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.

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 susceptible to L. major infection, the Th1 CD4+ T cell subsets are preferentially activated with production of type 1 lymphokines (e.g. interferulin 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, the 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 parasitophorous vacuole and aggravation of the disease. The cell mediated immune response is depressed with negative DTH.


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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 (Inter test 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)**


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 antimicrobial therapy. Surprisingly, when a combined *Leishmania* antigen-immunotherapy associated with antimonial was tried (W Mayrink et al. 1992 Parasitologia 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 tendency 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.