeling based upon existing NMT crystal structures are in progress to identify parasite-specific features of functional importance that can be exploited for the design of selective inhibitors.

In order to identify the downstream targets that cause lethality in the absence of NMT activity, we have searched the genomes of *L. major* and *T. brucei* for proteins carrying *N*-myristoylation motifs. Several *N*-myristoylated proteins have been cloned and characterised, including ADP-ribosylation factor (ARF) proteins, a *Leishmania* specific serine/threonine protein kinase and a novel serine/threonine protein phosphatase present in both *L. major* and *T. brucei.* Genetic approaches are being used to establish whether parasite death in NMT mutants and inhibitor-treated parasites is associated with depletion of one or more of these proteins *in vivo.*

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MCCT1 - DIAGNOSIS AND TREATMENT OF CHAGAS DISEASE AND TOXOPLASMOSIS IN PEDIATRIC PATIENTS

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A) Chagas disease

The accurate diagnosis is made by *T. cruzi* detection in blood, in CSF or in tissues. If this is not possible, diagnosis must be made through the study of specific antibodies. On the other hand, it is important to differentiate those procedures which can be performed in low-complexity health care centers and those centers where research and development tasks are performed.

Low complexity health care centers

a) In patients under 6 months of age, diagnosis should be exclusively made through the presence of parasites in blood. We recommend the microhematocrit (MH) technique, its sensitivity depends on the operator. According to our experience, sensitivity was 93% during the first weeks of life. Serological tests are not useful due to the presence of maternal antibodies during this period. Every child born of a serologically reactive mother with negative MH, should undergo serological testing as from the 7 months of life.

b) After the seventh month, diagnosis is confirmed when the patient´s serum presents 2 reactively different serological techniques (SC), ELISA, IHA, IFI.

High complexity centers

a) Parasitological techniques: both Xeno diagnosis and hemoculture are complex and expensive techniques. Their advantage is that they are highly sensitive while their disadvantage is that they take a long time to show results. PCR is still not a validated procedure. Our experience is as follows: in patients under 6 months of age, sensitivity(S) was 100% and specificity(E) was 97%. In patients over 7 months of age, S was 73.8% and E was 100%. We consider that all these procedures are useful only for research work.

b) Although many *T.cruzi* antigens have been assayed for serologic tests, only a few have proved to be useful. SAPA proposed as an acute infection marker was positive in about 30% of patients infected during the undetermined stage.

At present we have only one drug, benznidazol for treatment. Suggested dosage is from 5 to 10 mg/kg/d and duration of treatment varies from 30 to 60 days. Cure criteria is obtaining negative SC in two successive samples. The cure percentage of 203 children from 1 month old to 15 years of age showed the following results: a) 100% for patients younger than 3 months, b) 97% for children under 3 year old and c) 87% out of the total.

The older the patient is at the start of treatment, the longer it takes for negative tests to show. We have observed that negative testing occurs between three months and eight years after treatment. In order to shorten this period, we have developed an ELISA using antigen F2/3 obtained from trypmastigotes (Almeida). The results showed a significant difference (p=0.0025) comparing negative testing of anti-F2/3 antibodies with antibodies detected by SC in patients older than 8 months of age.

B) Toxoplasmosis

Human infection by *T.gondii* is very frequent, generally asymptomatic and only in immunodeficient patients or fetus that suffered acute infection during pregnancy, it can cause diseases of different severity. Most frequent pediatric patients are children born with congenital infection (CT). Only 40% of children born from *T.gondii* infected mothers acquire the infection.

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About 70% of prenatally infected infants are clinically asymptomatic at birth. Clinical findings in 23 infants with CT in our service were: chorioretinitis (82%), retinal detachment (26%), prematurity (21%), hidrocephalus (43%), microcephaly (43%), hepatomegaly (34%).

Parasitological diagnosis is expensive and has low sensitivity. The PCR is not properly standardized and shows false negatives and positives.

In the medical practice, diagnosis is made by evaluating specific antibodies to IgG, IgA and IgM. The most frequently used technique is ELISA, which uses different antigens. Other techniques are IHA and IFI.

The presence of maternal IgG in the newborn delays diagnosis in the first months of life. The detection of anti-*T.gondii* IgG after 8 months is the gold standard to diagnose CT. IgM and IgA are considered markers of CT in a newborn, but the S varies among authors. Table 1 shows our results.

Treatment of CT results in a more favorable outcome, the sooner the therapy is given, the better the results are. Unfortunately, many infants receive treatment, therefore we are not sure if they are infected. We tested some recombinant antigens (rRop2, rGra4, rGra7) with Dr Sergio Angel, (INTECH, Buenos Aires) in order to improve the diagnosis, at least to verify whether infants born from infected mothers are not infected. Table 2 shows the results in the follow-up of 59 infants and their mothers at risk of CT.

Antidodies against recombinant antigens became negative earlier than antibodies detected by conventional serology in the follow-up of not infected infants.

Table 1. CS analysis of the first serum samples from all infected and non infected ifants and their mothers

	IgG +/n (%)	IgM +/n (%)	IgA +/n (%)	IgM/IgA +/n (%)
Infected				
Mother	18/18 (100)	10/18 (55)	6/18 (33)	11/18 (61)
Newborn	23/23 (100)	15/23 (65)	17/23 (74)	19/23 (83)
Non Infected				
Mother	27/27 (100)	7/27 (26)	2/27 (7)	7/27 (26)
Newborn	36/36 (100)	3/36 (8)	3/36 (8)	5/36 (14)

Table 2. Negativization age of non-infected infants by using commercial (c)-, rRop2-, rGra4- and rGra7-ELISA

Technique	Average of negativization age+	Confidential limit (CI) 95% range	*p* value vs cELISA (*p*<)
CELISA	5.88	5.1 – 6.6	NC
rRop2*	3.43	2.6 – 4.3	0.05
rGra4	2.08	1.4 – 2.8	0.001
rGra7*	0.99	0.6 – 1.4	0.001

NC, not corresponding; +, in months; * $p<0.001$ between them

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MCCT2 - GLYCOLIPID ANTIGENS OF *LEISHMANIA* AND *TRYPANOSOMA CRUZI* AS POTENTIAL TARGETS TO CHEMOTHERAPY

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Leishmania is an intracellular parasite, and macrophages are its primary targeted host cell. Contrasting with the number of information available regarding the phagocytic processes of promastigote forms, few works in this area have been carried out with amastigotes, forms responsible for maintaining and disseminating the infection in the vertebrate host. In *L. (L.) amazonensis* amastigotes, glycosphingolipid (GSL) antigens were first described by Straus et al. (1) and shown to be related to macrophage invasion. Seventeen different neutral GSL antigens were identified by their reactivities with monoclonal antibodies (mAbs) ST-3, ST-4 and ST-5, directed to carbohydrate moieties of GSLs, and the chemical quantity of these antigenic GSLs would account for $1x10^6$ molecules/cell. The structure of the smallest GSL antigen was elucidated by ^{1}H NMR, GC/MS and use of exoglycosidase as: Galβ1-3Galα1-3-Galβ1-4Glcβ1-1Cer (henceforth referred to as β-Gal-globotriaosylceramide). The chemical quantity of β-Gal-globotriaosylceramide was estimated in $2x10^5$ molecules/cell.

Studies carried out in our lab, focusing in the biological roles of glycolipid antigens, demonstrated that the terminal β-Gal residue of *L. (L.) amazonensis* amastigote stage-specific glycosphingolipid antigens is a key component of the epitope recognized by the mAb ST-3. By *in vitro* assays 70-80% inhibition of macrophage invasion/binding by amastigotes of *L. (L.) amazonensis* was obtained with mAb ST-3. *In vivo* infectivity assays using amastigotes pre-incubated with either Fab fragments or intact antibody showed a delay in the development of BALB/c mice footpad lesions. The definitely evidence of the direct involvement of GSLs presenting β-Gal*p* in parasite-macrophage interaction was demonstrated by the observation that addition of the disaccharide Gal*p*b1-3Gal*p* (1mM) was able to inhibit about 48% of macrophage monolayers infectivity by amastigotes.

Thus, in order to identify the macrophage molecules involved in the *L. (L.) amazonensis* amastigote GSLs recognition, it was examined the binding of micelles prepared using amastigote purified GSLs to blots of the macrophage membrane lysate isolated from culture of mouse resident peritoneal macrophages. The micelles binding were probed using mAbs directed to parasite GSLs, it was observed that micelles containing *L. (L.) amazonensis* amastigote purified GSL fractions, bound strongly to a 30 kDa molecule. These results show that macrophage monolayers express a (glyco)protein able to recognize amastigote GSLs. In order to discard the possibility that glycosphingolipids containing one (CMH), two (CDH) or three (CTH) residues of carbohydrate, present in the amastigote GSLs fraction could be the responsible for this binding, a fraction containing only GSLs not reactive with mAb ST-3 (CMH, CDH, and CTH) were used to prepare micelles. Pre-incubation of micelles presenting CMH, CDH, and CTH with the nitrocellulose membrane did not interfere on the binding of micelles prepared with amastigote purified GSLs containing more complex carbohydrate chains reactive with mAb ST-3. As shown before these GSLs are present in the parasite surface, therefore instead of using GSLs micelles, a more biological assay was carried out using fixed amastigotes, and it was confirmed that the parasites were also able to bind to the 30 kDa (glyco)protein.

On the other hand, *Leishmania (Leishmania) major* do not express GSLs, but they express glycosylinositol phospholipids (GIPLs). Recently we demonstrated the role of glycosylinositol phospholipid-1 (GIPL-1) of *L. (L.) major* in interaction of promastigotes and amastigotes with macrophages. A mAb MEST-1 that recognizes glycolipids containing terminal galactofuranose (gal*f*)

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residue (2) was used to detect GIPL-1 in *Leishmania* by indirect immunofluorescence, and to analyze its role in macrophage infectivity. *L. (L.) major* promastigotes showed intense fluorescence with MEST-1, and GIPL-1 was detected in both amastigote and promastigote forms by HPTLC immunostaining using MEST-1. Delipidation of *L. (L.) major* promastigotes with isopropanol/hexane/water abolished the MEST-1 reactivity, confirming that only GIPL-1 is recognized in either amastigotes or promastigotes of this specie. The role of GIPL-1 and its gal*f* residue in the processes of binding and invasion of macrophage monolayers was analyzed by pre-incubating parasites with MEST-1 Fab fragments, which reduced macrophage infectivity by ~80% for promastigotes and 30% for amastigotes. The more modest inhibition of amastigotes adhesion than for promastigotes, could be due to GIPL-1 crypticity in amastigotes or due to a lower GIPL-1 concentration in amastigotes than promastigotes, as observed by immunofluorescence. The substantial inhibition (~80%) of macrophage infectivity by promastigotes in the presence of *p*-nitrophenyl-β-D-galactofuranoside confirmed the importance of terminal β-D-gal*f* residue. Significant inhibition (~65%) of macrophage infectivity was also observed when *p*-nitrophenyl-α-D-mannopyranoside was used, indicating involvement of a mannose receptor in *Leishmania*-macrophage interaction. No inhibition was observed when macrophages were preincubated with *p*-nitrophenyl-β-D-galactopyranoside. Cell-cell interactions and attachment processes are multi-step phenomena, and the present results indicate that one such receptor recognizes terminal residues of gal*f*. The receptor for terminal gal*f* residues in glycoconjugates of parasites and fungi is yet uncharacterized, but currently under investigation. Tsuji et al. (3) recently identified a new type of human lectin, termed human intelectin (hIntL), which binds to pentoses and to D-gal*f* residue in the presence of Ca^{2+}. A recombinant hIntL recognizes bacterial arabinogalactan of *Nocardia* containing D-gal*f*, thus, this lectin may be involved in recognition of various pathogens containing gal*f* residues, *e.g.*, *Nocardia, Mycobacteria, Streptococcus, Leishmania* and *Trypanosoma*. The present results suggest that terminal residues of β-D- gal*f* present in GIPL-1 from either promastigote or amastigote forms of *L. (L.) majo*r are recognized by a previously undescribed macrophage receptor involved in *Leishmania*-macrophage interaction.

Other glycoconjugates containing galactofuranose residues also have been described in fungi, bacteria, and trypanosomatides such as *Trypanosoma cruzi* and *Leishmania* (2, 4-6). Although the biological role of Gal*f* residues remains to be completely ascertained, the postulated absence of Gal*f* and galactofuranosidases in mammalian species suggests the intriguing hypothesis that terminal Gal*f* residues play a central role in survival of fungi and parasites by preventing the action of the host's glycosidases on their glycoconjugates. If this hypothesis is correct, Gal*f* residues are potentially useful as specific target molecules for therapy of parasitic diseases.

A lipopeptidophosphoglycan currently termed glycoinositol phosphoceramide (GIPC) has been described as the major glycoconjugate of the *T. cruzi* epimastigotes. *T. cruzi* GIPCs present well known carbohydrate structures containing one or two terminal Gal*f* residues (7,8). By indirect immunofluorescence it was demonstrated that mAb MEST-1 also recognizes *T. cruzi* epimastigotes and amastigotes, showing no/weak reactivity with trypomastigotes. Using different immunochemistry assays, it was verified that the antigens recognized by MEST-1 in these parasites are glycolipids and that the expression of these glycoconjugates varies as differentiation of amastigotes into trypomastigotes occurs, as noticed by the decrease of the MEST-1 reactivity with trypomastigotes. The glycolipid antigens labeled with MEST-1 are localized in epimastigotes on internal acidic structures (reservosomes), which are also stained with Lysotracker Red. In contrast, for amastigote forms, it was detected a strong fluorescence on the parasite surface, suggesting that GIPCs synthesis may be important for the intracellular amastigotes survival and division (9). Although there is no report about the efficacy of inhibitors of inositol phosphorylceramide (IPC) synthase in trypanosomatids, the effect of IPC synthase inhibitors in other organisms is well know, such as in *Aspergillus fumigatus* and *Aspergillus nidulans*,

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and these drugs are prime candidates for a more effective chemotherapy in deep mycosis (10). The data shown here indicates that search for efficient and highly specific inhibitors of galactofuranosyl, β-galactosyl transferases and IPC synthase of *Leishmania* and *T. cruzi* may represent a viable strategy for design new drugs for the chemotherapy of these major parasitic diseases.

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MCE1 - UNDERSTANDING THE GEOGRAPHY OF DISEASE TRANSMISSION USING ECOLOGICAL NICHE MODELING

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Disease transmission cycles can be seen as interacting sets of species that themselves interact with their environments in space and time. Ecological niche modeling (ENM) is a relatively new technique that uses known occurrences and GIS data sets to produce a predictive model of ecological niche dimensions. Using ENM, it is thus possible to reconstruct and predict geographic aspects of disease transmission cycles, based on species-specific ecological niche models. I illustrate the potential of this approach using examples from Chagas disease, leishmaniasis, West Nile virus, malaria, and ebola.

MCE2 - THE RAT SAT ON THE CAT: THE MANIPULATION OF HOST BEHAVIOUR BY *TOXOPLASMA GONDII.*

Joanne P. Webster

According to the Manipulation Hypothesis, a parasite may be able to alter the behaviour of a host for its own selective benefit, usually by enhancing its transmission rate. Classic examples concern transmission through the food chain, where a parasite is immature in the intermediate host, which must be eaten by a predatory definitive host before the parasite can reach maturity and complete its life-cycle. The parasite thus manipulates the behaviour of its intermediate host so as to ensure its transmission to the correct definitive host. *Toxoplasma gondii,* the causative agent of toxoplasmosis, is one such indirectly-transmitted parasite suggested to achieve such manipulation. Whilst it was traditionally believed that latent toxoplasmosis is asymptomatic and of no clinical or epidemiological interest within immunocompetent human or animal hosts, recent evidence now suggests this in unlikely to be true. Studies within naturally and experimentally infected rodents indicate that *T. gondii* can enhance the likelihood of predation by the feline definitive host through the subtle manipulation of intermediate host behaviour: infected rats are significant more active, more exploratory and less fearful of novel stimuli in their environment, and even show an attraction to areas indicative of predator presence. Moreover, this attraction towards areas of predator presence appears specific to only that of the feline definitive host, where infected rats continue to avoid non-feline predators such as mink. All behavioural traits and health indices unlikely to influence predation rate by the feline definitive host remain intact. A number of recent studies have also detected behavioural alterations within latently infected humans similar to those observed within rodents. Of current concern is a potential relationship between *T. gondii* infection in humans and some instances of first incidence psychosis or schizophrenia. Support for such a link is provided, in part, by the shared anti-protozoan properties of successful anti-schizophrenic drugs. The role of such chemotherapy on the development and maintenance of *T. gondii* induced behavioural alterations in its rodent intermediate host stage is also presented here, and the results discussed in terms of their theoretical and applied implications.

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MCIM1 - THE TRYPANOSOMA CRUZI PROLINE RACEMASE : AN ENZYME WITH MULTIPLE ROLES - FROM DEVELOPMENT TO PARASITE ESCAPE.

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We have previously proposed that mitogens and superantigens, moieties that are essential components of micro-organisms can be responsible for the initiation of nonspecific polyclonal lymphocyte activation and this, *per se*, may explain the strategy used by pathogens to avoid the host immune responses and to ensure persistence. We had shown that metacyclic infective forms of *T. cruzi* do secrete molecules responsible for such a mechanism of immune evasion. Using biochemical and molecular approaches we isolated from the supernatants of metacyclic cultures, a parasite protein involved in the polyclonal activation of B lymphocytes. This protein is the first eukaryotic proline racemase, *Tc*PRAC, previously annotated as *Tc*PA45, for *Trypanosoma cruzi* polyclonal activator of 45 kDa. By the analysis of the genomic organization and transcription of *TcPRAC* gene we showed the presence of two homologous genes (*TcPRACA* and *TcPRACB*) per parasite haploid genome. These two genes are located in two different chromosomal bands of which one contains more than 3 chromosomes of similar size. *Tc*PRAC mRNA is differentially transcribed in the different parasite stages. In fact, we showed that the parasite can differentially express intracellular and secreted isoforms of the *Tc*PRAC protein, respectively of 39 kDa in epimastigote (non-infective) forms of the parasite and of 45 kDa in metacyclic (infective) forms. The mapping of the 5' region of the transcribed genes during parasite development allowed us to demonstrate that both *TcPRACA* and *TcPRACB* transcripts are present in all parasite stages. Furthermore, we confirmed that although *TcPRACB* gene by itself only encodes the intracellular version of the protein and that *trans*-splicing signals embedded within *TcPRACA* paralogue enable the gene to generate both cytoplasmic and secreted isoforms of *Tc*PRAC by alternative *trans*-splicing mechanism. We then produced two recombinant isoforms of the *Tc*PRAC protein by overexpressing *TcPRACA* or *TcPRACB* paralogous genes in *E. coli* and purified the 45kDa and 39kDa isoforms of the protein tagged at the C terminus by a polyhistidine tail. By biochemical assays we showed that *Tc*PRACA and *Tc*PRACB isoforms do indeed posses proline racemase activities racemizing both L- and D- proline and no other amino acid. This activity is co-factor independent and closely resembles that of the proline racemase of the protobacterium *C. sticklandii (Cs*PRAC). Biochemical studies revealed that both isoforms possess distinct V_{max} and K_{max} values, specific buffer and pH requirements that are compatible with the enzyme being functional in either internal or external cellular compartments. However, assays with pyrrole-2-carboxylic acid (PAC), the specific and competitive inhibitor of proline racemase, revealed that PAC is relatively effective (in the micromolar range) in inhibiting both enzymes. Western blot analysis using total or cytosolic extracts from non-infective (epimastigote) and infective (metacyclic) forms of the parasite, and the corresponding culture media, confirmed that *TcPRACA* and *TcPRACB* paralogous genes are functional *in vivo* since both intracellular (39 kDa) and the secreted (45 kDa) isoforms display proline racemase activities. By *in vitro* and *in vivo* proliferation assays we showed that *Tc*PRACA, the parasite secreted proline racemase, is a T-cell independent B-cell mitogen. Moreover, the *Tc*PRACA mitogenic activity is abolished, or severely compromised whenever enzymatic activity is inhibited by specific inhibitors or substrate excess. Furthermore, we showed that B-cell triggering depends on conformational epitopes of *Tc*PRACA rather than on the proline racemase activity (see M. Goytia and coll, in these proceedings). Crystallography of *Tc*PRACA clearly showed that saturation of *Tc*PRACA by its specific inhibitor (PAC) induces a conformational change in the protein and that any direct interaction of *Tc*PRACA with a membrane B-cell receptor (postulated for the polyclonal B cell

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activation) is unambiguously dependent on conformational epitopes displayed by the protein. To better approach the biological relevance of proline racemases in the metabolism of *T. cruzi*, and consequently the influence of the enzyme on parasite differentiation, we over-expressed *TcPRACA* and *TcPRACB* paralogue genes in non-infective parasite forms using appropriate vectors to obtain stable chromosomal integration of these genes (in sense and antisense orientationsas verified by chromoblots). The first important observation was that non-infective forms of the parasite expressing full length antisense *TcPRACB* RNA (functional knock outs, KO) are not viable whereas functional *TcPRACA-KO* survive only poorly even under low selection pressure for recombinant parasites. We also determined that *Tc*PRAC enzymes are differentially involved in metacyclogenesis and that fully differentiated metacyclic parasites over-expressing *TcPRAC* genes were more virulent to host cells, as ascertained by invasion and release of the parasites in host cell co-cultures. These data showed that *Tc*PRACA proline racemase is both a mitogen and a virulence factor. Since we observed an important conservation of critical residues between *Tc*PRAC we then used available databases (SWISS-PROT and TrEMBL to define a minimal motif (Motif III), (DRSPCGX[GA]XXAXXA), sufficiently conserved to yield several predicted protein sequences with high similarity to proline racemases from 9-12 organisms including organisms of medical importance (i.e. *Bacillus anthracis, Brucella suis, Clostridium botulinum, Aspergillus fumigatus, Leishmania* (*L.*) *major, Trypanosoma brucei,* amongst others, but not *Plasmodium)* suggesting that proline racemases are widespread but not ubiquitous in more complex organisms. Biochemical and functional characterization of these putative proline racemases are in progress.

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MCIM2 - THE ROLE OF APOPTOTIC MIMICRY IN HOST- PARASITE INTERPLAY

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Multicellular organisms can clearly benefit from the non-inflammatory elimination by apoptosis of unnecessary and potentially harmful cells. Recently accumulated data show that unicellular organisms such as pathogenic trypanosomatids can also take advantage of different apoptotic features. Examples are the capacity of *Trypanosoma cruzi* and *Leishmania* spp to take advantage of different features of the apoptotic process to establish a host-parasite relationship, without death as the necessary outcome. Indeed, we have described that amastigotes of *Leishmania* (*L.*) *amazonensis* display phosphatidylserine (PS) on the outer leaflet of their cellular membrane. This molecular moiety induces an anti-inflammatory response of the macrophages through the activation of a specific receptor. Macrophages are the obligatory habitat of *Leishmania* spp. in the mammalian hosts and, paradoxically, are also the effector leishmanicidal cells when properly stimulated by the adaptive immune response of the host . The PS-dependent anti-inflammatory response is characterized by inhibition of nitric oxide synthesis and activation of TGFß and IL-10 production; the net result is an increase in internalization and a facilitated intramacrophagic proliferation of the parasite (1). PS exposure is one of the most precocious and widespread features of a cell primed for apoptosis; it is essential for the non-inflammatory disposal of dying cells and thus allows apoptosis to occur without disturbing normal organogenesis and normal adult cell renewal. This same feature, in an apoptotic-mimicry fashion, seems to be important for the establishment of *Leishmania* spp as an obligatory intramacrophagic parasite of mammalian hosts. Interestingly, *Trypanosoma cruzi,* a parasite displaying just a transient intramacrophagic phase in the vertebrate host, is endowed with the capacity of inducing apoptosis in the host's T lymphocytes [2]. This parasite, which displays only traces of PS in its lipid composition, takes advantage of the interaction with the macrophages of the PS exposed by the T lymphocytes, in a very similar way to what *Leishmania* (*L.*) *amazonensis* does with PS on its own surface. PS exposure can be thus viewed as an important phenotype for the establishment and maintenance of the present day *Leishmania*/host relationship. In spite of the fact that the PS-exposing amastigotes are endowed with the capacity of undergoing typical apoptotic oligonucleosomal DNA cleavage, the question of whether PS exposure by the parasite is an independent phenomenon or is part of the molecular demands of a fully operational cell death program remains unanswered. This, and several other related questions will be discussed in this presentation. First, we still have no clear-cut evidence whether the PS in the parasite's surface is synthesized by the parasite itself, or is derived from the host. The following are strong evidence indicating that the former is the case: (a) axenically grown metacyclic promastigotes expose PS; (b) plasma membrane translocases, which are capable of regulating PS exposure, have been described in promastigotes of *Leishmania* (*L.*) *tropica* [3]; and (c) amastigotes derived from susceptible BALB/c mice systematically expose more PS on their outer surface than amastigotes derived from resistant C57Bl/6 mice (manuscript in preparation). This fact induces important differential behavior regarding *in vivo* and *in vitro* infectivity of amastigotes derived from the two different strains of mice. Finally, and central to the present discussion, it is not clear either if the PS-exposing parasites are the truly infective forms, or if they only contribute to infectivity due to their PS-depended macrophage inactivation properties. If, indeed, PS exposure is part of a cell death

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program, and the PS positive parasites are the infective ones, they must be rescued from death within the phagolysosomal environment. If, on the contrary, the infective forms are the PS negative ones, with the PS positive forms facilitating infectivity, a truly cooperative system is operating here. In both situations, however, PS exposure, with its consequent macrophage inactivation, is the relevant phenotype for parasite persistence in the host. Parasite death by apoptosis is, in the present situation, just a side effect.

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MCMB1 - WIDESPREAD OPERONS IN THE *C. ELEGANS* GENOME: WHY AND HOW

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Unlike other animals but similar in some ways to trypanosomes, *C. elegans* and its relatives have polycistronic transcription units. These operons resemble bacterial operons. We analyzed the*C. elegans* genome to investigate which genes are contained in operons (Blumenthal et al., 2002, Nature 417:851), and determined that more than 15% are in operons. These multi-gene assemblages range from 2 to 8 genes in length and can extend over 50 kb. We have analyzed the list of ~1000 operons, containing ~2,600 genes, to determine what types of genes they contain and whether functionally related genes are co-transcribed in operons.

Some classes of genes are highly represented in operons, especially mitochondrial genes and the genes for the basic transcription, splicing and translation machinery. In general, genes whose mRNAs are enriched in the female germ line have the strongest tendency to be contained in operons. In contrast, genes that are highly regulated in a particular tissue are generally excluded from operons. Examples are genes for transcriptional activators, collagens, sperm proteins, intermediate filament proteins, cytochrome P450s, immunoglobulin domain proteins and basement membrane proteins.

Many operons encode two or more functionally related proteins. Recently, genes related to a gene of interest have been identified by their presence together in a *C. elegans* operon. The operon list suggests many unsuspected relationships among proteins, but whether most operons contain functionally related genes is not yet known.

The worm operons are processed much like trypanosome polycistronic transcripts. RNA polymerase makes a multicistronic pre-mRNA that is processed by cotranscriptional 3' end formation and trans-splicing . In *C. elegans* there are two spliced leader RNAs (the donor in trans-splicing). SL1 is used at 5' ends of most genes, just to trim off the outron, the sequence between the promoter and the trans-splice site. SL2 is specialized for use at trans-splice sites between genes in operons. 3' end formation generally occurs about 100 bp upstream of operon trans-splice sites, but unlike in trypanosomes, it is not dependent on trans-splicing or on the polypyrimidine tract used for trans-splicing. Instead, it is signaled by the conventional AAUAAA and GU rich signals that bind CPSF and CstF, as in mammals, and 3' end formation precedes trans-splicing.

We have identified the key signal between genes that results in efficient SL2-specific trans-splicing. It is a U-rich sequence, the Ur element, that is very likely to be the site at which CstF binds to facilitate 3' end formation as well. We have shown that CstF binds to the SL2 snRNP and this binding strongly correlates with function. Binding of protein to the Ur element serves two separable purposes. First, it blocks the progress of an exonuclease that gains entry to the pre-mRNA due to 3' end formation just upstream. Second, it attracts the SL2 snRNP. Preliminary evidence will be presented for a protein that is specific to the SL2 snRNP that appears to be responsible for this interaction.

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MCMB2 - MOLECULAR BASIS OF CHLOROQUINE RESISTANCE IN *PLASMODIUM VIVAX* MALARIA.

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Plasmodium vivax is the most widely distributed human malaria and responsible for 70-80 million clinical cases each year and large socio-economical burdens for countries such as Brazil where it is the most prevalent species. Unfortunately, due to the impossibility of growing this parasite in continuous *in vitro* culture and the low parasitemias obtained directly from infected patients, research on *P. vivax* remains largely neglected. We have therefore undertaken a genomics approach to study this human malaria by constructing a genomic library in yeast artificial chromosomes from which to obtain unlimited parasite material (Camargo AA, Fischer K, Lanzer M & del Portillo HA. 1997. Genomics, 42:467).

Chloroquine (CQ) has been the drug of choice for eliminating *P. vivax* infections but resistance has been an increasing problem since it was first reported from Papua New Guinea in the late 80's. In fact, the geographic distribution of CQ resistance (CQR) in *P. vivax* has now extended to India, Myanmar, Guyana and Brazil. The gene putatively responsible for CQR resistance in *P. vivax*, *pvcg10,* has been recently identified and mapped with the aid of the *P. vivax* YAC library (Nomura T, et al. 2001. J. Inf. Dis. 183:1653). Significantly, unlike its orthologous gene in *P. falciparum*, *pvcg10* mutations are not associated with CQR suggesting that CQR in *P. vivax* could be associated with expression levels adjustments or another yet to be discovered mechanism. We have thus generated transgenic lines of *P. falciparum* expressing *pvcg10* at different levels in order to determine whether a high expression of this transgene alters the IC50 and the global gene expression of the recipient 3D7 CQ sensitive clone.

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MCVE1 - OLD AND NEW CHALLENGES FACING THE CONTROL OF CHAGAS' DISEASE IN THE AMERICAS

Antônio Carlos Silveira

Human Chagas' disease is associated to poverty or to an unprotected relationship between the man and the vector. This is undoubtedly the primary challenge, which refers to the origin of the disease production.

Moreover, there are other challenging situations that are not old or new, but related to epidemiology of the disease, or to its natural history, which limit the possibilities of its control: I) since Chagas' disease is primarily an enzootics, it is not extinguishable; ii) due to the number of animal hosts, there is no way to act with the purpose of extinguishing infection sources; iii) at the acute phase, the disease is unapparent most of the times, which obstructs the detection of the major number of recent infection cases, when specific chemotherapy is definitely effective. Thus, both primary prevention of natural transmission and secondary prevention at the precocious pathogenic phase have limitations. In addition, it is relevant to mention the apparently low power of the available means of control, particularly due to the inexistence of a vaccine that protects populations under risk, and also due to the absence of pharmacies that can be used at a large scale. Nevertheless, although the vector is the only factor susceptible to sanitary procedures — through periodic and systematic use of residual-action pesticides at the infested houses —, the answer seems to be controlled transmission. This is enabled by some attributes of the vector: stable populations, low mobility and very slow reposition. Additionally, main vector species are completely domiciliary.

Even though Chagas' disease is vulnerable to control policies, in spite of its magnitude and transcendence, the greatest challenge of all, the oldest and longest one is the low level of priority that has been given to it. This problem comes from the chronic quality of the disease, from its large and silent clinical path and from the fact that populations under risk don't demand or have few opportunities of requesting services; moreover, they are predominantly rural and not influent or even heard. This is another challenge, which is the fact that these populations live at the edge of the development in many regions of Latin America, where poverty remains constant, or even worse.

Environment degradation, and/or predatory exploration of natural spaces, which took place at the origin of the vectors' domiciliation, still exists in considerable portions of the American continent as well.

New challenges result, to a great extent, from environmental changes. In the Amazon region an increasing number of autochthonous cases have been registered. These cases are almost always attributed to other means of transmission, derived from different actions, such as oral transmission and extra-domiciliary vector born transmission, this one related to labor activities, specially "piaçaba" extraction, main habitat of *Rhodnius brethesi.* These new strategies of control are not formulated. *Rhodnius pallescens*, for instance, is a very particular case in Panama, where it is proved to be an important vector, even if it doesn't make colonies inside the houses, because of its presence at palm trees that are part of the enclosed areas, because of its frequent "visits" to the houses and also because of the very high rates of this species' natural infection.

Part of the changes is consequence of the control itself. The elimination of domiciliary species, such as *Triatoma infestans'* in some of the South American countries, brought species that used to be sylvan or peridomiciliary, like *Triatoma rubrovaria,* to the south of Brazil. In addition, even partial control of domiciliary species may have caused the domiciliation of other ones. This is what apparently may have happened to *Panstrongylus lutzi* , in substitution of *Triatoma brasiliensis*, in the northeast of Brazil. In Central America, the expected goal is the perspective of eliminating *Rhodnius prolixus,* an

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introduced species, not autochthonous, and there should be awareness to this kind of reality, too.

The greater mobility of human population and the large migration from rural places to urban centers, which have been happening for the last decades, also imply new challenges. The possibility of passive dispersion of those vectors that are predominantly associated to humans has increased, and the risk of transmission by transfusion at non-endemic areas has greatly increased as well.

One final question to be considered is how to uphold epidemiologic vigilance of Chagas' disease in situations of minimum infestation and risk, which resulted from the success achieved, with the interruption of transmission in many areas, especially in some countries of "South Cone" region.

MCVE2 - *WOLBACHIA* BACTERIAL ENDOSYMBIONTS AS THE TOOLS AND TARGETS OF DISEASE CONTROL

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Wolbachia are intracellular bacterial endosymbionts of arthropods. As a group they show a diverse range of associations with their hosts, ranging from reproductive parasitism to a strict mutualism in filarial nematodes. One common feature of *Wolbachia* is its remarkable ability to influence host reproduction to facilitate its spread through populations. *Wolbachia* manipulate reproduction through parthenogenesis, feminization, male-killing and cytoplasmic incompatibility. Exploiting the ability of *Wolbachia* to spread through populations has been proposed as a means of introducing genetic modifications to pests and vectors of disease. In filarial nematodes, the mutualistic dependence on *Wolbachia* has been used in a new approach for the control of filariasis. Antibiotics have been used to clear the endosymbionts, which leads to an inhibition of larval development, moulting and in adult female worms a complete block in embryogenesis. The depletion of endosymbionts can also lead to the eventual death of adult worms, suggesting the bacteria may be essential for their long-term survival. The first trials using antibiotics against human filariasis have been encouraging, with long-term sterilization in onchocerciasis and potent macrofilaricidal activity in lymphatic filariasis. Growing evidence also points towards the bacteria contributing to disease pathogenesis in filariasis. When the bacteria are released from drug-damaged worms they cause inflammatory reactions associated with the adverse reactions to chemotherapy. Similar activation of innate inflammation by the bacteria causes the inflammatory responses that lead to river blindness. The use of antibiotics, in addition to perturbing the symbiotic relationship, could therefore also eliminate the cause of inflammatory-mediated disease.