Cellular response to leishmaniasis.

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SUMMARY.

Leishmaniasis is classified by the World Health Organization as one of the six major parasitic diseases. Over 12 million people are infected with Leishmania spp. The disease has a wide spectrum of clinical manifestations varying between self-healing cutaneous ulcers, disseminated cutaneous disease, non-resolving mucocutaneous lesions or fatal visceral disease. The clinical manifestations of leishmaniasis depend primarily on the species of parasite, but also involves genetically controled aspects of the host’s ability to develop an effective cell mediated immune response. In cutaneous leishmaniasis the role of CD4+ Th1 subsets is associated with resistance, whereas CD4+ Th2 is with susceptibility.

The evidence from studies on the immunology of leishmaniasis have clearly shown that both CD4+ and CD8+T lymphocytes are induced and are required to control the disease.
asociación de los linfocitos CD4+ Th1 con resistencia y Th2 con susceptibilidad. Sin embargo, es claro que tanto linfocitos CD4+ como CD8+ se inducen y requieren para controlar la enfermedad.

Palabras clave: Leishmaniasis, linfocitos CD4+, linfocitos CD8+, Células NK (Natural Killer).

CELLULAR RESPONSE IN LEISHMANIASIS

The disease

Clinical leishmanial infections range from self healing cutaneous to uncontrolled diffuse cutaneous disease, and from subclinical to fatal systemic visceral disease. Patients with active visceral leishmaniasis lack Leishmania specific delayed hypersensitivity responses during acute disease, when specific antibody titres are high, and their lymphocytes fail to proliferate, in vitro, to parasite stimulation. After resolution of symptoms, lymphocytes proliferate and produce cytokines in vitro in response to leishmania antigens. Although, leukopenia occurs in patients with acute visceral leishmaniasis, there may or may not be a severe reduction in the number of circulating lymphocytes, indicating that the depressed responsiveness is not due merely to the lack of available lymphocytes.

In cutaneous leishmaniasis, strong delayed hypersensitivity and in vitro proliferative responses occur both during disease and after healing. At the other end of the immunological spectrum is diffuse cutaneous leishmaniasis, characterized by uncontrolled cutaneous lesions and significant Leishmania specific antibody production in the absence of T cell proliferation or delayed hypersensitivity responses to the parasite. Mucosal leishmaniasis is frequently refractory to treatment and may be persistent or recurrent. With active mucosal disease, the intradermal skin test and lymphocyte proliferative responses are often exaggerated. The pathogenesis of the mucosal lesions may result from a hypersensitivity reaction to Leishmania, which could explain some of the features of the disease such as the destructive attack on host tissue and the relative paucity of the parasites in mucosal lesions (1).

T lymphocytes

There are two main types of lymphocytes: B cells which develop in the bone marrow and which may subsequently differentiate into antibody-producing plasma cells, and T cells which differentiate in the thymus. T cells consist of two major subsets, CD4+ and CD8+ cells. CD4+ cells provide help for antibody production, mediate the delayed type hypersensitivity responses, and are restricted by class II MHC antigens, whereas CD8+ cells are commonly associated with cytotoxic or suppressor functions, and recognize antigen in the context of MHC class I antigens. Both CD4+ and CD8+ T cell subsets are specifically involved in the clearance of intracellular pathogens, but under certain circumstances they are also involved in pathology and tissue damage. CD4+ T-cell clones can be divided into two subsets, Th1 and Th2, based upon the repertoire of cytokines they produce. Th1 cells produce interleukin-2 (IL-2), interferon-γ (IFN-γ) and lymphotoxin (LT), whereas Th2 cells produce IL-4, IL-5, IL-6 and IL-10 (2-5). Different functions have been associated with these populations; Th1 cells mediate delayed type hypersensitivity (6) and, although both Th1 and Th2 cells can provide help for antibody production, Th2 are much more efficient at this (7-10). T cell activation also appears to be affected by the type of APC; Th1 cells are preferentially stimulated by macrophages whereas Th2 cells are stimulated by B cells (11, 12). CD8+ T cells mainly produce the Th1 cytokine pattern (13).

CD4+ T cells in leishmaniasis.

Although mouse models do not faithfully reproduce the range of clinical leishmaniasis, they have provided important insights into
immunoregulation of leishmaniasis, particularly with regard to the role of T cell subsets and cytokine production in determining disease outcome and the role of T cells in leishmaniasis has been shown.

T cell deficient mice are unable to control *Leishmania* infection (14, 15) while reconstitution of BALB/c nude mice with CD4+ T cells from syngeneic euthymic donors resulted in resistance to infection (15-17). Susceptible BALB/c mice, exposed to sublethal whole body γ-irradiation before infection (18), or injection of anti-CD4 mAb (19), or cyclosporin A (20), renders them resistant to *L. major* infection. They developed a classical DTH response to *Leishmania* antigen, and antigen-specific culture supernatants could activate normal resident peritoneal macrophages to kill *L. major* amastigotes (21). However, sustained depletion of CD4+ T cells by mAb’s results in an inability to control the disease (22).

The nature of the T cell response determines the outcome of infection. CD4+ T lymphocytes mediate both resistance and susceptibility following a primary *L. major* infection, whereas CD8+ T lymphocytes play a limited role in controlling the disease. In contrast, CD8+ T cells may be as important as CD4+ T cells in mediating resistance to infection with *L. major* and *L. donovani*. It is now known that the outcome of *L. major* infection in mice is dependent on which of the CD4+ T cell subsets predominates: Th1 cell development leads the resistance, whereas the Th2 confers susceptibility.

Lymphoid tissue from resistant C57BL/6 and healed susceptible BALB/c mice contained predominantly mRNA for IFN-γ, whereas cells from susceptible BALB/c mice contained mainly mRNA for IL-4 and IL-10 (23-26). Protection of BALB/c mice by treatment with anti-IL-4 or anti-CD4 resulted in decreased levels of IL-4 mRNA and increased IFN-γ mRNA levels in the draining lymph node (26, 27). Culture supernatant from spleen cells of BALB/c mice recovered, or protected from infection, could activate macrophages to kill intracellular amastigotes due to the presence of INF-γ (21). In contrast, spleen cells from BALB/c mice with progressive disease, when stimulated in vitro, produced IL-3 and IL-4 which inhibited macrophage activation (28). Transfer of a Th1 cell line secreting IL-2 and IFN-γ protected BALB/c (29) or scid (30) mice against *L. major* infection. In contrast, transfer of a Th2 line secreting IL-4 resulted in an exacerbated infection. BALB/c mice inoculated with parasites plus IFN-γ produced higher levels of IFN-γ and had significantly smaller lesions than mice inoculated with parasites alone (31). In contrast, it has been reported that the transfer of an IFN-γ secreting cell line resulted in exacerbated infection (32, 33). However, different strains of the parasite could require other mechanisms as well as, or additional to, the induction of Th1 CD4+ T cells to control the disease. In cutaneous leishmaniasis, C57BL/10 mice heal following infection with *L. major*, but they fail to heal following infection with *L. amazonensis* (34) suggesting that resistance to *L. major* was mediated by Th1, but susceptibility to *L. amazonensis* was not exclusively controlled by Th2 cells. IL-4 production was restricted to the first few weeks of infection, but when lesions were evident IL-4 was not detected and only a slight reduction in lesion sizes and parasite numbers was observed with anti-IL-4 treatment one day before infection (34). Th1 and Th2 populations have not been clearly demonstrated in murine *L. donovani* infection (35). *L. donovani* infection in both cure C57BL/10 (Lsh+, H-2b) and non-cure B10.D2/n (Lsh-, H-2b) mice failed to produce IL-4 and IL-5 and had an early cytokine profile similar to that of naturally resistant (B10.L-Lsh+, H-2b) mice (36). Susceptible BALB/c mice are unable to show DTH response [related to Th1 (2)], at the active stage of the disease, whereas resistant BALB/b mice show strong DTH at any point of the infection (37). In BALB/c mice infected with *L. donovani*, IFN-γ secreting T cells predominate at both the early and late stages of infection,
whereas IL-4 secreting T cells predominate in between. In contrast, BALB/c and C57BL/6 mice show predominance of IFN-γ secreting T cells at any point post-infection (38). The isolation of Th1 and Th2 L. donovani-specific clones from humans recovered from visceral leishmaniasis after antimonial treatment has also been reported (39).

CD8+ T cells in leishmaniasis

The majority of research into immunity to leishmaniasis has concentrated on the IFN-γ producing Th1 CD4+ cell subset (40). Macrophages activated by cytokines such as IFN-γ show an elevated level of leishmanicidal behaviour to L. donovani (41,42) and L. major (43). However, anti-leishmanial capacity has also been achieved by granulocyte-macrophage colony-stimulating factor (GM-CSF) and tumor necrosis factor-α (TNF-α) (44). An increasing body of evidence suggests that Leishmania infection also induces CD8+ T cell responses.

In L. major infection, three weeks after infection at the onset of lesion healing, resistant CBA mice were found to have three times as many parasite-specific CD8+ T cells in the lymph nodes draining the cutaneous lesions as did susceptible BALB/c mice (22,45). CD8+ T cells from L. major-infected resistant CBA mice transferred Leishmania-specific DTH reactions to syngeneic recipients, providing evidence for the triggering of parasite-specific CD8+ T cells during infection with L. major (45). The possible role of these cells in infection was further elucidated using an anti-CD8 monoclonal antibody (mAb). Administration of anti-CD8 mAb exacerbated cutaneous L. major lesions in both susceptible and resistant strains of mice (22) and resulted in substantially greater numbers of parasites in the lesions compared to untreated, infected controls. However, even though the CD8+ cells were severely depleted, the genetically resistant strain still exhibited lesion healing. Deletion of CD8+ T cells in BALB/c mice made resistant by sublethal irradiation, by vaccination with killed L. major promastigotes, or by treatment with anti-CD4 or anti-IL-4 mAb, intravenous immunization, also led to enhanced disease, these cells having also found to mediate protective immunity (46-48). In isoprinosine-treated mice, increase in resistance to L. major has been associated with an increase in CD8+ T cell number, and increase in IFN-γ synthesis (49). It may be that in certain experimental models in which BALB/c mice are rendered resistant to infection (i.e. thymectomy, lethal irradiation and reconstitution with syngeneic bone marrow cells or by injection with anti-CD4 mAb), removal of suppressive CD4+ T cells allows the expansion of a protective CD8+ T cell population (48,50). These CD8+ T cells can transfer Leishmania-specific delayed test hypersensitivity (DTH) responses. In contrast to normal immune resistant mice which develop only small, rapidly healing secondary lesions, animals that receive anti-CD8 mAb at the time of reinfection develop severe, slowly healing secondary lesions (51). Contribution to the rapid resolution of secondary lesions was due to the INF-γ produced by memory CD8+ T cells in both resistant (CBA/J) and susceptible (BALB/c) mice. The latter are rendered resistant by anti-CD4 mAb treatment in the early phase of the primary infection (52). However, a clear need for CD8+ T cells has not been consistently demonstrated. In nude mice of both resistant and susceptible strains, the adoptive transfer of CD8+ T cells had no effect, whereas CD4+ T cells completely restored immunity to infection with L. major (17). Attempts to demonstrate a substantial role of CD8+ T cells in cutaneous leishmaniasis have been unsuccessful. Immunization with nonamer peptides based on H-2Kd motifs and derived from the L. major gp63 sequence, failed to induce a protective response even when two of the peptides elicited antigen-specific CD8+ T cells expressing elevated levels of IFN-γ mRNA and with cytotoxic activity against P815, sensitized either with peptide or lysate from L. major (53). Recent studies using β2m deficient
“knockout” mice that lack mature CD8+ T cells have demonstrated that the control of *L. major* infection is dependent on INF-γ in both normal or deficient mice failing to support CD8+ T cells in defence against *L. major* infection (53).

Killing of *Leishmania*-infected macrophages by a *Plasmodium* circumsporozoite protein-specific CD8+ T cell clone in a long 51Cr release assay which was partially abrogated by addition of anti-INF-γ mAb, suggests that CD8+ T cells in leishmaniasis could exert their activity by IFN-γ production rather than cytotoxic effects (54). The contribution of IFN-γ producing CD8+ T cells in a CD4+ T cell-dependent fashion, has also been demonstrated in *L. m. amazonensis*-infected C57BL/6 mice. Depletion of CD8+ T cells by mAb’s and complement results in 2/3 reduction of the total amount of IFN-γ (55).

In visceral leishmaniasis, the ability of nude BALB/c mice to control *L. donovani* infection was achieved only by the adoptive transfer of both CD4+ and CD8+ T cells (56). The ability of CD8+ T cells isolated from infected mice to produce IFN-γ (41,56,57), their direct relationship in infiltration into the liver and parasite clearance, as well as their predominance at the time of cure (56,58) have all been demonstrated. The requirements for these cells has been attributable to their role in the efficient generation of tissue granuloma during the curative phase of the disease (56-58). Acquired immunity in visceral leishmaniasis has been associated with MHC class II genes, but recent studies on class II transgenic mice also support the theory that altered patterns of resistance are due to IFN-γ production by CD8+ T cells (57). On the other hand, it has recently been shown that BALB/c mice infected with *L. donovani* produce functional cytotoxic CD8+ T lymphocytes (59).

**Natural killer cells.**

Little work has been done on the possible role of NK cells in leishmaniasis. The involvement of NK cells has been examined using C57BL/bg/bg mice which are profoundly deficient in NK cell activity. The mice showed less capacity to eliminate *L. donovani* infection than the phenotypically normal controls. However, there was no difference seen in the course of *L. major* infection (60, 61).

The possible role of natural killer cells (NK) in the initiation of CD4+ T cell subset differentiation and in controlling early resistance to *L. major* infection, has recently been reported. In resistant C3H/HeN mice, NK cell activity was more prominent than in susceptible BALB/c mice during the first week of infection, and removal of these cells significantly decreased IFN-γ levels and promoted IL-4 production in both, draining lymph nodes and spleen cells (62). In visceral leishmaniasis, studies performed in immunodeficient SCID mice reported that *L. donovani*-infection does not trigger IFN-γ production by NK cells. Furthermore, *in vitro* infection inhibited the IFN-γ response of scid spleen cells to simultaneous *Listeria monocytogenes* challenge (57).

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