

TDR-2 PATHOGENESIS AND IMMUNOPATHOLOGY OF LEISHMANIASIS

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The principal aspects of human cutaneous and mucocutaneous leishmaniasis that occur in Brazil will be discussed as well as some data regarding experimental murine infection by Leishmania major and Leishmania mexicana.

At least three subspecies of Leishmania are capable of causing American cutaneous and/or mucosal leishmaniasis in Brazil (Lainson, 1983): Leishmania mexicana amazonensis, Leishmania braziliensis braziliensis and Leishmania braziliensis guyanensis.

American cutaneous and/or mucosal leishmaniasis (ACL) generally occur in one of the following three principal clinical forms:

Cutaneous Leishmaniasis (CL): is the most frequent form of ACL and may be caused by any of the three Leishmania subspecies. The cutaneous lesions are generally localized where the sandfly has inoculated the parasite by its bite; however, multiple lesions may occur. Cure may be either spontaneous or induced by antimonial therapy.

Mucosal or Mucocutaneous Leishmaniasis (MCL): is caused by Leishmania braziliensis braziliensis (Lainson, 1983), although cases caused by Leishmania mexicana have been reported. Severe secondary lesions of the facial mucosa (nose, mouth) may occur even several years after the primary skin lesion has been cured. Despite the extension and severity of such mucosal lesions, very few parasites are found and generally there is a poor response to therapy. In the majority of such patients, the immune response appears exacerbated in comparison to that of the CL patients.

Diffuse Cutaneous Leishmaniasis (DCL): is caused by Leishmania mexicana amazonensis in Brazil (Lainson, 1983). Numerous nodular skin lesions, disseminated over the body, with abundant parasites are found. This is a rare and severe form of the disease that also shows poor response to therapy. While the cell-mediated immune response may be normal for other antigens, it is depressed to Leishmania-antigens.

Histopathological Aspects

With regard to the histopathological aspects, Bryceson (1969) classified the spectrum of the disease in Ethiopian patients, which ranged from an anergic pole with abundant macrophages and parasites (found in DCL patients), to a hyperergic pole with a tuberculous reaction and parasites practically absent. "Intermediary" alterations reveal phagocytes, lymphocytes, and a moderate quantity of parasites. Poor responses to therapy are found in both the polar forms, similar to that observed in leprosy (Turk & Bryceson, 1970; Convit & Pinardi, 1974). However, the histopathological picture found in the New World cases of cutaneous and mucocutaneous leishmaniasis reveals particularities that led Ridley et al (1980) as well as Magalhães et al (1986a, 1986b) to suggest other classifications.

One important aspect that differentiates the cutaneous leishmaniasis of the Old World from that of the New World is the fact that, in the latter, a striking reaction of the connective tissue is superposed to the inflammatory infiltrate. This reaction is characterized by fibrinoid necrosis of the connective tissue that frequently affects small vessels, and by the proliferation of fibroblasts during the repair process (Ridley et al., 1980). The granulomatous reaction tends to be disorganized, and related to a post-necrotic reaction of the tissue (Ridley, 1979; Magalhães et al., 1986a). Due to the scarcity of parasites at the lesions, it is difficult to interpret pathogenesis of MCL caused by L.b.b. However, such necrotic lesions may occur parallelly to the destruction of the parasites, thus contributing towards the cure or aggravation of the lesions.

Characterization of the cellular population present in the inflammatory infiltrate shows a discrete excess

of the CD 8 phenotype cells which have a non-organized distribution in the inflammatory reaction (Modlin et al., 1985).

Cutaneous Leishmaniasis

In most cases of this clinical form, spontaneous or therapy-induced healing is apparently related to a well-modulated cellular immune response.

Mendonça et al. (1986) have shown that partial resistance to antimonial therapy in patients with recent cutaneous lesions, was associated with a low *in vitro* lymphoproliferative response to parasite's antigens. We also reported that the specific lymphoproliferative response increases, during antimonial therapy when the healing process starts. This, in addition to previous reports of various authors (Witzum et al., 1978; Castes et al., 1983; 1984; Carvalho et al., 1985) point to the relevance of the T lymphocyte-mediated cellular immune response in the healing process of cutaneous lesions. By using mice as experimental model, it has been shown that the principal protective immunity against *L. major* infection is mediated by T cells (Mitchell et al., 1980; Liew et al., 1982) that produce gamma interferon (γ -IFN) and macrophage activating factor (MAF) leading to the activation of macrophages and the destruction of internalized parasites (Titus et al., 1984; Nacy et al., 1985).

The importance of the host's genetic background in the outcome of *Leishmania* infections, has become evident by the existence of mouse strains that are susceptible (Balb/c) or resistant (CBA, C57) to the parasite (Handman et al., 1980; Behin et al., 1979; Perez et al., 1979; Howard et al., 1980).

The severity of clinical forms may be related as well to the species or subspecies of *Leishmania* involved. In this connection different parasite strains may show different degrees of resistance to the destruction by macrophages (Scott et al., 1983).

Diffuse Cutaneous Leishmaniasis (DCL)

A severe impairment of the cell-mediated immune response to *Leishmania* antigens, occurs in this clinical form, leading to high number of parasites and macrophages in the nodular lesions which resemble those of lepromatous leprosy (anergic pole).

Both the delayed-type hypersensitivity (DTH) as well as the lymphoproliferative response are specifically negative (Castes et al., 1983; 1984). However, Leishmania specific serum antibodies can be found, which apparently are not effective to determine a favorable outcome of the disease.

The Balb/c mouse has been used as an animal model for infection by L. major (Howard et al., 1980; Louis et al., 1982). Balb/c is highly susceptible to this parasite and develops a disseminated cutaneous disease with high parasite load together with an impaired cell-mediated immunity, very similar to that which occurs in human DCL. The experimental manipulations of the cell-mediated immunity carried out on less susceptible mice (CBA, C57) leading to aggravation of lesions also resulted in an increased parasite load.

Petersen et al. (1982, 1984) demonstrated that the adherent cell depletion of DCL patients restored the in vitro lymphoproliferative response to Leishmania-antigens. The mechanisms involved in the immunosuppression induced by macrophages could also be related either to the synthesis of prostaglandins, since indometacin reverts such activity or to a defective presentation of antigens to T cells. As regards this last hypothesis, Mitchell and Handman (1985) refer that different T cell subsets lacking the Lyt 2 surface antigen may possess either resistance-promoting or disease-promoting effects (exacerbation of lesions) in murine cutaneous leishmaniasis depending on the recognition of different carbohydrate epitopes on the same molecule, according to how this molecule is presented by macrophages. In this connection, Russel and Alexander (1987) demonstrated that two membrane antigens isolated from L. m. mexicana (the glycolipid "excreted factor" and the glycoprotein gp 63) reconstituted into liposomes and inoculated in Balb/c and CBA mice induced a strong protection in the absence of any exacerbative response. According to these authors, the liposome packaging enables the antigen preparations to be inoculated in their native, non-denatured conformation, anchored in the phospholipid bilayer by their hydrophobic regions.

In addition, depending on the inoculation route for immunization, the Balb/c mice may achieve a substantial resistance by intravenous (i.v.) procedure, in contrast

to the subcutaneous (s.c.) immunization which, in addition to not conferring protection, exacerbates the lesions when the challenge infection is performed (Liew et al., 1984; Liew et al., 1985). Interestingly s.c. immunization also induces strong DTH contrary to i.v. immunization, showing a dissociation between strong DTH and protection (Dhaliwal & Liew, 1987).

Titus et al. (1984) demonstrated that L. major-specific mouse T cell lines and clones which express the L3T4+, Lyt 1+ 2- phenotype can also enhance the development of the disease when transferred to infected recipients.

In these cases where mouse lesions are aggravated by the manipulation of the cellular immunity, the number of macrophages in the lesions as well as the parasite load increases.

Such macrophage increase at the site of the lesion may provide parasite "save target cells" (Mirkovich et al., 1986), that are recruited to the lesion by the L3T4+, Lyt 1+ 2- cells (Louis et al., 1986) which are in excess in susceptible Balb/c mice (Millon et al., 1986). Immunization procedures that cause aggravation (eg: s.c.) may as well induce the expansion of L3T4+ T cell subsets which mediate DTH and possibly recruit target cells less hostile to the invading parasites (Dhaliwal & Liew, 1987).

The protective immunity due to either immunization procedures or to convalescence of infection must not only attract, but also activate the macrophages to kill parasites by means of the production of gamma interferon and MAF by L3T4+ Lyt 1+ 2- T lymphocyte subsets with helper/inducer activity.

Cells of the CD8 phenotype in the lesion of CDL patients may impair the development of an effective granulomatous response, by either inhibiting IL-2 production (Modlin et al., 1985) or consuming the IL-2 produced by CD4 lymphocytes.

In the case of visceral leishmaniasis caused by L. donovani, a situation of immunosuppression similar to that of DCL occurs. Low IL-2 and gamma interferon production levels have been found by Carvalho et al. (1985).

Mucocutaneous Leishmaniasis (MCL)

Leishmania braziliensis braziliensis is the principal parasite which produces the mucosal and mucocutaneous forms of leishmaniasis in America. In general the delayed-type hypersensitivity (DTH) (Montenegro's intradermal test) is enhanced and may even produce phlyctenules or necrosis at the site where the antigen was inoculated. The in vitro lymphoproliferative response to Leishmania antigens is greater than in the case of cutaneous leishmaniasis patients (Castes et al., 1983; 1984; Carvalho et al., 1985). The level of anti-Leishmania serum antibodies is also high (Cuba et al., 1984).

In the case of Leishmania b.braziliensis infection, the study of the parasite-host relationship has been difficult due to the non-availability of a suitable experimental model. Mice, including the Balb/c strain, are resistant and hamsters develop a visceral form of the disease that is different from the human disease.

It has been verified that dogs become naturally infected in endemic areas where transmission of L.b.b. occurs, (Coutinho et al., 1985) and that the cutaneous lesions are clinically and histologically similar to those of the human disease (Firmez et al., 1987) even as regards the occurrence of mucosal lesions. Probably experimental studies using dogs as animal model would contribute to a better understanding of the operational mechanisms determining the severe forms (mucosal) of the disease. The studies on infection by L.b.b. (and the mucosal and mucocutaneous forms of the disease) have basically been restricted to human cases. Based on the fact that, in the great majority of cases involving the mucosal form of the disease, an extraordinary scarceness of parasites occurs at the lesions, together with exacerbated cellular and humoral immune responses (Castes et al., 1983; 1984; Carvalho et al., 1985), it is probable that a specific hypersensitivity reaction to leishmanial antigens may be involved in the pathogenesis of such lesions. A long period of exposure of patients to the parasite's antigens should be involved in the development of hypersensitivity reactions, both in patients with a long history of the disease (Carvalho et al., 1985) as well as in persons living in endemic areas who are constantly exposed to sandfly bites. It should be

stressed that Firmez et al. (1987) were able to isolate L.b.b. from the scars of naturally infected and clinically cured dogs, showing that the parasite is able to keep in balance with the host, which could explain the occurrence of secondary lesions after the clinical cure of a primary lesion produced by L.b.b.

In tuberculoid leprosy the cell-mediated immunity is capable of practically eliminating bacilli from lesions where, however, severe nerve damage is clearly evident (Bloom, 1986). An explanation for such lesions is that activated macrophages secrete neutral proteinases, which, by acting on the plasminogen, produce plasmin that would have the ability locally to degrade myelin proteins (Bloom, 1986).

It is possible that in mucosal leishmaniasis, a similar mechanism related to the cell-mediated immune response at the site of the lesions, may be relevant for the destruction of parasites, but it could also contribute to necrosis of the connective tissue.

Amongst such mechanisms, it may be recalled that activated macrophages produce tumour necrosis factor (TNF), a cytokine that is capable of destroying tumoral cells without affecting normal cells (Caswell et al., 1975). TNF has an *in vitro* effect on the Malaria parasites (Taverne et al., 1981) and on *Trypanosoma cruzi* (Titto et al., 1986). It could be inferred that cells, whether parasitized or not, that express parasite antigens on their surfaces, could become targets for TNF, contributing to the destruction of internalized parasites and also to necrosis of tissue.

Another possible mechanism, which, however has not been confirmed by us *in vitro* (Coutinho et al., 1985) - could be related to parasitized macrophages, as well as to other cells present at the site of the lesion and expressing the parasite's antigens, which could be specifically recognized by cytotoxic T lymphocytes (Andrade et al., 1984), leading to the destruction of the parasites and to a cure or aggravation of the necrotic lesions.

The aggravation of Balb/c mouse lesions due to infection by *L. major* (Titus et al., 1984) or by *L. m. mexicana* (Rodrigues et al., 1986) by adoptive transfer of *Leishmania*-specific L3T4 T lymphocytes

cannot be correlated to the severe lesions of human mucosal leishmaniasis caused by L.b.b. The physiopathology of these severe lesions is quite different in each case: in the animal model with an extraordinary parasite load and, in human MCL, with parasites being practically absent.

We have succeeded in adapting the limiting dilution assay (LDA) in order to quantify T lymphocytes that recognize Leishmania antigens in the blood of ACL patients (Dorea et al., 1987). Recent results obtained at our laboratory using LDA have shown that there are no significant differences between the frequencies of T cells which recognize leishmanial antigens in the blood of patients having the mucosal or the cutaneous forms of the disease. As regards the T cell phenotypes in the blood - whether CD4 or CD8 - significant differences have not been found between CL and MCL patients (Carvalho et al., 1986; Dorea et al., in preparation).

It is possible, however, that qualitative differences do exist between T cell populations and clones that would recognize different epitopes of the parasite's antigens (Mitchell & Handman, 1985; Russel & Alexander, 1987) and that could contribute towards the cure or aggravation of the disease. Melby et al. (1987) demonstrated that T cells from patients with mucosal and cutaneous leishmaniasis respond in a profoundly heterogeneous pattern to antigens of molecular weights ranging from 5-200 KD. Peak proliferative responses occurred to low molecular weight antigens (10-20KD). Results from our laboratory (Conceição-Silva et al., 1987) in collaboration with Dr. J. Scharfstein have demonstrated that Leishmania-specific T cell populations derived from CL patients with moderately positive DTH recognize L.b.b. antigenic fractions of low molecular weights (< 30 KD), while MCL patients (strong DTH), and CL patients but with strong DTH, recognize greater molecular weight fractions. However, it is still too early to speculate on the value of such data in the prognosis of secondary mucosal lesion occurrence in CL patients with strong DTH.

Tissue necrosis may also be caused by antibody dependent mechanisms. Immunocomplexes have been detected in the lesions (Ridley & Ridley, 1984; Magalhães et al., 1986b), and anti-laminin antibodies in serum samples from ACL patients (Avila et al., 1984).

For the analogy between the hyperergic pole of leprosy (tuberculoid) and the mucosal form of leishmaniasis to be more complete, in addition to the exacerbated cell-mediated immune response and scarcity of bacilli or parasites in both cases, one should also expect to encounter a tuberculoid histological picture in mucocutaneous leishmaniasis. According to Magalhães et al. (1986a) tuberculoid reaction occurs in 4.6% of the MCL patients while a cellular exudative reaction, also with an extraordinary scarcity of parasites but in the absence of granulomatous reactions occurs in 75.2% of MCL patients. Ridley (1980) refers to similar findings in MCL patients, infected by L.b.b. where both these types of tissue reaction also occurred: type I (non-reactive cellular exudate) or type V (hypersensitivity) lesions.

However the cellular exudate detected in mucosal lesions would not indicate non-reactivity, since, in most of these cases, the cell-mediated immune response is preserved or even enhanced.

Such data makes it difficult to establish a clear clinical-histopathological-immunological correlation in mucosal leishmaniasis. Although the absolute majority of MCL patients show an exacerbated cell mediated immune response, Ferreira et al. (1979) have found a few cases of mucosal leishmaniasis with negative DTH.

Intercurrent factors such as alcoholism, malnutrition, and other concomitant infections or parasitic diseases may also influence the dynamics of the host-parasite relationship.

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