Human infection with *Leishmania* results in diverse clinical and immunopathological situations. The capacity of the parasites to cause this wide range of disease manifestations depends upon their ability to evade the immune defense mechanisms by performing a well-tuned orchestra of host-parasite interactions inside the macrophages. While updated knowledge focus on the key role of cell-mediated immunity (CMI) in protection, the survival strategies of the parasites leads to the suppression of CMI which can further be aggravated by the co-infections with HIV, tuberculosis *etc*.

The present review describes the immune mechanisms in human leishmaniasis with a special attention to visceral leishmaniasis or kala-azar, one of the most important epidemiological health problems in Indian subcontinent. Modulations of the both humoral and cell-mediated immune responses during asymptomatic infections, active disease and after successful chemotherapy are discussed. The components responsible for the regulation of the critical balance of Th1/Th2 type of responses are re-evaluated. Co-infection of HIV and visceral leishmaniasis and their interdependence has been addressed. Although the specific role of an elevated humoral response in kala-azar is yet to be established, attempts for its application in diagnosis, precisely for the development of field diagnostic techniques, are presented. Also discussed are attempts to utilize the immunogenic potentials of different leishmanial antigens in the development of anti-leishmanial vaccines.

**Key words** Cutaneous leishmaniasis - CD4+ T cell - CD8+ T cell - IFN-γ - IL-10 - IL-12 - immune response - macrophage - serodiagnosis - Th1 response - Th2 response - visceral leishmaniasis

Leishmaniasis represents a spectrum of diseases with important clinical and epidemiological diversity. There are three major forms of leishmaniasis in human *viz.*, cutaneous (CL), mucocutaneous (MCL) and visceral leishmaniasis (VL). The diseases are caused by the obligate intracellular protozoan parasites belonging to various species of the genus *Leishmania*. Leishmaniasis is endemic in 88 countries, of which 72 are developing countries, including 13 of the least developed countries. Ninety per cent of CL occurs in seven countries - Afghanistan, Algeria, Brazil, Iran, Peru, Saudi Arabia and Syria, while 90 per cent of VL occurs in five countries - Bangladesh, India, Nepal, Sudan and Brazil. CL is caused by a wide range of species, including *Leishmania major*, *L. aethiopica* and *L. tropica* in the Old World, and *L. mexicana*, *L. braziliensis*, *L. amazonensis*, *L. pifanoi*, *L. garnhami*, *L. venezuelensis*, *L. guyanensis*, *L. peruviana*, and *L. panamensis* in the New World. The disease, which is usually localized and heals spontaneously, is characterized by skin lesions rich
in parasites. MCL is most commonly caused by the New World species, *L. braziliensis*<sup>2,3</sup>, though *L. aethiopica* has also been reported to cause this syndrome. A persistent cutaneous lesion that eventually heals characterizes initial infection. Several years later, oral and respiratory mucosal involvements occur, causing inflammation and mutilation of the nose, mouth, oropharynx, and trachea. Progressive disease is difficult to treat and often recurs. With prolonged infection, death occurs from respiratory compromise and malnutrition<sup>4</sup>. VL, commonly known as kala-azar, is caused by *L. donovani* and *L. infantum* in the Old World and *L. chagasi* in the New World. It is characterized by fever, cachexia, hepatosplenomegaly, and blood cytopaenia, and is usually fatal without specific chemotherapy. VL is of higher priority than CL since anthroponotic VL foci are the origin of frequent and deadly epidemics. The name ‘kala-azar’ has originated from India, meaning ‘black-fever’, which refers to the hyperpigmentation of skin during the course of the disease. Alternatively, the term might be derived from the word ‘kal’ meaning ‘death’, which signifies the fatality of the disease<sup>5</sup>. Patients, cured of VL from Sudan and India, often develop post-kala-azar dermal leishmaniasis (PKDL), which appears as a dermatotropic form of *L. donovani* infection. According to recent reports, there are 1.0-1.5 million cases of CL and 500,000 cases of VL each year, and a population of 350 million is at risk. Disability-adjusted life years (DALYs) lost due to leishmaniasis are close to 2.4 million all over the world<sup>1,6</sup>.

*Leishmania* are protozoa belonging to the order Kinetoplastida and the family Trypanosomatidae. The parasites have a digenetic life cycle and exist in two distinct morphologies, the promastigote in the sand fly vector, and the amastigote in the mammalian host. The motile flagellated promastigotes exist, multiply and develop extracellularly in the alimentary tract of the blood sucking female sand fly vectors and are transmitted during the blood meal into mammalian hosts. Inside the mammalian hosts they infect macrophages of the reticuloendothelial tissue<sup>7,8</sup> and differentiate into nonmotile amastigotes and multiply as such in the phagolysosomal vacuoles. Macrophages play a primary role in the host defense and regulation of immune responses upon activation<sup>9</sup>. The parasites perform a complex host-parasite interaction inside the severe environment of the phagolysosomes and eventually evade this immune defense mechanism<sup>10</sup>. Infection of macrophages with *Leishmania* results in impaired microbialicidal machinery as evidenced by decreased responsiveness to the lipopolysaccharide required for induction of interleukin (IL)-1 production<sup>11</sup>. Although most of the informations on the immunologic mechanisms upon infection and protection from the *Leishmania* parasites were accumulated from the studies in mice, some critical findings of murine CL have been confirmed in humans in recent years. However, the immune response due to VL and the pathogenesis of the disease in human deviates considerably from the murine model.

Although it is evident that cell-mediated immune response (CMI) plays a very important role in the susceptibility or resistance and prophylaxis in response to chemotherapy, the role of humoral immune response cannot be ruled out as VL is marked by high levels of *Leishmania*-specific antibodies<sup>12,13</sup> which appear soon after infection and before the development of cellular immunologic abnormalities. The role of these elevated antibodies in resolution of the disease and protective immunity is mostly unknown. The occurrence of subclinical or asymptomatic infections among a large population of individuals residing in the endemic areas with development of specific antibodies and/or T-cell response to leishmanial antigens, however, suggests naturally acquired immunity<sup>14-17</sup>.

**Humoral responses in leishmaniasis**

Infection of *Leishmania* in human is characterized by the appearance of anti-leishmanial antibodies in the sera of the patients. In CL, usually they are present at low levels during the active phase of the disease<sup>18</sup>. However, in some studies the presence of antibodies against *L. braziliensis* infection in the sera of infected patients has been critically monitored and utilized for the diagnosis and prognosis of the disease<sup>19-23</sup>. Contrastingly, strong anti-leishmanial antibody titres are well documented in VL<sup>12,13</sup>. Although it is known that the Th1 cytokine
interferon-gamma (IFN-γ) probably upregulates isotypes IgG1 and IgG3, and the Th2 cytokines IL-4 and IL-5 stimulates the production of IgG4 in human24,25, the role of the elevated anti-leishmanial antibodies in kala-azar patients towards protection or pathogenesis is still unclear. Critical analysis of *Leishmania* antigen-specific Ig isotypes from our laboratory and others has revealed the elevated levels of IgG, IgM, IgE and IgG subclasses during disease26–31. To establish a correlation of these isotypes with progression and resolution of infection, another report from our laboratory has shown that antimonial drug resistance is associated with a reduction in IgG2 and IgG3 antibodies, with no significant change in the titres of IgG, IgM, IgA, IgE, and IgG4. However, a marked elevation of IgG1 was observed in all the patients studied32. Another study attempted to utilize the relative abundances of the anti-leishmanial IgG subclasses to discriminate among the immunity of the active and cured patients as well as the endemic healthy individuals and showed that a low CMI, in terms of delayed type hypersensitivity (DTH), is correlated with high IgG1, IgG3 and IgG4 and vice versa33. As it is still debatable whether these antibodies have any role in the protection of the disease, a recent experimental study postulated that IgG not only fails to provide protection against this intracellular pathogen, but it actually contributes to disease progression. Passive administration of anti-leishmanial IgG resulted in larger lesions in BALB/c mice with greater amount of IL-10 production14. This result can be correlated with the highly elevated titres of anti-leishmanial antibodies during the active phase of the disease and a consecutive fall in the antibody titre after a successful cure. The elevated antibody titres against promastigote or amastigote antigens, their fractions or recombinant antigens have been extensively exploited for specific serodiagnosis in last two decades in the form of direct agglutination test (DAT), ELISA, dot-ELISA, immunoblot, strip test, indirect immunofluorescence test (IFAT), indirect haemagglutination antibody (IHA) etc. However, diagnosis of leishmaniasis remains problematic. While differential diagnosis of symptomatic kala-azar from other fever related diseases with splenic or hepatic disorders like malaria, typhoid, tuberculosis etc., is difficult, CL, MCL and PKDL are often confused with leprosy and numerous other primary and secondary skin diseases like psoriasis, vitiligo, ringworm, lupus vulgaris, etc.35,36. Although the serological tests have been evaluated with various degree of sensitivity and specificity, a universal method of field diagnosis to replace the gold standard of kala-azar diagnosis by histological demonstration of parasites from the splenic or bone marrow aspirate is still lacking35,37–39. A recent systematic analysis from our laboratory on the serodiagnostic potential of *L. donovani* promastigote membrane antigen (LAg) has revealed differences in the recognition pattern among VL and PKDL sera of Indian origin40. While the IgG- and IgG1-specific reactivity for both VL and PKDL is 100 per cent, PKDL sera from the patients with varied degree of disease manifestation showed a wide range of anti-leishmanial Ig titres. IgE and IgG4, which were elevated in VL were negligible or absent in PKDL. Moreover, Western blot studies revealed that IgG specific recognition of 67 kDa band of LAg is specific for PKDL while IgG-specific recognition of 31 kDa band is specific for VL40. ELISA, immunoblot, IFAT and IHA are too sophisticated for field use. DAT with different leishmanial antigens and strip test based on the detection of serum-IgG against recombinant K39 (rK39)41 antigen have so far been proved to be the best options for serological field diagnosis. Determination of cut-off titre due to fluctuation of sensitivity and specificity at different serum dilutions, relatively high cost (US$ 4.5 per test), storage of liquid antigen at 4°C, and a lengthy method (18 h) are the constraints for DAT42. Although fast agglutination screening test (FAST), a modified version of DAT performed with freeze-dried antigen, has overcome the problem of antigen storage and assay time (3 h), it is costlier. Strip test using rK39, which is a 39 amino acid repeat within the 230 kDa LcKin protein of kinesin superfamily, can be used to diagnose VL caused by *L. donovani*, *L. chagasi* and *L. infantum*. It has been shown to produce very high sensitivity and specificity among the patients of the Indian subcontinent42–48. However, for African, Latin American and Mediterranean VL patients, the sensitivity and specificity of this test are lower49–53. Appearance of anti-K39 IgG in the healthy individuals of endemic regions and long persistence of these antibodies in the patients after successful
<table>
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<tr>
<th>Reference</th>
<th>Country</th>
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<td>VL 184, Suspected VL 13, NonVL 113.</td>
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<td>VL 68 (Gr1: from Minas Gerais 48, Gr2: from Bahia 20) MCL 13, CL 33, OD 40,</td>
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Ag, antigen; HC, Healthy controls; OD, other diseases; EC, Endemic controls; LAg, *L. donovani* promastigote membrane antigen; DAT, direct agglutination test; Ld, *L. donovani*; Li, *L. infantum*; Lc, *L. chagasi*; s. Ag, soluble antigen; Pro, promastigote; Am, amastigote; ND, not done; IFA, indirect immunofluorescence assay; SLA, soluble *Leishmania* antigen; CL, cutaneous leishmaniasis; MCL, mucocutaneous leishmaniasis. Superscript numerals denote reference numbers.
treatment (up to 2 yr), are other constraints of using this method\textsuperscript{54,55}. Another field diagnostic approach based on latex agglutination test (KAtex), to detect the leishmanial antigens in the urine of the patients has shown very high sensitivity and specificity. Reports of the performances of different diagnostic methods of VL based on the humoral responses in the last seven years are summarized in the Table.

**Cell mediated immune responses in human visceral leishmaniasis**

**Immune suppression and Th1/Th2 paradigm:** Despite the differences between CL and VL, resistance to disease in both the forms of leishmaniasis is marked by a dominant Th1 response. Severe manifestation of CL is associated with a strong Th2 compared to a predominant Th1 response in the mild manifestation of the disease\textsuperscript{69}. On the other hand, it is well documented that VL is characterized by suppression of CMI, which is proved from the unresponsiveness of the patients to the Leishmanin skin test (LST) or Montenegro test. This test measures a DTH reaction to an intradermal injection of leishmanial antigens\textsuperscript{70}. Containment of the disease following a successful treatment is associated with a strong cell mediated DTH response\textsuperscript{71}. The cell mediated immune suppression is also evident from blastogenesis assay of the peripheral blood mononuclear cells (PBMCs) (lymphoproliferation) from untreated VL patients from Brazil, Africa and India\textsuperscript{72-75}. Reduction in proportion of the helper T cells and the immunosuppression\textsuperscript{76} is rapidly reversible with effective chemotherapy. The control and protection of VL, in general, is dependent on the IFN-\(\gamma\) induced innate and adaptive cellular immune responses, which induce the intracellular killing by activated macrophages\textsuperscript{77}. That this immunosuppression due to VL is antigen specific is evident from the positive in vitro response as observed from lymphoproliferation or IFN-\(\gamma\) production from the PBMCs from both the infected and cured patients stimulated with Phytohemagglutinin (PHA) or an unrelated antigen, purified protein derivative of tuberculin (PPD)\textsuperscript{77,74,78}. It has long been strongly suspected that this immunosuppression in the active stage of the disease is regulated by a population of suppressor T cells, different from the conventional CD8+ T cells\textsuperscript{17,79,80}. It was suspected that this suppressor activity could be mediated by a subpopulation of the CD4+ T cells and could be distinguished by the CD45RB\textsuperscript{low} phenotype\textsuperscript{81,82}.

In genetically predisposed murine models of *L. major* infection, a clear Th1/Th2 polarization with distinct patterns of cytokine production by CD4+ T cells, *i.e.*, IFN-\(\gamma\)/IL-2 associated with resistance and IL-4/IL-10 associated with susceptibility was determined\textsuperscript{83}. The existence of such a distinct immunologic spectrum in human VL remains unclear. Different reports suggest that mixed Th1 and Th2 kind of responses are elicited upon *Leishmania* infection in humans\textsuperscript{84-87}. Under this mixed Th1/Th2 milieu, a suppressed Th1 response along with an elevated Th2 is the hallmark of the active disease while protective immunity is achieved by upregulation of Th1 response after a successful chemotherapy\textsuperscript{33,37}. Inability of the PBMCs of the VL patients to produce IL-2 and IFN-\(\gamma\) and their restoration after chemotherapy in response to the leishmanial antigens is also documented elsewhere\textsuperscript{88,89}. A generalized scheme of disease development and associated immune status in human kala-azar infection is shown in the Fig.

It is well known that IFN-\(\gamma\) induces microbicidal activity against both the promastigote and amastigote of *L. donovani* in monocyte derived human macrophages, in H\textsubscript{2}O\textsubscript{2}-dependent\textsuperscript{90,91} as well as in NO mediated pathway\textsuperscript{92}. Therefore, why high parasite burden is favoured in active VL patients can be explained by the deficiency of IFN-\(\gamma\) during disease. Upon infection with *L. donovani*, the monocytes from healthy individuals, pretreated with IFN-\(\gamma\), were shown to produce increased tumour necrosis factor-alpha (TNF-\(\alpha\)) and IL-1, two important elements in the host response to microbial infection. This suggested a potential mechanism by which IFN-\(\gamma\) mediates its effect\textsuperscript{11}. There are reports that show PBMCs isolated from all active VL patients fail to produce IL-1 and TNF-\(\alpha\) equally, upon stimulation with *L. chagasi* antigen lysate\textsuperscript{93,94}. This led to the postulation that only the populations of the parasitized reticuloendothelial cells were responsible for the production of these circulating IL-1 and TNF-\(\alpha\) while these two cytokines were not produced.
by the nonparasitized antigen stimulated PBMCs. Serum level of IL-4 was found to be high in the Brazilian VL patients compared to no or very low level of IFN-γ. However, considerable level of IFN-γ is secreted at the very initial stages of the exposure to the parasites as observed in the seroconverted or subclinically infected individuals in the endemic area. Although the reasons behind...
high levels of IFN-\(\gamma\) secretion at the initial phase of infection and the decline in its production at the active stage of the disease is not clear, this IFN-\(\gamma\) is found to come from the natural killer (NK) cells and might be important as a component of innate immune mechanism for the macrophage activation\(^{96}\). Reports on the administration of IFN-\(\gamma\) in the VL patients for therapy imply the functional importance of this cytokine to establish the immune environment for the clearance of the parasites\(^{97,105}\).

**Importance of IL-10:** IL-10 was initially characterized as a Th2 cytokine but later on it was proved to be a pleiotropic cytokine, secreted from different cell types including the macrophages\(^{106}\). Experimental evidences indicate that IL-10 plays an important regulatory role in the progression of VL. The suppressive ability of IL-10 on the IFN-\(\gamma\) mediated microbicidal activity of macrophages is well established for other diseases\(^{106,107}\). Recombinant IL-10 is shown to have inhibitory effects towards NO mediated killing of *L. infantum*, *L. major* and *L. braziliensis* in human macrophages, derived from the monocytes of healthy individuals\(^{92}\). Investigation on Brazilian patients showed that IL-10 production from *L. chagasi* antigen stimulated PBMC cultures of acute VL was significantly higher than in cured individuals, whereas asymptomatic leishmanin skin test (LST) positive individuals had no release of IL-10\(^{108}\). In active VL patients of Sudan and India, production of high levels of IL-10 mRNA is reported from the bone marrow aspirate, lymph nodes, PBMCs, and splenic aspirates, and the levels decrease after successful chemotherapy\(^{109-111}\). These studies also showed detectable levels of IFN-\(\gamma\) mRNA in the bone marrow, lymph node, PBMC or the splenic aspirate samples from the infected individuals though their specific cellular sources were not determined. This is interesting because, the production of IFN-\(\gamma\) at mRNA level is in contrast with the studies where evidences suggest that there is suppression of the Th1 kind of response, observed from the inability to produce IFN-\(\gamma\) by the PBMCs in active VL. Carvalho et al\(^{78}\) showed that while a combination of exogenous IL-2 and IFN-\(\gamma\) were able to restore only the lymphoproliferative response, a combination of anti-IL-4 and anti IL-10 mAb were able to restore both the lymphoproliferative response and the IFN-\(\gamma\) production of leishmanial antigen stimulated cultures of PBMCs from VL patients to a large extent. These observations clearly indicate that the suppression of the *Leishmania*-specific Th1 kind of response in the patients is the main reason for the disease susceptibility and this suppression is regulated by IL-10\(^{79}\). Decline in the level of IL-10 mRNA after successful chemotherapy therefore supports the fact that persistence of high levels of IL-10 in the host cells is beneficial for the parasite survival and pathology. Besides its possible role in susceptibility and progressive immunopathology in VL infection, high levels of endogenous IL-10 correlate with in the progression of PKDL. Along with PBMCs, dermal keratinocytes of the African PKDL patients who eventually developed PKDL produced significantly higher amounts of IL-10 than the cured VL patients who did not develop PKDL\(^{112}\).

**Importance of IL-12:** IL-12, known as a natural killer (NK) cell stimulating factor, cytotoxic lymphocytes maturation factor, and a central immunoregulator of the initiation and maintenance of the Th1 response, plays an important role in the induction of IFN-\(\gamma\) production by T and NK cells\(^{113-117}\). IL-12 is shown to be a potent inducer of the Th1-type response and protective immunity in murine *L. major* infection\(^{118,119}\). Along with IFN-\(\gamma\), IL-12 was reported to be produced from the PBMCs of cured VL patients but not from the active VL patients following stimulation with *Leishmania* lysate. That IL-12 plays a counter-regulatory effect against IL-10 in *Leishmania* infection, is proved from the observation that addition of recombinant IL-12 or neutralizing anti-IL-10 mAb could restore the IFN-\(\gamma\) production as well as the lymphoproliferative response in the *Leishmania* lysate stimulated PBMCs of active VL patients\(^{120,121}\). Conversely, neutralizing anti-IL-12 or rIL10 inhibits the production of IFN-\(\gamma\)\(^{20}\). As there are strong evidences that IL-10 inhibits IFN-\(\gamma\) production in human PBMCs by suppressing IL-12 synthesis from the accessory cells like macrophages, B cells or dendritic cells\(^{122}\), overproduction of IL-10 from different immune cells is most likely the key driving force to decline the Th1 environment and therefore establishing a Th2 type response in the active human VL. Further studies prove that this counter-regulatory activity of
IL-10 and IL-12 plays a fundamental role in modulating the immune responses of Leishmania infection in human towards Th2 or Th1 probably by modulating the B7/CD28 costimulatory interaction respectively. Recent experimental observations on L. major revealed that these two counteractive functions, i.e., IL-12 and IFN-γ-dependent host-protective immune responses and IL-10-dependent progressive Leishmania infection, are differentially regulated by CD40 signaling. While weak CD40 signals induce extracellular stress-related kinase-1/2 (ERK-1/2)-dependent IL-10 expression, stronger signals induce p38 mitogen-activated protein kinase (p38MAPK)-dependent IL-12 production. Induction of ERK-1/2 inhibits p38MAPK and vice versa. Upon Leishmania infection, CD40 signaling is skewed towards ERK-1/2, therefore inducing IL-10 and inhibiting IL-12 and iNOS-2 expression. Conversely, ERK-1/2 inhibition or IL-10 neutralization restores CD40-induced p38MAPK activation and parasite killing.

Involvement of different cells in immune regulation of human VL: Studies on the involvement of the different immune cells in the maintenance of Th1/Th2 environment through the secretion of different cytokines help to understand the nature of immunomodulation in VL. Most of the T cell clones derived from the PBMCs of the active VL patients and asymptomatic subjects (seropositive and DTH positive) were CD8+ with negligible CD4+, while the situation is reversed in the cured individuals with more numbers of CD4+ than CD8+ cells. CD8+ T cells isolated from the asymptomatic subjects produced high amounts of IFN-γ, which strongly suggests a role of CD8+ cells in human resistance to Leishmania infection. However, a unique population of CD4+ cells producing both IFN-γ and IL-5 is found to contribute in the control of infection in asymptomatic subjects. An earlier study demonstrated higher number of CD4+ clones than CD8+ clones in the asymptomatic DTH positive individuals, although clones isolated from active VL were mostly CD8+. Addition of the CD8+ clones isolated from acute kala-azar patients, augmented IL-10 production from the PBMC cultures of same patients after recovery, suggesting that CD8+ T cells may have a role in mediating IL-10 production during active disease.

Further observations suggested that CD8+ cells are likely to mediate the endogenous IL-10 secretion rather than antigen-specific IL-10, and thus the immunodominance of CD8+ T cells could probably play an important role in progressive VL. An earlier study by the same author postulated that CD8+ suppressor T cell (Ts) population and Th1 CD4+ populations have counter-regulatory effects for susceptibility and resistance, and predicted that the basic immunoregulatory mechanism during infection is the prevention of endogenous IL-10 secretion mediated by CD8+ Ts cells by the activity of leishmanial antigen-specific Th1 CD4+ cells. Symptomatic infections occur when this immune regulatory cycle fails due to antagonistic demands on the immune system by, malnutrition, stress or other causes. Epidemiological and clinical investigations suggested association of undernutrition with the development of clinically apparent VL and that the disease itself has a profound effect on nutritional status. There are evidences that PBMCs from healthy individuals, not exposed to Leishmania, show some natural reactivity to the leishmanial antigens by production of IFN-γ and IL-4 as well as proliferation of the PBMCs. This might be caused as a consequence of cross-activation by other micro-organisms or some evolutionarily conserved crossreacting antigens like heat shock proteins (HSP). As mentioned earlier, Th1/Th2 dichotomy is much debated in human VL mostly at the cured stage. Flowcytometric investigation for the specific T cells producing these cytokines in the culture of PBMCs of the cured VL patients revealed that these cytokines were produced mainly from the CD4+ cells, while a very low percentage of CD8+ cells were also involved in cytokine production. Examination of the co-expression of the cytokines revealed three types of cytokine producing cells viz. (i) IFN-γ producers, (ii) IL-4 producers, and (iii) IFN-γ and IL-10 producers; IFN-γ and IL-4 were never co-expressed. This study demonstrated that besides conventional Th1 and Th2 subsets there is existence of a T cell population producing both IFN-γ and IL-10, which could be functionally important as the regulatory subset that allows a balance between Th1 and Th2 cells in the cured VL patients. Comparative flowcytometric analysis on the PBMCs of healthy individuals and CL patients infected with L. aethiopica, in response to
*L. aethiopica* antigen reveal that the CD16+/CD56+ NK cells in the healthy individuals are the main responder cells rather than the T cells in the CL patients while the few T cells which responded in the healthy individuals were identified to be CD8+. Elevated levels of IFN-γ were produced in similar cultures of PBMCs from healthy individuals. Further studies show that live *Leishmania* promastigotes are able to stimulate human NK cells to secrete IFN-γ as an early response. This stimulation is contributed largely by the *Leishmania* homologue of receptors for activated C-kinase (LACK).

**Involvement of other cytokines in immuneregulation of human VL:** IL-4 is considered to be the signature cytokine of Th-2 response. Although known to correlate with disease in murine CL but not murine VL, IL4 is reported to be present in the sera, PBMC supernatant or as mRNA in human VL. But there are reports that IL-4 is not always produced in VL patients and that it has no immunomodulatory effect in downregulating the Th1 response during disease. However, these inconsistent detections of IL-4 in VL patients might be due to the fact that there are disease specific soluble IL-4 receptors in the serum of VL patients, which can neutralize both the bioactivity and immunologic detection of this cytokine. Other Th-2 cytokines like IL-13 and transforming growth factor-beta (TGF-β) have been reported to be produced in VL though their biological role in modulating the *Leishmania* specific immune responses is not well defined. Studies on regulatory T cells (CD4+ CD25+), which function through TGF-β and IL-10 production could help to understand the role of TGF-β. However, it is evident that parasite survival is favoured by the conversion of latent TGF-β of the host to active TGF-β by some parasite-derived factors, which help to create its immediate microenvironment to its own survival advantage.

**Implications of immunogenecity of different leishmanial antigens towards human cells**

The development of vaccines is the essential aim of studies on leishmaniasis. In the endemic areas of visceral leishmaniasis, a significant population of individuals does not manifest any clinical symptoms of the disease, but show elevated antileishmanial antibodies and/or a T cell response to leishmanial antigens. Moreover, patients recovered from kala-azar are usually immune to re-infection. These observations provide the idea that these individuals have somehow gathered life long protection from VL, possibly through the appropriate stimulation of protective host response by the *Leishmania* antigens. As discussed previously, it is known that the immunosuppression observed in VL is antigen-specific. The in vitro stimulation of immune cells from active or successfully treated VL patients by different leishmanial antigen preparations or purified antigen fractions provides idea about the potential immunogenicity of those antigens as possible vaccine candidates. While there is still no effective form of immunoprophylaxis against this disease, extensive investigations in this field include human vaccine trials with killed promastigotes and immunization of mice with attenuated, killed, and crude parasite fractions, as well as purified and recombinant antigens and their DNA. The impressive recent advances in this area may soon result in the development of a safe and effective vaccine. Search for the effective vaccine candidates basically involves screening of the different immunodominant antigens, studies on the inherent mechanisms of host immune responses and evaluation of selected antigens as vaccine candidates either in native or recombinant forms.

To identify some potential vaccine candidate antigens, screening of immunogenicity of *L. donovani* promastigote antigen fractions, isolated by electroelution, revealed that different antigen fractions of 14-80 kDa were almost equally potent to stimulate the PBMCs of the Kenyan cured VL patients to proliferate and produce IFN-γ. A more recent study from our laboratory has revealed that seven polypeptides of *L. donovani* promastigote membrane antigen preparation (LAg) of approximate MW 31, 34, 51, 63, 72, 91, and 120 kDa were recognized by 100 per cent kala-azar patient sera. The promising potentiality of these antigens as vaccine candidates against VL is under investigation. LPG (lipophosphoglycan) and glycoprotein (gp63), which are involved in the attachment of the parasite to the host cells, are two abundant molecules on the surface of all species of *Leishmania*. Observations on CL
patients caused by *L. braziliensis* and *L. mexicana* showed that LPG is a potent stimulator for lymphoproliferation of the PBMCs of these patients while gp63 is unable to stimulate similar cultures\(^{156,157}\). Supportive results were found from the studies on Kenyan VL patients where LPG isolated from *L. major* were able to specifically proliferate the T cells of the VL patients\(^{158}\). On the other hand, gp63 could not even stimulate the PBMCs of cured VL or healthy individuals while crude *L. donovani* sonicate could stimulate both. This study demonstrated that the clonal expansion of LPG-specific T cells might contribute to the defense against infection\(^{158}\). However, information regarding the immunogenicity of gp63 is conflicting, since cells from spontaneously healed Sudanese CL patients were reported to proliferate in response to purified native gp63 from *L. major*\(^{141}\). Moreover, gp63 stimulated T cell clones isolated from the cured CL patients, infected with *L. major*, were found to be of Th1 type and culture supernatants of these cells inhibited the *L. major* infection in human macrophages\(^{159}\).

Analysis of the host immune responses towards the parasite antigens is important for vaccine strategies. Evidences from the studies of CL caused by *L. aethiopica* suggested that there are differences in responsiveness towards *L. aethiopica* antigens between T cells from local cutaneous leishmaniasis (LCL) and diffused cutaneous leishmaniasis (DCL) patients. While the progressive DCL is characterized by nonresponsiveness, LCL, which is self-healing, shows antigen specific cellular immune function\(^{133}\). In case of VL, though mixed Th1/Th2 is characteristic of cure, an essential Th1 bias at this stage has to be achieved by an effective vaccine. This could be confirmed from the observations that nonspecific antigens like PPD presents a positive stimulation in the PBMCs of cured VL patients while tetanous toxoid (TT) could not stimulate the similar cultures\(^{87,160,161}\). It is known that PPD generally induces Th1-like and TT induces Th2-like responses in human\(^{162}\). A recent study on human trial of vaccination with alum-precipitated autoclaved *L. major* (Alum/ALM) ± bacille Calmette-Guerin (BCG), has postulated that cellular immune response to human leishmaniasis is dichotomous\(^{163}\). An early production of IFN-γ precedes over a positive LST and it declines with time while LST positivity persists. These indicate that IFN-γ production and elevated LST, two markers of protective Th1 response, probably measure two different facets of cellular immunity. One is mediated by IFN-γ production through less differentiated T cells or NK cells, while the other is mediated by more specialized and differentiated T cells capable of induction of a varied inflammatory reaction through chemotactic mediators.

There are several reports in the literature, which focus on the immunogenic potential of different synthetic or recombinant peptides. Among these, kinetoplast membrane protein-11 (KMP-11) of molecular mass 11 kDa, is physically associated with LPG (therefore previously known as LPGAP) in *L. donovani*. It was found to stimulate the production of both Th1 and Th2 type of T cell clones from the cured African VL patients with vigorous lymphoproliferation\(^{160}\). Three 38-mer synthetic peptides KMP-11-1 (a.a. 1-38), KMP-11-2 (a.a. 28-65) and KMP-11-3 (a.a. 55-92) had been shown to be potent immunogen with variable antigenicity for B and T cells as observed through humoral and cell mediated immune response during disease. However, these synthetic peptides presented weaker proliferative responses than the native KMP-11, which might be the result of either different processing and presentation of native protein and peptide by APCs, or the lack of association with the glycolipid LPG, thereby losing a natural adjuvant\(^{164}\). Promastigote surface antigen 2 (PSA-2), which is basically a complex of three polypeptides of molecular mass 96, 80 and 50 kDa and tethered to the promastigote membrane with glycoinositol phospholipid anchors\(^{165}\) was tested for its immunogenic properties on the T cells isolated from the cured Sudanese CL patients. While responses towards native PSA-2 isolated from *L. major* as well as *L. donovani* was a clear Th1 type as compared to the parallel TT stimulated cultures, there was a lack of response to the recombinant *Escherichia coli* derived PSA-2. This might be due to importance of glycosylation in defining the T cell epitopes of the antigen which probably determine the glycosylated antigens as better vaccine candidates for leishmaniasis in human\(^{161}\). In contrast to crude parasite lysate, which induced a mixed Th1/Th2 response, recombinant LeIF was shown to induce a dominant Th1 type of immune response, which is largely IL-12 mediated, in the PBMCs of CL patients infected with...
L. braziliensis. This is important in relation to the observation that crude L. amazonensis lysate was able to give rise to Leishmania-specific T cell lines with potent Th1 response from the PBMCs of healthy individuals in presence of IL-12. Moreover, these cell lines contained high percentage of CD8+ populations, which could lyse autologous Leishmania-infected but not uninfected macrophages. Other recombinant leishmanial antigens rHSP-70, Leishmania type 2C serine/threonine protein phosphatase (rLcPP2C) and rgp63 were found to induce Th1 type of response in asymptomatic and cured VL infected with L. chagasi, with elevated IFN-γ production and very low IL-10. Lymphoproliferation in response to all these three antigens were comparatively low than the crude LAg. Interestingly, response in the PBMCs of the healthy controls in this study is contrasting to those as reported earlier. Recombinant papLe22, a 22 kDa potentially disease-aggravating protein of L. infantum has been found to induce the production of IL-10 in the PBMCs of active VL patients. Leishmania homologue of receptors of activated protein kinase C (LACK), a 36kDa protein, is known to induce an early induction of IL-4 secreting cells in L. major susceptible BALB/c mice and drive the immune response towards Th2 phenotype. LACK was found to stimulate the proliferative response of CD8+ and NK cells in healthy individuals as well as active CL patients. Both IFN-γ and IL-10 were secreted in the supernatants of the LACK-induced PBMC cultures of these individuals. Therefore, the vaccine strategies with this antigen in human CL might be through directing the LACK response away from Th2 towards Th1. However, the absence of any response towards LACK in cured VL patients suggests that LACK might not be the right candidate for vaccination against VL. Further studies on phenotyping of LACK stimulated PBMCs of healthy individuals revealed that stimulation with recombinant LACK induced production of IFN-γ from CD8+ naïve (CD45RA+) cells and IL-10 from the CD4+ memory (CD45RA-, CD45RO+) cells.

Immune responses in kala azar patients with HIV co-infection

Among all types of leishmaniasis, VL is the most frequent potential opportunistic disease associated with HIV-1 since the mid 1980s. Although ninety per cent of the reported cases are from southwestern Europe, incidences of co-infection are increasing in eastern Africa and the Indian subcontinent. VL is found to promote the clinical progression of AIDS and it was suggested that Leishmania parasites could be seen as potential co-factor in HIV-1 pathogenesis. Studies on latently HIV-infected U1 moncytoid cell line, treated with live L. donovani or LPG, showed very significant elevation of reverse transcriptase activity as well as production of TNF-α, a potent inducer of HIV-1 expression, in the culture supernatants. L. donovani-induced immunosuppression is also enhanced by HIV-1. When PBMCs from healthy individuals are costimulated with both the antigens, addition of HIV antigens did suppress L. donovani-induced proliferation in a dose-dependent fashion compared to only L. donovani antigen stimulation. On the other hand, increased production of IL-6 and TNF-α, potent inducers for HIV-1, was found to be associated with L. donovani-induced viral replication. Further, L. donovani promastigotes and LPG were proved to mediate CD4+ T cell activation induced HIV-1 replication. Leishmanial antigen-induced TNF-α produced in the culture was important for the replication. The mutual interdependence as the agents for co-infection between these two pathogens is also reported. Co-infection in THP-1 macrophage cell line increases the multiplication of L. donovani amastigotes in the macrophages. Again, killed HIV preparation was shown to abrogate the proliferative response as well as IFN-γ production from the L. donovani antigen induced PBMCs of healthy individuals. Moreover, anti-IL-10 could not enhance IFN-γ production in HIV-VL co-infected patients as is generally found in only VL patients.

Role of regulatory T cells

Evidences from experimental murine models of L. major infection suggest that endogenous CD4+ CD25+ T cells (Treg) play an important role in the infectivity of this parasite. These cells are unique in expressing CD25 molecule, which is the IL-2 receptor α-chain, on their surface and characterized by their ability to control the excessive or misdirected immune response to microbial or self-antigens.
Constituting 5-10 per cent of the peripheral T cell populations in both human and mice at the steady state, CD4+CD25+ cells execute their suppressive or regulatory function either by cell contact dependent manner or by secretion of regulatory cytokines IL-10 and TGF-β\textsuperscript{185,186}. Activation of these cells is also triggered to some extent by the stimulation of toll-like receptor-4 (TLR-4) with its ligand\textsuperscript{187}. Production of high levels of IL-10 and TGF-β in the patients with acute infection of *Leishmania* and the resultant immunosuppression in them led to the investigation of the function of these cells in leishmaniasis and is in fact shown to accumulate in huge number in the cutaneous lesions of mice infected with *L. major* and therefore suppress the CD4+ CD25- effector cell functions to eliminate parasites\textsuperscript{188}. Another report tends to impart that CD4+ CD25+ cells in murine infection functions in exclusively IL-10 independent manner rendering their suppressive effect both on Th1 and Th2 cells and thereby playing important role in suppressing disease development of *L. major* infection in SCID mice reconstituted with naïve CD4+ CD25- cells. This study also postulates that Th2 cells are more susceptible to the inhibitory effect of CD4+ CD25+ cells than the Th1 cells\textsuperscript{189}. These informations strongly indicate that CD4+ CD25+ T cells may have similar immunomodulatory role in human VL, which needs to be investigated.

**Immune response in PKDL**

As a sequel to kala-azar, PKDL, first described by Brahmachari\textsuperscript{190} appears in a dermatotropic form of *L. donovani* infection in >50 per cent patients in Sudan and 10-20 per cent patients in India. The immunopathogenesis of the disease is still ill understood. Although patients of PKDL often bear the different forms of PKDL lesions for years and hence remain as reservoir for *L. donovani*\textsuperscript{191,192}, there are limited attempts to reveal the underlying immune mechanisms for the appearance of the disease\textsuperscript{192}. Studies, which have so far been made, indicate that unlike VL there is positive cell mediated immune response against leishmanial antigen in PKDL in terms of DTH (LST), lymphoproliferation and production of IFN-γ by the PBMCs\textsuperscript{74,193,195}. PKDL patients manifest an aggravation of the disease with time in form of nodular lesions or erythematous plaque formation. Again, PKDL patients with early infection show better CMI than the chronic patients. These indicate that there are different domains of PKDL patients according to the immunopathogenesis of the disease. Though there is generalized prominence of Th1 in PKDL, as found from IFN-γ production, high levels of IL-10 in the lesions as well as plasma of the patients\textsuperscript{112} indicate the importance of Th2 response in chronic PKDL. Preponderance of the CD8+ cells over CD4+ cells in PKDL lesions of Indian patients\textsuperscript{196,197} is suggestive of persistence of parasites as CD8+ cells might block the IL-2 production locally and in turn inhibit the IFN-γ production\textsuperscript{198} favouring disease.

**Conclusion**

Till date, the studies of the immunology on human infection of *Leishmania* emphasize the regulatory role of the cell-mediated immune response. Although anti-leishmanial antibodies are abundant in the sera of the patients during disease, a correlation between the elevation of Th1/Th2 response and the stimulation of these antibodies could not be established. As there is tremendous ecological and genetic diversity among the different human populations exposed to the parasite, conclusive understanding of the parameters of resistance versus control in human is difficult. Moreover, the immunological data available are still scarce. There is also an urgent need for a better experimental model mimicking human infections. It is evident that there is a marked occurrence of both Th1 and Th2 components of CMI response during VL as documented through the detection of serum and tissue cytokines. Recent reports suggest the involvement of immune cells other than the Th1 and Th2 subsets of CD4+ T cells. Among these, CD8+ cells macrophages and NK cells play major roles. In addition, recent experimental data obtained with studies on CD4+ CD25+ Treg cells point to a probable regulatory function of these cells in maintaining the immune homeostasis in human leishmaniasis. In contrast to the earlier ideas that antagonistic functions of IFN-γ and IL-4 determine the outcome of protection or pathogenesis of the disease, recent studies emphasize the importance of the balance of the two regulatory cytokines IL-12 and IL-10, critical for the regulation of the immune modulation during infection,
pathogenesis, and chemotherapy. To understand the nature of human infection with these parasites and to develop better chemotherapeutic and vaccine strategies, further in depth studies focused on the immune modulation in the subclinical and asymptomatic individuals needs attention. Immune status of the patients demonstrating antimony resistance and those suffering from relapse of infection should also be a major area of investigation as these patients might have developed some impairment towards protective immunity. Moreover, immunopathogenesis in patients with PKDL, so far a neglected field of research, deserves attention. As the patients with PKDL come up with varied degrees of disease manifestation and there are also considerable differences in the PKDL patients of Indian and African origin, systematic analysis of immune responses of this sole human reservoir of the disease will definitely help to clear our vision of leishmaniasis as a whole.

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