Acta Medica Mediterranea, 2011, 27: 7

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-g 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_H2 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_H1/T_H2 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_H1 and T_H2 cells. Finally, the involvement of $CD8^+$ T cells has been described, but the modality of their role and function, in this kind of infection, has not been outspreed so far.

Key words: Cutaneous leishmaniasis, Visceral leishmaniasis, T_H1/T_H2 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-γ 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_H2) siano in grado di promuovere la progressione della malattia nella leishmaniosi cutanea, mentre l'IL-4 sembra aumentare l'effetto protettivo delle risposte T_H1 nella leishmaniosi viscerale. Quindi, la risposta immunologica nei confronti di questi parassiti intracellulari non sembra adattarsi perfettamente al noto paradigma T_H1/T_H2 di resistenza/suscettibilità, 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_H1 e T_H2. Infine, nelle leishmaniosi, è stato descritto, anche, il coinvolgimento delle cellule T CD8+, 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_H1/T_H2, citochine, chemochine, apoptosi*

Introduction

Supervisory of public health should consider leishmaniasis a severe, increasing, health problem[1-2].

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[3].

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[4].

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[5].

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.) aethiopica*, and dermotropic *Leishmania (L.) infantum*, of the *Leishmania* subgenus; in the New World, by *Leishmania (L.) mexicana, Leishmania (L.) amazonensis, Leishmania (L.) venezuelensis,* and dermotropic *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[6,7].

Leishmania are digenetic parasites, developing as flagellated motile promastigotes in the gut of blood-sucking female sand flies (*Phlebotomus spp.* in the Old Word, *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[8,9].

Leishmania can infect mice quite easily, granting useful *in vivo* models to evaluate host immune response and regulation, and genetic control of infection[10,11].

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[8,12].

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 $CD4^+$ and $CD8^+$ 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[13-15].

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[16,17]. 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[16,17].

IFN-g-induced macrophage activation induces

T helper (T_H) 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[18].

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_H1- and T_H2-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[19,20]. Change in T_H1-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[19,20].

Resolution of infection: the T_H1 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_H1 cells, as innate mechanism response, and induces IFN-g secretion by T_H1 cells and NK cells, as antigen-specific acquired cell-mediated response[21-23].

Proper activation of immune T_H1 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_H1 cell, respectively)[24,25].

Signals able to regulate superoxide (O_2^-) and nitric oxide (NO) production and parasite killing are induced by IFN-g from T_H1 cells, and probably, to a lesser extent, from $CD8^+$ 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[26,27]. 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[28,29].

Another important pathway that seems to be important to develop adequate T_H1-mediated resistance to *Leishmania* infection is Fas/FasL interaction. Activated T_H1 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 $CD4^+$ T cell-induced apoptotic death[30-31].

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[30-31].

Antigen-specific $CD4^+$ $CD25^+$ T-regulatory cells, producing IL-10 and TGF-b, moderate activity of T effectors when infection break off[32,33]; so, in absence of persistent infection, protective immunity is significantly reduced[32-33].

Non-healing cutaneous Leishmania major infection: T_H2-dependent or defective T_H1 response

The T_H1-T_H2 paradigm of resistance/susceptibility to intracellular infection is largely based on investigations using *Leishmania major*. Initial studies suggested primary role of distinct $CD4^+$ T cell subsets, T_H1 and T_H2, 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_H1 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*[36,37]. Particularly, IL-13 has been found to act independently from IL-4, and the effects of IL-13 and IL-4 might be additive[37].

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 $CD4^+$ 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_H1 responses. Furthermore, T regulatory cells are significant producers of TGF-b, which is also partly able to suppress protective responses[36-38].

Impossibility to produce or respond to IL-12, and intrinsic defects in APC function or in T_H1 cell development have been hypothesized to explain inability to mount effective T_H1 response, irrespective of T_H2 response. Defective APC function, due to a failure to produce IL-12, may also underlie in a deficient APC IL-1 production[39]. 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_H2 response[39].

Contrariwise, intrinsic defective T_H1 cell development, originally attributed to unfair response to IL-12, has been associated with down-regulation of T_H1 cell IL-12Rb2 expression (40). It is well established that IL-12Rβ2 expression can be downregulated by IL-4 produced by T_H2, even though IL-4-indipendent mechanisms have also been described[40]. Commitment of immune system to T_H2 response has been recently explained by defective co-polarization of the T cell receptor (TCR) and the IFN-g receptor complex in naïve $CD4^+$ T cells during the APC/T precursor interaction[39,40].

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[41]. The Cathepsin L-like cysteine peptidase (CPB) has been identified as the likely *Leishmania mexicana* virulence factor inducing IL-4 production[42].

The role of IL-4 in subverting development of T_H1 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[43,44]. In this setting, ability of CPB to proteolytically degrade the NF-kB family of signalling proteins might be directly responsible of T_H1 responses inhibition (43,44). Thus, IL-4-independent mechanisms might prevail[43,44].

Paradox of T_H1 and T_H2 in visceral leishmaniasis

The dichotomy of T_H1 and T_H2 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_H1 response, rather than to T_H2 cell proliferation[45]. 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[41].

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[46,47]. 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[46,47]. 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[46]. IL-2 is significantly reduced in supernatants of actively infected patients, returning to normal level after recovery[46,47]. 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[48].

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-γ[49-51]. Furthermore, IL-10 production correlates with the progression of VL[52], and its neutralisation, with specific monoclonal antibody, restores T cell proliferation and IFN-g production in PBMC from acute VL patients[53]. *In vitro* studies have also demon-

strated that IL-12 shifts the responses toward T_H1-type and enhances IFN-g production[54].

Therefore, these findings suggest that, in patients with active VL, the cytokine profile is not clearly polarized, and both T_H1-like and T_H2-like cells appear to proliferate and be activated[46,50,55]. 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_H1 and T_H2 responses[56,57], although other unknown factors might influence the cellular immune behaviour. Altogether, data indicate that in symptomatic patients T_H1 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[58] and its ability to enhance both T_H1 responses, by increasing IFN-γ production from NK and T cells[59], and T_H2 responses, by augmenting IL-5 and IL-13 production[60]. In particular, it has been demonstrated that endogenous IL-15 plays a role in the suppression of T_H2 cytokines in acute VL patients, even though it does not enhance the production of T_H1 cytokines[61], indicating a potential protective role of IL-15 against leishmaniasis caused by the indirect effect on T_H1, due to the restriction of T_H2 proliferation[61]. Furthermore, IL-15 *in vitro* activation of macrophage cultures determines a significant anti-*Leishmania* activity, comparable to IFN-γ-induced one[62]. *Leishmania* macrophages intracellular killing, primed by IL-15, is followed by IL-12 synthesis increase[62]. 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_H2-independent?

As already mentioned, disease progression and exacerbating role of T_H2 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_H1 and T_H2 cytokines[45], even thought production of IFN-g correlates with resistance and T_H2 cytokines are not so clearly responsible of susceptibility[63].

According to this point of view, studies in mice suggested IL-4 may be protective in some circumstances, 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[64]. 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[65]. As evidence, in absence of IL-4, type-1 responses and IFN-g production fail to be maintained following chemotherapy or to be induced by vaccination[64,65].

Though TGF-b has significant disease promoting and progression activity, several studies indicate IL-10 as major immunosoppressive cytokine in VL[66]. Activation of T_H1 cell responses and priming of parasite killing is allowed by experimental IL-10 inhibition[66,67].

Furthermore, while healing process in susceptible mice is IL-12-dependent, IL-10, but not IL-4 or TGF-b, appears to blunt T_H1-type responses and determine disease fatal outcome[21].

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 $CD3^+$, $CD4^+$ and $CD8^+$ T cells, in patients affected with symptomatic *Leishmania infantum* infection, are within normal range[46,50]. Whereas, acute VL patients have markedly reduced levels of memory T cells ($CD3^+/CD45RO^+$) compared with healthy controls, which come back to normal levels as result of successful chemotherapy[46,50,68,69]. 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 $CD4^+$ T[68]. Importance of increased memory $CD4^+$ T cells level in this group of patients and the relationship with positive leishmanin skin test and acquired immunity needs further explanation[68,69]. Elevated number of $CD3^+$ HLA-DR^+ lymphocytes and increased expression of HLA-DR antigen on these cells show strong activation of cell-mediated immune response in acute VL patients[46].

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[70-73]. In experimental VL it has been demonstrated that infection of susceptible host results in CD4+ T cell apoptosis and reductions of T_H1 cytokine production[73].

In particular, it has been demonstrated that apoptotic mechanisms, mainly operating through the Fas (APO-1/CD95) pathway, grant resistance to *Leishmania major,* and singeneic *gld* and *lpr* mice, lacking of functional Fas system, fail healing their lesions[74]. However, mice infected by *Leishmania donovani* parasites show increased incidence of T cell apoptosis in liver and spleen[75]. 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[31]. T cells were mainly of CD4+ phenotype[31,76]. In particular, T_H1 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_H1-like subset (15-20% of CD4+ cells), correlate with studies, carried out in susceptible hosts, showing enhancement of CD4+ T cell apoptosis, especially T_H1-like cells subtype, associated to reduction of T_H1 cytokine production[73]. Deletion of CD4+ T_H1-like cells could contribute to depress cell-mediated immunity in acute VL patients, since T_H1 cytokines appears to be involved in protection against leishmaniasis, both in experimental models[27,73] and in human infections[57-59].

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[76]. 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[77]. 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[76-78].

Moreover, CD8+ lymphocytes secrete various cytokines (especially IFN-γ) 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_H1 cell development[78].

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[76].

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[79]. The inflammatory cells were mainly $CD3^+$ and IL-10 was the most prominent cytokine founded in lesions[80]. However, IFN-g was found in all and IL-4 in most lesions in varying amounts[79-80].

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_H2 cytokines, in non-healing infections, requires careful exploration. As a matter of fact, appropriate CL models show IL-4 and IL-13 (T_H2 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_H1/T_H2 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_H1	*Leishmania major, mexicana, amazonensis:* IL-12, IL-1α, IL-18, IL-23, IL-27, IFN-γ, TNF-α, MIF, IFN-α, IFN-β, GM-CSF, IL-10, TGF-β	*Leishmania major:* excessive TH2 response and/or defective APC function and/or defective TH1 response	IL-4 (?), IL-13, IL-10, TGF-β
			Leishmania mexicana: excessive TH2 response and/or defective APC function and/or defective TH1 response	IL-4, IL-13 (?), IL-10 (?)
			Leishmania amazonensis: excessive TH2 response and/or defective APC function and/or defective TH1 response	IL-4 (?)
Visceral leishmaniasis	T_H1 T_H2 (?)	IL-12, IL-4 (?) (experimental visceral leishmaniasis)	*Experimental visceral leishmaniasis:* defective T_H1 response (?) excessive T_H2 response (?)	IL-4 (?), IL-10, TGF-β (?)
	T_H1 T_H2 (?)	IL-2, IFN-γ, IL-12, IL-15, IL-4 (?) (human visceral leishmaniasis)	*Human visceral leishmaniasis:* defective T_H1 response (?) excessive T_H2 response (?)	IL-4 (?), IL-10, TGF-β (?)

CD: cluster of differentation
T_H1: T helper 1
T_H2: T helper 2
APC: antigen presenting cells
IL: interleukin
IFN-α: interferon-α
IFN-β: interferon-β
IFN-γ: interferon-γ
MIF: Migration Inhibitor Factor
TGF-β: Transforming Growth Factor-β
TNF-α: Tumor Necrosis Factor-α
GM-CSF: Granulocyte Macrophage-Colony Stimulating Factor

ry T cell subsets may provide an environment that contributes to the balance between T_H1 and T_H2 cells.

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