

MC001 - T Regulatory Cells and Persistence of Chronic Leishmaniasis Due to Infection with *L. (Viannia) panamensis*

EHRlich, A.*; CASTILHO, T.; GOLDSMITH-PESTANA, K.; SPARWASSER, T.; MCMAHON-PRATT, D.

YALE UNIVERSITY SCHOOL OF PUBLIC HEALTH, NEW HEAVEN, ESTADOS UNIDOS.

e-mail:diane.mcmahon-pratt@yale.edu

Given the limitations of current drugs, immunomodulatory approaches for the treatment of *Leishmania (Viannia)* infection are being sought. However, due to the expense and time involved, it would be useful if clinical trials could be targeted. The chronic infection BALB/c mouse model of *L. (V.) panamensis* infection mimics the immune response profile that is found for human patients and therefore should provide useful for screening/exploring immunomodulatory approaches and mechanisms related to the control of infection. The TLR9 agonist, unmethylated CpG, was evaluated for treatment of established *L. (V.) panamensis* infection. Mice treated with CpG had significantly reduced lesion sizes compared to control mice. Surprisingly, when the immune response responses were analyzed directly after treatment, IFN γ , IL-10, IL-13 and IL-17 levels were significantly reduced in comparison to control infected mice; further, increased TGF- β levels were observed for the CpG treated mice. Notably, the FoxP3+CD25+CD4+ Treg cells in *L. (Viannia) panamensis* infected mice were found to express IFN γ , suggesting a deficiency in regulatory function; this population disappeared after CpG treatment. These results suggested that the down-regulation of immune and inflammatory responses by T regulatory cells were involved in disease amelioration. Further experiments involving the depletion and reconstitution of T regulatory cells (CD25+CD4+) have confirmed this observation. To further examine the functional role of T regulatory cells in chronic infection, Treg cells were selectively depletion in transgenic DEREg mice. Depletion of Treg cells resulted in disease exacerbation. Similar results were found for infected mice treated with the indoleamine 2,3-dioxygenase (IDO) inhibitor, 1-methyl tryptophan (1-MT), which is known to inhibit regulatory T cell development. Mice treated with 1-MT, developed larger lesions, higher parasite burdens, and increased cytokine production (IFN γ , IL-10, IL-13 and IL-17). Further, the transfer of Treg cells (from uninfected mice) to infected mice resulted in disease amelioration; this was accompanied with a significant reduction in IFN γ , IL-10, IL-13, TNF α , IL-6 and IL-17. Moreover, cellular recruitment to the site of infection and draining lymph node were diminished in the CpG treated mice. These results suggest that a defect in T regulatory cells may facilitate the ongoing non-resolving T cell response (Th1-Th2) in *L. (Viannia) panamensis* infection. Consequently, CpG treatment appears to act by inducing a beneficial Treg response that dampens lesion site cellular recruitment and pathology of leishmaniasis. These results are consistent with human studies suggesting that inflammatory responses contribute to disease caused by *L. (Viannia)* parasites. Consequently, this model may prove useful for the screening of potential immunomodulatory agents for treatment.

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MC002 - Promise and Considerations for Cysteine Protease Inhibitors as Anti-Chagastic Therapies

NICOLL-GRIFFITH, D.A.
MERCK, ESTRANGEIRO, USBEQUISTAO.
e-mail:deborah_nicoll-griffith@merck.com

Introduction and Objectives: The roles of cysteine protease enzymes in the biochemistry and infectivity of *Trypanosoma cruzi*, the causative agent of Chagas' disease, have been elucidated over the last 3 decades. Inhibitors of these enzymes, which act through trapping the cruzipain active site cysteine with an electrophilic war-head, hold huge potential as therapeutic agents but the promise of these has yet to be realized in clinical studies. This presentation will cover learnings from cysteine protease research in the trypanosomal field and recent advances in developing the cysteine protease inhibitor, odanacatib, which inhibits the human cathepsin K enzyme. Cathepsin K is a cysteine protease enzyme implicated in osteoporosis. The discovery of reversible nitrile-containing inhibitors with low nM potency in cruzipain enzyme assays will be presented (Beaulieu et al. Identification of potent and reversible cruzipain inhibitors for the treatment of Chagas disease. *Bioorg.Med Chem.Lett.* 20:7444-7449 (2010)). Key inhibitors were tested versus the *T. cruzi* parasites in vitro against both the epimastigote and amastigote forms. Two compounds with acceptable in vitro potency, bioavailability and pharmacokinetics were tested in a murine model of *T. cruzi* infection. The study objectives were to examine whether basic and neutral inhibitors were cidal in these systems and to determine the tolerability of effective doses in the murine model of infection.

Results: Several reversible nitrile-containing cruzipain inhibitors with IC50s as potent as 1 nM were tested in epimastigote and amastigote assays and showed cidal activity, as measured by trypan blue exclusion, 3H-thydimine incorporation or Giemsa staining. There was a correlation between potency against cruzipain and the parasite EC50. However, an almost 1000-fold shift was observed. Compounds which were nM against the enzyme were in the low μ M range when tested against the parasites in vitro. Both neutral and basic compounds showed similar efficacy. Two key compounds, Cz007 and Cz008 with cruzipain IC50s of 1.6 and 1.7 nM, respectively, were tested in an in vivo murine study using the Brazilian strain of *T. cruzi*. After infection, the compounds were dosed orally by administration in chow for 28 days (Day3 to Day30) with nominal dosing of 3, 10 and 50 mg/kg.day. This was followed by ~2 months of no drug treatment. Finally, inhibition of the immune system with cyclophosphamide treatment (200 mpk) was effected on Day100 and the mice were followed to Day120 looking for recrudescence. Efficacy was demonstrated by measurement of blood parasitemia though-out the experiment and by quantitative PCR in blood, heart and esophagus at sacrifice. Blood parasitemia was reduced in all treatment groups but had not reached undetectable levels at the termination of treatment on Day30. Real time PCR of plasma, esophagus and plasma collected at sacrifice demonstrated reduced parasitemia in all treatment groups. Results were similar to, or better than, the control (50 mg/kg.day benznidazole). The best treatment was Cz007, a neutral compound, at 3 mg/kg.day. Bioanalysis of blood from satellite animals indicated that the Cz007-treated mice have <50 nM blood levels in the 3 mg/kg.day treatment group. Cz007 was well tolerated with no mice deaths at any point in the study. Harvested blood and esophagus showed no PCR signal for *T. cruzi*. One animal showed positive PCR in heart tissue near the limit of detection. The other dose groups of Cz007 and the basic compound, Cz008, were not as effective by PCR.

Conclusions: This study indicates that reversible nitrile-containing cruzipain inhibitors offer promise as anti-Chagasic agents. Cz007, a neutral compound, was well tolerated and showed good efficacy at 3 mg/kg.day with oral dosing in a murine model of infection.

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**MC003 - The *Trypanosoma cruzi* Infection in Humans and Experimental animals:
Parasite-free Chagas Heart Disease in the chicken model**

TEIXEIRA, A.R.L.C.; NITZ, N.; HECHT, M.M.
UNB, LAGO NORTE, DF, BRASIL.
e-mail:ateixeir@unb.br

The *Trypanosoma cruzi* autochthonous in America is now present in all Continents. The human acute *T. cruzi* infections can be asymptomatic but chronically infected individuals die of Chagas disease. The parasite mitochondrial kDNA minicircle transfer to the genome of chagasics can explain the pathogenesis of the disease; in Chagas cases with evident cardiomyopathy the kDNA minicircles integrate mainly in retrotransposons at several chromosomes, but the minicircles are detected also in coding regions of genes that regulate cell growth, differentiation, and immune responses. An accurate evaluation of the role played by the genotype alterations in the autoimmune rejection of self-tissues in Chagas disease is achieved in the crosskingdom chicken model system refractory to the *T. cruzi* infections. The inoculation of *T. cruzi* in embryonated eggs prior to incubation generates parasite-free chicks, which retain the kDNA minicircle sequence mainly in the macrochromosomes coding genes. The crossbreeding transfers the kDNA mutations to the chicken progeny. The kDNA-mutated chickens develop severe cardiomyopathy in adult life and die of heart failure. The phenotyping of the lesions reveals cytotoxic CD45, CD8 $\gamma\delta$, CD8 α T-lymphocytes carry out rejection of the chicken heart. These results suggest that the inflammatory cardiomyopathy of Chagas disease is a genetically driven autoimmune disease.

Biography

Antonio R. L. Teixeira received his MD degree from the Faculty of Medicine of the Federal University of Bahia, Brazil. From 1971 to 1974 he was a Research Fellow in Pathology at the New York Hospital, Cornell University Medical College. He trained at the L' Institut d'Imunogénétique et Cancerologie in Villejuif, France, and received his PhD Degree in Pathology from the Federal University of Minas Gerais, Brazil. Dr. Teixeira conducted Post-Doctoral studies at the National Institutes of Health, USA. His main research interest is epidemiology, clinics, and pathogenesis of the human Chagas disease. Dr. Teixeira is the founder of the genetically driven autoimmune theory in Chagas disease.

MC004 - Characterization of *Lutzomyia longipalpis*-pathogen interaction
TELLERIA, E.L.¹; DI-BLASI, T.¹; NUNES, B.T.¹; SANT ANNA, M.R.V.²; PROBST, C.M.³;
PITALUGA, A.N.¹; RAMALHO-ORTIGÃO, J.M.⁴; DILLON, R.²; BURLEIGH, B.⁵; TRAUB-
CSEKO, Y.M.¹

1. INSTITUTO OSWALDO CRUZ, FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL; 2. SCHOOL OF HEALTH AND MEDICINE, LANCASTER UNIVERSITY, LANCASTER, ENGLAND, REINO UNIDO; 3. INSTITUTO CARLOS CHAGAS – FIOCRUZ, CURITIBA, PR, BRASIL; 4. KANSAS STATE UNIVERSITY, KANSAS, ESTADOS UNIDOS; 5. HARVARD SCHOOL OF PUBLIC HEALTH, HARVARD UNIVERSITY, BOSTON, ESTADOS UNIDOS.

e-mail: ytraub@ioc.fiocruz.br

Lutzomyia longipalpis is the major vector for visceral leishmaniasis in Brazil. We are studying immune responses of this vector. Insect innate immune response pathways are: Toll, JAK-STAT and IMD. We are characterizing these pathways both in insects and in the *L. longipalpis* LL5 cell line. We have determined a role for the IMD pathway in the vector infection by *Leishmania* through the silencing of the repressor Caspar. We have also identified an IMD response in LL5 cells through the silencing of genes in the three pathways and the effect of this silencing on the expression of the anti-microbial peptides defensin, cecropin and attacin. We also investigated the expression of a defensin along insect development and in response to bacteria and *Leishmania* infection (both oral and injected). We observed a differential modulation of defensin expression in relation to Gram-positive and negative bacteria and also to route of infection.

We have previously identified a nonspecific antiviral response in LL5 cells in response to transfection with double stranded RNA (dsRNA). This was the first report of this kind of response in an insect cell line. We are presently identifying the mechanisms by which LL5 cells recognize dsRNA, using various approaches. We have performed deep-sequencing of transcripts from cells transfected with dsRNA or mock-transfected (control). These data are under analysis. We have also investigated the involvement of exosomes in the anti-viral response. Exosomes were observed both in transfected and mock-transfected cells by electron microscopy. No gross differences in protein composition were seen by one dimensional protein gels. The exosomes protein composition was determined by mass spectroscopy and these data are under analysis. miRNAs were detected in the exosomal fractions. These are presently being sequenced in search for differential small RNAs expression in cells transfected with dsRNA.

In previous experiments a *Leishmania* protein was identified with the potential to interact with the sandfly gut. A monoclonal antibody against this protein was obtained. We used this antibody in attachment inhibition assays, using dissected guts (ex-vivo) and artificial infection (in vivo) with *Leishmania*. When the New World pair, *L. longipalpis* and *Leishmania chagasi*, was used, no inhibition of attachment or infection were observed. On the other hand, in the Old World pair *Phlebotomus papatasi* and *Leishmania major*, a significantly lower ex-vivo attachment and in-vivo infection were observed, thus confirming a role for this protein in some sandfly-parasite interactions.

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MC005 - Development of an oral vaccine platform based on the protective properties of surface proteins of the intestinal parasite *Giardia lamblia*.

LUJAN, H.-

UCC - CONICET, ESTRANGEIRO, ARGENTINA.

e-mail:hlujan@ucc.edu.ar

Despite the impact of world-wide vaccination programs, which have significantly reduced the incidence and mortality of infectious diseases, there is still a great necessity to develop novel, cheap and safe innovative vaccination strategies inducing long-lasting immunity. Promising approaches include innovative delivery systems for efficient antigen-presentation. Since most infectious agents invade the organism via mucosal surfaces, adaptive mucosal immunity plays a central role in protecting the host against infections. Oral administration of vaccines represent a very attractive option, notably because it is non invasive and suitable for mass vaccination. However, the main impediment for oral vaccine development has been that orally administered antigens are easily destroyed by the gastrointestinal tract or potentially capable of inducing immune tolerance. The intestinal parasitic protozoan *Giardia lamblia* expresses at its surface variant-specific surface proteins (VSPs) that are extremely resistant to the low pH of the stomach as well as to intestinal proteases, allowing the parasite to survive in the harsh environmental conditions of the small intestine. These VSPs are able to induce potent mucosal and systemic immunity against this diarrhea-causing parasite upon immunization via the oral route. In addition, It has been reported that retrovirus-based virus-like particles (VLPs) given by injection are efficient immunogens to induce both cellular and humoral responses, particularly inducing potent neutralizing antibody responses. We thus hypothesized that the expression onto VLPs of *Giardia* VSPs should shield these particles for oral administration. This should result in efficient cellular immune responses against epitopes presented inside the particles and neutralizing antibody response against heterologous envelope protein pseudotyped onto the particles together with the shielding VSPs. To obtain a proof of principle and, simultaneously, to develop a potential vaccine candidate, we used Influenza Hemagglutinin (HA) as a vaccinal antigen, for which we have already established all the procedures to monitor cellular and humoral anti-HA immune responses, including challenge experiments with live virus. We produced our vaccines composed of VSP-HA chimeric proteins or HA-expressing VLPs covered with VSPs and HA and the corresponding controls. Our results clearly demonstrated that *Giardia* VSP can protect vaccinal antigens in the gastrointestinal track for oral administration of vaccines, generating strong T and B cell-mediated mucosal and systemic protective responses. The development of this universal platform for oral delivery of vaccines should have a broad application to different infectious diseases.

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MC006 - Blastocystis – friend or foe?

STENSVOLD, C.R.

STATENS SERUM INSTITUT, COPENHAGEN, DINAMARCA.

e-mail:run@ssi.dk

Blastocystis belongs to the genetically heterogeneous group of Stramenopiles and is the most common, non-yeast, eukaryotic organism found in the human intestine. We estimate that > 1 billion people host *Blastocystis*. Morphologically identical *Blastocystis* organisms exhibit remarkable genetic diversity and currently comprise 13 ribosomal lineages (the so-called subtypes (STs)), that differ genetically by up to 15% and therefore could be considered discrete species. In most countries humans are primarily colonised by ST3, followed in prevalence by ST1, ST2 and ST4. ST5—ST9 appear to be zoophilic with occasional transmission to humans while the others (ST10—ST13) have not been detected in humans to date. Molecular epidemiological studies of patients with gastrointestinal symptoms and healthy individuals are invaluable in determining the role of *Blastocystis* in health and disease. Recently, we and a Spanish research team independently found strong ST4 predominance among patients presenting with acute diarrhoea and data generated in the UK and Denmark show an excess of ST4 among IBS clinic patients.

Whole genome sequencing (WGS) and comparative genomic analysis are essential and appropriate tools for identifying species- and strain-specific differences in virulence/attenuation, population structure, host-pathogen interactions, mechanisms of drug resistance and many other topics of clinical, epidemiological and evolutionary significance. The complete genome of *Blastocystis* sp. ST7 was published recently. However, ST7 is common in birds but uncommon in humans, and given the extreme genetic diversity of *Blastocystis*, analysis of the ST7 genome may be of limited value in studies aiming to study virulence genes and clarify the clinical significance of *Blastocystis* in humans. We are planning to perform WGS of *Blastocystis* sp. ST1—ST4 with a view to identifying genetic evidence of subtype-dependent differences in pathogenicity by comparative genomic analysis.

MC007 - Genetic manipulation of the mosquito's innate immunity to fight malaria
DONG, Y.¹; DAS, S.¹; CIRIMOTICH, C.¹; SOUZA-NETO, J.A.²; MCLEAN, K.¹; DIMOPOULOS, G.¹
1. *JOHNS HOPKINS BLOOMBERG SCHOOL OF PUBLIC HEALTH, BALTIMORE-MD, ESTADOS UNIDOS*; 2. *CDTS/FIOCRUZ, RIO DE JANEIRO, RJ, BRASIL*.
e-mail: jneto@cdfs.fiocruz.br

A causative agent of human malaria, *Plasmodium falciparum*, is transmitted by *Anopheles* mosquitoes. The malaria parasite is under intensive attack from the mosquito's innate immune system during its sporogonic development. We have used genetic engineering to create immune-enhanced *Anopheles stephensi* mosquitoes through blood meal-inducible expression of a transgene encoding the IMD pathway-controlled NF- κ B Rel2 transcription factor in the midgut and fat-body tissue. Transgenic mosquitoes showed greater resistance to *Plasmodium* and microbial infection as a result of timely concerted tissue-specific immune attacks involving multiple effectors. The relatively weak impact of this genetic modification on mosquito fitness under laboratory conditions encourages further investigation of this approach for malaria control.

MC008 - New insights concerning P21-His6 biological activity and protozoa intracellular traffic
SILVA, C.V.¹; CLEMENTE, T.M.; RODRIGUES, A.A.; MACHADO, F.C.; DOS SANTOS, M.A.
UNIVERSIDADE FEDERAL DE UBERLANDIA, UBERLANDIA, MG, BRASIL.
e-mail: silva_cv@yahoo.com.br

Trypanosoma cruzi P21 was recently characterized and its biological activity during parasite cell invasion has been studied. For that purpose, its recombinant form (P21-His6) was used in vitro during phagocytosis assays. We observed that the recombinant protein was able to upregulate phagocytosis of *T. cruzi* extracellular amastigotes, *Leishmania amazonensis* promastigotes, *Toxoplasma gondii* tachyzoites and also zymosan. This pro-phagocytic activity relied on host actin polymerization induced by the protein. Moreover, our results showed that P21-His6 binds to CXCR4 chemokine receptor and triggers signaling pathways dependent on PI3-kinase, AKT, m-Tor, ERK 1, MEK 1/2 and n-RAS. Also, we have observed that this protein shows a chemokine-like activity recruiting neutrophils and macrophages in vitro and in vivo. Taking together, these data revealed that P21 might be an important component for *T. cruzi* evasion from host immune system and also that its recombinant form represents an important tool for phagocytosis and chemotaxis assays. Financial Support: FAPEMIG, CAPES and CNPq

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