Part II. Leishmaniasis: Cell Biology and Immunology

Chaired by Professor D J Bradley

**Part II.I *Leishmania*/Macrophage Interactions:
their role in determining Host Response
to Leishmanial infection.**

J M Blackwell

**Department of Tropical Hygiene
London School of Hygiene and Tropical Medicine
London WC1E 7HT**

Early workers identified host macrophages as the site of intracellular parasitisation by leishmanial parasites. Several phases in the interaction between these parasites and macrophages have since been identified which may be crucial in determining the outcome of an infection.

Firstly, since *Leishmania* species are obligate intracellular parasites of the monocular phagocyte system, they must first be targetted to and gain entry into a permissive subpopulation of macrophages. This depends upon receptor/ligand binding between parasite and macrophage and transmission of transmembrane signals to promote a phagocytic response. Certain receptor/ligand interactions may also promote transmission of signals which trigger a respiratory burst response and the release of potentially harmful oxygen metabolites.

In their interaction with murine resident peritoneal macrophages, promastigote forms of the parasite bind via complement and lectin-like receptors and elicit a strong respiratory burst response. Amastigotes appear to be less dependent upon entry via these receptors and fail to elicit a significant respiratory burst response. They are also more resistant to the products of respiratory burst activity.

After internalisation, genetically controlled variations in the ability of host macrophages to control parasite multiplication may also have a profound effect upon the subsequent course of infection. Innately resistant macrophages control parasite proliferation keeping the parasite load to a level which promotes development of an effective T-cell mediated immune response, resulting in production of gamma interferon and other lymphokines which activate macrophages to kill the parasite. This, in turn, is dependent upon presentation of parasite antigen in association with MHC products by macrophages and macrophage-like dendritic cell populations.

Polymorphism of genes encoding MHC class II molecules also influences disease profiles during the course of infection. Innately susceptible macrophage populations yield high parasite loads which may, in association with particular MHC class II molecules, paralyse the host's immune response by shifting the balance towards the suppressor arm of T-cell regulated responses.

Part II.II Immunological Control and Vaccination

J Alexander

**Department of Bioscience and Biotechnology
The University of Strathclyde
Glasgow G4 0NR****Introduction**

Vaccination against the cutaneous forms of leishmaniasis has long been practised. Accounts in the literature suggest that from as early as the 19th century children in Baghdad had been vaccinated against "Oriental sore", by inoculating material from active human lesions by excoriating the skin of the arm or thigh (Wenyon, 1911). In this way long-lasting immunity could be induced and the face could be saved from disfiguring scars. Until recently a similar form of vaccination using replicating cultured *L. major* promastigotes had been widely used in the USSR and Israel. However, the use of live material is subject to obvious drawbacks, scarring is inevitable at the site of inoculation and there is always the possibility of a particularly susceptible recipient developing a chronic or severe infection. For this reason the above two programmes have been largely discontinued and large scale vaccination using living organisms remains progress only in Iran.

The Protective Usefulness of Attenuated Vaccines

The problems inherent in the use of virulent vaccines have stimulated trials using attenuated parasites or parasite fractions. Although these have proved disappointing with regard to Old World cutaneous leishmaniasis, recent field trials in South America have produced promising results. Using a vaccine consisting of equal parts heat-killed and sonicated promastigotes from five different parasite isolates, Mayrink *et al.* (1985) successfully and significantly protected from infection approximately 70% of the participants that became skin-test positive following vaccination.

There are few vaccination studies on human visceral leishmaniasis caused by *L. donovani*. However, Manson-Bahr (1961) has reported that a ground squirrel strain of *L. donovani* did not visceralise following intradermal infection and protected three volunteers against subsequent challenge with human *L. donovani*. The ground squirrel parasite has, in fact, been identified as *L. major* using biochemical and serological methods (Chance *et al.*, 1978). Cross-immunity between species of *Leishmania* is not uncommon and is a point on which I shall elaborate further.

The Susceptibility of the Host

Ultimately the development of successful vaccination protocols is dependent on a thorough understanding of the immunological mechanisms determining the growth of *Leishmania* parasites in the host. For obvious reasons it is difficult to study such phenomena in man. This has necessitated the use of laboratory models, particularly inbred mice, for the dissection and investigation of immunological control in leishmaniasis. A wide spectrum of disease profiles develops in mice depending on the genetic background of the host. Although immunity to cutaneous leishmaniasis, *L. major*, and visceral leishmaniasis, *L. donovani*, is under the control of different genes, innate susceptibility to both diseases is related to a primary macrophage defect (Gorczynski & MacRae, 1982; Crocker *et al.*, 1984) which in turn gives rise to the generation of T-suppressor cells (Howard *et al.*, 1981; Blackwell & Ulczak, 1984). The overwhelming evidence also indicates that protective immunity is cell mediated as it can be adoptively transferred with T-cells but not B-cells (Preston & Dumonde, 1976; Rezai *et al.*, 1980). The humoral response is, at best, irrelevant or it may be detrimental as the generation of T-suppressor cells has been shown to be B-cell dependent in non-healing *L. major* infections in BALB/c mice (Sacks *et al.*, 1984). Although monoclonal antibodies or their Fab fragments have been shown to be protective if inoculated together with the parasites into the host (Anderson *et al.*, 1983; Handman & Mitchell, 1985), they have no effect if inoculated separately and would therefore appear to have little protective effect in controlling leishmaniasis.

Numerous laboratory studies have now shown that immunity can often be induced in normally non-curing leishmaniasis either by selectively depleting T-suppressor cells by prophylactic whole-body irradiation or by promoting the growth of a protective T-cell population by vaccination (reviewed by Liew, 1986). Most studies have used as vaccines heat-killed, avirulent or radio-attenuated promastigotes and protection against cutaneous leishmaniasis could be induced by repeated intravenous or intraperitoneal inoculation. Surprisingly, vaccination via the subcutaneous route was often ineffective, or worse, exacerbated infections. Two exceptions to this observation have been reported: subcutaneous vaccination with avirulent promastigote mutants of *L. braziliensis* protected BALB/c mice against homologous challenge (Gorczynski, 1985), and radioattenuated *L. major* protected against *L. mexicana*, depending on the mouse strain used (Alexander & Blackwell, 1986).

As previously mentioned, there are many examples of cross-immunity between different species of *Leishmania*. A prior infection with *L. major*, for example, protects both man (Adler & Gunders, 1964) and mice (Alexander & Phillips, 1978) against subsequent infection with *L. mexicana*. Similarly, Howard *et al.* (1982) demonstrated that intravenous

vaccination with radioattenuated *L. major* or *L. donovani* protect BALB/c mice against cutaneous infection with *L. m. mexicana*, *L. m. amazonensis* and *L. braziliensis panamensis*. Conversely, vaccination with *L. major* or *L. donovani* by any route could not protect susceptible mice against intravenous infection with *L. donovani* (Blackwell, unpublished observations). In a similar manner a prior infection with *L. major* failed to limit the number of parasites in the viscera following an intravenous challenge with *L. m. mexicana*. It did, however, inhibit the subsequent development of cutaneous metastases which developed in non-vaccinated mice (Alexander & Blackwell, unpublished observations). As *L. donovani* infections are initiated in the dermis and some time elapses before the parasites migrate to the viscera (Manson-Bahr, 1961), by intravenously inoculating parasites we may be bypassing a normal pathway of resistance which operates exclusively in the cutaneous tissues.

Vaccine Development: The Future

In a comparatively recent study, Gorczynski and MacRae (1982) demonstrated that although BALB/c skin macrophages infected with *L. major* induced T-suppressor cell proliferation, the same macrophage population incubated with soluble excretion factor (EF) induced the proliferation of a protective T-cell population. As a result, certain purified parasite antigens have been, and are being, tested as candidate vaccines. So far varying degrees of success have been achieved with EF (Steinberger *et al.*, 1984) and a plasma membrane glycolipid (Handman & Mitchell, 1985). Success in both studies was dependent on the presence of adjuvant. Interest is now, however, centering on a major surface glycoprotein (GP 63) (Alexander & Russel, 1985) which not only plays a pivotal role in the surface interactions between the parasite and its host cell, but is common to many *Leishmania* species.

Finally, from our current knowledge of leishmaniasis in man and what we have recently learned from the mouse model, it may be feasible to develop vaccines for use in humans.

REFERENCES

- ADLER S and GUNDERS A E. (1964) *Trans R Soc Trop Med Hyg*; **58**: 274-277.
- ALEXANDER J and BLACKWELL J M. (1986) *Ann Parasit*; in press.
- ALEXANDER J and PHILLIPS R S. (1978) *Exp Parasitol*; **45**: 93-100.
- ALEXANDER J and RUSSEL D G. (1985) *Curr Top Microbiol Immunol*; **120**: 43-67.
- ANDERSON S, DAVID J R. and MCMAHON-PRATT D. (1983) *J Immunol*; **131**: 1616-1618.
- BLACKWELL J M and ULCAZAK O M. (1984) *Infect Immunol*; **44**: 97-107.

- CHANCE M L. *et al.* (1978) *Ann Trop Med Parasitol*; **72**: 533-542.
- CROCKER P R, BLACKWELL J M and BRADLEY D J. (1984) *Infect Immunol*; **43**: 1033-1040.
- GORCZYNSKI R M. (1985) *Cell Immunol*; **94**: 11-20.
- GORCZYNSKI R M and MACRAE S. (1982) *Cell Immunol*; **67**: 74-89.
- HANDMAN E and MITCHELL G F. (1985) *Proc Natl Acad Sci USA*; **82**: 5910-5914.
- HOWARD J G, HALE C and LIEW F Y. (1981) *J Exp Med*; **153**: 557-568.
- HOWARD J G. *et al.* (1982) *J Immunol*; **129**: 2206-2210.
- LIEW F Y. (in press) In: Host Resistance Mechanisms. Rockefeller Univ. Press.
- MANSON-BAHR P E C. (1961) *Trans R Soc Trop Med Hyg*; **55**: 550-555.
- MAYRINK W. (1985) *Ann Trop Med Parasitol*; **79**: 259-269.
- PRESTON P M and DUMONDE D C. (1976) *Clin Exp Immunol*; **23**: 126-138.
- REZAI H R, FARREL J and SOULSBY E L. (1980) *Clin Exp Immunol*; **40**: 508-514.
- SACKS D L. *et al.* (1984) *J Immunol*; **132**: 2072-2077.
- STEINBERGER A. (1984) *Exp Parasitol*; **58**: 223-229.
- WENYON C M. (1911) *Parasitology*; **4**: 273-344.

Part II.III *Leishmania* Antigen Presentation and T Cells

R S Bray

Department of Pure and Applied Biology
Imperial College of Science and Technology
London SW7

The method by which antigen is presented to the T-cell domain by antigen presenting cells is obviously critical to the T-cell response where T-cells of the same phenotype are capable of expressing both helper and suppressor functions. There is evidence that the configuration of the antigenic molecule on presentation can alter the T-cell response.

In our hands the only effective antigen presenting cell *in vitro* is the macrophage although spleen cells have been effective with others. While whole soluble antigen is an effective inducer of T-cell proliferation, the fractions coming off first from a Sephadex 100 column contain most of the effective stimulatory antigens. Following infection in CBA/Ca mice the antigen still present in lymph node cells is sufficient to initiate primary T-cell proliferation. In our hands colonies of T-cells can be transferred and kept alive for several weeks but colonies do not continue to proliferate beyond the initial growth despite the presence of interleukin II.

Part III. *Leishmania* Biochemistry and Chemotherapy

Chaired by Dr D L Goodwin

Part III.I Antileishmanial Chemotherapy Reviewed

R A Neal

London School of Hygiene and Tropical Medicine
St Albans, Herts AL14 0XQ.

Introduction

The early development of chemotherapy was stimulated by the realization that some dyes such as methylene blue were able preferentially to stain particular cells and this idea developed into the concept of chemical substances which could unite with and inhibit parasitic agents of disease – the so-called magic bullet theory of Paul Ehrlich. There was next a quantum leap to the use of arsenic compounds against African trypanosomes, particularly a supposedly less toxic compound atoxyl, originally prepared by Bechamp in 1863 and used clinically at that time for treatment of skin conditions and anaemia.

A smaller conceptual leap in thinking was then to the use of antimony compounds such as tartar emetic which like the arsenic compounds was also trypanocidal. The first use of antimonials in leishmaniasis in man is credited to Vianni who used tartar emetic in Brazil to treat a patient with cutaneous leishmaniasis. Since that time other antimonial drugs have been studied such as urea stibamine, sodium antimony tartrate, stibophen, anthiomaline, stibamine, neostibophen, neostibamine, sodium stibogluconate, meglumine antimoniate and many others.

From about 1950 it became apparent that the pentavalent compounds were much less toxic clinically than the trivalent antimonials, and today treatment is confined primarily to two pentavalent antimonials, sodium stibogluconate and meglumine antimoniate. The organo derivatives of oxo-antimony anions are ill-defined and exhibit various degrees of polymerisation which makes complete chemical quality control of the products impracticable. However, modern antimonial drugs, with standardization of conditions of synthesis, chemical analysis of pentavalent antimony combined with biological control of toxicity and efficacy, give the best possible standard compound. Sodium stibogluconate in aqueous solution has a mean molecular weight of about 400.

The Clinical Situation

From the clinical point of view, the drugs currently available for treatment of all forms of leishmaniasis are the two pentavalent antimonial compounds, sodium stibogluconate and meglumine antimoniate, the polyene antibiotic amphotericin B used primarily as second line treatment for Latin-American cutaneous leishmaniasis