First World Congress on Leishmaniasis-World Leish1

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The leishmaniases are a widespread group of parasitic diseases, which pose serious health problems that still remain unsolved in the 1990's. The parasite *Leishmania*, is responsible for a wide spectrum of morbidity, in over 12 million people. It is estimated that 350 million people are at risk, and that some 1.5-2 million people are infected annually. This parasite has proven to be an excellent model for studies on host-parasite and host-vector interactions, immunological mechanisms, molecular biology, and epidemiology. The aim of the congress was to promote interaction and cooperation among scientists resulting in new solutions to regional or global problems on leishmaniasis. The place chosen for this first world meeting on leishmaniasis, Istanbul, is the only city in the world on two continents, Asia and Europe. This eclectic city provided for all the participants an unforgettable scientific and cultural experience. The Congress benefited from the active participation of scientists from at least 36 countries. This participation is fully reflected by the content of the 411 abstracts published.

It is not possible to comment on all the presentations, and this report is a selective list of some of the highlights.

CL Jaffe (Hebrew University-Hadassah Medical School, Jerusalem, Israel) opened the proceedings with a conference about the protein kinases (PK) and parasite survival. These proteins have important roles in the regulation of cellular metabolism, differentiation, growth and signal transduction. Little is known regarding their host-parasite interactions. Environmental changes, such as temperature and pH affect PK activity. These proteins can also affect host response to the parasite. Jaffe showed some assays related with these enzyme activities and discussed how the intra- and extracellular PK function, in parasite survival, growth and response to host environmental changes.

Vaccine Development

The development of a vaccine against *Leishmania* has been a complex process and one of the principal aims of studies on leishmaniasis. Recombinant DNA technology has been successfully applied to the development of vaccines against a number of diseases. Different methods to select leishmanial antigens as vaccine candidate were presented. YAW Skeiky (Corixa Corp. Seattle, WA) with other collaborators (J Webb, R Badaró, A Campos-Neto & SG Reed) have tested recombinant antigens, identified by expression cloning or by reverse genetic approaches. The selection was carried out by the ability of these antigens to stimulate immune responses in cells from patients as well as to elicit Th1 associated responses from lymphocytes from infected BALB/c mice. They showed the results of protective immune responses against *L. major* in BALB/c mice with a combination of three antigens. SCF Mendonça (Fiocruz, Brazil) reported the results of comparison between autoclaved and non-autoclaved preparations of a vaccine composed of whole antigens from killed promastigotes of *L. amazonensis* in two groups of immunized individuals from an endemic area in Brazil. Promastigotes of autoclaved *L. major* were used as a vaccine in volunteers in endemic areas infected with *L. major*, *L. tropica* and *L. donovani* (Iran, Pakistan and Sudan). RF Hashemi (Razi Research Vaccine and Serum Institute, Iran) showed that the immunogenicity of the vaccine was satisfactory and adverse effects in vaccinated volunteers were not observed. The efficacy of an autoclaved *L. major* vaccine (ALM), mixed with BCG was showed in volunteers against natural cutaneous leishmaniasis in Isfahan (AZ Momeni, Isfahan University of Medical Sciences, Iran) and in schoolchildren, in Bam, Iran (I Sharifi et al., Medical School, Kerman University of Medical

Sciences). In Baluchistan, Pakistan, where both anthroponotic and zoonotic cutaneous leishmaniasis are endemic, a randomized double-blinded-placebo-controlled vaccine efficacy trial using this killed *L.major* vaccine was conducted among the individuals tested. A Firooz et al. (Pasteur Institute, Iran) reported that the use of three injections of ALM with BCG, 30 days apart, are completely safe and significantly more immunogenic than BCG alone in adults.

Several animal models have been tested with different vaccination schedules and vaccine preparations. D Rivier et al. (University of Lausanne, Switzerland) showed the importance of adjuvants and the site of immunization for vaccination using CBA (inbred strains of mice, that develop a cutaneous ulcer caused by *L. major*, such as in the human disease) as a model animal. The animals were immunized with a purified amphiphilic leishmanolysin gp63 and infected with *L. major,* the results show that the protection against *L. major* could be obtained following subcutaneous injection of gp63 at a distance from the site of infection only when adjuvants (*C. parvum* or BCG) were omitted from the vaccine. Currently, *Leishmania* antigens have been tried in several primate models as well as in humans, however, they have been ineffective as vaccines. RT Kenney, AA Gam and DL Sacks, reported the immunogenic effect of a vaccine that combines a killed preparation of *L. amazonensis* (produced by Biobras) and recombinant interleukin-12, that has a marked effect as an adjuvant in the BALB/c model of CL adsorbed to aluminum hydroxide fluid gel and tested in *Macaca mulatta*. They suggest the need for an adjuvant to stimulate an effective immune response in this primate model. The relative value of different animals models for *Leishmania* vaccine trials was discussed. Two aspects of the model system in particular need consideration: the first of these is whether the host animal is capable of mimicking human infection and subsequent immune response sufficiently closely; the second is whether the appropriate parasite stage and method of delivery is used.

Canine Leishmaniasis

Visceral leishmaniasis (VL), or kala-azar is a zoonosis in most regions where it occurs. Dogs are the most important vertebrate reservoir of the disease and are mainly responsible for the persistence of VL in the Paleartic and Neotropical regions. Canine leishmaniasis is a viscerocutaneous disease and therefore it is incorrect to call it VL. As in man, there are some cases of assymptomatic infection. No specific *Leishmania* drug exists to treat canine visceral leishmaniasis (CVL). However, several human drugs were tested. The development of effective treatments for canine visceral leishmaniasis is essential to eradicate the disease in humans, nevertheless, very little is known about the immune cells implicated in CVL and its protective role. P Abranches (Tropical Medicine and Hygiene Institute, Portugal) stressed the importance of knowing when canine cutaneous parasitism becomes infectious to the insect vector. JA Rioux indicated later periods of the disease, J Alvar showed by xenodiagnosis that it is possible that transmission might occur earlier. The finding of animals without humoral response means that the prevalence of canine leishmaniasis found in epidemiological surveys by search of antibodies may be underestimated, and that the spontaneous development of resistance (related with active cellular immunity) in these infected animals is interesting for research studies aiming at a canine vaccine. Some presentations showed studies of the humoral response against *L. infantum* in vaccinated or treated dogs. Results obtained with the use of autoclaved *L. major* mixed with BCG as adjuvant revealed a partial protection in vaccinated dogs in which a cell-mediated response was established as well as asymptomatic dogs spontaneously recovered from leishmaniasis. Animals treated with pentamidine reveal that after treatment the immune cellular response which is involved in protection against *L. infantum* infection was established (A Rhalem et al., Institut Agronomique et Veterinaire Hassan II, Morocco).

HIV and Leishmaniasis

The occurrence of human individuals infected with both diseases has increased. HIV-VL coinfection is usual in some Spanish regions. The diagnosis is hampered by its coexistence with other opportunistic infections as well as the poor sensitivity of serology versus *Leishmania* spp. This coinfection is accompanied by severe immunodepression stronger than that caused by other diseases. The presence of p24 antigen and the bicytopenia (platelets and leucocytes) are discriminatory criteria in the diagnosis of HIV-VL (J Hernandez, Andalusian Group for Study of Infectious Diseases, Spain).

Kinetoplast DNA

Studies of kinetoplast-DNA (KDNA), showed that this extrachromosomal DNA structure, have associated proteins (histone H1-like), where genes have been cloned (DS Ray, University of California, USA). Replication of the individual kDNA minicircle initiates at a 12-nucleotide sequence, termed the universal minicircle sequence (UMS), which was conserved in all the trypanosomatids

species studied. Computer analyses confirm the location of the origin-associated UMS within a local sequence-directed curved DNA structure, and revealed the potential of the DNA double-helix at the minicircle origin region to generate a stable intrastrand secondary structure. Kinetoplast segregation is co-ordinated with other structural events in the cell cycle. The segregation is mediated by attachment of the mitochondrion/kinetoplast complex to the flagellum basal bodies. Through sequence analysis the mitochondrial genome is encoded in the maxicircle while the minicircle has been shown to encode small RNA molecules (guides RNAs) which are involved in the process of RNA editing. Models of kDNA replication were also discussed (Y Tzfati et al., The Hebrew University, Israel; K Gull et al., University of Manchester, UK; K Stuart et al., University of Washington, USA).

Taxonomy of Leishmania

JA Rioux and N Leger (Montpellier-Reims, France), discussed nomenclatural problems, where considerations about species and genus were raised. K Victoir et al. (Laboratoire de Genetique Moleculaire des Parasites et des Vecteurs, Montpellier) showed that the combination of analysis by restriction enzymes (RFLP) and PCR amplification of the conserved part of the gp63 gene, when compared with MLEE data, might be an epidemiological and diagnostic tool to differentiated between *L.* (*V.*) *peruviana* and *L.* (*V.*) *braziliensis* (two genetically close species with different pathogenicity). Studies to show the differences between both species was carried out using molecular karyotyping, the limits for using this method were discussed. Results were presented suggesting mating between both species which are considered to reproduce mainly clonally (JC Dujardin et al., Prince Leopold Institute of Tropical Medicine, Belgium). Through the MLEE and RAPD analysis the data support the view that these species correspond to two closely related but distinct phylogenetic lines (clades).

Sand Fly Vectors and Non-vector Transmission

Female sandflies salivate as they probe the skin for blood. Sand fly saliva is known to supress immune functions of macrophages and exarcebate experimental cutaneous leishmaniasis. The saliva of *Phlebotomus papatasi*, the vector of *L. major* contains a potent inhibitor of protein phosphatase (PP)-1 and PP-2A. Saliva of this sand fly also, down-regulated the expression of the inducible nitric oxide synthase gene and reduced the production of nitric oxide by activated macrophage. J Waitumbi and A Warburg (Hebrew University, Israel) suggest that the supression of nitric oxide production caused *by P. papatasi* saliva, is achieved via the inhibition of PP activity in macrophages. Another interesting subject is the presence of apyrase (an ATP/ADPase that inhibits platelet aggregation) and maxadilan (a potent vasoactive peptide with proved pharmacological and immunomodulatory activites) in the saliva of some species of sandflies (including all *L. longipalpis* spp.). Both facilitate the location of blood vessels in the skin, prevent hemostasis and assure an adequate blood supply to the bite site. The same authors discussed the properties and functions of different sand fly saliva among *P. papatasi* and *Lu. longipalpis* spp. They suspected that maxadilan may be the exacerbating factor in saliva, in fact, their studies showed that lesion exacerbation is not attributable to any single salivary component. Many studies have suggested *L. longipalpis* as a complex of species. Azevedo et al. (Fiocruz, Brazil) compared populations from different regions in Brazil by morphological methods, suggesting a polymorphism among specimens from these areas (PA, MA, RN, CE and MG) in Brazil. The transmission of the *Leishmania* parasite without the sand fly vector has been reported, by blood transfusion, sexual intercourse, direct contact or congenital transfer. Meinecke et al. (Olgahospital, Germany) reported a rare case of VL where the mother of an infected child never showed signs of the disease but was shown to be subclinically infected. The authors emphasize that congenital infection with VL may be much more common than is thought.

Diagnosis

Different methods of *Leishmania* diagnosis were presented, such as, parasitological, immunological (IFAT, ELISA, TR-FIA/time resolved fluoro immuno assay, TRALd/rapid test antibody *L. donovani*, SPEEDLEISH/Bio veto test), DNA hybridization and PCR. PCR-based methods, have been used with the intention of identification of different species of *Leishmania* by detection of DNA polymorphisms in *Leishmania* and the use of sequences from the conserved region of minicircle kDNA (G Schönian et al., Humboldt University, Berlin; Barker et al., Cambridge University, UK; O Fernandes et al., Fiocruz, Brazil; OF Osman et al., University of Khartoum, Sudan; R Piarroux, University of French Country, France). In conclusion, PCR-based methods have become an essential tool for the diagnosis of leishmaniasis. This rapid and sensitive method allows the detection of parasite DNA from different material, and can be used for a wide variety of situations.

Finally, MA Özcel presented the economic

importance of parasitic diseases. The information of prevalence and incidence on the the various aspects of the parasitic diseases in certain areas in the world, such as transmission, disease characteristics and control measures, provide baseline points for social and economic studies to determine the role of human behaviour in the incidence, transmission and control of diseases.

The full proceedings of the conference are published in a supplementary issue of *Acta Parasitologica Turcica* (the official Journal of the Turkish Society for Parasitology).

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