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PROTIST NEWS

Meeting Report: WorldLeish 2, Crete, Greece, May 20–24, 2001

A Provocative Foreword (L.S.)

For historical reasons the study of the major parasitic trypanosomatids such as the Old World and New World *Leishmania* and the African and South American trypanosomes has always been somewhat insular. This insularity was caused by the peculiarities of the diseases caused by these parasites, the specific vector biologies and epidemiology involved, and the geographical localization of the diseases (which of course determines consumer interest!). With the advent of molecular analysis it has become clear that there are no major biological or molecular differences between trypanosomatids and that cross fertilization between these fields is beneficial and even imperative. I would argue for functional or problem-oriented meetings rather than genus-specific meetings. However when a field reaches a certain density of researchers, a realistic compromise must be found between inclusiveness and exclusiveness, and it is beneficial to clinicians and field biologists to be able to talk to biochemical and molecular types and vice-versa. And of course there is that warm, fuzzy feeling of being surrounded by your peers who are working on the same organism.

The Meeting (L.S.)

The first international symposium of "leishmaniacs" (copyright, I believe, is jointly held by Chuck Greenblatt and K.P. Chang!) was held in Istanbul in 1997 and it was extremely successful, so that a second meeting was held in Crete, May 20–24, 2001. The Crete Symposium meeting (had over 400 participants, showing that there are a lot of people working on various *Leishmania* parasites and diseases throughout the world. It was especially nice to see so many scientists from countries where leishmaniasis is endemic or epidemic. Part of the problem of Leishmanial insularity was solved by inviting people to talk on results using other trypanosomatids such as *Trypanosoma* and *Crithidia*.

There were 8 general lectures on topics ranging from genomics, vaccine development, chemotherapy and evolution. Following the general lectures, there were three simultaneous sessions with short talks on cell and molecular biology, immunology and epidemiology, chemotherapy and drug development, diagnosis, reservoirs, and sand fly vectors. There were also a large number of posters in afternoon sessions. From the initial "Leishmaniac Manifesto" of K.P. Chang to the breaking of dishes during the Greek dancing in the hotel Taverna, this was a extremely well organized and interesting meeting in a fantastic locale. The meeting and audio-visual facilities were also excellent, including computer projectors for Powerpoint presentations. Thanks and appreciation should be given Ketty Soteriadou, Ziya Alkan and K.P. Chang for a great job!

I (L.S.) have created a web site (with permission of the Organizing Committee) with pdf files of the abstracts from this meeting.

The URL is: http://www.rna.ucla.edu/crete/index.html

I have also taken the liberty of entering all the attendees into the database of the Molecular Parasitology Network at http://www.arc.ucla.edu/mpn/faculty.cfm. Each person can log on and enter and revise their own information.

A Modest Proposal (L.S.)

An interesting poster (I. Bauer, James Cook University, Australia) showed that few tourists to endemic countries actually knew anything about Leishmaniasis, and this probably extends to most researchers other than true leishmaniacs, including members of competitive grant review committees. This reviewer suggests that perhaps this lack of recognition is related to the non-descriptive name of the disease, which most people can not spell or even pronounce.

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I don't want to cast any aspersions on the good Dr. Leishman, but look for example at *Sleeping Sickness*, the common name for African trypanosomiasis, or *River Blindness*, the common name for onchocerciasis. A major problem of course is that leishmaniasis has multiple syndromes, depending on the parasite species and the host response. But I feel that we desperately need a new name (or names) for our disease so we can better communicate with people outside our field! Trends in Parasitology may be the venue for a competition to seek an appropriate and useful new name.

The Science

There was a lot of really good science at this meeting. One problem was that we were torn between the interesting sessions that we would have attended, but could not because of the three concurrent streams. This took away somewhat from the purpose of the meeting which was to cross fertilize all these fields and start a dialogue between the cellular, the molecular and vector biology folk. However there was plenty of time during the poster sessions and meals to meet colleagues and discuss science. We cannot begin to cover all of the 257 different presentations, but L.S. in addition to provocative proposals will attempt to present the highlights from his point of view, and E.H. will provide a summary of interesting vaccine-related developments and problematic issues.

Genomics and Proteomics (L.S.)

The various genome sequencing projects have brought a revolution to biology and this is also occurring in parasitology. The *Leishmania* genome sequencing is progressing, (although not as fast as the *T. brucei* and the *Plasmodium* sequencing projects) and a *T. cruzi* project is just initiating. The analysis of several small chromosomes and random genomic fragments from *Leishmania* (Peter Myler and Ken Stuart, Seattle Biomedical Research Institute; Al Ivens, Sanger Center, UK) has already led to new ideas on gene organization and regulation. Approximately 24 Mb of the 33.5 Mb genome of *L. major* has already been sequenced. Fifty percent of this sequence is coding, with one gene approximately every 3.7 kb. There is extensive synteny between trypanosomatid species, which augurs well for cross species comparisons. Apparent large transcriptional units, both divergent and convergent organized were observed in several chromosomes. UV inactivation kinetics is being used to zero in on the elusive Pol II promoter (P. Myler, SBRI) and the equally elusive centromere sequences (C. Blaineau, Lab Genome des Parasites, Monpellier). Preliminary proteomic analyses were performed using two dimensional gels and even mass spectrometry (K. Stuart, SBRI; P. Nugent and D. Smith, Imperial College, U.K.; and M. Ouellette, CNTR, Quebec). Unfortunately the technical problems associated with performing mass spectrometry identification of proteins from spots on 2D gels have not yet been solved (M. Ouellette, CNTR), but some promising results have been obtained for differentiation of *L. donovani* (M. Thiel, Bernard Nocht Inst., Hamburg). And microarray technology is being applied to problems of stage specific gene expression in *Leishmania* even before completion of the genome sequence (J. Blackwell, Welcome Trust Lab., Cambridge).

The Kinetoplast-Mitochondrion (L.S.)

An novel localization of a protein that binds to the origin of replication of the kinetoplast DNA minicircle in *Crithidia fasciculata* (a model organism for *Leishmania*!) was reported (J. Shlomai, Hebrew Univ.). This protein localized in a cell cycle-specific manner to two regions on the flagellar side of the kDNA nucleoid body where minicircles are removed from the network for replication. The kDNA replication story continues to amaze and perplex the viewer! Another novel finding in a *Crithidia* system was the demonstration that a single nuclear gene for RNase H (RNH1) produces proteins targeted either to the nucleus or the mitochondrion, depending on which AUG codon is used for translation initiation (D. Ray, UCLA).

The long standing problem of the existence of a mitochondrial translation system and mitochondrial ribosomes was solved (D. Maslov, UC Riverside). The problem appeared to be that the mitochondrial translation products are extremely hydrophobic and migrate abnormally in gels. Protein synthesis was also demonstrated in isolated mitochondria.

A mitochondrial 3′ terminal uridylyl transferase was isolated from *Leishmania tarentolae* and shown to be a member of the nucleotidyl transferase superfamily, and RNAi was used to show that this enzyme is involved in U-insertion RNA editing (L. Simpson, UCLA). Several proteins were identified by mass spectrometry analysis from a purified macromolecular complex from *T. brucei* competent for *in vitro* editing and the genes cloned (K. Stuart, SBRI). Two of these were the RNA ligases involved in editing and others were of as yet unidentified function.

Regulation of gene expression during the parasite life cycle (E.H.)

An important observation in *Leishmania* research has been the differential expression of many functionally important proteins and glycolipids during the parasite differentiation from the promastigote to the intracellular amastigote. The question addressed at this meeting by several investigators has been the regulation of this developmental gene expression. Is there a single mechanism which determines expression in amastigotes? B. Papadopoulou (Centre de Recherches, Canada) has identified a short 150 base pairs sequence present in 3′ UTRs of several open reading frames which were specifically expressed in amastigotes. Moreover, this short sequence could direct stage-specific expression of a luciferase reporter. The 150 bp sequences varied in their degree of conservation among the various genes and some seem to be located at the 5′ rather than the 3′ end of the genes they regulate. This sequence most likely works by regulating the stability of the RNAs. However, this is not the only mechanism regulating amastigote-specific expression because not all amastigote-specific genes contain this sequence. D. Smith (Imperial College, UK) has mapped the 3′ UTRs of several genes which are differentially expressed during the promastigote differentiation from the less infective procyclic form to the infective metacyclic form and found that the metacyclic-specific genes have a conserved 286 bp 3′ UTR and precise polyadenylation sites. As with the amastigote-specific mRNAs, it is at the level of mRNA stability that the regulation of gene expression occurs. It would be of interest to know if the 150 bp sequence described by Papadopoulou's group is contained within the 286 nucleotides of Smith's group. Similar messages about the importance of the 3′ UTRs for regulation of gene expression come from studies on HSP83 and HSP100 expression (M. Shapira (University of Beer Sheva, Israel) and J. Clos (Bernard Nocht Institute, Germany), as well as cysteine proteases (J. Mottram and G. Coombs, University of Glasgow, U.K).

On a more general and practical note, studies by Mensa-Wilmot's group (University of Georgia, USA) have examined the role of sequences in the 5′ UTRs of genes in the regulation of the level of protein expression. Using an interesting approach of synthetic UTRs containing motifs from the Shine-Dalgano box, the *E. coli* lacZ spacer, and nucleotides preceding the translation initiation site, they have generated a series of sequences, which confer dramatically different degrees of expression of a reporter gene. The productive sequences termed **translational enhancers** have been engineered into expression vectors. Moreover, such translational enhancers have been identified in some highly expressed *Leishmania* genes.

Evolution of Leishmania and other Trypanosomatids (L.S.)

Someone once told me that evolution is something you do when you get old. But Fred Opperdoes (Research Unit for Tropical Diseases, Brussels), who is certainly still in his prime, presented a very interesting hypothesis on the evolution of kinetoplastid protists and actually backed it up with some new facts and some old facts that had not fit into the paradigm previously. He found that several enzymes for carbohydrate metabolism tree with chloroplast and cyanobacterial enzymes. As was recently suggested independently by Krepinsky et al. (Eur. J. Biochem. 268, 2678, 2001) and also P. Beech (Protist 151, 299–305, 2000), the ancestor of *Euglenozoa* protists may have had a plastid organelle which became a chloroplast in the Euglenoids and was lost in the kinetoplastids after the genes from the plastid moved to the nucleus. One problem in this hypothesis is that the plastid proteins would have to have somehow acquired a signal sequence for the peroxisome-like organelle, the glycosome. Also the known occurrence of rampant lateral gene transfer in evolution raises a flag of caution. This hypothesis however makes many seemingly aberrant facts understandable, such as the plant-like alternative oxidase (G. Hill, Meharry Medical School) present in blood stream African trypanosomes.

Chemotherapy (E.H.)

There were two informative lectures on treatment of visceral and cutaneous Leishmaniasis (H.W. Murray, N.Y. Presbyterian Hospital; J.D. Berman, Walter Reed Army Institute of Medicine). Multiple agents are now available for the treatment of visceral disease other than the old standard, pentavalent antimony. These include different formulations of amphotericin B, aminosidine and oral miltefosine. Immunochemotherapeutic approaches are also in clinical trial phases. However, we were somewhat crestfallen by the review of the current state of treatment for cutaneous leishmaniasis and the prospects for the future. It was stressed that the cure rate must be greater than a placebo cure rate in controlled studies for a new drug to be considered effective. And therein lies the problem in a spectrum of diseases, some of which cure naturally and some of which

may not cure. So, it is important to first determine the parasite type, emphasizing the importance of classification of the *Leishmania* species by isoenzyme or molecular methods. Old World disease caused by *L. major* and *L. tropica* and New World disease caused by *L. mexicana* usually show natural cure. Disease caused by *L. brasiliensis* complex cells (causing disease which may reactivate even years after initial cure) species is still treated by pentavalent antimony. There was a comprehensive review of the current drugs including those developed on a rational basis such as allopurinol, and these mostly do not work. However, there is a suggestion that oral miltefosine may be effective. We did not go away feeling extremely confident about the future prospects of drug development for cutaneous disease.

Issues related to the development of a *Leishmania* vaccine (E.H.)

Several presentations dealt with the results from clinical vaccine trials and the consensus seems to be that the killed vaccine has not been as successful as hoped when tested rigorously in the field. The major question then is where should we go from here ? Having invested in this model, should we just simply ditch it and move on to new approaches? If so, maybe it is time to address the fundamental questions below, which have been flagged in several presentations.

How to select vaccine candidates?

Some vaccine candidates selected with human or mouse immune serum have been successful in the mouse model, but others have not worked out. This approach seems to have been useful for identification of protective secreted antigens. Reed (Corixa) indicated that selection of antigens based on reactivity of T cell lines turned out not to be useful in the *Leishmania* system.

One example of a failed antigen was presented by Chenik et al. (Inst. Pasteur of Tunis). This was a 20 kDa antigen from *L. infantum* (conserved in *L. major*) which induced excellent Th1 responses in vitro in human monocytes, yet did not protect against infection with *L. major* in mice. Vaccination was done in BALB/c mice using a recombinant BCG expressing the antigen and it seemed to induce an exclusive Th1 response. Is the problem with the antigen, the adjuvant or the delivery or the use of an inappropriate animal model? Is it the large parasite dose used for infection?

On a more positive note, data presented by several members of the Reed group (Corixa) indicated that a multisubunit vaccine including the stress-inducible LmSTI1 protein, LeIF and the thiol specific antioxidant peroxidoxin induced protection in the mouse model when administered as a polyprotein (Leish-111f) and in mice and monkeys, when administered as a polygenic plasmid DNA. Interestingly, a major contribution of LeIF to the vaccine seemed to be an increased production of IFN-γ.

Sacks (Lab. of Parasitic Diseases, NIH) also presented a success story demonstrating that DNA vaccination with three antigens LACK, MAPS and M15 was far more potent than vaccination with the protein antigens with adjuvant, both in terms of reducing pathology and in terms of eliminating the parasites from the skin and thus preventing further transmission. The success of the DNA vaccination may depend on the induction of CD8+ T cells.

Is the mouse a good model for testing human vaccines? Which mouse model?

Sacks advocated a mouse model which combines several features of natural transmission: a very low dose of metacyclic parasites injected intradermally in the ear. The BALB/c mouse is probably best suited to assess the pathology of leishmaniasis which is induced by a combination of host immune responses. For example, Sacks demonstrated that CD8+ T cells are required for the formation of the lesion and b-microglobulin null mice which lack CD8 T cells display no pathology despite the presence of large numbers of parasites in the skin. Moreover, the persistence of parasites in the clinically cured immune individual appeared to be supported by the presence of IL-10; mice lacking IL-10 or mice treated with neutralizing antibodies to the IL-10 receptor eliminated all parasites. Studies presented by Farrell and colleagues (Univ. of Pennsylvania) indicated a similar role for IL-10 in susceptibility to visceral leishmanias.

What parasite to use for challenge infection?

Discussions in the corridors indicated that much work has focused on the host, but not enough thought has been given to the parasite. Isolation of parasites and in vitro culture selects for organisms which grow well under these conditions. Selection of metacyclic parasites using lectins in the case of *L. major*, and antibodies for *L. mexicana* introduces another conundrum. A most interesting presentation indicating the importance of the quality of the challenge infection was given by Rogers and Bates (Liverpool School of Tropical Medicine) who showed that parasite-derived glycoconjugates in the sandfly saliva introduced with the parasites increases their virulence and promotes lesion development. However, saliva itself had only a marginal role in virulence.

What about long-term memory induced by vaccination and parasite persistence in the immune individual?

Unfortunately, as demonstrated by Sacks and colleagues, once the persistent parasites were eliminated, immunity waned. Although often postulated, this is the first demonstration, to my knowledge, for the need of constant, "trickle" antigen presence for the maintenance of long-term T cell memory to *Leishmania*. A major question for future vaccine development is how should long-term T cell memory be induced? How should it be maintained? Would frequent infectious bites in the field be sufficient for boosting immunity? Should the vaccine aim to prevent infection, to eliminate the parasites or to prevent host pathology?

Summary

This meeting showed once again that the study of *Leishmania* parasites and parasite-host/vector interactions at the molecular, biochemical, cellular, immunological, epidemiological and clinical levels is alive and well. There will be another Worldleish Symposium in 2005 but the location has not yet been decided.

What are we to expect from the next Worldleish III? Research on microbial pathogenesis and immunology is changing rapidly and fundamentally, from the characterization of individual host or parasite factors to a comprehensive analysis of host-pathogen interactions. In our field, this will become possible with the completion of the *Leishmania* genome sequence and the availability of the human and mouse genomes. We will be able to obtain a global picture of gene content, expression and regulation thus allowing us to focus detailed studies on relevant aspects. We will be able to elucidate the organization and dynamics of the metabolic, signalling and regulatory networks through which parasites establish infection and subvert the function of the host immune system. The power of this approach derives from a combination of genomics and proteomics and we have already seen the beginnings of these approaches this year from Blackwell, Nugent and Smith, Ouellette, Stuart and Forget.

A missing area has been structural biology. However, a combination of rapid advances in X-ray crystallographic techniques in the last few years and the availability of large amounts of sequence information has resulted in new thinking in the field, namely "structural genomics". No doubt we will see more of this next time around.

So, the message for leishmaniacs is that there is a lot to do and much fun to be had doing it!

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