

## IMMUNOLOGY OF HUMAN VISCERAL LEISHMANIASIS AND PERSPECTIVE OF THE USE OF IMMUNOMODULATORS

E.M. CARVALHO, A. BARRAL, R. BADARO & M. BARRAL-NETTO

Serviço de Imunologia, Hospital Prof. Edgard Santos, Universidade Federal da Bahia, Rua João das Botas s/nº, Canela, 41140 Salvador, BA, Brasil

*Leishmania* are unique protozoa that cause a broad spectrum of acquired immunological disorders in the mammalian host (Castes et al., 1983; Carvalho et al., 1985; Andrade et al., 1982; Haldar et al., 1983; Carvalho et al., 1981; Petersen et al., 1982). The species and sub-species of *Leishmania* are usually correlated with typical pictures of clinical disease (Thakur, 1984; Marsden & Jones, 1985; Beach et al., 1984; Ayele et al., 1984). However, a single species of *Leishmania*, as *L. mexicana amazonensis* can cause diseases in humans representing the whole spectrum of leishmaniasis: single skin ulcer, mucosal disease, diffuse cutaneous leishmaniasis, and visceral leishmaniasis (Barral et al., 1986; Carvalho, 1986; Deane & Grimaldi, 1985). Visceral Leishmaniasis (VL) is usually caused by *L. donovani* although sporadic cases have been reported as due to *L. aethiopica* or *L. mexicana amazonensis* (Schnur et al., 1981; Barral et al., 1986a); the disease is endemic in South America, Asia and Africa (Badaro et al., 1986b; Thakur et al., 1984; Southgate, 1964; Yan-jia, 1982; Napier, 1924) and foci have also been documented in Europe (Pampiglione et al., 1974). In both New and Old World the immunological hallmark of visceral leishmaniasis is a specific immunosuppression of responses to leishmanial antigen (Carvalho & Bacellar, 1983; Ho et al., 1983; Rezai et al., 1978; Manson-Bahr, 1961). Several attempts to understand the immunological abnormalities seen in VL patients have been made. Recently effort has been focused in identifying the lack of production of some lymphokines during the interaction of the parasite with macrophages and lymphocytes. Because unresponsiveness to leishmania antigen is observed before clinical disease is apparent and because all the immunological abnormalities are reversible following successful therapy (Carvalho et al., 1981; Carvalho, 1985) the potential use of immunomodulators and recombinant lymphokines needs to be explored. This report is concerned in reviewing immunological aspects of human visceral leishmaniasis and is discussing the perspectives of the use of immunomodulators in this disease.

### IMMUNOSUPPRESSION TO LEISHMANIA ANTIGEN IN VISCERAL LEISHMANIASIS

Depression of the cellular immune response is a characteristic of visceral leishmaniasis (VL) and this disorder is related to the pathogenesis of some complications observed in the course of *L. donovani* infection. Delayed hypersensitivity skin test responses are negative during active VL (Manson-Bahr, 1961; Manson-Bahr et al., 1959; Andrade et al., 1982), peripheral blood mononuclear cells do not proliferate in the presence of leishmania antigen (Carvalho, 1985; Carvalho et al., 1981; Ho et al., 1983; Haldar et al., 1983) and supernatants of VL lymphocytes stimulated with leishmanial antigen do not contain gamma interferon or interleukin-2 (Carvalho et al., 1985). In addition, serum from visceral leishmaniasis patients suppress lymphocyte reactivity to mitogen and unrelated antigens (Carvalho & Bacellar, 1983; Barral et al., 1986b). In contrast to a poor cellular immune response, VL patients have high levels of specific antibody to leishmanial antigens. However, rather than antibodies, responses mediated by T cells and by mononuclear phagocytes are of fundamental importance in resistance to leishmaniasis (Preston et al., 1972; Behin et al., 1977; Bryceson & Turk, 1971, Skov & Twohy, 1974).

As a probable consequence of the suppressed immune function, patients have an inability to control the replication of *Leishmania* in macrophages, and in many cases also develop secondary bacterial or viral infections (Guerreiro et al., 1985). The mechanisms of the immunosuppression in visceral leishmaniasis are unknown. Although leukopenia occurs in patients with active VL, there is no significant decrease in total numbers of circulating lymphocytes (Carvalho, 1985). It has been suggested that antigen reactive lymphocyte may become sequestered in liver and spleen, decreasing the number of circulating cells capable of recognizing leishmanial antigen (Sacks et al., 1987). Another plausible explanation for the inability of cells to proliferate to leishmanial antigen would be the presence of suppressor cells and/or sup-

pressor factors preventing proliferation of antigen reactive cells. One of the important issues in understanding the mechanism of immune suppression in VL is to enable to improved management of the disease.

The depression of the cellular immune response in visceral leishmaniasis is reverted after therapy. Table I shows that of 19 patients with active VL none had lymphocyte proliferative response to leishmanial antigen before therapy (mean cpm  $\pm$  standard deviation of unstimulated cultures was  $400 \pm 57$ ). After treatment, 9 out of 17 patients had a significant lymphocyte proliferative response to leishmanial antigen (mean cpm of the responses was  $17,875 \pm 16,221$ ). Restoration of lymphocyte reactivity to leishmanial antigen could be documented as soon as ten days after therapy. However lymphocyte reactivity was seen more commonly 20 days after therapy when 6 out of 8 patients had responses to leishmanial antigen. In contrast, when studies were performed in the first 20 days after therapy only a small number of patients (3 out of 9) had significant 3H thymidine uptake when stimulated by leishmanial antigen. In five patients without reactivity immediately after therapy, another lymphocyte blastogenesis test was done more than 6 months after therapy, and in all cases strong lymphocyte reac-

tivity could be documented. In the other 4 such subjects no follow-up evaluation could be done.

The mechanisms of the immunosuppression in visceral leishmaniasis are not completely understood, but necessary events for cellular proliferation, as IL-2 production, is impaired since this product is absent in supernatants of VL lymphocyte cultures stimulated with leishmanial antigen (Table II). In these experiments, CTLL cells were used to measure IL-2 production in supernatants of VL lymphocytes stimulated with leishmanial antigen or PHA, and the results are expressed in cpm of 3H thymidine uptake. While supernatants of VL lymphocytes stimulated with PHA induced  $9904 \pm 3746$  cpm, supernatants of VL lymphocytes stimulated with leishmanial antigen induced  $653 \pm 130$  cpm. In contrast supernatants of leishmanial antigen stimulated mucocutaneous lymphocytes induced a large 3H-Thymidine uptake by CTLL-2 cells (cpm  $12,190 \pm 3,357$ ). The inability of VL lymphocytes to produce IL-2 when stimulated by leishmania antigen explains the low lymphocyte reactivity to leishmanial antigen observed in the blastic transformation test, due to the central role of IL-2 in lymphocyte proliferation.

TABLE I

Lymphocyte Reactivity to *Leishmania* antigen before and after therapy of visceral leishmaniasis

Patient number	Number of days after therapy evaluation was performed	3H thymidine Before therapy	Uptake before therapy
1	05	$863 \pm 78$	$174 \pm 11$
2	07	$389 \pm 47$	$114 \pm 50$
3	10	$719 \pm 41$	$4350 \pm 1198$
4	10	$153 \pm 16$	$494 \pm 54$
5	10	$203 \pm 74$	$944 \pm 244$
6	12	$191 \pm 20$	$6570 \pm 498$
7	13	$615 \pm 127$	$6455 \pm 758$
8	14	$645 \pm 203$	$636 \pm 126$
9	14	$545 \pm 28$	$287 \pm 16$
10	20	$452 \pm 9$	$3178 \pm 45$
11	22	$855 \pm 70$	$18028 \pm 2258$
12	27	$188 \pm 50$	$544 \pm 102$
13	29	$570 \pm 151$	$1803 \pm 1678$
14	35	$422 \pm 15$	$25415 \pm 486$
15	76	$277 \pm 51$	$12323 \pm 525$
16	85	$288 \pm 25$	$27574 \pm 2219$
17	85	$355 \pm 56$	$56980 \pm 191$

Peripheral blood mononuclear cells ( $2 \times 10^5$  cells on 0,2ml of RPMI containing 15% heat inactivated AB serum) were stimulated with *Leishmania donovani* antigen in a concentration of  $5 \mu\text{g/ml}$ . After 5 days of incubation ( $37^\circ\text{C}$ ,  $5\%\text{CO}_2$ ) 1uCi of 3H thymidine was added per well (New England Nuclear Corp. Boston, MA) and the cells harvested 45h later.

TABLE II

Production of Interleukin-2 by visceral leishmaniasis, mucocutaneous leishmaniasis and healthy subjects lymphocytes \*

Groups	IL-2 production in cultures stimulated with	
	PHA	Leishmania antigen
Visceral leishmaniasis	9904 ± 3746	653 ± 130
Mucocutaneous leishmaniasis	15470 ± 6780	12910 ± 3357
Healthy subjects	11940 ± 5560	585 ± 117

\*  $3 \times 10^6$  mononuclear cells in 1ml RPMI were stimulated with PHA (final dilution 1:10) and leishmania antigen (20 µg/ml). After 24 hours the supernatants were harvested filtered (Milipore 0,45) and stored at -20°C. IL-2 was measured using IL-2 dependent CTLL-2 cells. The data represents the mean ± SD of cpm of 7 experiments.

Attempting to better understand the immunosuppression in VL we decided to perform co-culture experiments with pre-treatment (non-responders) cells obtained previously and cells obtained after antimonial therapy. In these studies we took frozen T cells obtained during active disease and co-cultured these T cells with fresh cells obtained after successful therapy when restoration of the lymphocyte reactivity proliferative response to leishmanial antigen is achieved.

Table III shows the lymphocyte reactivity of VL patients to leishmanial antigen, and PWM, before and after therapy as well as the 3H thymidine uptake of co-cultures. Mononuclear cells before therapy had  $460 \pm 76$  cpm whereas after therapy they had  $4,293 \pm 1,422$  cpm. Co-culture of T frozen cells obtained prior to chemotherapy with autologous mononuclear cells obtained after treatment reduced the response of posttreatment mononuclear cells to *Leishmania* by 80%. This data show that T cells from patients with visceral leishmaniasis can suppress lymphocyte blastogenesis of autologous cells obtained after therapy when restoration of the immune response is observed. The phenotype of the T cells involved in the suppression of the lymphocyte blastogenesis needs to be determined.

The importance of an intact cellular immune response against *Leishmania* antigens to control progression of leishmanial infection has been well documented in experimental animals (Preston et al., 1972; Behin et al., 1977; Murray et al., 1983) and in diffuse cutaneous leishmaniasis (Bryceson, 1970; Petersen et al., 1982; Petersen et al., 1984). In the present study, we infected "in vitro" 6 days-cultivated human macrophages with promastigotes of *L. donovani*. To determine the ability of supernatants of

leishmanial antigen-stimulated lymphocytes from VL patients to increase macrophage microbicidal activity, the percentage of infected macrophages and the number of amastigotes per 100 macrophages were determined 48 hours after infection with *L. donovani* (Table IV).

Supernatants from VL lymphocytes stimulated with leishmanial antigen failed to decrease the infection on human macrophages. In contrast, supernatants from mucocutaneous lymphocytes decreased the percentage of infected macrophages and the number of amastigotes per 100 cells.

Previous studies *in vitro* have documented that gamma interferon is an important macrophage activating factor and that macrophages in the absence of gamma interferon have decreased ability to kill *Leishmania* (Murray et al., 1983; Hoover et al., 1985; Murray & Cartelli, 1983; Murray et al., 1985). The inability of VL lymphocyte to activate macrophages to kill *Leishmania* correlates with the observation shown on Table V that summarizes the experiments in which gamma-interferon levels were measured in lymphocyte supernatants from VL and mucocutaneous leishmaniasis patients and in the supernatants of lymphocytes from healthy subjects. The level of gamma interferon was measured by the protection of the cytopathic effect of VSV on WISH cells. Antiviral activity was measured against a laboratory gamma interferon standard and is expressed as international units per milliliter (Rubin et al., 1983). In these studies, gamma-interferon levels in supernatants from VL lymphocytes stimulated by leishmanial antigen were of  $15 \pm 4$  IU and in mucocutaneous leishmaniasis lymphocytes the gamma-interferon levels were  $704 \pm 163$  IU ( $p < 0.01$ ). The inability of visceral leishmaniasis lymphocytes to produce gamma-interferon was only docu-

TABLE III  
Immunosuppression in visceral leishmaniasis – evidence for the role of T cells

Cells source	3H thymidine uptake*	
	Leishmania antigen	PWM
Mononuclear cells before antimony therapy	460 ± 76	11346 ± 891
Mononuclear cells after antimony therapy	4293 ± 1442	8072 ± 592
Co-cultivation of pre- and pos-therapy cells**	530 ± 149	5917 ± 571

\* Results shown are the mean cpm x 10<sup>-3</sup> ± SEM of studies of patients.

Mononuclear cells obtained after treatment (1,5 x 10<sup>5</sup>) were co-cultured with 5 x 10<sup>4</sup> previously frozen pre-treatment T cells from the same patients for 5 days in the presence of antigen or mitogen.

TABLE IV  
Inability of lymphocytes from visceral leishmaniasis patients to kill *Leishmania* in macrophages

Lymphocytes used to supernatant production	Percentage of infection	Number of amastigotes per 100 cells
Visceral leishmaniasis	40 ± 12	344 ± 112
Mucocutaneous leishmaniasis	15 ± 6	136 ± 63

TABLE V  
γ-Interferon level in supernatants of visceral leishmaniasis mucocutaneous leishmaniasis and healthy subjects lymphocytes\*

Groups	Interferon production	
	PHA	Leishmania antigen
Visceral leishmaniasis	204 ± 86	15 ± 4
Mucocutaneous leishmaniasis	389 ± 97	704 ± 163
Healthy subjects	267 ± 48	10 ± 6

\*Gamma interferon was determined in the same supernatants used for IL-2, using a cutophathic effect inhibition assay with vesicular stomatitis virus in WISH cells.

mented in the presence of leishmanial antigen and the production of gamma interferon in cultures stimulated with PHA was similar to that observed with lymphocytes from healthy subjects.

#### SERUM SUPPRESSOR FACTORS IN VISCERAL LEISHMANIASIS

Besides the evidence for an antigen specific immunosuppression in visceral leishmaniasis, we

have also shown that visceral leishmaniasis sera is a potent inhibitor of cellular immune response (Carvalho & Bacelar, 1983; Barral et al., 1986a, b). Visceral leishmaniasis sera inhibit even mitogen driven lymphocyte proliferation, causing reductions of the response to Con A, PHA or PWM in the order of 67.2%, 71% and 75% respectively (mean from determinations in several AVL sera in comparison to sera from normal volunteers). Serum from AVL does not

interfere to the Con A binding to cell surfaces. The suppressive effect is not due to either cytotoxicity or inadequate nutritional support. Altered kinetics of DNA synthesis in the presence of AVL serum are also not responsible for the observed difference between cultures performed with VL or normal human serum-NHS (Barral et al., 1986a, b).

We have determined that PHA-stimulated Interleukin-2 (IL-2) production by normal human peripheral blood mononuclear cells (PBMC) is impaired in the presence of VL serum. Cells were stimulated in the presence of 10% VL serum or in 10% NHS and the IL-2 production was tested in a IL-2 dependent CTLL maintenance assay. At a dilution of 1:4 supernatant from cultures with NHS gave  $2,628 \pm 613$  cpm, whereas those in VL serum gave  $451 \pm 413$  cpm. The number of cells expressing IL-2 receptors was not affected by VL serum. Cells were stimulated in the presence of NHS or VL serum and then submitted to indirect immunofluorescent assay using anti-Tac antibodies. The percentage of reactive cells was  $54 \pm 9.5\%$  with VL serum and  $61.8 \pm 12.9\%$  with NHS. Additionally it was possible to restore the Con A responsiveness of cells cultivated in the presence of 10% VL serum by adding exogenous IL-2. One hundred units of IL-2 increased the proliferation of such cultures 4.4 to 7.3 times, but stimulated similar cultures in NHS by only 0.6 to 1.1 times. Such results show that sera from VL impair the production of, but not the induction of receptors, or responsiveness to IL-2 in human PBMC.

Serum from VL also affects interferon production by Con A stimulated human PBMC. Cultures with NHS gave  $7.7 \pm 0.58$  units/ml (log mean  $\pm$  S.D.) and cultures with VL serum reduced it  $5.7 \pm 0.58$  U/ml. Addition of IL-2 reversed the suppressive effects. In two experiments, cultures receiving 100 IL-2 U/ml produced equal amounts of Interferon ( $6.33 \pm 0.58$  U/ml) regardless of serum source. We therefore concluded that impairment of interferon production induced by VL is secondary to the defect of IL-2 production.

The nature of the serum suppressive factor is poorly characterized. The capacity to suppress mitogen-driven responses argues against the possibility of the stimulant being complexed by antibodies; and, furthermore, the binding of Con A to PBMC is not affected by VL serum (Barral et al., 1986a, b). Sera from VL patients exhibit a remarkable hypertriglyceridemia, and lipids have been implicated in immunosuppres-

sion in other situations (Chisari, 1977; Curtiss & Edington, 1981). We have no indication for the participation of parasite products in the observed immunosuppression. Another possibility which deserves consideration is the suppressive participation of immunoglobulin, either alone or as immunocomplexes, since this has been documented (Mehra et al., 1979; Tsuyugushi et al., 1980) and immunoglobulin levels are extremely elevated in VL sera. Recently we have obtained evidence that IgG-depleted VL sera (by absorption to Protein A-Sepharose) partially lose their suppressive activity. It is most likely that the suppressive effect observed in VL sera represents the end result of several different factors.

#### DIAGNOSIS AND HUMORAL IMMUNITY

Classical visceral leishmaniasis or Kala-azar is characterized by fever, hepatosplenomegaly, anemia, leukopenia and hyperglobulinemia. More recently we have described a subclinical form of the disease where very few symptoms are present, and either progression to classical disease or a self healing course may occur (Badaro et al., 1986a). The diagnosis of classical visceral leishmaniasis is made by the demonstration of amastigotes in the Giemsa stained smear of bone marrow or splenic aspirates (Ho et al., 1948). Both techniques are painful and require previous clinical and laboratorial information to be performed. The sensitivity of the bone marrow aspiration to demonstrate *Leishmania* is close to 80% in the best series (Carvalho & Prata, 1966) but it is less than 30% in subclinical patients (Badaro, personal observation). Splenic aspiration is a very sensitive method for demonstrating *Leishmania* in hepatosplenic visceral leishmaniasis patients, but it is only feasible in patients with spleen enlargement at least 3 cm below the costal margin (Lightner et al., 1983). In contrast, available serological tests are practical, quick, very sensitive and quite specific. In our opinion three tests can be used for routine diagnosis: the modified direct agglutination test (DAT), the indirect immunofluorescent assay (IFA) and the enzyme linked immunosorbent assay (ELISA).

The DAT was first proposed by Allain and Kagan for American cutaneous leishmaniasis (Allain & Kagan, 1975). This test is very simple and does not require sophisticated equipment. The antigen is stable (trypsinized promastigotes). The limitation is the cross reactivity with other hemoflagellates such as *Trypanosoma*

*cruzi* and African trypanosomes. However, sera with titers greater than 1/4096 are definitely related to *Leishmania* infection (Harith et al., 1986). The IFA for sero-diagnosis of visceral leishmaniasis has been in use since 1964 (Duxbury, 1964). Log phase promastigotes from a recently isolated culture is the best antigen source. There is no advantage in using amastigotes as antigen in this test (Badaro et al., 1983). The cross reactivity with *T. cruzi* and other hemoflagellates in the IFA is less critical than in the DAT. The sensitivity of this test is good enough that it can be used in epidemiological surveillance and for routine diagnosis. Titers equal to or greater than 1/256 are definitely positive and all titers over 1/1024 are strictly related to leishmanial infection. Kala-azar patients' sera usually have titers greater than 1/4096.

The micro-ELISA is currently the test of choice for serodiagnosis in many tropical diseases (Houba et al., 1980). For visceral leishmaniasis it is possible to use a soluble antigen and a single dilution to define a positive serum (Badaro et al., 1986c). The cross reactivity is very low (less than 5%) even when sera from Chagas' disease patients are tested. The ELISA is the most sensitive, specific, convenient and least expensive test for the diagnosis of visceral leishmaniasis.

Antibodies to leishmanial antigens are produced early after infection and their documentation is useful to the diagnosis of subclinical visceral leishmaniasis (Pampiglione et al., 1974; Badaro et al., 1986c). In patients with classical disease there is a polyclonal activation and besides antibodies to parasite antigens other antibodies as against DNA and rheumatoid factor can be easily detected, associated with the hyperglobulinemia observed in these patients (Carvalho et al., 1983; Galvão-Castro et al., 1984; Pearson et al., 1983).

High titers of circulating immunocomplexes are also observed during the disease (Carvalho et al., 1983; Galvão-Castro, 1984) and the possible participation of these compounds in the pathogenesis of the anemia and in the bleeding disorders observed in visceral leishmaniasis has been considered (Pontes de Carvalho et al., 1986).

#### POTENTIAL USE OF IMMUNOMODULATORS IN VISCERAL LEISHMANIASIS

We have reported that patients with visceral leishmaniasis have suppressed cellular responses and we have associated such suppression to an

inability of the patients' lymphocytes to produce both IL-2 and gamma interferon when stimulated by leishmanial antigen. Cure of the disease is associated with restoration of the immune response to leishmanial antigen. More recently we have documented that children infected with *L. donovani* may or may not develop clinical visceral leishmaniasis (Badaro et al., 1986a). Immunological studies in children infected with *L. donovani* suggest an association between development of the disease with depression of cellular immunity to leishmanial antigen in the initial phase of the infection. In contrast, children who have a strong lymphocyte reactivity to *Leishmania* antigen and positive skin test do not progress to classical visceral leishmaniasis. These data indicate that depression of cellular immunity to parasite antigens contribute first, to the appearance of clinical manifestations, and second, to the progression of the disease, and consequently the occurrence of several complications. Since immune dysfunction is intimately related to persistence and multiplication of the parasite in the host, immunomodulators are potential agents to be used in visceral leishmaniasis. In this area virtual candidates are interferon gamma, granulocyte-monocyte colony-stimulation factor (GM-CSF), Interleukin-1 and Interleukin-2 (IL-1 and IL-2).

Interleukin-1 is a cytokine produced by monocytes and represents one of the initial steps of the immune response (Scala & Oppenheim, 1983). IL-1 has *in vitro* a number of immunological effects, and it has been shown that human macrophages infected with *L. donovani* and stimulated with PMA produce less IL-1 than uninfected macrophages stimulated by PMA (Reiner, 1987). However because IL-1 does not revert *in vitro* the depression of lymphocyte reactivity to leishmanial antigen (Carvalho et al., unpublished data) and due to the pyrogenic and cytotoxic effects of this cytokine, IL-1 should not have priority to be tested in this disease.

Interleukin-2 is a T cell growth factor and its production is reduced in visceral leishmaniasis. Many T cell functions are dependent of IL-2 such as Interferon gamma and lymphotoxin production (Smith, 1984; Kasahara et al., 1983; Farrar et al., 1981; Vilcek, 1985). In mice infected with *T. cruzi* IL-2 reverts the inability to produce IgG antibodies to T dependent antigens (Reed et al., 1984, a, b), *in vitro* IL-2 restores the lymphocyte reactivity to *Mycobacterium*

*leprae* antigen in leprosy patients (Kaplan et al., 1985). In leishmaniasis we have reported here that IL-2 restores mitogen-driven lymphocyte proliferation and interferon production suppressed by VL sera. Thus the potential value for IL-2 therapy may be considered in leishmaniasis since it may restore the immunocompetence. Via Interferon gamma production, IL-2 may increase microbicidal activity (Sharma et al., 1985). In humans IL-2 has been used in cancer and in AIDS patients (Rosenberg et al., 1983) and no major side effects have been documented.

Granulocyte-monocyte colony stimulating factor is a lymphokine which cause division of stem hematopoietic cells and induce cell maturation and activation (Metcalf, 1985; Gasson et al., 1984; Grabstein et al., 1986b). It has recently been shown to have antiprotozoal and anti-viral activity associate with its functional effect as macrophage activating factor. *In vitro* GM-CSF inhibits the intra-cellular replication of *T. cruzi* in both human and mouse macrophages (Reed, personal communication), and it is capable of activating macrophages to become tumoricidal and to mediate a number of cellular activities (Grabstein et al., 1986a).

Visceral leishmaniasis is associated not only with depression of the cellular immunity but also with hematopoietic defects characterized by neutropenia and consequently leukopenia, anemia and bleeding disorders. Bacterial infections are the most important cause of death in visceral leishmaniasis. In spite of depression of cellular immunity the aethiological agents of infections associated to Kala-azar are *Escherichia coli* and gram-positive cocci; complications due to opportunistic pathogens traditionally associated with immunocompromised hosts are not recognized. Infections in VL patients may be dependent on the decrease in neutrophil numbers observed during the disease (Carvalho, 1985) or alteration of neutrophil function (Fernandes & Rocha, 1967; Lazzarin et al., 1976). It would be of great potential value if some of life threatening hematopoietic defects could be reversed early in the disease. Additionally, a decrease in parasite burden by GM-CSF through a macrophage activation would be very important.

Interferon gamma is the most important macrophage activating factor and its capability in inducing intracellular parasite killing by mac-

rophages have been well documented. *In vitro* interferon gamma induces macrophages to kill different species of *Leishmania* (Murray et al., 1982), *T. cruzi* (Nogueira et al., 1981) and *T. gondii* (Nathan et al., 1983). In experimental animals protection of mice against *Listeria monocytogenes* can be obtained by recombinant immune interferon (Kiderlen et al., 1984) and the development of leishmaniasis in infections mediated by *L. donovani* is associated to inability of the mouse strain to produce interferon gamma (Murray et al., 1982). Furthermore, visceral leishmaniasis patients' lymphocytes do not produce interferon gamma when stimulated by leishmanial antigen. Use of interferon gamma in human beings is associated with mild side effects and peripheral blood monocytes are activated with parenteral (Nathan et al., 1985) or even intradermal administration of gamma-Interferon (Nathan et al., 1986). Indeed recombinant human gamma-Interferon has been safely used in cancer (Nathan et al., 1985; Vadhan-Raj et al., 1986) and in AIDS patients (Odajnyk, 1984).

Available drugs in Brazil for treatment of visceral leishmaniasis are pentavalent antimonials and Amphotericin B, whose limitations and adverse reactions are well documented (Thakur, 1984; Marsden et al., 1984). In our experience antimonial therapy fails in about 10% of hospitalized patients. In such cases the drug of choice is Amphotericin B, despite its several side-effects. We think that before administration of amphotericin B the association of gamma-Interferon with antimonial drugs would be a rational approach to evaluate the efficacy of this lymphokine in visceral leishmaniasis. Determination of parasite burden by spleen puncture is necessary in controlling the therapeutic response. Another possible use of gamma-Interferon would be on clinical trials is sub-clinical visceral leishmaniasis. In this situation, without full manifestation of the disease, the side effects of pentavalent antimonials limits the acceptance of therapy by the patients. Additionally, since progression of subclinical to full-blown disease takes months (Badaro et al., 1986a, b, c) interferon gamma could be used alone. The ideal patients in such trials would be children with negative skin tests and depression of lymphocyte reactivity to leishmanial antigen. In this case monitoring of the immune response with lymphocyte blastogenesis and skin tests; and reduction of liver and spleen size would be the indicated criteria to evaluate therapeutic responses.

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