Opening Conferences

OC001 - QUARENTA ANOS DE UMA INICIATIVA QUE DEU CERTO

COLLI, W.[*1]
[1.] *IQUSP, SAO PAULO, SP, BRASIL.*
e-mail:walcolli@iq.usp.br

No início da década de 70 contavam-se nos dedos da mão os grupos que procuravam desenvolver uma Parasitologia moderna com abordagens moleculares que fugissem um pouco do simples diagnóstico microscópico dos parasitas. Essa década foi rica em iniciativas de apoio à ciência. Havia uma fermentação que tinha a ver com o desenvolvimento da Bioquímica, Biologia Molecular, Imunologia e Biologia Celular como ferramentas para aprofundar o conhecimento do que, até então, era apenas descritivo.

Nessa época foram duas as principais iniciativas que resultaram no fortalecimento da pesquisa em Protozoologia: a instituição do PIDE (Programa Integrado de Doenças Endêmicas) e a organização de reuniões que transformaram nossa protozoologia, eminentemente clássica em molecular.

Uma primeira reunião foi programada para a sede do CNPq no Rio de Janeiro em 20-21 de junho de 1974 e convocada por José Ferreira Fernandes, à época Professor do Departamento de Bioquímica do recém instalado Instituto de Química da USP. Essa convocação formalizada por Ferreira tinha na retaguarda a ação estratégica de Zigman Brener, da UFMG e do Centro de Pesquisas René Rachou e Firmino Torres de Castro, do Instituto de Biofísica da UFRJ.

Desse encontro nasceu a ideia de promover reuniões anuais e Zigman Brener foi indicado para organizar a primeira. Ele escolheu o Hotel Glória de Caxambu na esteira de seus colegas da Sociedade Brasileira de Bioquímica Carlos Ribeiro Diniz, Marcos Mares-Guia e Giovanni Gazzinelli que já organizavam, desde 1972, reuniões anuais nesse mesmo hotel. Os motivos eram vários e simples: aproximada equidistância dos três maiores centros produtores de ciência na área, uma cidade bucólica em que, à exceção de visitar o Parque das Águas, pouco há a fazer a não ser ficar no Hotel discutindo ciência e, finalmente, a tradição do Hotel Glória de receber convenções e congressos. A primeira reunião que ocorreu entre 3 e 5 de novembro de 1975 teve 25 participantes.

A partir daí formou-se um pequeno comitê de ex-organizadores que escolhiam os próximos. Esse comitê foi conhecido pelo nome de Comitê Fantasma. Após as 11 primeiras reuniões verificou-se que não era mais possível continuar a organizá-las sem uma sociedade que lhes desse suporte administrativo. Havia necessidade de um CNPJ, ainda que as agências financiadoras nunca tivessem glosado as prestações de contas dos organizadores. Decidiu-se, então, fazer uma campanha para que os membros da comunidade que frequentavam as reuniões de doença de Chagas se associassem à Sociedade Brasileira de Protozoologia. Em novembro de 1985, os novos sócios dessa sociedade compareceram em massa à assembleia geral e elegeram Wanderley de Souza como primeiro presidente da nova era. Durante um tempo as reuniões do grupo de Chagas e da SBPz eram mantidas separadas, mas no mesmo período e no mesmo Hotel. Em 2003, o Comitê Fantasma se auto-extinguiu e a partir de 2004 as diretorias da SBPz assumiram o congresso em definitivo. Nesse ínterim mudou-se o Estatuto a fim de manter a secretaria e a tesouraria na cidade de São Paulo e introduzir a figura de um novo Diretor além do Presidente e do Vice-Presidente, representado no primeiro ano pelo Presidente Anterior e no segundo ano pelo Presidente Eleito.

Com o tempo o Congresso firmou-se internacionalmente, muitos pesquisadores do exterior pediam para ser convidados e pagavam suas despesas, ganhou um Hino, passou a outorgar 3 prêmios a fim de reconhecer a qualidade de seus membros e de seus estudantes e desfrutou de uma das grandes qualidades arquitetônicas do Hotel Glória que é o bar da piscina. Por lá passam todos, param para conversar e até para tocar violão e atabaque.

OC002 – MEMÓRIAS DO PRÊMIO ROITMAN

ROITMAN, I.[*1]
[1.] *NÚCLEO DO FUTURO DA UNB, BRASILIA, DF, BRASIL.*
e-mail:iroitman@uol.com.br

O prêmio Roitman foi instituído na decada de 70 do século passado, com o objetivo de proporcionar um ambiente informal nas Reuniões de Pesquisa Básica em Doença de Chagas que se realizavam anualmente em Caxambú, Minas Gerais. Essa informalidade criou um ambiente propício para a interlocução entre os pesquisadores e o grande número estudantes que compareciam ao evento. Os prêmios nas categorias nacionais masculino e feminino e na categoria internacional eram concedidos baseado no percurso acadêmico dos/as candidatos/as e pela ingestão de bebida alcoólica durante o evento. No início da reunião era dado conhecimento público das regras do prêmio. Ao final em cerimônia concorrida o tema do prêmio (rótulo de bebida alcoólica que era entregue ao vencedor) era objeto de análise e reflexão antes do solene momento de entrega dos prêmios. O prêmio Roitman foi encerrado no final do século XX.

Closure Conference - CC001 - LEISHMANIASIS: A PURINERGIC POINT OF VIEW

AFONSO, L.C.C.[*1]
[1.] *UFOP, OURO PRETO, MG, BRASIL.* - e-mail:afonso@nupeb.ufop.br

Due to the lack of a de novo pathway for the biosynthesis of purines, *Leishmania* parasites depend on the uptake of these molecules for their survival and proliferation. On the other hand, extracellular ATP and adenosine exert an important role in the control of inflammatory response in the host. ATP is generally perceived as a danger signal that stimulates inflammation while adenosine, acting on specific receptors, presents anti-inflammatory properties. Over the last twelve years our laboratory has focused on the role of purines on the control of the immune response during infection by *Leishmania* parasites and, more recently, on the influence of these molecules on metacyclogenesis development. Our results have shown that the lack of purines, which may occur in the midgut of infected sandflies after the passage of the blood meal, may trigger the development of metacyclic promastigotes in the insect vector. To obtain purines from the extracellular medium, *Leishmania* parasites express on the cell membrane at least two enzymes involved in the hydrolysis of phosphate groups from extracellular nucleotides leading to the production of adenosine which is, then, transported into the cytosol to feed the purine salvage pathway. We also have shown that the presence of ectonucleotidases on the parasite's membrane correlates with the ability of the parasite to infect macrophages in vitro and also with lesion development in the murine model of infection. This interference is probably related to the ability of extracellular adenosine to inhibit the inflammatory response. In addition to the participation of parasite's molecules in the modulation of the immune response, our data also show that infection by *Leishmania* promastigotes modifies the host immune response by increasing the ability of the infected cell to produce extracellular adenosine. Thus, dendritic cells and macrophages infected with *Leishmania* increase the expression of CD39 and CD73 on their surfaces. These enzymes are involved in the extracellular hydrolysis of ATP to adenosine and have been shown to contribute to down modulation of the immune response. In fact, our results show that infection of dendritic cells by *L. amazonensis* leads to a decreased capacity of these cells to present antigen to T cells by a mechanism that involves the expression of CD39 and CD73 and activation of the A2b adenosine receptor. Finally, we have recently demonstrated that *Leishmania braziliensis* isolates obtained from mucosal lesions present higher ectonucleotidase activity than those from derived from cutaneous lesions. In the murine model, these isolates cause a delay in the establishment of the immune response, probably due to the inhibition of dendritic cell activation. A significant correlation between the level of ectonuclotidase activity and the delay in the onset of the immune response was observed. In summary, our results point to an important role of purines in *Leishmania* biology, modulating not only the differentiation of the parasite into infective metacyclic promastigotes but also interfering with the establishment of the immune response in the infected host via the production of extracellular adenosine by the parasites enzymes as wells as by the induction of adenosine production by the infected cells and activation of specific adenosine receptors. The study of the molecules involved in these phenomena may provide future targets for prophylactic as well as therapeutic protocols. **Supported by:**CNPq, FAPEMIG, CAPES, UFOP

CO001 - RISK ASSESSMENT OF INFECTION BY *GIARDIA* AND *CRYPTOSPORIDIUM* IN DRINKING WATER

SATO, M.I.Z.[*1]
[1].*CETESB, SAO PAULO, SP, BRASIL.*
e-mail:minesato@osite.com.br

Microbial risk assessment has been a valuable tool for setting health-based targets and consequently for validation of water safety plans. These risk assessment models take into account the raw water quality, treatment effects, water quality changes during the distribution and/or storage and drinking-water consumption to provide an estimate of consumer exposure to contaminants. The World Health Organization (2011) is presently encouraging the countries to use the risk assessment coupled with risk management as a more effective tool for the control of water safety in addition to compliance with end-products standards. This new approach focuses all the critical points since the watershed to the tap water (Water Safety Plans).

The quantitative microbiological risk assessment (QMRA) approach is now well established for several pathogens (bacteria, viruses and protozoan) including *Giardia* and *Cryptosporidium* and it allows to health authorities and water suppliers to manage decisions based on quantitative estimates. The USEPA recommends that any drinking water treatment process should be designed to ensure that human populations are not subjected to risk of infection greater than 1:10,000 for a yearly exposure. (Hunter & Fewtrell, 2001).

Among the waterborne pathogens, protozoa pose major challenges to design and maintenance of safe water supplies (Zmirou-Navier *et al*. 2006). The protozoan parasites *Giardia lamblia* and *Cryptosporidium hominis* are recognized as important waterborne disease pathogens and are associated with severe gastrointestinal illness. Recent reviews about waterborne outbreaks reported these parasites were the most frequently identified etiologic agents in the last 12 years in the United States (Craun *et al.* 2006) and were responsible for the higher numbers of waterborne parasitic protozoan outbreaks around the world (Baldursson & Karanis, 2011) In Brazil cryptosporidiosis and giardiasis represent an important cause of morbidity in children from 0 to 5 years (Franco & Cordeiro 1996, Carvalho Almeida *et al.* 2006, Gonçalves *et al.* 2006).

These pathogen parasites are of special concern for health and environmental authorities in developing countries considering the poor sanitary conditions even in more developed regions and the highlight of these organisms in waterborne outbreaks around the world. Data about these pathogens in source and drinking waters in Brazil are scarce (Sato et al, 2012).

This lecture will outline the QMRA approach and will report the application of this tool to assess risk of *Giardia* and *Cryptosporidium* infection through drinking water consume. The use of QMRA for the Water Safe Plans and in establishing drinking water quality criteria will also be discussed.

References

- Baldursson & Karanis 2011. *Water Research* , *45* (20): 6603-6614.
- Carvalho-Almeida *et al.* 2006. *Rev Inst Med Trop S. Paulo*; *48*(1): 27-32.
- Craun *et al.* 2006. *J Water Health*; *4* (Suppl. 2): 19-30.
- Franco & Cordeiro 1996. *Rev Soc Bras Med Tropical*; *29*(6), 585-91
- Gonçalves *et al.* 2006. *Clinics*; *61*(2): 119-26.
- Hunter & Fewtrell 2001. *Acceptable risk. In: Water Quality: Guidelines, Standards and Health*. Eds. Fewtrell L. & Bartram. IWA Publishing, London
- Sato *et al.* 2013. *Science of Total Environment, 442*: 389-396.
- World Health Organization 2011 *Guidelines for Drinking Water Quality: Volume 1 Recommendations*, 4rd ed. World Health Organization, Geneva
- Zmirou-Navier et al. 2006. *BMC Public Health 6,* 122. Published online 2006 May 3. doi: 10.1186/1471-2458-6-122.

CO002 - WATER AND PARASITES: CURRENT STATE AND FUTURE ASPECTS - MORE THAN 20 YEARS RESEARCH ON WATER-BORNE PROTOZOAN

KARANIS, P.[*1]
[1].*FACULTY OF MEDICINE UNIVE, NETTERSHEIM-HOLZMÜLHEIM, ALEMANHA.*
e-mail:panagiotis.karanis@uk-koeln.de

Water plays a role in the transmission of a significant number of waterborne parasites. The significance of giardiasis, cryptosporidiosis and toxoplasmosis has increased worldwide causing the highest disease burden among waterborne and foodborne pathogens. The ubiquitous protozoan Giardia, Cryptosporidium, Toxoplasma and other protozoan are subject of interest, due to the spread of (oo)cysts via water causing waterborne outbreaks in different parts of the world. The purpose of this presentation is to call attention to the evolution of research in the field of waterborne protozoan parasites and it consists of several parts:

a) Water detection and the relation of the possible accumulation of the parasites in water matrices: the different methods for the detection of Giardia, Cryptosporidium, Toxoplasma in drinking and environmental waters considering validations and applications of convetional and molecular tools for diagnosis and monitoring of waterborne protozoan pathogens. Beyond this, we offer an alternative strategy for the simple detection of the (oo)cysts in water matrices for routine screening and outbreaks investigations. It includes a combination of conventional and molecular tools (LAMP assay) for effective (oo)cyst recovery and detection in water sources following by PCR for genotyping of species. This application will be practical for the pre-detection of pathogens and screening of water samples followed by species identification by PCR and sequencing in the positive samples. The possible interactions, accumulation and distribution of the above protozoa in biofilms and water organisms will be discussed.

b) Waterborne outbreaks: the presentation will provide a comprehensive review of worldwide waterborne parasitic protozoan outbreaks that occurred and were published globally between 1900 – today. At least 534 outbreaks of human diseases due to the waterborne transmission of parasitic protozoa occurred and were reported during the time period from 1900 - 2011. However, in those countries that are likely affected most a lack of surveillance systems is noticeable. Countries that established surveillance systems did not establish an international standardization of reporting systems.

c) Clima changes and its impact to the distribution of waterborne parasitic diseases. The change of climate is an accepted fact of science and had affected the world's system in all times Climatic alteration may act as an enhancer of the parasitic protozoan infections and climate change will lead to a worldwide increase of the incidence of waterborne outbreaks of parasitic protozoan diseases during the next decades. However, the impact of global climate change on human waterborne protozoan diseases will be not the same in the different continents.

d) Cryptosporidium taxonomy: Genetic polymorphism within Cryptosporidium spp. is being detected at a continuously growing rate, owing to the widespread use of modern molecular techniques. The current status of taxonomy, genotyping, molecular phylogeny of cryptosporidia will be discussed.

e) Establishment of in vitro axenic culture of Cryptosporidium: The presentation will describe novel findings from culturing Cryptosporidium spp. in axenic culture system as well as the factors limiting the development of Cryptosporidium in cultivation systems. There is a natural pressure of Cryptosporidium parasite to develop outside of the host and the results suggest that long term culture and maintenance of Cryptosporidium stages is possible, if appropriate conditions could be defined. Nevertheless, this seems not to be a general phenomenon for Cryptosporidium isolates, and it needs further investigation in order to achieve the complete cycle and make possible the mass production of the parasite for in in vitro systems for further investigations and drug screening and to avoid animal experiments.

In the sum, we can say that a 'new era' in the research field of water-borne parasites and especially for Cryptosporidium is the horizont.

CO003 - MECHANISMS OF IMMUNITY AFTER GENETIC VACCINATION AGAINST EXPERIMENTAL *TRYPANOSOMA CRUZI* INFECTION.

VASCONCELOS, J.R.[*1]; DOMINGUEZ, M.R.[1]; ERSCHING, J.[1]; ARAUJO, A.F.S.[1]; VIRGILIO, F.S.[1]; RODRIGUES, M.M.[1]
[1].*UNIFESP, SAO PAULO, SP, BRASIL.*
e-mail:mrodrigues@unifesp.br

Heterologous prime-boost vaccination using plasmid DNA followed by replication-defective adenovirus vector provide long term immunity against experimental infections with a variety of pathogens including the human protozoan parasite *Trypanosoma cruzi*. Long-lived $CD8^+$ T cells with an effector memory (TEM) phenotype ($CD11a^{High}$, $CD44^{High}$, $CD127^{High}$ and $CD62L^{Low}$) are important mediators of the protective immunity. In the past few years, we have been studying the characteristics of the TEM cells activated after the infectious challenge with *T. cruzi*.

After challenge, we observed an increase in the frequency of specific $CD8^+$ T cells in the spleen. We confirmed that these cells were indeed TEM induced by vaccination, not recently activated naïve T cells, using the gzmBCreERT2/ROSA26EYFP transgenic mouse line which indelible labels with enhanced yellow fluorescent protein cells expressing Granzyme B after immunization. Not only these TEM were activated but they also strongly inhibited the activation of naïve specific T cells. These TEM cells are $KLRG1^+CD27^-CD43^-CD183^-$ and produce the anti-parasitic mediators IFN-g and/or TNF. The increased frequency of TEM in the spleen or the protective immunity they mediate were independent on T cell proliferation as treatment with the cytostatic toxic agent Hydroxyurea did not inhibit either one. In contrast, recirculation was critical for protective immunity as the administration of the drug FTY720 led to a strong accumulation of specific T cells in the lymph node and reversed protective immunity induced by vaccination.

Based on evidences that recirculation after challenge is critical for T-cell mediated protective immunity, we tested whether certain integrins, cell adhesion molecules (CAM) and chemokines were key mediators of this process playing a critical role during immunity against infection. Mice vaccinated with the heterologous prime-boost vaccine and challenged with parasites were treated with blocking antibodies to LFA-1 and/or VLA-4. Immunized mice treated with control Rat IgG or anti-VLA-4 antibodies controlled the parasitemia and survived the lethal challenge. In contrast, immunized mice treated with anti-LFA-1 or anti-LFA-1 and anti-VLA-4 displayed high parasitemia and all of them died after challenge. LFA-1 blockage neither diminished the frequency nor compromised the capacity of specific $CD8^+$ T cells to respond *in vitro* (IFN-γ and TNF). We performed similar experiments by vaccinating *icam-1*$^{-/-}$ or *cccr5*$^{-/-}$ mice. These mice controlled the infection at similar extension as wild type animals.

CONCLUSION: Recirculation possibly mediated by LFA-1, but not proliferation, is important for the protective function mediated by antigen-experienced $CD8^+$ TEM cells.

Supported by:CNPq, FAPESP

CO004 - THROWING LIGHT INTO DARK CORNERS &NDASH; INVESTIGATING THE CRYPTIC LIFE OF *TRYPANOSOMA RANGELI*

GRISARD, E.C.[*1]
1.UFSC, FLORIANOPOLIS, SC, BRASIL. e-mail:edmundo.grisard@ufsc.br

Trypanosoma rangeli is a hemoflagellate protozoan parasite infecting humans, wild and domestic mammals in an extensive geographical area in Central and South America. Presenting a sympatric distribution with *T. cruzi*, a controversial taxonomic position and a unknown life cycle in mammalian hosts, *T. rangeli* has an interesting and intriguing biology that lead us to investigate distinct aspects on the host-parasite and vector-parasite interaction and, ultimately, sequencing the parasite genome. Due such importance, studies from several distinct groups have pointed out the genetic plasticity of this taxon and characterized a variety of strains, genes, proteins or molecules.

Upon publication of the TriTryps genomes (*T. brucei*, *T. cruzi* and *Leishmania major*), as well as other pathogenic and non-pathogenic trypanosomatids, sequencing the non-pathogenic *T. rangeli* genome was in urgent need for large-scale investigations of the complex biology of these human parasites, allowing deeper investigations on the genetic, evolutionary and phylogenetic basis of pathogenicity by comparative genomics. Thus, along former studies on the cellular and molecular biology of *T. rangeli*, we have assessed the parasite genome to perform a comparative analysis of gene content, genome architecture and other characteristics aiming the understanding the biological behavior of this non-pathogenic trypanosome parasite.

Among the striking new features unveiled by genomic comparisons of *T. rangeli* SC-58 strain with *T. cruzi*, its closer relative, are the reduced number of copies of members of the MASP, Trans-sialidases and Mucins multigene families that are considered key virulence factors in the *T. cruzi* infection. Special emphasis was also dedicated to elucidate unknown biological aspects and key pathways related to host-parasite interactions such as immune evasion, RNAi machinery and anti-oxidant defense. Comparative phylogenomic analysis with the TriTryps genomes using over 1,500 orthologs has undoubtedly pointed out the proximity of this taxon to *T. cruzi* than to *T. brucei*.

The *T. rangeli* haploid genome is estimated to have ~27.7Mb, presents shorter subtelomeric sequences compared to *T. cruzi* and *T. brucei*, reveals intraspecific karyotype variability and the absence of minichromosomes. Among the 7,613 coding sequences described, 2,416 of which had their functional annotation validated, ~65% were hypothetical proteins and 44 were proteins of unknown function, for which, proteomic data is available.

Based on the generated *T. rangeli* genomic, transcriptomic and proteomic data, several genes/proteins are being study in detail, especially those showing differences from pathogenic trypanosomatids. Among these, we point out proteins involved in anti-oxidant defenses (Cystein synthase – CS and Cystathionine β-synthase - CBS), several proteins involved on host-parasite interactions (gp63, gp82, gp85, gp90, Oligopeptidase B) and proteins involved on cell cycle (Type II topoisomerase, Polo-like and Aurora kinases). Regarding RNAi, *T. rangeli* presents orthologs to *T. brucei* RNAi machinery genes but, except for DCL2, the other four components are truncated and the pathway is non-functional in this taxon.

Furthermore, transfection of *T. rangeli* with *T. cruzi* or Leishmania spp. genes involved on crucial steps on the host-parasite interaction has been used as a tool to assess the precise involvement of these proteins. So far, genes coding for the ornithine decarboxylase (ODC) from *L. brasiliensis*, trans-sialidase and gp82 from *T. cruzi* were transfected and their expression by *T. rangeli* promoted phenotypic changes on this non-pathogenic trypanosome while interacting with host-cells *in vitro*.

The sequencing of the *T. rangeli* genome allowed us to move onto large-scale proteomic approaches to assess similarities and differences with pathogenic trypanosomatids as well as to address unknown aspects of the parasite life cycle. At this point, two major points of interest are under study, i) the proteomic maps of the parasite epi-to-trypomastigote differentiation and ii) the surfaceome of trypomastigote forms. The surfaceome *in vitro* differentiated *T. rangeli* trypomastigotes was assessed by both gel-free (LC–ESI-MS/MS) and gel-based (GeLC–ESI-MS/MS) approaches. A total of 138 *T. rangeli* proteins were identified, among which, 42 were exclusively identified for this species. In addition, immunoblotting assays using sera from experimentally infected allowed identification of *T. rangeli*-exclusive proteins (GP63-related and flagellar calcium-binding protein) as promising diagnostic targets. **Supported by:**CNPq, FINEP, CAPES and UFSC