

## ADVANCES IN LEISHMANIASIS IMMUNOPATHOGENESIS

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[Recenti progressi nell'immunopatogenesi delle leishmaniosi]

### SUMMARY

Nowadays leishmaniasis is an increasing, severe, public health problem, and its control is strictly connected to vaccine development and therapeutic manipulations of the immune system. The clinical outcome of leishmaniasis is related to cytokine response profile. Immune system type-1 response and IFN- $\gamma$  production provide well-known protective activity against *Leishmania*, whereas role of T helper ( $T_H$ ) 2 cytokines in non-healing infections requires further exploration. As a matter of fact, IL-4 and IL-13 ( $T_H$ 2 cytokines) promote disease progression in cutaneous leishmaniasis, whereas IL-4 seems to enhance protective type-1 responses in visceral leishmaniasis. Thus, immune response to intracellular parasites should dismiss the  $T_H$ 1/ $T_H$ 2 paradigm of resistance/susceptibility, embracing theory of a more complicated network of regulatory/counter regulatory interactions. Moreover, the presence of antigen specific regulatory T cell subsets may provide an environment that contributes to the balance between  $T_H$ 1 and  $T_H$ 2 cells. Finally, the involvement of CD8<sup>+</sup> T cells has been described, but the modality of their role and function, in this kind of infection, has not been outspread so far.

**Key words:** Cutaneous leishmaniasis, Visceral leishmaniasis,  $T_H$ 1/ $T_H$ 2 paradigm, citokines, chemokines, apoptosis

### RIASSUNTO

Le leishmaniosi rappresentano, attualmente, un grave problema di salute pubblica, epidemiologicamente in aumento, ed il loro controllo è strettamente connesso allo sviluppo di vaccini e di particolari manipolazioni farmacologiche del sistema immunitario. Il risultato clinico delle leishmaniosi, guarigione o meno, è legato al profilo citochinico della risposta immune. Le risposte immunologiche di tipo 1 e la produzione di IFN- $\gamma$  forniscono un potente effetto di protezione contro le leishmanie, mentre il supposto ruolo peggiorativo dei linfociti T helper ( $T_H$ ) 2 e delle citochine da essi prodotte, nelle infezioni da leishmanie non guarite, richiede ulteriori approfondimenti. È un dato di fatto che l'IL-4 e l'IL-13 (citochine  $T_H$ 2) siano in grado di promuovere la progressione della malattia nella leishmaniosi cutanea, mentre l'IL-4 sembra aumentare l'effetto protettivo delle risposte  $T_H$ 1 nella leishmaniosi viscerale. Quindi, la risposta immunologica nei confronti di questi parassiti intracellulari non sembra adattarsi perfettamente al noto paradigma  $T_H$ 1/ $T_H$ 2 di resistenza/susceptibilità, teoria semplificata di una certamente più complessa rete di interazioni fra meccanismi regolatori e contro regolatori immunologici. Inoltre, la presenza di cellule T antigene-specifiche a funzione regolatrice potrebbe creare un peculiare milieu, che può, a sua volta, contribuire all'equilibrio funzionale tra cellule  $T_H$ 1 e  $T_H$ 2. Infine, nelle leishmaniosi, è stato descritto, anche, il coinvolgimento delle cellule T CD8<sup>+</sup>, ma loro ruoli e funzioni, in questo particolare tipo di infezione, non sono stati ancora ben definiti.

**Parole chiave:** Leishmaniosi cutanea, leishmaniosi viscerale-paradigma  $T_H$ 1/ $T_H$ 2, citochine, chemochine, apoptosi

### Introduction

Supervisory of public health should consider leishmaniasis a severe, increasing, health problem<sup>(1-2)</sup>.

Infective diseases affect more than 50 million people who travel from industrialized to developing countries each year; leishmaniasis, which is endemic in the areas of tropics, subtropics, and southern Europe, is one<sup>(3)</sup>.

Therapeutic manipulations of the immune system, and vaccine development, to obtain better

tools, and more cost-effective strategy for vector control and case management, are research areas offering the greatest prospective<sup>(4)</sup>.

Leishmaniasis comprises a miscellaneous group of diseases caused by protozoan parasites of the *Leishmania* genus. Different clinical manifestations of leishmaniasis depend both on host genetic control and infecting species, which are more than 20<sup>(5)</sup>.

The parasites, which cause heterogeneous clinical pathway in humans, can be divided in the subgenus *Leishmania* (*L.*) or *Viannia* (*V.*).

Cutaneous leishmaniasis (CL) is caused, in the Old World, by *Leishmania (L.) major*, *Leishmania (L.) aethiopic*, and dermatropic *Leishmania (L.) infantum*, of the *Leishmania* subgenus; in the New World, by *Leishmania (L.) mexicana*, *Leishmania (L.) amazonensis*, *Leishmania (L.) venezuelensis*, and dermatropic *Leishmania (L.) infantum*, of the *Leishmania* subgenus, and *Leishmania (V.) braziliensis*, *Leishmania (V.) guyanensis*, *Leishmania (V.) lainsoni*, *Leishmania (V.) naiffi*, *Leishmania (V.) panamensis*, *Leishmania (V.) peruviana*, and *Leishmania shawi*, of the *Viannia* subgenus. Mucocutaneous leishmaniasis (MCL), or *espundia*, is caused by *Leishmaniasis (V.) braziliensis*, and *Leishmania (V.) panamensis*, whereas visceral leishmaniasis (VL), also named 'Kala Azar', is caused by *Leishmania (L.) infantum* in the Mediterranean area, Middle-East and Asian countries, and *Leishmania (L.) chagasi* in Latin America. *Leishmania (L.) mexicana* and *Leishmania (L.) amazonensis*, due to their genotypic relationships, are now regarded as a single specie within *Leishmania mexicana* complex, whereas *Leishmania (L.) infantum* and *Leishmania (L.) chagasi*, together with *Leishmania (L.) donovani*, are now considered, due to their genotypic relationships, as a single specie, within the *Leishmania donovani* complex. Sometimes, *Leishmania* infection may evolve, after recovery, in a chronic CL form, called 'Post-Kala Azar Dermal Leishmaniasis' (PKDL), which usually need long and expansive treatments<sup>(6,7)</sup>.

*Leishmania* are digenetic parasites, developing as flagellated motile promastigotes in the gut of blood-sucking female sand flies (*Phlebotomus spp.* in the Old World, *Lutzomyia spp.* in the New World), which may be transmitted to the vertebrate host via dermis during blood-meal, and as obligate intracellular non-motile amastigotes in phagolysosomes of macrophages and dendritic cell of vertebrate hosts.

Leishmaniasis is mainly zoonotic disease: rodents, edentates and marsupials in CL, wild canines and domestic dogs in zoonotic VL being the most widespread reservoirs, but others mammals might be too, with the exception of anthroponotic VL (widespread in India), in which man is the only source of infection<sup>(8,9)</sup>.

*Leishmania* can infect mice quite easily, granting useful *in vivo* models to evaluate host immune response and regulation, and genetic control of infection<sup>(10,11)</sup>.

## Immune responses in animal and human Leishmaniasis

### General aspects

Pathogenesis of leishmaniasis relays to both parasite factors and host mechanisms, which are closely linked and interdependent. Inoculation of promastigotes, via sandfly saliva, is first point of *Leishmania* infection, which continues entering macrophages to escape host responses. Progressive intracellular infection by amastigotes depends on the maintenance of macrophages in an inert, inactivated state. Nevertheless, the immunocompetent hosts develop both non-specific (innate) and antigen-specific (adaptive) cell-mediated responses, able to control disease evolution, producing (self-healing disease or asymptomatic infection) or not (non-healing disease) the desired clinical end-result<sup>(8,12)</sup>.

At site of infection, innate responses include Pattern Recognition Receptors (PPRs, i.e. toll-like receptors), soluble products (complement, and released cytokines, including interleukin [IL]-1a, IL-12, Tumor Necrosis Factor [TNF]) and cells (neutrophils, monocytes, macrophages, natural killer [NK] cells, and dendritic cells), which may, especially IL-12 production, drive induction of acquired cell-mediated immunity. This complex set of mechanisms leads to the activation of specific CD4<sup>+</sup> and CD8<sup>+</sup> T cells, which, via adhesion molecules and chemokine mechanisms, are actively recruited to cutaneous or visceral sites, and, sharing with influxing blood monocytes, direct local inflammatory responses, including granuloma staging and lesion development<sup>(13-15)</sup>.

Massive infiltration of macrophages and chemokines, such as Monocyte Chemoattractant Protein-1 (MCP-1), monokine induced by interferon (IFN)-g (MIG), IFN-g-inducible protein-10 (IP-10), and only low amount of macrophage inflammatory protein 1a (MIP-1a) invade sites of *Leishmania* infection in dermis of patients affected by CL<sup>(16,17)</sup>. MCP-1 expression, in skin lesions of patients with self-healing CL, help healing process proportionally to increase of its concentration, whereas high local levels of MIP-1a are associated with non-healing form of CL. Moreover MCP-1 and IFN-g cooperate to enhance monocytes clearing of intracellular parasites, whereas IL-4 inhibits effect of MCP-1<sup>(16,17)</sup>.

IFN-g-induced macrophage activation induces

T helper (T<sub>H</sub>) 1-type cell responses by development of a pleiotropic cytokines network, in which IL-12, produced by activated antigen presenting cells (APC), i.e. macrophages and dendritic cells, generates basic response, whereas IFN-g and other cytokines enhance it<sup>(18)</sup>.

Nevertheless, immune system response is still partially misunderstood: as a matter of fact, patients with clinically apparent infections, especially acute VL (see below), develop not characteristically polarised T<sub>H</sub>1- and T<sub>H</sub>2-type responses, and both activating (i.e. IL-12, IFN-g,) and suppressive (i.e. IL-4, IL-10, IL-13, Transforming Growth Factor [TGF]-b) cytokines patterns may be detected (19,20). Development of normal counterbalance mechanisms to curtail inflammation, like in any other inflammatory environment, may be pointed out also in acute leishmaniasis<sup>(19,20)</sup>. Change in T<sub>H</sub>1-type responses and inactivation of macrophages may be due to IL-4, IL-10, IL-13 and TGF-b secretion, thereby causing reduction of tissue injury, but enhancement of intracellular infection<sup>(19,20)</sup>.

#### *Resolution of infection: the T<sub>H</sub>1 response*

Nowadays, the role of a strong type-1 response, as protective immune response against CL, has been clearly established (15).

Evidences demonstrated that IL-12, from activated APC, maybe enhanced by other cytokines, such as IL-1a, IL-18, IL-23 and IL-27, drives differentiation and proliferation of T<sub>H</sub>1 cells, as innate mechanism response, and induces IFN-g secretion by T<sub>H</sub>1 cells and NK cells, as antigen-specific acquired cell-mediated response<sup>(21-23)</sup>.

Proper activation of immune T<sub>H</sub>1 cell response needs both Major Histocompatibility Complex (MCH) class II antigen presentation and ligation of co-stimulatory molecules (i.e. B7-1/B7-2 and CD40 on the APC with CD28 and CD40L on the T<sub>H</sub>1 cell, respectively)<sup>(24,25)</sup>.

Signals able to regulate superoxide (O<sub>2</sub><sup>-</sup>) and nitric oxide (NO) production and parasite killing are induced by IFN-g from T<sub>H</sub>1 cells, and probably, to a lesser extent, from CD8<sup>+</sup> T cells, as part of the antigen-specific acquired cell-mediated immune response, but also by IL-12-activated NK cells, as part of the innate response. Although molecular mechanism of NO action on *Leishmania* is unknown so far, early data suggested that it could

be directly cytotoxic to the parasites<sup>(26,27)</sup>. The NO-pathway, thus, appears to be a common mechanism of *Leishmania* killing.

Moreover, other cytokines, such as TNF-a, Granulocyte Macrophage-Colony Stimulating Factor (GM-CSF), Migration Inhibitor Factor (MIF) and type-1 interferons (IFN-a subtypes and IFN-b subtype), demonstrated potential ability to enhance macrophage leishmanicidal activity, induced by IFN-g and CD40/CD40L interactions<sup>(28,29)</sup>.

Another important pathway that seems to be important to develop adequate T<sub>H</sub>1-mediated resistance to *Leishmania* infection is Fas/FasL interaction. Activated T<sub>H</sub>1 lymphocytes induce apoptotic death in target cells expressing the Fas protein (38). Furthermore, IFN-g promotes Fas up expression on membrane surface of macrophages infected with *Leishmania*, making them susceptible to CD4<sup>+</sup> T cell-induced apoptotic death<sup>(30-31)</sup>.

Thus, Fas-induced apoptotic death of infected macrophages might limits the number of host cells at the site of infection which are required for amastigote replication<sup>(30-31)</sup>.

Antigen-specific CD4<sup>+</sup> CD25<sup>+</sup> T-regulatory cells, producing IL-10 and TGF-b, moderate activity of T effectors when infection break off<sup>(32,33)</sup>; so, in absence of persistent infection, protective immunity is significantly reduced<sup>(32-33)</sup>.

#### *Non-healing cutaneous Leishmania major infection: T<sub>H</sub>2-dependent or defective T<sub>H</sub>1 response*

The T<sub>H</sub>1-T<sub>H</sub>2 paradigm of resistance/susceptibility to intracellular infection is largely based on investigations using *Leishmania major*. Initial studies suggested primary role of distinct CD4<sup>+</sup> T cell subsets, T<sub>H</sub>1 and T<sub>H</sub>2, producing the counter regulatory cytokines, IFN-g and IL-4 respectively, on the resolution or progression of disease (34,35). However, contradictory reports have been published indicating a disease progression role for IL-4 (36). These studies lead to two possible condition: first, if other regulatory cytokines may be responsible for immunosuppressive activity previously attributed to IL-4, and/or if well known defective T<sub>H</sub>1 response plays a major role in progressive disease.

Recent studies, using gene-deficient and transgenic mice, have clearly identified other cytokines, in addition to IL-4, having major roles in non-heal-

ing response to *Leishmania major*<sup>(36,37)</sup>. Particularly, IL-13 has been found to act independently from IL-4, and the effects of IL-13 and IL-4 might be additive<sup>(37)</sup>.

Other studies suggest *Leishmania major* disease progression may be promoted by IL-10, such as IL-13 and IL-4. Suppression of IL-12 production by APC and of IFN-g by CD4<sup>+</sup> T and NK cells, macrophage inactivation and inhibition of their leishmanicidal functions, identify IL-10 key role in the pathogenesis of leishmaniasis, especially in the down-regulation of T<sub>H</sub>1 responses. Furthermore, T regulatory cells are significant producers of TGF-b, which is also partly able to suppress protective responses<sup>(36-38)</sup>.

Impossibility to produce or respond to IL-12, and intrinsic defects in APC function or in T<sub>H</sub>1 cell development have been hypothesized to explain inability to mount effective T<sub>H</sub>1 response, irrespective of T<sub>H</sub>2 response. Defective APC function, due to a failure to produce IL-12, may also underlie in a deficient APC IL-1 production<sup>(39)</sup>. As a matter of fact, IL-1 upregulates IL-12 production, as well as MHC class II co-stimulatory molecule expression, and mediates the ability of IFN-g to inhibit T<sub>H</sub>2 response<sup>(39)</sup>.

Contrariwise, intrinsic defective T<sub>H</sub>1 cell development, originally attributed to unfair response to IL-12, has been associated with down-regulation of T<sub>H</sub>1 cell IL-12R $\beta$ 2 expression (40). It is well established that IL-12R $\beta$ 2 expression can be downregulated by IL-4 produced by T<sub>H</sub>2, even though IL-4-independent mechanisms have also been described<sup>(40)</sup>. Commitment of immune system to T<sub>H</sub>2 response has been recently explained by defective co-polarization of the T cell receptor (TCR) and the IFN-g receptor complex in naïve CD4<sup>+</sup> T cells during the APC/T precursor interaction<sup>(39,40)</sup>.

#### *Non-healing cutaneous Leishmania mexicana/Leishmania amazonensis infection: IL-4-dependent*

IL-4, unlike IL-13, IL-10 and TGF-b, which are only partially involved, seems to make the greatest contribution in non-healing cutaneous *Leishmania mexicana* infection<sup>(41)</sup>. The Cathepsin L-like cysteine peptidase (CPB) has been identified as the likely *Leishmania mexicana* virulence factor inducing IL-4 production<sup>(42)</sup>.

The role of IL-4 in subverting development of T<sub>H</sub>1 response has been clearly identified in *Leishmania mexicana* infection by several studies; contrariwise similar studies, on the closely related parasite *Leishmania amazonensis*, have no evidence, bordering on secondary role IL-4 in non-healing *Leishmania amazonensis* infection<sup>(43,44)</sup>. In this setting, ability of CPB to proteolytically degrade the NF-kB family of signalling proteins might be directly responsible of T<sub>H</sub>1 responses inhibition (43,44). Thus, IL-4-independent mechanisms might prevail<sup>(43,44)</sup>.

#### *Paradox of T<sub>H</sub>1 and T<sub>H</sub>2 in visceral leishmaniasis*

The dichotomy of T<sub>H</sub>1 and T<sub>H</sub>2 cell response, induced by *Leishmania* parasites, is even less clear in VL. Disease progression, in experimental VL by *Leishmania donovani*, is due to failure of appropriate T<sub>H</sub>1 response, rather than to T<sub>H</sub>2 cell proliferation<sup>(45)</sup>. As a matter of fact, there is no evidence of IL-4 exacerbating role in murine VL, and, under certain circumstances, it can prime for IL-12 production and type-1 response<sup>(41)</sup>.

In the sera, at beginning of infection, patients infected by *Leishmania donovani* show high concentrations of IFN-g and IL-10, which fall down within the normal range after undertaking successful chemotherapy<sup>(46,47)</sup>. When performed at time of diagnosis, peripheral blood mononuclear cells (PBMC) *in vitro* stimulation with *Leishmania* antigens produces low levels of IFN-g and IL-10, but normal level when same cells are assayed after recovery<sup>(46,47)</sup>. High concentration of IL-4 may be detected in supernatants in all the phases of the disease, whereas IL-4 and IL-2 are undetectable in sera<sup>(46)</sup>. IL-2 is significantly reduced in supernatants of actively infected patients, returning to normal level after recovery<sup>(46,47)</sup>. IL-4 low sera levels might be related to IFN-g high production, whereas IL-2 sera levels reduction might be due to high sera concentration of soluble IL-2 receptor (sIL-2R), which binds IL-2 with an high affinity mechanism<sup>(48)</sup>.

In spite of the similar secretion pattern of IFN-g and IL-10 above reported, the control of infection or complete recovery seems to be associated with an increased production of IL-2 and IFN- $\gamma$ <sup>(49-51)</sup>. Furthermore, IL-10 production correlates with the progression of VL<sup>(52)</sup>, and its neutralisation, with specific monoclonal antibody, restores T cell proliferation and IFN-g production in PBMC from acute VL patients<sup>(53)</sup>. *In vitro* studies have also demon-

strated that IL-12 shifts the responses toward T<sub>H</sub>1-type and enhances IFN- $\gamma$  production<sup>(54)</sup>.

Therefore, these findings suggest that, in patients with active VL, the cytokine profile is not clearly polarized, and both T<sub>H</sub>1-like and T<sub>H</sub>2-like cells appear to proliferate and be activated<sup>(46,50,55)</sup>. The balance of cytokines at the site of primary activation of *Leishmania*-specific cells appears to be of major importance for the development of T<sub>H</sub>1 and T<sub>H</sub>2 responses<sup>(56,57)</sup>, although other unknown factors might influence the cellular immune behaviour. Altogether, data indicate that in symptomatic patients T<sub>H</sub>1 cytokine production is not depressed, but there is an unresponsiveness to the stimuli of these cytokines, also caused by the high production of IL-10. Recent studies attention another lymphokine, IL-15, for its role against infectious diseases<sup>(58)</sup> and its ability to enhance both T<sub>H</sub>1 responses, by increasing IFN- $\gamma$  production from NK and T cells<sup>(59)</sup>, and T<sub>H</sub>2 responses, by augmenting IL-5 and IL-13 production<sup>(60)</sup>. In particular, it has been demonstrated that endogenous IL-15 plays a role in the suppression of T<sub>H</sub>2 cytokines in acute VL patients, even though it does not enhance the production of T<sub>H</sub>1 cytokines<sup>(61)</sup>, indicating a potential protective role of IL-15 against leishmaniasis caused by the indirect effect on T<sub>H</sub>1, due to the restriction of T<sub>H</sub>2 proliferation<sup>(61)</sup>. Furthermore, IL-15 *in vitro* activation of macrophage cultures determines a significant anti-*Leishmania* activity, comparable to IFN- $\gamma$ -induced one<sup>(62)</sup>. *Leishmania* macrophages intracellular killing, primed by IL-15, is followed by IL-12 synthesis increase<sup>(62)</sup>. Altogether, these data indicate IL-15 could, directly or indirectly, have role as activator of leishmanicidal activity, by inducing IL-12 production.

#### *Non-healing visceral leishmaniasis: T<sub>H</sub>2-independent?*

As already mentioned, disease progression and exacerbating role of T<sub>H</sub>2 response and IL-4 production during *Leishmania donovani* infection have no absolute evidence yet. Early studies suggest no cure rate control due to differential production of T<sub>H</sub>1 and T<sub>H</sub>2 cytokines<sup>(45)</sup>, even though production of IFN- $\gamma$  correlates with resistance and T<sub>H</sub>2 cytokines are not so clearly responsible of susceptibility<sup>(63)</sup>.

According to this point of view, studies in mice suggested IL-4 may be protective in some cir-

cumstances, and can promote resistance, rather than susceptibility, to intracellular pathogens: in effect, IL-4 and IL-4Ra deficient animals are more susceptible to disease than their wild-type counterparts<sup>(64)</sup>. IL-4 and IL-4Ra signalling is essential not only for optimal clearance of *Leishmania donovani* from liver and limiting infection in spleen, following primary infection, but also for effective T cell-dependent chemotherapy, and for vaccine-induced resistance<sup>(65)</sup>. As evidence, in absence of IL-4, type-1 responses and IFN- $\gamma$  production fail to be maintained following chemotherapy or to be induced by vaccination<sup>(64,65)</sup>.

Though TGF- $\beta$  has significant disease promoting and progression activity, several studies indicate IL-10 as major immunosuppressive cytokine in VL<sup>(66)</sup>. Activation of T<sub>H</sub>1 cell responses and priming of parasite killing is allowed by experimental IL-10 inhibition<sup>(66,67)</sup>.

Furthermore, while healing process in susceptible mice is IL-12-dependent, IL-10, but not IL-4 or TGF- $\beta$ , appears to blunt T<sub>H</sub>1-type responses and determine disease fatal outcome<sup>(21)</sup>.

#### *T cell subset modifications in visceral leishmaniasis*

Although data indicate *Leishmania donovani* parasites cause immunodepression, exact mechanism they can induce suppression is not clear. As a matter of fact, levels of CD3<sup>+</sup>, CD4<sup>+</sup> and CD8<sup>+</sup> T cells, in patients affected with symptomatic *Leishmania infantum* infection, are within normal range<sup>(46,50)</sup>. Whereas, acute VL patients have markedly reduced levels of memory T cells (CD3<sup>+</sup>/CD45RO<sup>+</sup>) compared with healthy controls, which come back to normal levels as result of successful chemotherapy<sup>(46,50,68,69)</sup>. Contrariwise, all patients affected with asymptomatic infection and positive leishmanin (or Montenegro) skin test, intradermically performed using culture of promastigotes as antigen, show marked increment of memory CD4<sup>+</sup> T<sup>(68)</sup>. Importance of increased memory CD4<sup>+</sup> T cells level in this group of patients and the relationship with positive leishmanin skin test and acquired immunity needs further explanation<sup>(68,69)</sup>. Elevated number of CD3<sup>+</sup> HLA-DR<sup>+</sup> lymphocytes and increased expression of HLA-DR antigen on these cells show strong activation of cell-mediated immune response in acute VL patients<sup>(46)</sup>.

#### *The role of apoptosis and CD8+ T cells in*

## *Leishmania infection*

As aforesaid, defective host-cellular responses to challenge with pathogenic infectious agents may be caused by induction of T cell apoptosis, either in mice or in humans<sup>(70-73)</sup>. In experimental VL it has been demonstrated that infection of susceptible host results in CD4+ T cell apoptosis and reductions of T<sub>H</sub>1 cytokine production<sup>(73)</sup>.

In particular, it has been demonstrated that apoptotic mechanisms, mainly operating through the Fas (APO-1/CD95) pathway, grant resistance to *Leishmania major*, and singenic *gld* and *lpr* mice, lacking of functional Fas system, fail healing their lesions<sup>(74)</sup>. However, mice infected by *Leishmania donovani* parasites show increased incidence of T cell apoptosis in liver and spleen<sup>(75)</sup>. Leukocyte apoptosis seems to be involved in VL patients too, both in acute and in healed phases of disease.

In fact, monocytes and T lymphocytes from acute VL patients show significantly higher apoptosis levels compared to observed in healed subjects ones. T lymphocytes rate of apoptotic cells was far greater than monocytes one<sup>(31)</sup>. T cells were mainly of CD4+ phenotype<sup>(31,76)</sup>. In particular, T<sub>H</sub>1 subset, evaluated by chemokine receptor-5 (CCR5), is involved in this process and used CD95-mediated mechanism (31). These data, pointing out significant increase in apoptosis of T<sub>H</sub>1-like subset (15-20% of CD4+ cells), correlate with studies, carried out in susceptible hosts, showing enhancement of CD4+ T cell apoptosis, especially T<sub>H</sub>1-like cells subtype, associated to reduction of T<sub>H</sub>1 cytokine production<sup>(73)</sup>. Deletion of CD4+ T<sub>H</sub>1-like cells could contribute to depress cell-mediated immunity in acute VL patients, since T<sub>H</sub>1 cytokines appears to be involved in protection against leishmaniasis, both in experimental models<sup>(27,73)</sup> and in human infections<sup>(57-59)</sup>.

Contrariwise, higher apoptosis frequency in CD8+ T cells rather than in CD4+ T ones has been pointed out in active human localised CL, and these apoptotic events were very low in patients with self-healing lesions<sup>(76)</sup>. Thus, cell-mediated immunity failure, responsible of severe immunodepression in various forms of leishmaniasis, can be attributed to inappropriate and missregulated T-cell death.

The amount of available data suggests CD8+ T cells are involved in leishmanicidal activity, and this last one seems to be modulated by IFN-g and IL-2 secretion<sup>(77)</sup>. Variety of effector mechanisms by

these CD8+ T cells, involving direct cytotoxic activity, via perforin/granzyme pathway, and apoptosis induction, via Fas/FasL interaction, have been highlighted in several reports<sup>(76-78)</sup>.

Moreover, CD8+ lymphocytes secrete various cytokines (especially IFN- $\gamma$ ) and chemokines (especially ones belonging to C-C group, i.e. Regulated upon Activation, Normal T-cell Expressed, and presumably Secreted [RANTES]), which might have key role in acquired cell-mediated immunity against parasite, i.e. by promoting CD4+ T<sub>H</sub>1 cell development<sup>(78)</sup>.

Perforin/granzyme-mediated direct cytotoxicity and parasitized macrophages apoptosis induction are candidate mechanisms employed by CD8+ T cells in their effort to limit parasite multiplication<sup>(76)</sup>.

## *Post Kala Azar dermal leishmaniasis*

In most cases of PKDL, parasites or parasites antigens were observed in all the lesions and were able to induce the formation of an inflammatory infiltrate, consisting of a mixture of macrophages, lymphocytes and plasma cells<sup>(79)</sup>. The inflammatory cells were mainly CD3+ and IL-10 was the most prominent cytokine founded in lesions<sup>(80)</sup>. However, IFN-g was found in all and IL-4 in most lesions in varying amounts<sup>(79-80)</sup>.

## **Conclusions**

Cytokines response pattern and clinical *Leishmania* outcome are strictly connected. Type-1 lymphocyte immune response and IFN-g production have been clearly identified as protective immune factors against *Leishmania*, whereas definite role of T<sub>H</sub>2 cytokines, in non-healing infections, requires careful exploration. As a matter of fact, appropriate CL models show IL-4 and IL-13 (T<sub>H</sub>2 cytokines) can promote disease progression, whereas IL-4 role in VL is not well defined, as its ability to enhance protective type-1 response.

Thus, immune response to intracellular parasites should dismiss T<sub>H</sub>1/T<sub>H</sub>2 paradigm of resistance/susceptibility, embracing theory of a more complicated network of regulatory/counter regulatory interactions, and IL-4 role in influencing *Leishmania* infection is waiting for future re-evaluation, according to *Leishmania* species involved, host organism used and tissue site examined. Moreover, the presence of antigen specific regulato-

**Table 1:** Types of CD4+ T-cells and cytokines involved in different forms of leishmaniasis

Disease	Healing		Non healing	
	CD4+ T-cell	Cytokines	CD4+ T-cell	Cytokines
Cutaneous leishmaniasis	T <sub>H</sub> 1	<i>Leishmania major, mexicana, amazonensis</i> : IL-12, IL-1 $\alpha$ , IL-18, IL-23, IL-27, IFN- $\gamma$ , TNF- $\alpha$ , MIF, IFN- $\alpha$ , IFN- $\beta$ , GM-CSF, IL-10, TGF- $\beta$	<i>Leishmania major</i> : excessive TH2 response and/or defective APC function and/or defective TH1 response	IL-4 (?), IL-13, IL-10, TGF- $\beta$
			<i>Leishmania mexicana</i> : excessive TH2 response and/or defective APC function and/or defective TH1 response	IL-4, IL-13 (?), IL-10 (?)
			<i>Leishmania amazonensis</i> : excessive TH2 response and/or defective APC function and/or defective TH1 response	IL-4 (?)
Visceral leishmaniasis	T <sub>H</sub> 1 T <sub>H</sub> 2 (?)	IL-12, IL-4 (?) (experimental visceral leishmaniasis)	<i>Experimental visceral leishmaniasis</i> : defective T <sub>H</sub> 1 response (?) excessive T <sub>H</sub> 2 response (?)	IL-4 (?), IL-10, TGF- $\beta$ (?)
	T <sub>H</sub> 1 T <sub>H</sub> 2 (?)	IL-2, IFN- $\gamma$ , IL-12, IL-15, IL-4 (?) (human visceral leishmaniasis)		

CD: cluster of differentiation

T<sub>H</sub>1: T helper 1T<sub>H</sub>2: T helper 2

APC: antigen presenting cells

IL: interleukin

IFN- $\alpha$ : interferon- $\alpha$ IFN- $\beta$ : interferon- $\beta$ IFN- $\gamma$ : interferon- $\gamma$ 

MIF: Migration Inhibitor Factor

TGF- $\beta$ : Transforming Growth Factor- $\beta$ TNF- $\alpha$ : Tumor Necrosis Factor- $\alpha$ 

GM-CSF: Granulocyte Macrophage-Colony Stimulating Factor

ry T cell subsets may provide an environment that contributes to the balance between T<sub>H</sub>1 and T<sub>H</sub>2 cells.

## References

- Dantas-Torres F, Brandao-Filho SP. *Visceral leishmaniasis in Brazil: revisiting paradigms of epidemiology and control*. Rev Inst Med Trop Sao Paulo 2006; 48: 151-6.
- Bern C, Chowdhury R. *The epidemiology of visceral leishmaniasis in Bangladesh: prospects for improved control*. Indian J Med Res 2006; 123: 275-88.
- Steffen R, Rickenbach M, Wilhelm U, Helminger A, Schar M. *Health problems after travel to developing countries*. J Infect Dis 1987; 156: 84-91.
- Murray HW, Berman JD, Davies CR and Saravia NG. *Advances in leishmaniasis*. Lancet 2005; 366: 1561-77.
- Croft SL, Vivas L and Brooker S. *Recent advances in research and control of malaria, leishmaniasis, trypanosomiasis and schistosomiasis*. East Mediterr Health J 2003; 9: 518-33.
- Thomaz-Soccol V, Lanotte G, Rioux J A, Pratlong F, Martini-Dumas A and Serres E. *Phylogenetic taxonomy of New World Leishmania*. Ann Parasitol Hum Comp 1993; 68: 104-6.
- Gramiccia M And Gradoni L. *The current status of zoonotic leishmaniases and approaches to disease control*. Int J Parasitol 2005; 35: 1169-80.
- Bates PA and Rogers ME. *New insights into the developmental biology and transmission mechanisms of Leishmania*. Curr Mol Med 2004; 4: 601-9.
- Colmenares M, Kar S, Goldsmith-Pestana K and McMahon-Pratt D. *Mechanisms of pathogenesis: differences amongst Leishmania species*. Trans R Soc Trop Med Hyg 2002; 96: S3-7.
- Gumy A, Louis JA and Launois P. *The murine model of infection with Leishmania major and its importance for the deciphering of mechanisms underlying differences in Th cell differentiation in mice from different genetic backgrounds*. Int J Parasitol 2004; 34: 433.
- Louis JA, Gumy A, Voigt H, Launois P and Rocken M. *The use of the murine model of infection with Leishmania major to reveal the antagonistic effects that IL-4 can exert on T helper cell development and demonstrate that these opposite effects depend upon the nature of the cells targeted for IL-4 signaling*. Pathol Biol 2003; 51: 71.

- 12) Vannier-Santos MA, Martiny A and de Souza W. *Cell biology of Leishmania spp.: invading and evading*. Curr Pharm Des 2002; 8: 297.
- 13) McSorley S, Proudfoot L, O'Donnell CA and Liew FY. *Immunology of murine leishmaniasis*. Clin Dermatol 1996; 14: 451.
- 14) Barbieri CL. *Immunology of canine leishmaniasis*. Parasite Immunol. 2006; 28: 329.
- 15) Roberts MT. *Current understandings on the immunology of leishmaniasis and recent developments in prevention and treatment*. Br Med Bull 2006 ; 75-76: 115-30.
- 16) Steigerwald M and Moll H. *Leishmania major modulates chemokine and chemokine receptor expression by dendritic cells and affects their migratory capacity*. Infect Immun 2005; 73: 2564-7.
- 17) Ritter U and Moll H. *Monocyte chemotactic protein-1 stimulates the killing of Leishmania major by human monocytes, acts synergistically with IFN-gamma and is antagonized by IL-4*. Eur J Immunol 2000; 30: 3111-20.
- 18) Constantinescu CS, Hondowicz BD, Elloso MM, Wysocka M, Trinchieri G and Scott P. *The role of IL-12 in the maintenance of an established Th1 immune response in experimental leishmaniasis*. Eur J Immunol 1998; 28: 2227-33.
- 19) Kemp M, Kurtzhals JA, Bendtzen K, Poulsen LK, Hansen MB, Koech DK, Kharazmi A and Theander TG. *Leishmania donovani-reactive Th1- and Th2-like T-cell clones from individuals who have recovered from visceral leishmaniasis*. Infect Immun 1993; 61: 1069-73.
- 20) Chtanova T and Mackay CR. *T cell effector subsets: extending the Th1/Th2 paradigm*. Adv Immunol 2001; 78: 233-66.
- 21) Bacellar O, D'oliveira A Jr, Jeronimo S and Carvalho EM. *IL-10 and IL-12 are the main regulatory cytokines in visceral leishmaniasis*. Cytokine 2000; 12: 1228-31.
- 22) Murray HW, Tsai CW, Liu J and Ma X. *Responses to Leishmania donovani in mice deficient in interleukin-12 (IL-12), IL-12/IL-23, or IL-18*. Infect Immun 2006; 74: 4370-4.
- 23) Artis D, Johnson LM, Joyce K, Saris C, Villarino A, Hunter CA and Scott P. *Cutting edge: early IL-4 production governs the requirement for IL-27-WSX-1 signaling in the development of protective Th1 cytokine responses following Leishmania major infection*. J Immunol 2004; 172: 4672-5.
- 24) Elloso MM and Scott P. *Expression and contribution of B7-1 (CD80) and B7-2 (CD86) in the early immune response to Leishmania major infection*. J Immunol 1999; 162: 6708-15.
- 25) Padigel UM and Farrell JP. *CD40-CD40 ligand costimulation is not required for initiation and maintenance of a Th1-type response to Leishmania major infection*. Infect Immun 2003; 71: 1389-95.
- 26) Holzmuller P, Bras-Goncalves R and Lemesre JL. *Phenotypical characteristics, biochemical pathways, molecular targets and putative role of nitric oxide-mediated programmed cell death in Leishmania*. Parasitology 2006; 132: S19-32.
- 27) Liew FY, Wei XQ and Proudfoot L. *Cytokines and nitric oxide as effector molecules against parasitic infections*. Phil Trans R Soc Lond B 1997; 352: 1311-5.
- 28) Al-Zamel F, Al-Shammery FJ, El-Shewemi S and Soliman R. *Enhancement of leishmanicidal activity of human macrophages against Leishmania major and Leishmania donovani infection using recombinant human granulocyte macrophage colony stimulating factor*. Zentralbl Bakteriol 1996; 285: 92-105.
- 29) Nunes MP, Cysne-Finkelstein L, Monteiro BC, de Souza DM, Gomes NA and DosReis GA. *CD40 signaling induces reciprocal outcomes in Leishmania-infected macrophages; roles of host genotype and cytokine milieu*. Microbes Infect 2005; 7: 78-85.
- 30) Eidsmo L, Nylen S, Khamesipour A, Hedblad MA, Chiodi F and Akuffo H. *The contribution of the Fas/FasL apoptotic pathway in ulcer formation during Leishmania major-induced cutaneous Leishmaniasis*. Am J Pathol 2005; 166: 1099-108.
- 31) Potestio M, D'Agostino P, Romano GC, Milano S, Ferlazzo V, Aquino A, Di Bella G, Caruso R, Gambino G, Vitale G, Mansueto S and Cillari E. *CD4+ CCR5+ and CD4+ CCR3+ lymphocyte subset and monocyte apoptosis in patients with acute visceral leishmaniasis*. Immunology 2004; 113: 260-8.
- 32) Belkaid Y. *The role of CD4(+)/CD25(+) regulatory T cells in Leishmania infection*. Expert Opin Biol Ther 2003; 3: 875-85.
- 33) Kariminia A, Bourreau E, Pascalis H, Couppie P, Sainte-Marie D, Tacchini-Cottier F and Launois P. *Transforming growth factor beta 1 production by CD4+ CD25+ regulatory T cells in peripheral blood mononuclear cells from healthy subjects stimulated with Leishmania guyanensis*. Infect Immun 2005; 73: 5908-14.
- 34) Aguilar-Torrentera F and Carlier Y. *Immunological factors governing resistance and susceptibility of mice to Leishmania major infection*. Rev Latinoam Microbiol 2001; 43: 135-42.
- 35) Sacks D and Noben-Trauth N. *The immunology of susceptibility and resistance to Leishmania major in mice*. Nat Rev Immunol 2002; 2: 845-58.
- 36) Noben-Trauth N, Lira R, Nagase H, Paul WE and Sacks DL. *The relative contribution of IL-4 receptor signaling and IL-10 to susceptibility to Leishmania major*. J Immunol 2003; 170: 5152-8.
- 37) Matthews DJ, Emson CL, McKenzie GJ, Jolin HE, Blackwell JM and McKenzie AN. *IL-13 is a susceptibility factor for Leishmania major infection*. J Immunol 2000; 164: 1458-62.
- 38) Li J, Hunter CA and Farrell JP. *Anti-TGF-beta treatment promotes rapid healing of Leishmania major infection in mice by enhancing in vivo nitric oxide production*. J Immunol 1999; 162: 974-9.
- 39) Shankar A H and Titus R G. *The influence of antigen-presenting cell type and interferon-gamma on priming and cytokine secretion of Leishmania major-specific T cells*. J Infect Dis 1997; 175: 151-7.
- 40) Chakir H, Campos-Neto A, Mojibian M and Webb JR. *IL-12Rbeta2-deficient mice of a genetically resistant background are susceptible to Leishmania major infection and develop a parasite-specific Th2 immune response*. Microbes Infect 2003; 5: 241-9.
- 41) Alexander J and Bryson K. *T helper (h)1/Th2 and Leishmania: paradox rather than paradigm*. Immunol Lett 2005; 99: 17-23.
- 42) Denise H, McNeil K, Brooks DR, Alexander J, Coombs GH and Mottram JC. *Expression of multiple CPB genes encoding cysteine proteases is required for Leishmania mexicana virulence in vivo*. Infect Immun

- 2003; 71: 3190-5.
- 43) Ueda-Nakamura T, da Conceicao Rocha Sampaio M, Cunha-e-Silva NL, Traub-Cseko YM and de Souza W. *Expression and processing of megasome cysteine proteinases during Leishmania amazonensis differentiation*. Parasitol Res 2002; 88: 332-7.
  - 44) Cameron P, McGachy A, Anderson M, Paul A, Coombs GH, Mottram JC, Alexander J and Plevin R. *Inhibition of lipopolysaccharide-induced macrophage IL-12 production by Leishmania mexicana amastigotes: the role of cysteine peptidases and the NF-kappaB signaling pathway*. J Immunol 2004; 173: 3297-304.
  - 45) Kaye PM, Curry AJ and Blackwell JM. *Differential production of Th1- and Th2-derived cytokines does not determine the genetically controlled or vaccine-induced rate of cure in murine visceral leishmaniasis*. J Immunol 1991; 146: 2763-70.
  - 46) Cillari E, Vitale G, Arcoledo F, D'Agostino P, Mocciano C, Gambino G, Malta R, Stassi G, Giordano C and Milano S. *In vivo and in vitro cytokine profiles and mononuclear cell subsets in Sicilian patients with active visceral leishmaniasis*. Cytokine 1995; 7: 740-5.
  - 47) Ansari NA, Saluja S and Salotra P. *Elevated levels of interferon-gamma, interleukin-10, and interleukin-6 during active disease in Indian kala azar*. Clin Immunol 2006; 119: 339-45.
  - 48) Vitale G, Reina G, Mansueto S, Malta R, Gambino G, Mocciano C, D'Agostino P, Dieli M and Cillari E. *The significance of serum soluble IL-2 receptor as a marker for active visceral leishmaniasis in Sicilian patients*. Clin Exp Immunol 1992; 90: 219-22.
  - 49) Cillari E, Liew FY, Lo Campo P, Milano S, Mansueto S and Salerno A. *Suppression of IL-2 production by cryopreserved peripheral blood mononuclear cells from patients with active visceral leishmaniasis in Sicily*. J Immunol 1988; 140: 2721-6.
  - 50) Cillari E, Milano S, Dieli M, Maltese E, Di Rosa S, Mansueto S, Salerno A and Liew FY. *Reduction in the number of UCHL-1+ cells and IL-2 production in the peripheral blood of patients with visceral leishmaniasis*. J Immunol 1991; 146: 1026-30.
  - 51) Carvalho EM, Badaro R, Reed SG, Jones TC and Johnson WD. *Absence of gamma interferon and interleukin-2 production during active visceral leishmaniasis*. J Clin Invest 1985; 76: 2066-9.
  - 52) Ghalib HW, Piuvezam MR, Skeiky YA, Siddig M, Hashim FA, el-Hassan AM, Russo DM and Reed SG. *Interleukin 10 production correlates with pathology in human Leishmania donovani infections*. J Clin Invest 1993; 92: 324-9.
  - 53) Carvalho EM, Bacelar O, Brownell CE, Regis T, Coffman RL and Reed SG. *Restoration of IFN-gamma production and lymphocyte proliferation in visceral leishmaniasis*. J Immunol 1994; 152: 5949-56.
  - 54) Ghalib HW, Whittle JA, Kubin M, Hashim FA, el-Hassan AM, Grabstein KH, Trinchieri G and Reed SG. *IL-12 enhances Th1-type responses in human Leishmania donovani infections*. J Immunol 1995; 154: 4623-9.
  - 55) Kurkjian KM, Mahmutovic AJ, Kellar KL, Haque R, Bern C and Secor WE. *Multiplex analysis of circulating cytokines in the sera of patients with different clinical forms of visceral leishmaniasis*. Cytometry A 2006; 69: 353-8.
  - 56) Kemp M, Theander TG and Kharazmi A. *The contrasting roles of CD4+ T cells in intracellular infections in humans: leishmaniasis as an example*. Immunol Today 1997; 17: 13-6.
  - 57) Romagnani S. *The Th1/Th2 paradigm*. Immunol Today 1997; 18: 263-6.
  - 58) Woldmann TA and Tagaya Y. *The multifaceted regulation of interleukin-15 expression and the role of this cytokine in NK cell differentiation and host response to intracellular pathogens*. Annu Rev Immunol 1999; 17: 19-49.
  - 59) Lauwerys BR, Garot N, Renauld JC and Houssiau FA. *Cytokine production and killer activity of NK/T-NK cells derived with IL-2, IL-15, or the combination of IL-12 and IL-18*. J Immunol 2000; 165: 1847-53.
  - 60) Loza MJ, Zamai L, Azzoni L, Rosati E and Perussia B. *Expression of type 1 (interferon gamma) and type 2 (interleukin-13, interleukin-5) cytokines at distinct stages of natural killer cell differentiation from progenitor cells*. Blood 2002; 99: 1273-81.
  - 61) Milano S, Di Bella G, D'Agostino P, Barbera C, Caruso R, La Rosa M, Ferlazzo V, Vitale G, La Russa C, Gambino G, Chifari N, Mansueto S and Cillari E. *IL-15 in human visceral leishmaniasis caused by Leishmania infantum*. Clin Exp Immunol 2002; 127: 360-5.
  - 62) D'Agostino P, Milano S, Arcoledo F, Di Bella G, La Rosa M, Ferlazzo V, Caruso R, Chifari N, Vitale G, Mansueto S and Cillari E. *Interleukin-15, as interferon-gamma, induces the killing of Leishmania infantum in phorbol-myristate-acetate-activated macrophages increasing interleukin-12*. Scand. J Immunol 2004; 60: 609-14.
  - 63) Lehmann J, Enssle KH, Lehmann I, Emmendorfer A and Lohmann-Matthes ML. *The capacity to produce IFN-gamma rather than the presence of interleukin-4 determines the resistance and the degree of susceptibility to Leishmania donovani infection in mice*. J Interferon Cytokine Res 2000; 20: 63-77.
  - 64) Satoskar A, Bluethmann H and Alexander J. *Disruption of the murine interleukin-4 gene inhibits disease progression during Leishmania mexicana infection but does not increase control of Leishmania donovani infection*. Infect Immun 1995; 63: 4894-9.
  - 65) Stager S, Alexander J, Carter KC, Brombacher F and Kaye PM. *Both interleukin-4 (IL-4) and IL-4 receptor alpha signaling contribute to the development of hepatic granulomas with optimal antileishmanial activity*. Infect Immun 2003; 71: 4804-7.
  - 66) Wilson ME, Young BM, Davidson BL, Mente KA and McGowan SE. *The importance of TGF-beta in murine visceral leishmaniasis*. J Immunol 1998; 161: 6148-55.
  - 67) Murray HW, Flanders KC, Donaldson DD, Sypek JP, Gotwals PJ, Liu J and Ma X. *Antagonizing deactivating cytokines to enhance host defense and chemotherapy in experimental visceral leishmaniasis*. Infect Immun 2005; 73: 3903-11.
  - 68) Gollob KJ, Antonelli LR and Dutra WO. *Insights into CD4+ memory T cells following Leishmania infection*. Trends Parasitol 2005; 21: 347-50.
  - 69) Hailu A, van Baarle D, Knol GJ, Berhe N, Miedema F and Kager PA. *T cell subset and cytokine profiles in human visceral leishmaniasis during active and asymptomatic or sub-clinical infection with Leishmania donovani*. Clin Immunol 2005; 117: 182-91.

- 70) Estaquier J, Idziorek T, Zou W, Emilie D, Farber CM, Bourez JM and Ameisen JC. *T helper type 1/T helper type 2 cytokines and T cell death: preventive effect of interleukin 12 on activation-induced and CD95 (FAS/APO-1)-mediated apoptosis of CD4+ T cells from human immunodeficiency virus-infected persons.* J Exp Med 1995; 182: 1759-67.
- 71) Dockrell DH. *Apoptotic cell death in the pathogenesis of infectious diseases.* J Infect 2001; 42: 227-34.
- 72) Sereti I, Herpin B, Metcalf JA, Stevens R, Baseler MW, Hallahan CW, Kovacs JA, Davey RT and Lane HC. *CD4 T cell expansions are associated with increased apoptosis rates of T lymphocytes during IL-2 cycles in HIV infected patients.* AIDS 2001; 15: 1765-75.
- 73) Das G, Vohra H, Rao K, Saha B and Mishra GC. *Leishmania donovani infection of a susceptible host results in CD4+ T-cell apoptosis and decreased Th1 cytokine production.* Scand J Immunol 1999; 49: 307-10.
- 74) Conceicao-Silva F, Hahne M, Schroter M, Louis J and Tschopp J. *The resolution of lesions induced by Leishmania major in mice requires a functional Fas (APO-1, CD95) pathway of cytotoxicity.* Eur J Immunol 1998; 28: 237-45.
- 75) Alexander CE, Kaye PM and Engwerda CR. *CD95 is required for the early control of parasite burden in the liver of Leishmania donovani-infected mice.* Eur J Immunol 2001; 31: 1199-210.
- 76) Bertho AL, Santiago MA, Da-Cruz AM and Coutinho SG. *Detection of early apoptosis and cell death in T CD4+ and CD8+ cells from lesions of patients with localized cutaneous leishmaniasis.* Braz J Med Biol Res 2000; 33: 317-25.
- 77) Tsagozis P, Karagouni E and Dotsika E. *Function of CD8+ T lymphocytes in a self-curing mouse model of visceral leishmaniasis.* Parasitol Int 2005; 54: 139-46.
- 78) Tsagozis P, Karagouni E and Dotsika E. *CD8(+) T cells with parasite-specific cytotoxic activity and a Tc1 profile of cytokine and chemokine secretion develop in experimental visceral leishmaniasis.* Parasite Immunol 2003; 25: 569-79.
- 79) Sharma MC, Gupta AK, Verma N, Das VN, Saran R and Kar SK. *Demonstration of Leishmania parasites in skin lesions of Indian post kala-azar dermal leishmaniasis (PKDL) cases.* J Commun Dis. 32: 67-8.
- 80) Ismail A, El Hassan AM, Kemp K, Gasim S, Kadaru AE, Moller T, Kharazmi A and Theander TG. *Immunopathology of post kala-azar dermal leishmaniasis (PKDL): T-cell phenotypes and cytokine profile.* J Pathol 1999; 189: 615-22.

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