RESEARCH NOTE

CD4+ and CD8+ T Cell Immune Responses of Immunocompetent and Immunocompromised (AIDS) Patients with American Tegumentary Leishmaniasis

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The majority of the *Leishmania* parasites which causes American tegumentary leishmaniasis (ATL) in Brazil belongs to the following species: *L. braziliensis*, *L. guyanensis* and *L. amazonensis.* However, in the area of Rio de Janeiro the only species that has been detected infecting human and dogs is *L. braziliensis* (H Momen et al. 1983 *J Cell Biochem 70* (Suppl): 28, G Grimaldi Jr et al. 1989 *Am J Trop Med Hyg 41*: 687). Transmission occurs when the respective Phlebotominae vector inoculates the promastigote forms of the parasite into the dermis of the mammalian host during its blood meal. The promastigote forms are then internalized by dermal phagocytic cells and transformed into amastigote forms. Macrophages represent the preferential habitat for the amastigote forms and the principal effector cells for parasite destruction as well.

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The host/parasite relationship can either be driven toward the cure and destruction of the parasite or toward active disease with production of tegumentary lesions, depending on the higher or lower macrophage capacity for parasite destruction. Many studies utilizing the mouse model (SG Coutinho et al. 1984 *Parasite Immunol 6:* 157, RG Titus et al. 1984 *Clin Exp Immunol 55:* 157, P Heinzel et al. 1989 *J Exp Med 169:* 59, FY Liew et al. 1989 *Eur J Immunol 19:* 1227, RG Titus et al. 1989 *J Exp Med 170:* 2097, RM Locksley et al. 1991 *Res Immunol 142:* 28, P Scott 1991 *Exp Parasitol 75:* 196, FP Heinzel et al. 1991 *Proc Natl Acad Sci USA 88:* 7011, SL Reiner et al. 1993 *Science 259:* 1457) have shown that the T cell-mediated immune responses play a pivotal role in these processes, either by activation of macrophages and killing of the parasites or by inhibition of the macrophage functions. In the first case, as observed in mouse strains resistant to *L. major* infection, the Th1 CD4+ T cell subsets are preferentially activated with production of type 1 lymphokines (e.g. interleukin 2 - IL-2, gamma interferon - IFN-γ and lymphotoxin) leading to activation of macrophages and destruction of intracellular amastigotes. A delayed-type hypersensitivity (DTH) to parasite antigens is elicited in resistant mice. In the second case, as observed in mouse strains susceptible to *L. major* infection, Th2 CD4+ T cell subsets will be preferentially activated leading to production of type 2 lymphokines (e.g. IL-4, IL-10) with inhibition of macrophage activation allowing parasite multiplication into the parasitophorus vacuole and aggravation of the disease. The cell mediated immune response is depressed with negative DTH.

There is evidence that CD8+ T cells may also play an important role in the mechanisms of cure and resistance to *Leishmania* infection, either by IFN-γ production and activation of macrophages (I Müller et al. 1993 *Infect Immun 61*: 33730, MMY Chan et al. 1993 *Eur J Immunol 23*: 1181, I Müller et al. 1994 *Infect Immun 62*: 2575, MM Stefani et al. 1994 *Eur J Immunol 24*: 746) or by a cytolitic effect of CTL cells upon parasitized macrophages (Coutinho et al. *loc. cit.*, F Conceição-Silva et al. 1994 *Eur J Immunol 24*: 2813) or by a combination of both effects.

ATL IN IMMUNOCOMPETENT PATIENTS

Studies in humans have not shown a such well delimited poles for the immune responses in ATL, in spite of some similarities with the mouse model (J Convit 1974 *Ethiopian Med J 12*: 187, M Castes et al. 1983 *Clin Immunol Immunopathol 27*: 176, EM Carvalho et al. 1985 *J Immunol 135*: 4144, SCF Mendonça et al. 1986 *Clin Exp Immunol 64*:

269, SG Coutinho et al. 1987 *Mem Inst Oswaldo Cruz 82* (Suppl I): 214, M Castes et al. 1988 *J Clin Microbiol 26*: 1207, F Conceição-Silva et al. 1990 *Clin Exp Immunol 79*: 221, C Pirmez et al. 1990 *J Immunol 145*: 3100, A Barral et al. 1993 *Proc Natl Acad Sci USA 90*: 3442, J Convit et al 1993 *Trans R Soc Trop Med Hyg 87*: 444).

Probably in the human cases of natural resistance to dermatropic *Leishmania* species, without lesions, a typical Th1 $CD4^+$ immune response may occurs with production of IFN-γ (type 1 lymphokine) like in resistant mouse strains (M Kemp et al. 1994 *Clin Exp Immunol 96*: 410). In endemic areas of leishmaniasis in South America, cases of positive DTH without lesion or typical scars have been found, pointing to the occurrence of naturally resistant individuals (WJS Souza et al. 1992 *Acta Tropica 52*: 111, J Weigle et al. 1993 *J Infect Dis 168*: 699).

On the other hand, a small percentage of patients can develop severe forms of ATL in endemic areas in Brazil: the diffuse cutaneous leishmaniasis (DCL) and the mucocutaneous leishmaniasis (MCL).

DCL is produced mainly by *L. amazonensis.* Nodular disseminated lesions appear, full of parasitized macrophages. A parasite specific cellular immune depression occurs, with production of type 2 lymphokines (IL-4, IL-10), as well as negative DTH, similarly to the *L. major* infection in susceptible mouse strains (Castes et al. *loc. cit.*, J Convit et al. *loc. cit.*, G Caceres-Dittmar et al. 1993 *Clin Exp Immunol 91*: 500).

MCL is produced mainly by *L. braziliensis*. Secondary metastatic mucosal lesions occur in the face (nose, mouth) with a extreme scarceness of parasites. The T cell-mediated immune response is frequently enhanced with exacerbated hypersensitivity (Carvalho et al. *loc. cit.*, Coutinho et al. 1987 *loc. cit.* Castes et al. *loc. cit.*). There are very few experimental studies on this clinical form, because mice are naturally resistant to experimental infection with *L. braziliensis*.

The most common clinical form of ATL is the localized cutaneous leishmaniasis (LCL) where a single or a few skin ulcers occur with a tendency toward self healing or susceptibility to the classical antimonial therapy. The scarceness of parasites in the lesions and the presence of hypersensitivity to leishmanial antigens (DTH) are the main immunoparasitological feature of LCL.

Studies from our laboratory (AM Da-Cruz et al. 1994 *Infect Immun 62*: 2614, SG Coutinho et al. manuscript in preparation) on LCL patients examined before antimonial therapy (active disease) and at the end of the therapy (cure) have shown that the lymphoproliferative responses (LPR) of peripheral blood mononuclear cells (PBMC) after stimulation *in vitro* with total *L. braziliensis* promastigotes antigens, as measured by 3H thymidine incorporation, were not significantly different before therapy (stimulation indices - SI = 31.9 ± 29.4) and at the end of the therapy (SI = 15.0 ± 16.0) although a tendency to decline has been observed.

The phenotypes of leishmanial antigen-reactive T cell-stimulated *in vitro* were also investigated in PBMC cultures. After five days in culture, blast cells were separated by centrifugation over a discontinuous Percoll (Sigma Chemical Co., MO, USA) gradient incubated in the presence of monoclonal antibodies for $CD3^+$ (T3-RD1, Coulter Immunology, FL, USA), $CD4^+$ (T4-FITC, Coulter Immunology) and $CD8^+$ (T8-RD1, Coulter Immunology) and finally analyzed by flow cytometry. The supernatant of each culture was also collected and stored at -70°C until use for determination of cytokine concentrations.

Comparing the proportions of $CD4^+$ and $CD8^+$ *L. braziliensis*-reactive blast T cells before therapy (BT) and at the end of therapy (ET) we observed an increase in the percentage of $CD8^+$ cells (BT=23.9 ± 11.7; ET=42.6 ± 21.7; $p < 0.05$), a decline in the proportion of $CD4^+$ cells (BT=61.2 18.3; ET=40.9 ± 21.7; $p < 0.05$) and a consequent reduction in the $CD4^+/CD8^+$ ratio (BT=2.5; ET=0.9).

These results suggested that $CD8^+$ T cells could be implicated in the mechanisms of cure of LCL. However it was not clear whether the process of cure was associated only with the increased percentages of $CD8^+$ *Leishmania*-reactive T cells or whether it also depended on the balance between $CD4^+$ and $CD8^+$ cells.

In this group of patients (and in another group of LCL patients further studied) the levels of IFN-γ and IL-4 production by *Leishmania*-reactive T cells were determined, by testing the supernatant of antigen-stimulated PBMC cultures. IFN-γ was measured by a solid-phase enzyme-linked immunosorbent assay (ELISA test kit for quantification of human IFN-γ; Holland Biotechnology, Holland), and IL-4 was also measured by an ELISA test (Intertest 4 - Genzyme Corporation, MA, USA). The mean levels of IFN-γ in supernatants from *Leishmania*-stimulated cell cultures were 123.7 ± 58.6 U/ml before therapy (active disease) and 193.4 ± 70 U/ml at the end of therapy (cure). The mean levels of IL-4 at the same occasions were respectively BT=415.8 ± 633.1 pg/ml and ET= not

detectable. Hence in association with the $CD4^+$-$CD8^+$ switch in cured patients it was also observed a slight, but not significant increase of IFN-γ production at the end of therapy, as well as a striking significant decrease in the IL-4 production in cured patients. Thus active LCL was characterized by predominance of a $CD4^+$ T cell response with production of a mixed type 1 (IFN-γ) and type 2 (IL-4) cytokine profile. On the other hand the process of cure was associated with a predominance of a $CD8^+$ T cell response, with production of IFN-γ and absence of IL-4, characterizing an apparently beneficial type 1 cytokine profile.

The lymphokine profiles determined in the skin lesions of active cases of ATL (C Pirmez et al. 1993 *J Clin Invest 91*: 1390, Cáceres-Dittmar et al. *loc. cit.*) have also shown a mixture of type 1 and type 2 lymphokines with relatively predominant of mRNA for type 1 lymphokines.

ATL IN IMMUNOCOMPROMISED PATIENTS (AIDS)

Patients in a state of immunosupression produced by the acquired immunodeficience syndrome (AIDS) when also infected with *Leishmania* parasites, display clinical signs and symptoms that are either novel or more severe than usual. There are several reports of AIDS patients who acquired visceral leishmaniasis apparently as an opportunistic disease (R Badaró et al. 1986 *Lancet I:* 647). Several cases of ATL and AIDS have been described, all of them displaying unusual immunopathological features (JR Coura et al. 1987 *Mem Inst Oswaldo Cruz 82*: 581, M Scaglia 1989 *Trans R Soc Trop Med Hyg 83*: 338, RMC Cunha et al. 1991 *Rev Soc Bras Med Trop 24* (Suppl 2): 109, E Machado et al. 1992 *Mem Inst Oswaldo Cruz 87*: 487).

We have studied two cases of ATL/AIDS associated diseases: the first one (AM Da-Cruz et al. 1992 *Trans R Soc Trop Med Hyg 86*: 511) displayed many lesions most of them with a pustulonodular aspect, and high parasite load. A clear depression of the T cell-mediated immune responses to *Leishmania*-antigens occurred as detected by negative intradermal test (DTH) and the absence of a lymphoproliferative response to leishmanial antigens (SI=1.2). This picture is similar to that observed in classical DCL. The HIV infection provokes a decrease in the pool of circulating $CD4^+$ cells leading to generalized immune depression (AIDS). Thus, the DCL-like picture in our patient was probably related to the inability of his T cell-mediated immune response to control the spread of the *Leishmania* infection.

The second case (AM Da-Cruz et al., manuscript in preparation) displayed most of the ATL lesions in the face and presented resistance to the classical antimonial therapy. Surprisingly, when a combined *Leishmania* antigen-immunotherapy associated with antimonial was tried (W Mayrink et al. 1992 *Parassitologia 34*: 159), the patient had clinical cure of the ATL lesions, in spite of no apparent clinical improvement of the HIV infection. The lymphocyte proliferative response induced by leishmanial antigens which was negative before the combined therapy (active ATL lesions) became positive after that therapy (healed ATL lesions). The majority of the antigen-responding cells after therapy belonged to the $CD8^+$ phenotype as measured by flow cytometry. The levels of IFN-γ in the supernatants of the antigen stimulated PBMC cultures were also not detectable before therapy and positive after therapy.

These results suggest that activation of $CD8^+$ T cells and production of IFN-γ may have a beneficial effect in ATL, although we can not reject the possibility that the IFN-γ detected in the culture supernatants would be produced by other cell types (e.g.: natural killer cells).

FINAL COMMENTS

We have shown that cure in LCL either in immunocompetent individuals or in immunocompromised patients (AIDS), can be associated with predominant $CD8^+$ T cell activation and production of IFN-γ *in vitro*. This does not mean that $CD4^+$ *Leishmania*-reactive T-cells are not important in the mechanisms for healing of lesions, because the observed decrease in the $CD4^+$ subpopulations in AIDS patients aggravates the parasitic disease.

Three hypothesis at least could arise to explain the immunological changes observed after therapy: (a) the effect of therapy and the $CD4^+$ T cells functions (production of mixed type 1 and type 2 lymphokines) led to decreased parasite load and healing of lesions. In this case $CD8^+$ T cells would represent an epiphenomenon, just replacing the actually effective $CD4^+$ T cells, which would suffer apoptosis after their activation. However, the second AIDS patient mentioned above had a tendence to cure associated with $CD8^+$ and not $CD4^+$ T cell responses. Moreover, results from our laboratory (SCF Mendonça et al. 1995 *Am J Trop Med Hyg 53*: 195) on vaccination of human volunteers with a crude promastigote leishmanial vaccine (W Mayrink et al. 1979 *Trans R Soc Trop Med Hyg 73*: 385) have shown that the majority of the *Leishmania*-responding T cells in assays of lymphoproliferative response to the parasite antigen belonged

to the $CD8^+$ phenotype. In this case the $CD8^+$ T cell response seems to be involved in the mechanisms of protective immunity since there is evidence that the vaccine is able to induce protection in approximately 50% of cases (CMF Antunes et al. 1986 *Int J Epidemiol 15*: 5732); (b) $CD8^+$ T cell would play an important role for cure of leishmaniasis by production of type 1 lymphokines leading to immunomodulation of hypersensitivity and/or activation of macrophages for parasite destruction; (c) there is also evidence that $CD8^+$ T cells can have a cytotoxic effect (CTL) on parasitized macrophages, with a beneficial effect on the follow-up of the disease (Conceição-Silva et al. *loc. cit.*). However, this effect when exacerbated, could be detrimental for the patient (A Barral et al. 1993 *Mem Inst Oswaldo Cruz 88* (Suppl): 29).

Transmission of ATL is mainly restricted to silvatic or periurban areas. However, because of the expansion of HIV infection in Brazil, a rise in the frequency of ATL/AIDS associated infections could occur and consequently higher number of severe forms of the disease.