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MEETING REPORT

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## The Net Closes on Trypanosomatids

Alberto MR Dávila, Kevin M Tyler\*

Departamento de Bioquímica e Biologia Molecular, Instituto Oswaldo Cruz-Fiocruz, Av. Brasil 4365, 21045-900 Rio de Janeiro, RJ, Brasil \*Dept. of Pathology, Northwestern University Medical School, 303 E. Chicago Ave., Chicago, IL, 60611, USA

At the turn of the last century, Africa faced an epidemic of sleeping sickness. For a while in the 70's, with the advent and targeted use of insecticides like DDT, it seemed the disease would be eradicated. Today, Africa faces another sleeping sickness epidemic, which it seems little better equipped to respond to than it was a century ago and sleeping sickness looks to be a disease which may be with us for a long time to come. For this reason, the *Third Internet Conference on Salivarian Trypanosomes and Trypanosomatids* was designed as a virtual world meeting specifically targeted at providing a real world focus for basic scientists working with trypanosomes. The conference was free, spread over a two-week period and consisted of six sessions, a focused debate and a poster session. The full proceedings are published in the *International Journal for Parasitology* 31(5-6), May, 2001 (<http://www.elsevier.nl/locate/ijpara>).

In statesman-like keynote addresses to conference, two of the most senior figures in the field, George Cross and J Richard Seed reviewed the key advances and failures of the last century. Their presentations tackled the potential impact of current new technologies and the political, social and economic realities in which the pipeline from science to public health must operate in order to tackle sleeping sickness. They also went on to address the future of trypanosome research and integrated control approaches in public health.

### BIOLOGY AND ULTRASTRUCTURE

Keith Gull kicked off the scientific proceedings with a philosophical review of kinetoplastid cell biology. He asked and answered questions about how to utilize the vast amounts of information and new tools generated by current genome projects to pursue fundamental biological questions associated with the kinetoplastida in a post genomic era. The mechanisms by which the catenated mass

of minicircles and maxicircles composing the kinetoplast is replicated and segregated with fidelity in each cell cycle has been one of the most intriguing areas of research in kinetoplastid biology. James Morris and colleagues from the Englund lab reviewed current thinking on kinetoplast replication going on to propose a model in which the kinetoplast actually spins to ensure a uniform distribution of minicircles in the periphery. Michel Dollet discussed the extreme heterogeneity within the genus *Phytomonas* (plant trypanosomatids) and described the characterization of a monophyletic group of phloem restricted trypanosomatids – the “phloemicola”. Peter Overath and colleagues discussed the evolutionary significance of their observation that the presence of highly immunogenic surface proteins was peculiar to the salivaria, with non-salivarian trypanosomes having weakly immunogenic mucin-like carbohydrate-rich glycoproteins on their surface instead. Finally, one of us (KMT) presented an updated life cycle for *Trypanosoma cruzi* highlighting unresolved areas of interest.

### BIOCHEMISTRY AND DRUG DEVELOPMENT

The first presentation of the session by Fred Opperdoes and Paul Michels reviewed the progress made in developing glycolysis enzymes as drug targets since the discovery that glycolysis is partially compartmentalized in the trypanosome's glycosome. They then moved on to describing current work characterizing the enzymes of the pentose phosphate pathway. Thomas Seebeck presented a digest of his lab's recent work investigating cAMP signalling in trypanosomes and highlighting the characterization of new genes in the trypanosomes cAMP signalling pathway (including a new family of phosphodiesterases) as possible therapeutic targets. A complimentary presentation from Mecia Oliveira demonstrated that disruption of inositol metabolism in *T. cruzi* was a valid target for chemotherapy. Together, these papers sparked considerable discussion as to the respective roles on inositol, calcium and cAMP signalling in trypanosome differentiation and prolifer-

eration. Herb Tanowitz presented data on the activation of the ERK 1/2, AP-1, ET-1 pathways by *T. cruzi* infection in the myocardium. He suggested that the vasculature plays a role in the pathogenesis of chagasic cardiomyopathy and discussed the implications for the use of palliative therapeutics in treatment of Chagas heart disease. Closing the session, Harry de Koning provided a comprehensive review of the manner in which current and prospective drugs for the treatment of sleeping sickness are believed to cross the parasite plasma membrane. In particular, emphasizing the role of the P2 adenosine transporter in the development of drug resistance and the difficulties which must be faced in order to overcome this resistance.

### MOLECULAR BIOLOGY

Piet Borst and Sebastian Ulbert started this session by reviewing the field which has, more than any other, popularized the study of *T. brucei*, the mechanism by which the trypanosome evades the host immune response-antigenic variation. His review focused on recent work on the mechanism of expression site switching and on the way inactive expression sites are kept silent. Expression sites were the primary topic of a timely and complementary review from Luc Vanhamme and colleagues of the Pays lab which explained what is known about the mechanism by which only one site is expressed and possible reasons for the multiplicity of expression sites. Wim Degraeve provided an update on the current status of the trypanosomatid genome projects (*Leishmania major* 'Freidlin', *T. brucei* TREU927/4, and *T. cruzi* CL-Brenner) helpful links for genome-related resources and key contacts. Damar da Costa-Pinto and colleagues in the Traub-Cseko lab presented work in *Leishmania* utilizing GFP as a fluorescent marker for the subcellular localization of constructs. In particular trafficking to a lysosomal compartment of a GFP/cysteine protease chimaeric protein was investigated. Hitherto the conference it has been widely speculated that no polymerase II promoters existed in trypanosomatids. However, in the final talk of the session Anish Das and colleagues from the Bellofatto lab were able to convincingly demonstrate that Spliced Leader RNA gene transcription (in *Leptomonas seymori*) is driven from a polymerase II promoter. They also provided a characterization of the promoter and its associated promoter binding proteins and details of the promoter's transcriptional activity.

### IMMUNOLOGY AND PATHOLOGY

The controversy over the mechanisms by which Chagas heart disease pathogenesis occurs was revisited by the first two talks of the session. It is

well established that both parasite directed immune and autoimmune responses are elicited during Chagas heart disease. It is also believed by most, that cardiac pathology is immune mediated. The causal linking of these observations is, however, still poorly construed –, as are the relative amounts of cardiac damage each immune component contributes. In the first presentation by Rick Tarleton, Occam's razor was invoked to argue that because parasites are persistent during heart disease, there is no reason to invoke autoimmunity as a mechanism of pathogenesis. However, in the session's second talk, Juan Leon and David Engman eloquently argued that, given the weight of circumstantial evidence that autoimmunity is present in some *T. cruzi* infected humans and mice it may have a role in pathogenesis. Indeed, they were able to demonstrate that the level of cardiac myosin autoimmunity induced during acute *T. cruzi* infection can be similar in character and magnitude to that of an established model of autoimmune cardiomyopathy. In another presentation addressing the pathogenesis of Chagas heart disease, Paola Minoprio reviewed the mechanisms, effects and implications of the polyclonal activation of lymphocytes which is characteristic of the disease. Sam Black and colleagues presented a review of the interesting and apparently novel mechanism of innate immunity of Cape buffalo to salivarian trypanosome infections in particular highlighting the respective roles of xanthine oxidase and serum catalase in parasite killing. Patrick de Baetselier from the Beschlin lab presented an extensive characterization of the effect that knocking out the trypanosome phospholipase C gene has on the immune response in a murine model. In essence, evidence was presented that the observed attenuation of disease caused by the knockout parasites resulted from a shift in the balance of the immune response towards a type II cytokine environment. Piscine cryptobiosis is a kinetoplastid disease of fish which causes significant damage to the annual catch world-wide, Patrick Woo's presentation reviewed the progress made in exploiting vaccines, drugs and transgenic fish to counteract the disease and highlighted parallels between cryptobiosis and the kinetoplastid diseases of mammals.

### EPIDEMIOLOGY

Michel Tibayrenc provided the rationale behind the creation of a centralized agency to counter emerging and re-emerging infectious diseases in Europe and set forth a triple mission for The European Center for Infectious Diseases: to provide surveillance and control, advanced research, training and teaching. Peter van den Bosche reviewed the epidemiological status of bovine trypano-

somosis in Southern Africa, emphasizing that control options vary in accordance with four different epidemiological situations that can be readily distinguished. Notably, control of tsetse with insecticide treated cattle is effective only when a large proportion of feeds are taken from cattle over a large area and when tsetse invasion can be sufficiently reduced. Discussion of the use of trypanosomosis vs trypanosomiasis took place after this presentation, an issue which has proved divisive between veterinary and medical fields. In keeping with the theme of increased communications between related disciplines, reconciliation was proposed by simply treating both terms as valid. Geoff Hide and Aimee Tilley described the use of mobile genetic elements (MGE) PCR for the molecular typing of *T. brucei* strains. MGE markers like RIME have the advantage that they are specific to trypanosomes, usually unlinked and have what can be approximated to a random distribution throughout the trypanosome genome. These advantages have potentiated a rapid, sensitive tool for discriminating between strains and investigating the epidemiology of sleeping sickness even in clinical isolates. Some discussion on the occurrence of mixed infections and selective pressure of field isolates in the lab took place with Wendy Gibson. John McDermott and Paul Coleman reviewed the current control strategies for tsetse-transmitted trypanosomosis in cattle in different geographic regions of sub-Saharan Africa. According to their comparison, the rankings of the effect of control strategies on reducing disease prevalence were vector control, vaccination, and drug use, in that order. Finally, Marc Desquesnes and one of us (AMRD) described the usefulness of a PCR-based assay to differentiate among trypanosomatid species using only one pair of primers.

#### VECTORS

In this session, Chris Schofield and Ian Maudlin called attention to the control of African trypanosomiasis. Referring to the declaration on the control of African trypanosomiasis, signed in the 36th session of the Organization for African Unity (OAU), they compared the operational challenges of that concerted effort with the Southern Cone initiative against Chagas disease. Charles Beard reviewed recent progress in a promising new area of control, the use of genetically modified endosymbiotic bacteria of the triatomine intestinal tract to make triatomine bugs refractory to infection by *T. cruzi* and hence break the life-cycle. A point about plasmid stability under field conditions and the possibility of recombination was raised in discussion which led to debate on the desirability of fixing such genes in the wild population since the

more stable the genetic transformation, the greater the potential efficiency and permanence of the approach. Complementary work on manipulating the microflora of the tsetse fly was presented by Colin Dale and Sue Welburn in which the endosymbiont "Sodalis" strain was engineered to avoid potentiating trypanosome susceptibility in tsetse. Francis Oloo reviewed the status and prospects for tsetse control in Eastern Africa. Discussion focussed on simple, cost-effective and user-friendly tactics that could be adopted by local communities, and highlighted the problems in maintaining such programs and the risk of reinvasion when programs lapse. Priscilla Machado and colleagues from the Edmundo Grisard's lab presented data on the susceptibility of *Rhodnius* species to different *T. rangeli* strains, suggesting the existence of high adaptation between strain and local vector. The last presentation of this session was the one from Alexandre Peixoto and colleagues describing new molecular markers for phlebotomine sand flies.

#### PHYLOGENY AND EVOLUTION

Hooman Momen opened this session reviewing some current problems in the systematics of trypanosomatids. His suggestion to include *Leishmania*, *Sauroleishmania* and *Endotrypanum* within a single genus because of their genetic affinity provoked considerable discussion. Wendy Gibson highlighted recent research on genetic exchange in *T. brucei* and called attention to the position of *T. brucei* in a molecular phylogenetic tree based on 18S ribosomal sequence, noting that the tree provided no clues to the likely existence of genetic exchange in trypanosome species other than Salivaria. The ensuing discussion focussed on molecular techniques for "trapping" the sexual stages of *T. brucei* as well as reports suggesting sexual process in several other trypanosomatids. Finally, Sergei Podlipaev offered us a review focussed on the diversity of insect trypanosomatids, which raised discussion on the lack of reference strains for detailed studies, the presence of endosymbionts, and the lack presence of trypanosomatids in early insects as Paleoptera.

#### POSTER SESSION

Notable among the posters was a phylogenetic comparison of elongation factor genes by Eric Leblanc and colleagues. Two reports by Brad McGwire first (with KP Chang) that loss of GP63 adversely affects *Leishmania* survival in macrophages and second (with DM Engman) that the mechanism of flagellar targeting for the flagellar calcium binding protein FCaBP is conserved between *Leishmania* and trypanosomes. A poster by Jeroen Saeij and Geert Wiegertjes showed evidence

of a role for NO in fish immunity to trypanoplasmas. Our pick for best poster, however, went to Edith Authié and colleagues which showed convincingly that vaccination with the cysteine protease of *T. congolense* (congopain) attenuated pathogenesis by neutralizing the enzymes activity without actually affecting the development of the infection.

#### CONCLUSIONS AND NEW INITIATIVES

The meeting was a great success with a good level of discussion. Transcending geographic barriers was one of our aims and conference presented research from 17 countries and from every continent. The level of attendance was beyond our expectations. In the first week alone we logged over 20,000 hits from about 1,500 machines (individual IP addresses) with 400-500 registering at the site. This kind of attendance is much higher than a specialist conference of this kind could ever realistically expect to field in the real world and was indicative of the huge amount of interest the conference generated. Detailed technical conclusions and recommendations are published in the proceedings.

The currency of science is information, and in recent years the internet has revolutionized the rate and scale at which information can be transferred. This is just as well since vastly more, pertinent information is now being generated and processed than ever before. The internet has a huge potential

to provide for enhanced communications between groups of scientists, between science and public health and between science and the public at large. For those interested in kinetoplastid disease the internet offers the prospect of an utopian vision for universally accessible, free, single site access to all kinetoplastid related internet resources. These could include textbooks, lecture series, genomic and proteogenomic databases, links to bioinformatics tools, conferences and symposia, specialist listings, discussion groups and even chat rooms. The conference was launched from trypanosome.com and we are hopeful that we will be able to use this site in the future as a staging point from which to unify and rationalize existing internet resources whilst adding more as the need arises. Our next step in realizing this aim will be to situate a new e-journal (*Kinetoplastid Biology and Disease*) at the site. This should allow the reporting on current projects and advances and should encourage debate of topical issues throughout the site. As for future conferences, the next will be held in 2002 but we will aim to hold at least one focussed symposium before then – the proceedings of which to be published in the new e-journal. In writing this article for *Memórias do Instituto Oswaldo Cruz* what we aim to report is not a *fait accompli* but rather the initiation of a project facilitating the use of the web to take aim at kinetoplastid diseases.