Symposium on the IMMUNOLOGY OF LEPROSY, Oslo, Norway, 1986
Lepr Rev (1986) 57, Suppl 2, 255-261

Attempts to assess the contribution of T lymphocytes from the L3T4$^+$ and LYT2$^+$ subsets in the immunological control of cutaneous leishmaniasis

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Introduction

The Leishmanias are protozoan parasites capable of infecting several mammalian species including man. After injection into the skin, by the bite of infected sandflies, the flagellate forms (promastigotes) of this microorganism are engulfed by mononucleated phagocytic cells where they transformed into aflagellate amastigotes. Upon feeding on infected hosts, the insect vector takes up parasitized macrophages and in their digestive tract the amastigotes transform into promastigotes. Infection of man with Leishmania results in a broad spectrum of disease profiles which is thought to depend both upon the characteristics (i.e. tissue tropism) of the different leishmania species and the host response.

Depending on their genetic background, the entire spectrum of clinical manifestations seen in humans can be reproduced in mice experimentally infected with *L. major*. For example, after infection with *L. major* CBA mice develop small local lesions which resolve after a few weeks whereas in BALB/c mice, the severe lesions which develop at the site of inoculation do not heal and visceralisation occurs (1, 2). It appears therefore that the parameters which give rise to resistance or susceptibility to infection with *L. major* can best be studied in the murine model of infection.

The results obtained by several groups of investigators indicate that specific antibodies do not play an important role in the control of experimentally-induced infection with *L. major* (3).

It is now well established that macrophages are important in the expression of disease induced by *L. major*. This contention is supported by several experimental observations. The demonstration that thioglycollate-induced macrophages obtained from the peritoneal cavity of genetically susceptible mice support the proliferation of intracellular *L. major* better than macrophages from resistant mice indicated that the expression of disease is influenced by the innate capacity of macrophages to eliminate *L. major* (1). Some degree of refractoriness of macrophages from genetically susceptible mice to become activated by lymphokine preparations *in vitro* has also been experimentally demonstrated (4). In addition, a specific resistance of some leishmania species (L. Mexicana Amazonensis) to the microbicidal action of macrophages activated by appropriate lymphokines has been documented (5). Finally, ob-

servations showing that compared to resistant mice, increased numbers of immature cells of the macrophage-granulocyte lineage presumably permissive for parasite growth are found in genetically susceptible BALB/c mice give further support for an important role of macrophages in determining the outcome of infection (6).

The pivotal role of T cells in the resolution of lesions induced in mice by *L. major* is now generally recognized. This role has been clearly established by the pioneering work of G. Mitchell and colleagues in Australia which showed that the severe generalized disease that normally develops in nu/nu mice after infection with *L. major* can be overcome by the transfer, before infection, of as few as 10^6 syngeneic T cells (7). The exact mechanism by which T cells participate in the healing of *L. major*-induced lesions in vivo is not yet known. However, it is generally assumed that the release by specific T cells of lymphokines capable of activating infected macrophages represents the main effector pathway for the elimination of these parasites. A possible role of T lymphocytes cytolytic for *L. major*-infected macrophages should also be considered (8).

It is likely that the comprehension of the paremeters of the T cell response necessary for the immunological control of cutaneous leishmaniasis will depend upon the characterization of the T cell response generated as a result of infection in genetically resistant mice. In addition, the identification of the defect(s) in the immune T cell response in susceptible mice might permit to devise ways of manipulating the immune system so as to induce protective immune responses. It is therefore not surprising that several laboratories have tried to analyse and compare the functional T cell responses in mice from resistant and susceptible strains. In adoptive cell transfer experiments using as recipients either normal or athymic mutant mice from resistant strains, protective immune response has been shown to be mediated by $Lyt1^+2^-$ T cells (9, 3). Furthermore, evidence has been obtained suggesting that specific suppressor T cells, also expressing the $Lyt1^+2^-$ cell surface phenotype, and capable to inhibit a potentially curative T cell response are induced during infection of genetically susceptible mice (3). These $Lyt1^+2^-$suppressor cells and their culture supernatants could inhibit an *L. major*-induced T cell proliferative response *in vitro* and the induction of specific delayed-type hypersensitivity (DTH) reactions *in vivo* (3). A defective antigen-presenting function of parasitized macrophages from susceptible mice has been postulated to play a role in the induction of these *L. major*-specific T cells (1, 10). Furthermore, it has been recently hypothesized that «protective» T cells and specific suppressor T cells (promoting the disease) recognize different carbohydrate determinants of the same lipid containing glycoconjugate present in the membrane of *L. major* (11). When enchored in the membrane of macrophages by its lipid moiety, the glycoconjugate would present determinants which in association with class II MHC gene products trigger protective macrophage-activating T cells. Delipidated glycoconjugate bound to its macrophage receptor would present determinants triggering suppressor T cells capable of inhibiting protective T cells presumably through interleukin 2 (IL. 2) consumption. According to this hypothesis, mice genetically resistant to infection with *L. major* should be non-responsive to suppressor cells-inducing carbohydrate determinants (11).

In this presentation, results obtained in our laboratory showing that the magnitude of the specific $L3T4^+$ T cell response might also determine the expression of murine cutaneous leishmaniasis will be reviewed. In addition, evidence will be shown indicating that $Lyt2^+$ T cells also play a role in the optimal control of infection with *L. major*.

Outline of methods

1. *L. MAJOR*-SPECIFIC $L3T4^+$ T CELL POPULATIONS

Lymph node cells from mice either primed with crude leishmania antigens or infected with 2

x 10^6 *L. major* were cultured in the presence of *L. major* antigens *in vitro* for five to six days. Blasts were isolated on discontinuous Percoll gradients and cultured for three days in IL-2-containing medium. Characterization of these cells by flow cytometry revealed that they expressed the Thy-1.2$^+$, L3T4$^+$, Lyt2$^-$ surface phenotype (12).

2. LIMITING DILUTION ANALYSIS (LDA) FOR THE ESTIMATION OF FREQUENCY OF *L. MAJOR* SPECIFIC T CELLS (MEDIATING DTH) IN LYMPH NODES OF INFECTED MICE

L. major-specific T cells able to transfer DTH reactions were enumerated and phenotypically characterized following an LDA procedure described preciously (13). In brief, serial dilutions of lymph node cells were injected together with *L. major* SC into one hind footpad of normal syngeneic mice. Sixteen hours later, positive transfer of DTH reactions were scored. Estimation of the frequency of specific T cells were obtained by minimum chi-squared analysis applied to Poisson distribution. To determine the surface phenotype of specific T cells, frequencies were determined after treatment with IgM cytolytic anti-L3T4, or Lyt2 monoclonal antibodies (MAbs) and complement.

3. DEPLETION OF L3T4$^+$ and LYT 2$^+$T CELLS IN VIVO WITH APPROPRIATE MABS

Two different rat Ig2b MAbs were used: anti-L3T4 MAb GKI. 5 (14) and anti-Lyt2 MAb H35-17.2 (15) were kindly donated by Drs. F. Fitch (Chicago, USA) and M. Pierres (Marseilles, France) respectively. These MAbs were administered *in vivo* in the form of culture supernatants of the relevant hybridoma line. The regimens used to selectively deplete T cells from a particular subset with MAbs have been detailed (16, and manuscript submitted for publication).

4. ASSESSMENT OF INFECTION WITH *L. MAJOR*

Various times after injection of living *L. major* promastigotes SC in one footpad, the size of the resulting lesions was obtained by measuring the increase in footpad thickness with a vernier caliper and by counting the numbers of living *L. major* in the infected footpad using a limiting dilution assay (17).

Results

1. EXACERBATION OF CUTANEOUS LEISHMANIASIS IN MICE ADOPTIVELY TRANSFERRED WITH *L. MAJOR* SPECIFIC L3T4$^+$ T CELLS

The *L. major*-specific L3T4$^+$ T cells were assessed functionally, after expansion in IL. 2, and demonstrated to perform a variety of immunological functions which could have been predicted to facilitate the resolution of lesions in infected mice. Namely these T cells where able to; (a) provide helper activity in antibody responses both *in vivo* and *in vitro* (18), (b) transfer DTH responses to syngeneic mice and and (c) produce, upon specific stimulation *in vitro,* macrophage activating factors/IFN and haemopoietic colony stimulating factors such as Interleukin-3 (18, unpublished observations).

Surprisingly, the intravenous injection of these L3T4$^+$ T cells twenty-four hours before infection with *L. major* exacerbated the course of cutaneous leishmaniasis in both susceptible and resistant mice as assessed by measuring the size of lesions and by enumerating parasites in the lesions (12, 17). All cloned T cell lines derived from these L3T4$^+$ T cell populations exhibited similar functional activities and also enhanced the development of lesions.

Although the exacerbation of cutaneous leishmaniasis by L3T4$^+$ T cells was immunologically specific, T cells specific for an irrelevant antigen were also able to enhance cutaneous

leishmaniasis but only when they were activated by their specific antigen and attracted to the site of the developing lesion. Recent observations showing that enhancement of lesion's development after injection of 10^7 *L. major*-specific L3T4$^+$ T cells does occur in either ATxBM mice or in mice rendered B cell deficient by treatment with anti-IgM antibodies suggest that this effect is not the consequence of either the production of suppressor cells or of specific antibodies. (Titus, Cerny, Zinkernagel and Louis, manuscript in preparation).

In as much as significant numbers of specific L3T4$^+$ T cells localize rapidly into the site of lesions, we had hypothesized that their activation could result in the continuous recruitment, at the site of infection with *L. major,* of blood derived phagocytes, the host cells required for the growth of leishmania (12, 13, 19). The importance of recruitable haemopoietic cells for the growth of *L. major* in infected tissues is exemplified by observations showing that four days after infection, the number of parasites present in the lesions of mice, which had been lethally irradiated forty-eight hours before infection, was ten times lower than that found in lesions of similarly infected mice (19). Moreover, the administration, in the site of the lesion, of bone-marrow cells (depleted of T cells) was shown to reconstitute normal levels of parasite growth in irradiated mice. While in situ administration of L3T4$^+$ specific T cells or intraveinous injection of bone-marrow cells had no effect on the number of parasites produced in lethally irradiated mice, administration of both cell populations resulted in a dramatic increase in the number of parasites found in these mice (Mendonca and Louis, manuscript in preparation).

These observations indicate that these L3T4$^+$ T lymphocytes, upon activation at the site of infection with *L. major,* favour the growth of parasites by recruiting myelomonocytic cells originating in the bone-marrow. In addition, these L3T4$^+$ T cells release molecules capable of influencing the proliferation and differentation of hemopoietic progenitor cells (unpublished). It is therefore possible that *in vivo* these molecules lead to increased numbers of circulating myelomonocytic cells recruitable to the site of lesions.

2. ENUMERATION OF PARASITE-SPECIFIC L3T4$^+$ AND LYT2$^+$ T CELLS IN SUSCEPTIBLE AND RESISTANT MICE DURING THE COURSE OF INFECTION WITH *L. MAJOR*

The observations described above indicated that an excess of L3T4$^+$ specific T cells may favour the development of lesions. Therefore, it was considered of interest to determine whether or not infection of susceptible BALB/c mice was accompanied by the induction of high numbers of specific L3T4$^+$ T cells. To this end, the frequency of *L. major*-specific T cells capable of initiating specific DTH responses was estimated in the lymph nodes draining the lesions of susceptible and resistant mice at various times after infection following a methodology outlined in the method section and detailed elsewhere (13). At all times points tested after infection, the frequency of specific T cells was significantly higher (ten to fifty times) in the lymph nodes of BALB/c mice than in CBA mice. Analysis of the surface phenotype of T cells mediating DTH revealed that ninety per cent of BALB/c specific T cells expressed the L3T4$^+$ surface antigen whereas in CBA mice approximately equal numbers of L3T4$^+$ and Lyt2$^+$ T cells were generated.

It appears therefore that the generation of considerable numbers of specific L3T4$^+$ T lymphocytes mediating DTH reactions characterizes the T cell response of susceptible mice to infection with *L. major*. Recent obervations showing that, at the time when cutaneous lesions begin to resolve in resistant mice, higher numbers of specific Lyt2$^+$ T cells are present in lymph nodes of these mice than in BALB/c mice indicate that specific Lyt2$^+$ T cells also might have a protective function in murine cutaneous leishmaniasis (manuscript in preparation).

3. EFFECT ON THE COURSE OF CUTANEOUS LEISHMANIASIS OF DEPLETING EITHER L3T4$^+$ T CELLS OR LYT2$^+$ T CELLS BY ADMINISTRATION OF RELEVANT MABS

Results reviewed above indicated that : (a) an excess of L3T4$^+$ T cells could be detrimental for the host and (b) the specific T cell response of susceptible mice is earmarked by an important L3T4$^+$ T cells response.

Therefore, the course of infection was studied in susceptible mice in which the pool of L3T4$^+$ T cells had been reduced by administration of anti-L3T4$^+$ MAbs. Elimination of approximately seventy per cent of L3T4$^+$ T cells from lymphoid tissues of BALB/c mice reversed the susceptibility of these mice to infection with *L. major* (16). Interestingly, similar reduction of L3T4$^+$ T cells in genetically resistant CBA mice was detrimental to the host (19).

One might speculate that reduction of the number of L3T4$^+$ T cells in BALB/c mice, in which an excess of specific L3T4$^+$ T cells are normally triggered as a result of infection, could facilitate the induction of optimal numbers of specific L3T4$^+$ T cells necessary for the immunological control of cutaneous leishmaniasis. In contrast, similar manipulation in CBA mice would interfere with the induction optimal numbers of specific L3T4$^+$ T cells normally triggered in these mice as a result of infection. It is important to emphasize that virtual elimination (more than ninety-five per cent) of L3T4$^+$ T cells by intensive treatment with MAb GK 1.5 resulted in the development of severe uncontrolled lesions in both susceptible and resistant mice, indicating the necessity of some specific L3T4$^+$ T cell responses for the immunological control of cutaneous leishmaniasis in mice.

Modulation of the Lyt2$^+$ T cell subset by administration of anti-Lyt2 MAbs also influenced the course of murine cutaneous leishmaniasis. Elimination of sixty-five to eighty-five per cent of Lyt2$^+$ T cells significantly enhanced the development of lesions induced by *L. major* in both genetically susceptible and resistant mice (manuscript in preparation). Therefore, it appears that both specific L3T4$^+$ and Lyt 2$^+$ T cells are necessary for an optimal control of infection with *L. major*.

Concluding remarks

Results obtained by several groups of investigators provided evidence for the requirement of specific L3T4$^+$, Lyt1$^+$2$^-$ T cells in the immunological control of murine cutaneous leishmaniasis. The destruction of intracellular parasites by macrophages activated by L3T4$^+$ T cell derived factors is likely to represent the main effector mechanism. However, the results obtained in BALB/c mice suggest that the protective role of L3T4$^+$ cells may depend on the actual number of such cells that are generated during infection. In excess, these L3T4$^+$ T cells may favour the recruitment and/or the proliferation of macrophages at the site of lesions. As a result of the local accumulation of macrophages, the number of parasitized macrophages will be considerable increased to that the infection becomes no more controlable by protective immune mechanisms (such as destruction of the parasites by activated macrophages). According to this hypothesis, the difference in the outcome of infection between susceptible and resistant strains may be explained, at least in part, by a quantitative difference in the number of parasite-specific L3T4$^+$ cells.

An alternative hypothesis would be that the L3T4$^+$ T cell response in susceptible and resistant mice is qualitatively different. It has been shown very recently that two functional subsets of L3T4$^+$ T cells exist that differs in their ability to produce distinct lymphokines (20). It is therefore also possible that one subset of L3T4$^+$ T cells has mainly a protective role (e.g. by secreting factors activating macrophages), whereas another may be deleterious (e.g. by producing factors implicated in the multiplication and/or the recruitment of phagocytes).

The outcome of infection, according to this hypothesis will depend on the balance between these two subsets.

The results summarized in this review also indicate that Lyt2$^+$ T cells might have a protective function in murine cutaneous leishmaniasis.

Acknowledgment

The experimental work from our laboratory referred to in the text is supported by the Swiss National Science Foundation and the UNDP/World Bank/WHO Special Programme on Tropical Diseases.

We thank Miss P. Adams for excellent secretarial assistance.

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