WORLD HEALTH ORGANIZATION

ORGANISATION MONDIALE DE LA SANTE

LEISH/WP/82.1

ENGLISH ONLY

EXPERT COMMITTEE ON LEISHMANIASES

Geneva, 10-16 November 1982

Draft agenda item 2.3.1

BIOLOGICAL CURE AND IMMUNITY: THE IMMUNE RESPONSE IN LEISHMANIASIS

by

J. Mauel, Ph.D.
WHO Immunology Research and Training Centre, CH-1066 Epalinges, Switzerland

CONTENTS

1. INTRODUCTION

1.1 Some features of the biology of Leishmania parasites that are relevant to host defence

Fresh normal serum from many animal species displays potent lytic activity against various Leishmania parasites, perhaps due to the occurrence of "natural" antibodies or to activation of complement by the alternative route. Parasite survival within the host may thus depend on the capacity of the microorganism to become intracellular. No particular organelle is detectable ultrastructurally that might facilitate cell invasion, and leishmaniae appear to be internalized by simple phagocytosis. Little is known regarding the nature of the molecular structures mediating attachment of the parasites to the phagocytes. Receptors for the Fc portion of immunoglobulins or complement factors do not appear to be involved and it is possible that parasites bind to macrophages via lectin-like structures on the host-cells and appropriate ligands on the microorganism, or vice versa.

The available evidence suggests that, following internalization, the parasitophorous vacuole undergoes fusion with lysosomes. In their amastigote form, the parasites do not appear to be harmed, as they have been shown to multiply in the phago-lysosomal environment. It has been suggested that the parasite surface may present "refractory" properties to lysosomal degradation. Alternatively, leishmaniae may release substances capable of inhibiting lysosomal enzymes as shown for the anti-β-galactosidase properties of the "excretory factor".

Phagocytosis of Leishmania parasites by macrophages in vitro induces in the latter cells a metabolic burst characterized by increased oxygen uptake, and the generation of toxic oxygen metabolites such as hydrogen peroxide. Leishmaniae (at least in their promastigote form) are very susceptible to killing by such compounds. This susceptibility may be linked to their known inability to synthesize heme, which constitutes the active group in enzymes such as catalase and peroxidase, which are required to detoxify oxygen intermediates. In vitro at least, most promastigotes are thus rapidly killed upon being phagocytized; a few may nevertheless transform to amastigotes, which appear to be more resistant to intracellular destruction, and may then initiate infection. Depending on the particular host-parasite combination however, even amastigotes may be susceptible to killing and digestion in macrophages that have become activated as the result of a cell-mediated immune response.

1.2 Genetic aspects of infection

The disease patterns produced by injection of Leishmania parasites into mice of different genetic backgrounds may vary widely, depending, presumably, on both host and parasite factors. For instance, evolution of the skin lesions induced by inoculation of L. tropica varies between mouse strains, which can be classified as "healers" or "non-healers" according to the outcome of infection. A similar situation prevails in L. donovani infections in the same host, and there are indications that susceptibility in man is also controlled by genetically determined factors. Although it has been demonstrated that the macrophage-parasite relationship plays a determining role in "acute" resistance or susceptibility (that is exercised before the onset of an immune response), the precise nature of the factors responsible for the "acutely resistant" and "acutely susceptible" phenotypes is still unclear.

2. HUMAN LEISHMANIASIS: MAIN IMMUNOLOGICAL FEATURES

Different clinical manifestations are known to occur in man following infection by Leishmania; they depend on the nature of the infecting species and, presumably, on the type of immune response mounted by the host (Table 1).

TABLE 1. A SIMPLIFIED TABULATION OF THE MAIN IMMUNOLOGICAL FEATURES OF LEISHMANIAL INFECTIONS IN MAN

Parasite	Disease type	Immune response		Parasites in lesions	Tendency to self-cure
		Cutaneous delayed hypersensitivity	Antibodies		
L. tropica	Cutaneous: oriental sore	Present	Low	Present	Yes
	DCL	Absent	Present	Abundant	No
	LR	Strong	Variable	Rare	No
L. donovani	Visceral: kala azar	Absent	Abundant	Abundant	No
	Cutaneous: PKADL	Variable	Variable	Present	No
L. brasiliensis	Cutaneous	Present	Present	Present	Yes
	Muco-cutaneous: "espundia"	Present	Present	Rare	No

DCL = diffuse cutaneous leishmaniasis; LR = leishmaniasis recidiva;
PKADL = post-kala azar dermal leishmaniasis.

2.1 Cutaneous leishmaniasis

The cutaneous ulcer (oriental sore) resulting from infection by L. tropica heals spontaneously. There follows what is said to be a permanent immunity against reinfection by the homologous parasite, although rare cases of reinfection (or activation of a latent infection) have been documented following immunosuppressive therapy. Oriental sore is characterized immunologically by strong delayed hypersensitivity reactions following intracutaneous injection of leishmanial antigen. Peripheral blood lymphocytes respond to antigen challenge in vitro by a marked proliferative activity. Antibodies, albeit in low titres, can usually be demonstrated by immunofluorescence. The self-healing forms of cutaneous leishmaniasis in South America share similar immunological characteristics.

Cutaneous leishmaniasis is sometimes considered to constitute a "polar" disease, akin in this respect to leprosy. The two (rare) polar forms would be diffuse cutaneous leishmaniasis (DCL) and leishmaniasis recidiva (LR). DCL is characterized by a cutaneous anergy to parasite antigens, that is said to be specific. It is not known whether such anergy should be considered as the result, or the cause, of the intense parasite proliferation observed in this disease. On the contrary, patients with LR display "exquisite sensitivity" to leishmanin as detected by skin testing, which correlates with the conspicuous scarcity of parasites in the lesions. Both conditions do not seem to evolve spontaneously towards healing. Classical oriental sore would appear to occupy, both histologically and immunologically, an intermediary position between the above two polar forms of the disease.

2.2 Visceral leishmaniasis

Contrary to oriental sore, visceral leishmaniasis (caused by infection with L. donovani and related species) is usually non-self-healing. Indian kala azar is characterized immunologically by high titres of circulating antibodies, concomittant with an increase in immunoglobulin levels (particularly of the IgM and IgG classes). Delayed hypersensitivity to parasite antigens is usually absent during the active phase of the disease; in addition, a generalized hyporeactivity to other antigens, contact sensitizers and mitogens is observed both in vivo and in vitro in kala azar patients. These parameters, however, return to control levels after cure. The acquisition of a positive delayed skin hypersensitivity reaction to leishmanin appears to correlate with protection against reinfection, which is said to be absolute and long-lasting. Cases of post kala azar dermal leishmaniasis (PKADL)

represent an immunological puzzle, in that immunity appears to control parasite proliferation in the viscera, but not in the skin. In this condition, various levels of immunoreactivity, of both the cellular and humoral type, can apparently be observed.

2.3 Mucocutaneous leishmaniasis

Delayed hypersensitivity as well as circulating antibodies have been demonstrated at least in some patients with "espundia". However, at present, it is still unclear why this immune response is not protective and whether immune reactions play a role in the progressive erosion of the soft tissues of the naso-pharyngeal regions, characteristic of this condition, or whether such erosion results from some unrelated pathological processes.

2.4 Immune response and biological cure in human leishmaniasis

Considering that (a) the self-curing forms of cutaneous leishmaniasis are characterized, from an immunological standpoint, by the presence of cell-mediated immune reactions, (b) acquisition of immunity in visceral leishmaniasis correlates with the emergence of a positive delayed skin test, and (c) the presence of antibodies does not seem to confer protection (cf kala azar), recovery and protection in human leishmaniasis have usually been regarded as resulting from cell-mediated rather than humoral immune processes. Cellular mechanisms may be necessary but not sufficient, however, since many types of leishmanial infections are accompanied by cellular hypersensitivity, yet no cure is observed.

3. IMMUNITY IN ANIMAL MODELS

Several experimental models of leishmanial infection have been studied. The main immunological features of two of them are given below together with the conclusions that have been reached regarding the mechanisms of immune recovery.

3.1 The guinea-pig

Inoculation of *L. enriettii* in bare tracts of guinea-pig skin produces a skin lesion somewhat similar to human oriental sore. Cellular immune reactions to parasite antigen (delayed skin hypersensitivity, blast transformation of lymph node lymphocytes *in vitro*, macrophage migration inhibition, etc.) can be demonstrated, as well as the presence of antibodies. Spontaneous healing is observed within several weeks; the animal is thereafter immune against reinfection. Histological studies suggest that, in the healing animal, parasites are destroyed within host macrophages. General or specific "paralysis" to parasite antigens can be induced by immunosuppressive means: this results in an increase in the severity of the lesions and delays or abrogates self-cure.

The study of effector mechanisms leading to parasite elimination strongly suggests that: (a) in the recovering animal, macrophages become activated following interaction between sensitized lymphocytes and parasite antigen; (b) activated macrophages can destroy *L. enriettii* parasites; (c) serum antibodies do not seem to affect the intracellular micro-organisms. These observations emphasize the role played by the activated macrophage in the process of immune recovery in this animal model; they do not preclude, however, a possible role for antibodies in protection against reinfection in the recovered host.

3.2 The mouse

Inoculation of *L. tropica* in the skin of mice from different strains produces an ulcer, that will eventually heal, or not heal, according to the genetic background of the recipient. The production of antibodies, as well as a strong cellular immune response, can be detected early in the infection in all the strains tested. The cellular response (as measured by skin testing) appears to decrease in "non-healer" animals as the disease progresses, whereas it remains strong in healer strains throughout the infection and after self-cure. Responding lymphocytes from animals inoculated with parasites in Freund's complete adjuvant have been cloned: the same clone can display all of the following functions: (a) proliferation in the presence of appropriately presented parasite antigen; (b) helper activity for antibody

production; (c) transfer of delayed-type hypersensitivity; and (d) activation of Leishmania-infected macrophages for intracellular killing of the parasite. Results from in vitro experiments suggest that in this model also, macrophage activation is an essential component of the mechanisms of recovery.

4. FAILURE TO HEAL IN ANIMAL MODELS

Experimental evidence indicates that at least three mechanisms may contribute to render an infected host unable to eliminate the parasite. These are:

(a) Deficient effector function of activated macrophages. Experiments in vitro have shown that activated macrophages from "non-healer" strains (with regard to L. tropica infection) are deficient in their capacity to destroy the microorganism, compared to activated macrophages from "healer" strains. These results establish a correlation between the non-healer phenotype on the one hand, and macrophage defect in leishmanicidal activity on the other.

(b) Deficient antigen-presenting capacity of macrophages. Based on in vivo and in vitro findings in the mouse, it has been claimed that infected macrophages (or at least a subpopulation thereof) from highly susceptible hosts, might both allow uncontrolled parasite proliferation, and show a defect in their capacity to present adequately leishmanial antigen to the immune system. This defect might reside in the low level of Ia antigens present on these phagocytes.

(c) Generation of suppressor T cells in "non-healer" animals. The intensity of the delayed skin response (to leishmanial antigens) of "non-healer" mice has been shown to decrease as the infection progresses. Moreover, spleen cells from heavily infected animals, when transferred to normal recipients, specifically suppress the response to a sensitizing parasite inoculum. The splenic suppressor cells are T lymphocytes, they are sensitive to certain treatments and for instance, highly susceptible animals can be converted to a "healer" phenotype when submitted to an appropriate course of X-irradiation before infection.

The relationships, if any, between the above three mechanisms, are unknown.

REFERENCES

Section 1

Mauel, J. (1980) The biology of the macrophage-Leishmania interaction. In: van den Bossche, H., ed, The host-invader interplay. Amsterdam, Elsevier-North Holland, pp. 165-178

Section 2

Mauel, J. & Behin, R. (1981) Immunology of leishmaniasis. In: Levandowski, M. & Hutner, S. H., eds, Biology and physiology of protozoa, 2nd ed., vol. 4, New York, Academic Press, pp. 385-429

Sections 3 and 4

Behin, R., Mauel, J. & Sordat, B. (1979) Experimental parasitology, 48, 81

Bryceson, A. D. M., Bray, R. S., Wolstencroft, R. A. & Dumonde, D. C. (1972) Clinical and experimental immunology, 7, 301

Gorczynski, R. M. & MacRae, S. (1982) Cellular immunology, 67, 74

Handman, E., Ceredig, R. & Mitchell, G. F. (1979) Australian journal of experimental biology and medical science, 57, 9

Howard, J. G., Hale, C. & Liew, F. V. (1980) Journal of experimental medicine, 152, 594

Louis, J. A., Zubler, R. H., Coutinho, S. G., Lima, G., Behin, R., Mauel, J. & Engers, H.D. (1982) Immunological reviews, 61, 215

Preston, P. M., Behbehani, K. & Dumonde, D. C. (1978) Journal of clinical and laboratory immunology, 1, 207

= = =