Immune response to *Leishmania* infection

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*Leishmania* is a protozoan parasite and the causative agent of the disease leishmaniasis. Anti-leishmanial immune response is shown to be host genotype dependent so that some inbred strains of mouse are susceptible while others are resistant. The resistance is conferred by T-helper type-1 (Th1) cells while the susceptibility is conferred by Th2 cells. Th1 cells secrete IL-2 and IFN-γ but Th2 cells secrete IL-4, IL-5 and IL-10. It has been shown that IFN-γ activates macrophages to express iNOS2, the enzyme catalyzing the formation of nitric oxide. Nitric oxide kills the intracellular amastigotes. In contrast, Th2 immune response limits the action of Th1 functions via IL-10 and IL-4, which deactivate macrophages helping intracellular parasite growth and disease progression. Being a parasite, *Leishmania* ensures its own survival by modulating host immune system either by inducing immunosuppression or by promoting pro-parasitic host functions. A detailed knowledge of this host-parasite interaction would help in designing prophylactic and therapeutic strategies against this infection.

**Key words** Animal models - antigen presenting cells - dendritic cells - host-parasite interaction - immuno-modulation - leishmaniasis - T cell response

**Brief history**

William Leishman and Charles Donovan first demonstrated the protozoan parasite in the spleen of patients suffering from a malaria-like illness, which became known as visceral leishmaniasis (VL), separately but simultaneously in 1903. The causative agent of VL was named as *Leishmania donovani* after its co-discoverers.

Human leishmaniasis is distributed worldwide, but mainly in the tropics and subtropics, with a prevalence of 12 million cases and an approximate incidence of 0.5 million cases of VL and 1.5 million cases of cutaneous leishmaniasis (CL) (http://www.who.int/tdr/disease/leish/diseaseinfo.htm).

*Leishmania* life cycle and metacyclogenesis

The sand fly vector of genus *Phlebotomus* (old world) or *Lutzomyia* (new world) becomes infected when feeding on the blood of an infected individual or an animal reservoir (Fig.1). The *Leishmania* parasites live in the macrophages as round, non-motile amastigotes (3-7 µm in diameter). The fly ingests the macrophages during the blood meal and the amastigotes are released into the stomach of insect1. Almost immediately the amastigotes transform into the motile, elongated (10-20 µm), flagellate promastigote form. The promastigotes then migrate to the alimentary tract of the fly, where they live extracellularly and multiply by binary fission2. Sand fly saliva selectively inhibits parasite killing by macrophages and nitric oxide production3. The major surface glycoconjugate lipophosphoglycan (LPG) constitutes a dense glyocalyx that covers the entire surface of the parasite including the flagellum. Immature organisms, termed procycls, express shorter LPG molecules but mature metacyclics bear the capping at the terminal β-galactose residues with α-arabinose and elongation by increasing the numbers of repeating disacharides unit by two to three folds. This mature metacylic form of the
organism is released from the midgut and migrates to the proboscis. Whereas procyclic organisms from log phase cultures are extremely sensitive to complement-mediated lysis through the alternative pathway, metacyclic organism activates the classical pathway but are not lysed. When the sand fly next feeds on a mammalian host, it transfers the metacyclic *Leishmania* promastigotes to the host along with the saliva. Once in the host, the promastigotes are taken up by the macrophages where they rapidly revert to the amastigote form, survive and multiply inside the macrophages, eventually leading to the lysis of the macrophages. The released amastigotes are taken up by additional macrophages and so the cycle continues. Ultimately all the organs containing macrophages and phagocytes are infected, especially the spleen, liver and bone marrow.

**Leishmaniasis in human and their types**

The leishmaniasis has been classified into different classes on the basis of the basic syndromes of the disease. Different species of *Leishmania* appear identical and are generally distinguished by clinical and geographic characteristics. Modern speciation by isozyme pattern, monoclonal antibodies, DNA hybridization, DNA restriction endonuclease fragment analysis, and chromosomal karyotyping is continuing to delineate new species, particularly in the new world, and to demonstrate the capacity of different species to cause similar clinical syndromes. There are four major syndromes-visceral leishmaniasis (kala azar), cutaneous leishmaniasis, monocutaneous leishmaniasis (espundia) and diffuse cutaneous leishmaniasis (Table I).

**Visceral leishmaniasis** (kala azar): *L. donovani* causes kala azar, a disease that may be endemic, epidemic or may be sporadic. African kala azar is found in the eastern half of Africa. Indian kala azar has an age and sex distribution similar to African kala azar. The manifestations appear generally in 3 months. Fever, typically nocturnal and occasionally double-quodid, is almost universal and is accompanied by tachycardia without sign of toxaemia. Diarrhoea and cough are frequent. Non-tender splenomegaly becomes dramatic by the third month. The liver enlarges conspicuously. Hypoalbuminaemia and polyclonal-hypergamma-globulinaemia (IgG and IgM) are constant features. Circulating immune complexes are frequently present. Immune-complex glomerulonephritis and interstitial nephritis have been described. Edema cachexia, and hyperpigmentation (kala azar means “black fever”) are late manifestations. After successful treatment, 3 to 10 per cent of cases develop post kala azar dermal leishmaniasis (PKDL) wart like nodules over the face and extensor surface of the limbs. In the Indian disease, PKDL appears after a latent period of 1 to 2 yr and may last for years.

**Cutaneous and mucocutaneous leishmaniasis**: This form of leishmaniasis is caused by a number of species in both the old and the new world. The disease is characterized by single or multiple localized lesions on exposed areas of skin that typically ulcerate. *L. tropica* and *L. major* cause old world whereas *L. mexicana* and *L. brasiliensis* cause new world cutaneous leishmaniasis. The incubation period ranges from 2 to 6 wk. The initial lesions are often multiple and located to lower extremities. Regional lymphadenopathy and satellite lesions are common. Mucocutaneous leishmaniasis and/or espundia, is caused primarily by *L. brasiliensis* which typically produces several lesions.
on the lower extremities that undergo extensive ulcerations. After months to year, metastatic lesions appear in the nasopharynx. Nasal obstruction and epistaxis are frequent presenting symptoms. Animal model of *Leishmania*-resistant and susceptible host

Using human for the characterization of a new drug and to understand its immunological aspect is unethical. Development of new chemotherapy for leishmaniasis and to understand the drug-immune interface, animal model is the best choice. Animals remain the best model for the characterization of the disease and its impact on to the host. The main prerequisite for choosing any animal model is that it should have the matching physiology with human and at the same time availability of the animal and its handling should not be difficult. Hamster and mouse are the two well-studied and suitable models for studying the infection and chemotherapy. But still monkey model is used for the vaccine trial. *Leishmania* infections vary markedly between individual to individual whereas an animal model may always present a uniform disease profile. Infection in BALB/c mouse, a susceptible host, is a well-studied model but this model is not suitable for trial of any chemotherapeutic purpose because the effective dose is much higher to cure *Leishmania* infection in this model compared to human. Although VL and CL can be studied in animal model, there is no animal model available for studying the PKDL (post kala azar dermal leishmaniasis).

**Animal model of *Leishmania*-resistant and susceptible host**

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**Table I.** Old world and new world *Leishmania* species and geographical distribution

<table>
<thead>
<tr>
<th>Species</th>
<th>Geographic distribution</th>
<th>Reservoir</th>
<th>Clinical syndrome</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. chagasi</em> (New world)</td>
<td>Mexico, Surinam, Brazil, Paraguay, Argentina, Venezuela, Brazil, Bolivia</td>
<td>Canine</td>
<td>VL, CL, PKDL</td>
</tr>
<tr>
<td><em>L. amazonensis</em> (New World)</td>
<td>Brazil, Costa Rica, Texas, Guyana Peru, Bolivia, Venezuela</td>
<td>Rodents, Marsupial</td>
<td>CL, VL, PKDL, MCL, DCL</td>
</tr>
<tr>
<td><em>L. major-like Isolates</em> (New world)</td>
<td>Colombia, Panama, Venezuela</td>
<td>Canines?</td>
<td>CL, DCL</td>
</tr>
<tr>
<td><em>L. mexicana</em> (New world)</td>
<td>Mexico, Guatemala, Texas, Costarica, Panama</td>
<td>Forest Rodent</td>
<td>CL, DCL</td>
</tr>
<tr>
<td><em>L. major</em> (Old world)</td>
<td>Middle East, Indian Subcontinent, northwestern China, Africa</td>
<td>Humans, rodents, mustelids, hedgehogs, rabbits</td>
<td>CL</td>
</tr>
<tr>
<td><em>L. tropica</em> (Old world)</td>
<td>Middle East, India, Mediterranean littoral, western Asiatic areas</td>
<td>Canids and perhaps some rodent</td>
<td>Dry cutaneous lesions</td>
</tr>
<tr>
<td><em>L. donovani</em> (Old world)</td>
<td>Africa, India, East Asia</td>
<td>primates, equids, rodents</td>
<td>VL, PKDL</td>
</tr>
</tbody>
</table>

VL, Visceral leishmaniasis; CL, cutaneous leishmaniasis; PKDL, post kala azar dermal leishmaniasis; MCL, mucocutaneous leishmaniasis; DCL, diffuse cutaneous leishmaniasis
observation extends to the murine *L. major* model where the strain of inbred mouse determines the outcome of infection. C57BL/6 mice being uniformly resistant and BALB/c consistently susceptible\(^\text{10}\). It is well documented that Th1 immune response is the key event to prevent *Leishmania* infection. Activated Th1 cells induce IFN-γ that in turns activates the macrophages and kill the parasites. C57BL/6 mice mount early Th1 immune response and prevent the further growth of the parasite causes self-healing phenotype\(^\text{11,12}\) whereas susceptible BALB/c strain mounts early Th2 response and results in non healing lesion and exaggeration of disease\(^\text{11,13,14}\). Respective resistance and susceptibility of C57BL/6 and BALB/c strains depend not only on the Th1 and Th2 type of immune response of CD4⁺ T cells but also on the genetic background of the host. Initially it was shown that resistance or susceptibility of the recombinant strains of mouse was dictated by the haplotype of the host\(^\text{15}\). Congenic mouse of a particular haplotype with either susceptible or resistant background could not correlate the susceptibility or resistance with the haplotype of the strain for the *Leishmania* parasites (Table II). It suggests that susceptibility or resistance of the host may be partly regulated by the haplotype with some other factors. Factors for susceptibility or resistance could be segregated by repetitive backcrossing of resistant B10.D2 and susceptible BALB/c strains. Loci on chromosomes 6, 7, 10, 11, 15, and 16 were associated with resistance, demonstrating the multigenic nature of this phenotype\(^\text{16}\). Moreover, F1 progeny of BALB/c and C57BL/6 mice were shown to intermediary phenotypes for *Leishmania* infection suggested the contribution of genes either in susceptibility or resistance of the host. Bone marrow macrophages derived under influence of granulocytes macrophage-colony stimulating factor (GM-CSF) or IL-3 or monocytes-colony stimulating factor (M-CSF) further increase the respective resistance and susceptibility of these macrophages to *Leishmania* infection\(^\text{17}\). These observations suggest the critical role of myeloid cells in the resistance or susceptibility to *Leishmania*. Resistance or susceptibility of myeloid cells to *Leishmania* needs to be characterized further.

**Hamster and Guinea pig:** While the mice are either intrinsically resistant or susceptible to *Leishmania* infection and offer a well-characterized genetic make-up, chiefly by the use of inbred, recombinant and naturally or experimentally mutated strains, hamsters provide an excellent model for an overtly susceptible host. Therefore, hamsters are used for histopathological studies, drug efficiency studies and vaccine studies despite the lack fine immunochemicals that limit the mechanistic exploration of immune responses to *Leishmania* infection\(^\text{18}\). Guinea pigs have been a traditional model for studies of delayed type hypersensitivity. They are the natural host of *L. enriettii* and have been experimentally infected with other species of *Leishmania*\(^\text{19}\). They have been used as a skin-test model to screen potential antigens for use in diagnostic tests for *Leishmania*\(^\text{19}\).

**Host parasite interaction and antigen presentation**

*Leishmaniasis* is an excellent example of a complex parasite-host interaction. *Leishmania* promastigotes bind to some of the surface molecules like complement receptor 1 and 3 (CR1&3) and C3b of macrophage before they are internalized. CR1 constitutes the major macrophage ligand for mature promastigotes\(^\text{20}\), though additional parasite surface glycoprotein (e.g., gp63 membrane protease) and other macrophage receptors (e.g., CR3, mannose fucose receptor) have been

<table>
<thead>
<tr>
<th>Strain</th>
<th>Haplotype</th>
<th>CD4⁺ T cell response</th>
<th>Susceptibility</th>
<th>Disease profile</th>
</tr>
</thead>
<tbody>
<tr>
<td>C57BL/6</td>
<td>H2b</td>
<td>Th1 response</td>
<td>No</td>
<td>Self curing</td>
</tr>
<tr>
<td>BALB/c</td>
<td>H2a</td>
<td>Th2 response</td>
<td>Yes</td>
<td>Non-healing</td>
</tr>
<tr>
<td>BALB/b</td>
<td>H2b</td>
<td>Th2 response</td>
<td>Yes</td>
<td>Non-healing</td>
</tr>
<tr>
<td>B10.D2</td>
<td>H2a</td>
<td>Th1 response</td>
<td>No</td>
<td>Self curing</td>
</tr>
<tr>
<td>C3H/HeN</td>
<td>H2b</td>
<td>Th1 response</td>
<td>No</td>
<td>Self curing</td>
</tr>
<tr>
<td>C3H/HeJ</td>
<td>H2a</td>
<td>Th1 response</td>
<td>No</td>
<td>Self curing</td>
</tr>
<tr>
<td>DBA/2</td>
<td>H2b</td>
<td>Th1 response</td>
<td>Intermediate</td>
<td>Self curing</td>
</tr>
</tbody>
</table>
implicated in various studies\textsuperscript{21,22}. Once internalized, promastigotes transform into intracellular amastigotes. Amastigotes replicate by binary fission, eventually rupturing the macrophage and spreading to uninfected cells. The internalization pathway of amastigotes to the macrophages is poorly defined. The identification of natural antibodies coating amastigotes \textit{in vivo} suggested that macrophage FcIg and CR3 receptor might contribute to phagocytosis\textsuperscript{22}. \textit{Leishmania} promastigotes are covered with a dense surface glycocalyx, composed largely of molecules attached by glycosylphosphatidylinositol (GPI) anchor\textsuperscript{23}. These GPI anchored molecules include proteins such as the parasite surface protease gp63 and proteophosphogycans (PPGs). The most abundant constituent is a large GPI-anchored phosphoglycan called lipophosphoglycan (LPG)\textsuperscript{24,25}. LPG and gp63 account for the virulence of the parasite. LPG has been implicated in many steps required for the establishment of macrophage infection and for the survival in insect vector\textsuperscript{26,27}. LPG does not play a role in the amastigotes stage, however, amastigotes continue to make structurally related glycoconjugates\textsuperscript{28,29}. On the other hand, gp63 also helps the parasite to enter in the host cells and for its survival. As an endoproteinase with a broad substrate spectrum, gp63 has the potential to degrade immunoglobulins, complement factors, and lysosomal proteins\textsuperscript{30}. Its proteolytic activity at pH 4 bears apparent relevance to the survival of amastigotes in the acidic environment of macrophage phagolysosomes\textsuperscript{31}. Further molecular basis of this mechanism remains to be deciphered.

Various markers have been used to study the intracellular compartment in which amastigotes replicate in macrophages. For example, a proton ATPase\textsuperscript{34} and LAMP-1. With time, the vacuole matures to a late endosomal compartment. Association of MHC class II molecules to parasitophorous vacuole\textsuperscript{35,36} suggesting a mechanism by which the immune response to this intracellular organism becomes class II and CD4-dependent.

Recognition of antigenic peptides of the parasite through the class II pathway was revealed by experiments using mice without MHC II or $\beta_2$-microglobulin genes that are deficient in MHC class II/CD4 cells or MHC class I/CD8 cells, respectively. MHC class II-deficient mice suffered fatal, uncontrolled infection\textsuperscript{37} whereas MHC class I-deficient mice controlled infection with \textit{L. major} in a manner similar to normal littermates\textsuperscript{38}, suggesting that CD4\textsuperscript{+} but not CD8\textsuperscript{+} T cells are required for controlling \textit{L. major} infection.

After internalization of organisms into phagosomes, secondary lysosomes are fused to form the complete parasitophorous vacuole. Metacyclic forms rapidly transform into intracellular amastigotes. This transformation takes the shedding of the promastigote LPG that migrated to the surface of the infected macrophages. LPG inhibited the respiratory burst, a natural process that occurs after phagocytosis, and the hydrolytic activity of the lysosomal enzymes, possibly through chelation of calcium and inhibition of protein kinase C\textsuperscript{33}.

**Fig. 2.** A model for the attachment of \textit{Leishmania} to macrophages: \textit{Leishmania} promastigotes bind to some of the surface molecules like complement receptor 1 and 3 (CR1&3) and C3b of macrophage before they are internalized. CR1 constitutes the major macrophage ligand for mature promastigotes. \textit{Leishmania} primarily binds to macrophages via surface glycolipid. The secondary binding of \textit{Leishmania} involves gp63 and C3-derivatives of serum which complex together and bind CR3 on the macrophages. Another possible binding of \textit{Leishmania} is perhaps possible via macrophage’s Toll-like receptors (TLRs).
Role of polymorphonuclear neutrophils granulocytes and chemokines in leishmaniasis

Neutrophils or polymorphonuclear neutrophils (PMNs), the first cells to migrate to the site of infection or injured tissue, function as a primary effector or phagocytic cells, phagocytosing Leishmania. Once ingested, foreign particles are destroyed by proteolytic enzymes stored in the special granules and by production of reactive oxygen species. Leishmania phagocytosed neutrophils start secreting the chemokines like IL-8, essential to bring the more neutrophils at the site of infection. Two to three days later, the second wave of cells, monocytes/macrophages, enters the site of infection. After infection with L. major, the chemokines MIP-2 and KC (the functional murine homologues of IL-8) are very rapidly produced in the skin. As a mechanism of parasitism, it is beneficial to Leishmania to escape from other cells of the immune system, for example, the leukocytes before establishing the infection completely and neutrophils were found to be beneficial for parasite survival in the infected tissue. It has been shown that Leishmania promastigotes can induce the migration of human PMNs by releasing a factor (Leishmania chemotactic factor, LCF) with potent chemotactic activity on neutrophils but not on other leukocytes, such as monocytes or natural killer (NK) cells. It was shown that co-incubation of Leishmania with PMNs inhibits the CXC-chemokine interferon gamma (IFN-γ)-inducible protein-10 (IP-10) suggesting that Leishmania inhibited the Th1 or NK cell activity. PMNs are intrinsically short-lived cells with half-life of approximately 6-10 h in the circulation, after which they go under spontaneous apoptosis. The life of mature neutrophils can be extended in vitro by incubation with either proinflammatory cytokines including GM-CSF and G-CSF, IL-8, IL-1β, glucocorticoids and bacterial products, such as LPS. It has been shown that Leishmania also extends the life span of neutrophils granulocytes, a mechanism that involves the inhibition of caspase-3, a known apoptosis inducing caspase in short lived PMNs. Leishmania delays but does not prevent the spontaneous apoptosis of PMNs. The infected cells become apoptotic after 2-3 days. The infected PMNs recruit monocytes/macrophages by inducing the chemokines MIP-1α and MIP-1β. These macrophages ingest the infected PMNs by identifying phosphatidyl serine on the surface of apoptotic cells. Ingestion of apoptotic cell does not activate microbicidal function of macrophages and would be an ideal way of silent entry of Leishmania to its main host cells, macrophages. Before going to apoptosis neutrophils also secrete different cytokines affecting the T cells activation and differentiation. In murine leishmaniasis, depletion of neutrophils modulated the anti-leishmanial T-cell response and tissue pathology. Resting macrophages and IFN-γ activated macrophages differ in their antigen presenting cells (APCs) functions. Likewise it has been observed the 47 per cent human peripheral blood neutrophils express CD28 and that CD28 signals through PI3 kinase and induces IFN-γ and also induces T cell chemotactic factors suggesting the role of neutrophils in initiation of T cell response against Leishmania. IFN-γ might result in augmentation of MHC class II expression in an autocrine manner and subsequent leishmanicidal function. These reports suggest that neutrophils may have quite contradictory function in Leishmania infection-direct interaction with Leishmania helps both phagocytic killing and transfer to macrophages for better survival whereas...
indirect interaction with *Leishmania*-infected macrophages results in macrophage activation and Th1 induction. In addition, neutrophils are recently proposed as efficient APCs (Fig. 3).

**Mast cells and leishmaniasis**

It is known that IL-4 is a cytokine, which influences the course of infection and disease progression. IL-4 is also produced by some other cell types like mast cells or by basophils together with T cells, although this process is usually stimulated by cross-linking with antibody bound to Fc receptors, an unlikely occurrence in naïve mice. The differential role of mast cells in *Leishmania* infection in a susceptible and a resistant mouse strain remains unknown. A previous study on genetically mast cell deficient mice with mutations in c-kit (c-kit ligand) loci demonstrated that these mice developed a smaller lesion and healed quicker than their wild-type littermates suggesting a role of mast cells. Since the mutated mice are semi-syngeneic with C57BL/6 mice, displaying a self-curing phenotype, the mast cells in the resistant mouse may be anti-parasitic or their pro-parasitic role could be masked by other factors that dictate resistance. Since *L. donovani* disseminates to lymphoid organs as opposed to *L. major* infection, which is restricted to skin lesion and the draining lymph nodes, mast cells may play different roles in VL and CL. It has been demonstrated that *Leishmania* regulates mast cell augmentation differentially in susceptible vs resistant mouse strains and this augmentation is dependent on IL-3 in BALB/c mice but not in C57BL/6 mice. Moreover, mast cells inhibited IFN-γ dependent restriction of parasite in susceptible BALB/c mice but not in resistant C57BL/6 mice suggesting the differential role of mast cells in leishmaniasis in susceptible and resistant mice.

**Dendritic cells as an efficient antigen presenting cells (APCs) and Leishmania infection**

Dendritic cells (DCs) are potent antigen presenting cells and can induce T cell activation efficiently. It has also been shown that DCs are the source of different cytokines such as IL-12, IL-10 and IFN-γ. Incubation of *Leishmania* promastigotes with dendritic cells induced early IL-12 production *in vitro*, which might be contributed from the pre existing pool of IL-12 p70 which was secreted soon after ligation of any microbial product, suggesting the role of DCs in the initiation of T cell immune response in *Leishmania* infection. It is also reported that uptake of *Leishmania* amastigotes by skin derived DCs induces IL-12 p70, upregulates of costimulatory molecules and vaccinates against *L. major* infection; in marked contrast, *L. major* inhibits IL-12 production in macrophages. It was shown the CC chemokines receptor (CCR) 2-/- mice are defective in DCs migration from marginal zone of lymph node to T cells area and markedly impaired in antigen specific T cell activation and make resistant mouse strain susceptible for *L. major* suggesting the regulatory role of chemokines receptors in parasitic infections. Another study suggested that down regulation of CCR7 by *L. donovani* impaired the DCs migration and used by the parasite as an immune evasion strategy, contributing to disease progression.

Dendritic cells are potent candidate for immunotherapy of leishmaniasis. Upon loading with microbial antigen (Ag) and adoptive transfer, DCs are able to induce immunity to infections. This offers encouragement for the development of DC-based vaccination strategies. However, the mechanisms underlying the adjuvant effect of DC are not fully understood, and there is a need to identify Ag with which to arm DC. It has been shown that DC produced IL-12 is the main cytokine that induced protection against *Leishmania* and *Toxoplasma*. Consistent with this finding, Ag-pulsed langerhans cells (LC) from IL-12-deficient mice were unable to release IL-12 and completely abrogated the capacity of LC to mediate protection against leishmaniasis. Another report suggested that CD40 ligand dependent IL-12 p70 secretion from dendritic cells during *Leishmania* infection was strain and species dependent. The intrinsic differences in the ability of *Leishmania* species to prime DCs for CD40 L-dependent IL-12 p70 secretion might account, at least in part, for the evolution of healing and non-healing forms of leishmanial disease. DC-based immunotherapy combined with chemotherapeutic agent for example sodium antimony gluconate conferred the protection against established *L. donovani* infection and induced Th1 immune response. Another intervention suggested the differential regulatory role of CD11c+ DC and CD11c- DC in *Leishmania* infection. The combination of LACK antigen and CpG ODN leads to the presence of CD11c+ DCs in the draining lymph node.
that are capable of vaccinating naive mice in the absence of further antigen and adjuvant suggesting the immunotherapeutic potential of CD11c+ DCs in *L. major* infection. These observations suggested the potential role of dendritic cells in immunotherapy of leishmaniasis.

**Effector function of macrophages in *Leishmania* infection and immune evasion mechanism**

Macrophage is a primary phagocyte that plays host for *Leishmania*. Activation of macrophage is a primary mechanism to eliminate the *Leishmania* parasite presumably mediated by toxic metabolites of oxygen, which may include super oxide anion \((O_2^-)\), hydrogen peroxide \((H_2O_2)\) and nitric oxide \((NO)\). A variety of stimuli can induce the morphological, biochemical and functional changes characteristic of activated macrophages. Activated macrophages produce different cytokines like TNF-\(\alpha\), IL-6, IL-18, IL-12 and IFN-\(\gamma\). IL-12 is an effective adjuvant and a prerequisite for Th1 type of immune response in most of intracellular parasitic infections. It has been reported that endogenous IL-12 is required to eliminate *Leishmania* growth in IFN-\(\gamma\) gene knock out mice whereas IL-12 knock out macrophages are lacking *Leishmania* preventive phenotypes. The main producers of IL-12 are antigen presenting cells for example macrophages and dendritic cells, which produce IL-12 through CD40 and CD40L interactions. CD40L present on the surface activated T cells interacts with CD40 on the macrophages and induces IL-12 expression and production. CD40L knock out mice are susceptible to *Leishmania* infection and have less IL-12. IFN-\(\gamma\) and nitric oxide production compared to its wild type counterparts suggesting that CD40-CD40L interaction is required for the clearance of the disease. Another recent study showed that treatment of susceptible BALB/c strain with CD40 monoclonal antibody was detrimental to *Leishmania* infection *in vivo* and *in vitro*. CD40 ligation induces IL-12, which in turn activates the T cell to produce IFN-\(\gamma\) and leishmanicidal function. Priming of susceptible BALB/c mice with exogenous rIL-12 during *Leishmania* infection also promises protection and gives self-healing phenotype. IL-18 is another proinflammatory cytokine that helped in evoking Th1 immune response particularly in collaboration with IL-12 most effectively. IL-12 and IL-18 both induced IFN-\(\gamma\) from murine macrophage in combination but either one alone was not sufficed to induce IFN-\(\gamma\) from the peritoneal macrophages. Another report suggested the protective role of IL-18 in *L. major* and *Staphylococcus aureus* infection. Treatment of mice either with rIL-18 or rIL-12 was unable to protect mice from cutaneous lesion but combination of both induced protective T cell response and less severe disease. Among macrophage activation cytokines, IFN-\(\gamma\) has been critically implicated. Otherwise resistant strains of mouse with targeted disruption of either IFN-\(\gamma\) or IFN-\(\gamma\) receptor gene were unable to restrict growth of *L. major* *in vivo* and suffered fatal infection. In a number of studies, recombinant IFN-\(\gamma\) was capable of activating infected macrophages from both resistant and susceptible mice to clear *L. major* *in vitro*. The final common pathway mediating parasite stasis or destruction by murine macrophages involves the production of nitric oxide (NO) from iNOS. Inhibition of NO production renders macrophages unable to restrict *L. major* replication *in vitro*. Administration of NO inhibitors to otherwise resistant mice abrogates the capacity to control infection. Such findings are consistent with the impaired production of NO by macrophages from IFN-\(\gamma\) or IFN-\(\gamma\) receptor gene knock-out mice. The importance of IFN-\(\gamma\) in NO induction was demonstrated by the finding that IFN-\(\gamma\) alone among a number of cytokines, was capable of independently enhancing iNOS transcription and NO release from stimulated mouse peritoneal macrophages. However, several cytokines enhance NO production in synergistic manner with IFN-\(\gamma\) and are likely to be involved in mediating parasite control *in vivo*. The most completely studied anti-leishmanial cytokine is TNF-\(\alpha\). TNF-\(\alpha\) synergizes with IFN-\(\gamma\) in the induction of iNOS and NO production by macrophages *in vitro*. Further, administration of recombinant murine or human TNF-\(\alpha\) to resistant or susceptible strains of mice infected with *L. major* ameliorated the course of disease. p55 TNF receptor transduced the TNF-\(\alpha\) response. Neutralizing anti TNF antibody transiently exacerbated disease in resistant CBA and C3H mice, although the course of infection in BALB/c mice was unaltered or affected minimally.

Additional cytokines that, with IFN-\(\gamma\), synergistically mediate activation of macrophage to clear *L. major* include IL-2, IL-4, and IL-7. Synergy usually requires the presence of infection and either prior or co-culture with IFN-\(\gamma\). Indeed, reversing the incubation
using IL-4 prior to IFN-\(\gamma\) abrogates the subsequent activation of macrophages\(^{123}\). Mice on genetically resistant background with constitutive IL-4 expression, however, developed persistent infection\(^{124}\). This suggests that at least IL-4 interferes with normal clearance of the parasite, as supported by \textit{in vitro} studies\(^{123}\) and as suggested by the genetic susceptibility of BALB/c mice\(^{125,126}\). IL-3 and GM-CSF, which with IL-5 also constitute a family of cytokines that shares a common signal transducing molecule\(^{127}\), were detrimental to \textit{L. major} infection \textit{in vitro} or \textit{in vivo} in various systems\(^{123,128,129}\).

Although \textit{Leishmania} infection in genetically resistant mouse strains results in complete disappearance of disease, viable \textit{L. major} can be recovered from the spleen, bone marrow, lymph nodes, and liver for the life of the animal\(^{130,131}\). Persistent parasites may be required to maintain memory T cells and effective immunity.

Though, the effector functions of macrophage promise to protect host from intracellular parasite (Fig.4), parasites still survive and persist inside the macrophages indicating that parasite deploys a mechanism to evade and modulate the host immune system for its own benefits. \textit{Leishmania} is an excellent example of it. Antigen presentation through MHC class II is hampered in \textit{Leishmania}-infected macrophages. It has been shown that \textit{Leishmania} amastigotes are internalized and degraded the MHC class II molecules associated with the parasitophorus vacuole and hampered the antigen presentation via MHC class II\(^{132}\). Degradation of MHC class II molecule has been prevented by pretreatment of amastigotes with specific protease inhibitor. MHC class II antigen presentation was sequestered more in late infected macrophages than early one\(^{133}\). A recent intervention revealed at the single cell level that MHC class II presentation was inefficient in \textit{L. donovani} infected macrophages and ineffective induction of T cell signaling essential for reorientation of the T cell microtubule organizing center and less IFN-\(\gamma\) production\(^{134}\). Interaction of B7.1 and B7.2 co-stimulatory molecule, with CD28 is required for activation of T cells apart from MHC-TCR interaction\(^{135}\). It was reported that \textit{L. donovani} infection down regulated the B7.1 expression and unable to deliver the co-stimulatory signal required for T helper cells differentiation\(^{136}\), suggesting the regulatory role of co-stimulatory molecule in parasitic infection. CD40 interacts with CD40L and helps in co-stimulation. Although CD40 induced \textit{Leishmania} killing through p38 mitogen activated protein kinase (p38 MAPK)\(^{98}\) but CD40 induced p38MAPK phosphorylation and iNOS2 expression inhibited in late infected macrophages with \textit{L. major}. Activation of p38 MAPK with an activator restored the CD40 induced leishmanicidal function both \textit{in vivo} and \textit{in vitro}\(^{98}\). Inhibition of CD40 induced p38MAPK phosphorylation by \textit{Leishmania} may be either by parasite’s direct inference with CD40 induced protective signaling pathway or by the activation of pro-parasitic signaling pathway which helps parasite to survive in the host. Moreover, the activation of phosphotyrosine phosphatases in \textit{L. donovani} and \textit{L. major} infected macrophages inhibited protective IFN-\(\gamma\)-responsiveness and impaired JAK-2 phosphorylation\(^{137,138}\). Some key survival strategies of \textit{Leishmania} are shown in Fig. 5.

**T cell response in leishmaniasis**

Since T cells play a major role in generating specific and memory T cell response in intracellular parasitic infections, T cells effector function has been
characterized most extensively in Leishmania infection. It was reported that non-healing BALB/c mice infected with L. major contained transcript of IL-4 in their draining lymph node cells, in marked contrast to C57BL/6 mice that expressed transcript for IFN-γ but not IL-411. This finding was confirmed by a kinetic analysis demonstrating the sustained expression of IL-4 mRNA in infected BALB/c mice with significant elevation of serum IgE levels that did not occur in C57BL/6125. Experimental data suggested the role of Leishmania specific CD4+ T cells to passively transfer the resistance or exacerbation of disease in immunodeficient or sublethally irradiated naive hosts, correlating with their production of Th1 or Th2 cytokines139-142. Some investigations suggested that the protective or non-protective immune response against Leishmania depends on the type of leishmanial antigen recognized by the T cells143. Such finding suggested that different antigens might drive Th1 or Th2 responses in susceptible BALB/c mice. Initial studies suggested the protective role of peptide epitopes from gp63, a conserved membrane protease that is immunogenic in most strains of mice144. Use of distinct peptide epitopes of gp63.

Fig. 5. Immune evasion and immune modulation mechanisms of Leishmania: Some key survival strategies of Leishmania are shown here. 1. Binding of Leishmania to macrophages receptors like CR1 and CR3 gives inhibitory signal that suppresses macrophages functioning necessary to eliminate parasite. 2. Once Leishmania promastigotes infect the macrophages, it downregulates the expression of CD80, a co-stimulatory molecule, resulting in unresponsive T cells. Leishmania amastigotes also sequester the MHC class II presentation, leading to T cell anergy. 3. Leishmania-infected macrophages interact with CD4+ T cells that expand early IL-4 producing population of CD4+ T cells, downregulating IFN-γ and Th1 immune response but promoting disease progression. 4. Cross linking of anti-CD40 antibody activates p38MAPK induced leishmanicidal function via iNOS2 induction. Leishmania infection downregulates CD40 induced p38MAPK phosphorylation and iNOS2 expression in macrophages. 5. Leishmania also activates phosphotyrosine phosphatases of the host macrophages that in turn affect the IFN-γ signaling pathway and inhibit JAK-2 phosphorylation and iNOS2 expression.
protected the BALB/c mice when administered with adjuvant\textsuperscript{142}; but the other study could not support the protective effect of this peptide when mice were infected with different strains of *L. major* and a higher infective dose\textsuperscript{143}. This discrepancy suggested the differential expression of gp63 by different strains\textsuperscript{146}.

$\text{V}\beta 4/\text{V}\alpha 8$ TCR usage has been identified in *L. major* infected draining lymph node CD4$^+$ T cells\textsuperscript{147}. A number of *Leishmania*-specific CD4$^+$ hybridomas cloned from early periods after infection of both the Th1 and Th2 phenotype was demonstrated to use this TCR. A protective Th1 clone from immunized BALB/c mouse also used this hetero dimer\textsuperscript{142}. It has also been seen that $\text{V}\beta 4/\text{V}\alpha 8$ CD4$^+$ T cells are the major producer of IL-4 in susceptible BALB/c mice and instruct the Th2 development\textsuperscript{148}. These initial observations suggested that Th1 and Th2 effector T cells could be derived from the same antigen but developed in either Th1 or Th2 depending upon the priming condition. The other report was unable to confirm the expansion of $\text{V}\beta 4/\text{V}\alpha 8$ population in uninfected or infected BALB/c or C57BL/6 mice\textsuperscript{149}. We have also failed to notice any alteration in T cell repertoire by staining CD4$^+$ T cells from uninfected or *L. major* infected draining lymph node with a panel of $\text{V}\beta$-TCR antibodies (M. Jadhav and B. Saha, unpublished observations). Therefore, any restricted use of $\text{V}\beta$-TCR in *Leishmania* infection remains unlikely; and so remains the role of *Leishmania*-specific TCR in early Th2 response dictating susceptibility.

It has been well documented that T cells can differentiate either in Th1 or Th2 type of effector cells and this plasticity of differentiation depends chiefly on the priming during differentiation\textsuperscript{150}. The finding was further substantiated using cells from transgenic mice expressing a single TCR\textsuperscript{151,152}. IL-4 was shown to induce Th2 whereas IL-12 induced Th1 differentiation\textsuperscript{153-156}. During early infection with *L. major* both resistant and susceptible host showed mixed response in CD4$^+$ cell population consisting of IL-2, IL-4 and IL-13 that peaked at 4 days\textsuperscript{157}. IFN-\(\gamma\) transcripts were variable in different strains of mice. Strikingly, IL-4 production in infected mice was similar to fully developed Th2 clones in all the strains of mice analyzed. Administration of anti-CD4 antibody\textsuperscript{158,159} and anti-IL-4 antibody\textsuperscript{126,160} was shown to heal the infection suggesting that CD4$^+$ population, which induces the IL-4 in early infection, plays critical role in disease progression. The observation gained further support from the susceptibility of IL-4 transgenic mice in resistant background\textsuperscript{124} and from the resistance of IL-4 gene deficient mice\textsuperscript{161} to *L. major* infection. These findings suggested the role of IL-4 in disease progression. However, the role of IL-4 as a susceptibility factor has come under suspicion due to several observations. Firstly, IL-4 induction in *Leishmania* infection was shown to be dependent on other T cell factors like IL-2. Administration of anti-IL-2 or anti-IL-2 receptor antibody ameliorated the *L. major* infection\textsuperscript{162} suggesting IL-2 as a susceptibility factor for leishmaniasis. This was further confirmed by a report showing IL-2 induced IL-4 production in CD4$^+$ T cells\textsuperscript{163}. Secondly, the IL-4-deficient mice raised from BALB/c embryonic stem cells remained susceptible to *L. major* infection\textsuperscript{164}. Thirdly, a report demonstrated a dual role of IL-4 in *L. major* infection where depending on the phase of response and the antigen-presenting cells, IL-4 promoted Th1 response\textsuperscript{165}. These results finally shifted focus from IL-4 to IL-10 as a susceptibility factor.

Initially IL-10 was categorized as a Th2 cytokine\textsuperscript{151} but with time and newly discovered suppressor population of T cells, its Th2 candidature has been diluted out. Recently it has been shown that IL-10 plays a suppressive or regulatory role in autoimmune diseases\textsuperscript{166}, host versus graft rejection\textsuperscript{167}, and parasitic infections\textsuperscript{168,169}. Administration of anti-IL-10 antibody during *L. major* infection further reduced the susceptibility of IL-4 receptor \(\alpha\) gene deficient mice\textsuperscript{170}. It has been shown that IL-10 dictates the susceptibility to *L. donovani* infection\textsuperscript{171,172} and is required for higher parasite persistence in both resistant C57BL/6 and susceptible BALB/c mice\textsuperscript{173,174}. Administration of anti-IL10 receptor antibody was shown to cure the *Leishmania* infection\textsuperscript{174,175}. Consistent with these findings, another report\textsuperscript{176} suggested the role of IL-10 by using IL-10 deficient mice of both BALB/c and C57BL/6. These mice were resistant to *L. donovani* infection\textsuperscript{176} and were producing more IL-12 and IFN-\(\gamma\) suggesting that IL-10 is the critical factor for disease progression. It has also been shown that co-administration of IL-10 plasmid with low dose of *L. major* inoculums, known to induce protective Th1 phenotype, promoted the disease in BALB/c mice\textsuperscript{177} further confirming the disease progressive role of IL-10\textsuperscript{168}. Induction of IL-10 increased by addition of IL-2 and the suppressive role of IL-10 in leishmaniasis are also demonstrated (M. Bodas and B. Saha, unpublished observations).
IL-12, produced by macrophages, and IFN-γ, produced by NK cells, are the potential candidate cytokines based on their known ability to influence Th1 development in vitro in various systems\textsuperscript{153,156}. Addition of IFN-γ to parasite inoculum decreased substantially the amount of IL-4 recovered after in vitro stimulation of lymph node cells isolated 3 days later from BALB/c mice\textsuperscript{178}. It has also been shown that neither single dose nor sustained delivery of IFN-γ during the course of infection was capable of reversing the ultimate progressive course of the disease\textsuperscript{126,178}. Consistent with this finding, *Leishmania* transfected to express the IFN-γ also did not alter the course of infection in BALB/c mice\textsuperscript{179}. These observations suggested that early IL-4 and IL-10 production in leishmaniasis was not due to lack of IFN-γ, though, IFN-γ does play a role in *Leishmania* infection by maintaining Th1 development. Neutralization of IFN-γ at the time of infection by anti IFN-γ antibody made resistant mice to susceptible\textsuperscript{180}. IFN-γ gene disrupted mice of resistant background failed to clear the parasite and also demonstrated the default Th2 development of CD4\textsuperscript{+} T cells\textsuperscript{102}. IFN-γ receptor deficient mice of resistant background were also susceptible for leishmaniasis\textsuperscript{103}.

NK cells, CD8\textsuperscript{+} T cells and γδ T cells have been demonstrated to produce IFN-γ in leishmaniasis. Since NK cells comprise the IFN-γ producing compartment and influence the Th1 development, they might play a role in resistance and susceptibility for *Leishmania* infection. The role of NK cells has been ruled by the study using beige mice that have abnormal NK cells function but unaltered disease pattern compared to normal mice\textsuperscript{181}. It has been shown that NK cells induce IFN-γ mediated early protective response in resistant C3H/HeN mice against *L. major* infection compared with the diminished activity of NK cells in susceptible BALB/c mice\textsuperscript{182} suggesting the possible role of NK cells in resistance or susceptibility of the host. Further, a marked exacerbation of infection was found in the NK-depleted C57BL/6 mice within the first two weeks of infection with less IFN-γ production\textsuperscript{183}. Poly I: C treatment in order to activate NK cell activity in vivo in BALB/c mice, which are genetically susceptible to *L. major* infection led to milder symptoms and to a significantly lower parasite burden in the early course of infection and induced higher IFN-γ production\textsuperscript{183}. The protective role of NK cells was completely abrogated with IL-12 neutralization in resistant C57BL/6 mice\textsuperscript{184} suggesting the role of IL-12 in early IFN-γ production from NK cells mediating Th1 immune response in resistant mice. BALB/c mice lack an early NK cell response or a Th1-type immune response after *L. major* infection due to simultaneous production of IL-12 inhibitory factors like IL-4, IL-10 and TGF-β\textsuperscript{184}. Consistent with these observations, another report suggested that depletion of NK1.1\textsuperscript{+} cells or neutralization of IFN-γ in C57BL/6 mice prior to infection led to rapid parasite spreading with kinetics similar to those seen in susceptible animals\textsuperscript{185}. Experiments with severe-combined immuno deficient mice provided further evidence that parasite containment depends on natural killer cells and IFN-γ, but is independent of T cells\textsuperscript{185}. All these observations suggested that NK cells were active participants in the non-specific phase or pre T cell-phase of anti-leishmanial activity in controlling the parasite multiplication early in the course of *L. major* infection in mice. A marked expansion of γδ T cells in chronically infected mice and the adverse effect of their depletion on disease progression have been demonstrated\textsuperscript{186} suggesting the anti-leishmanial functions of γδ T cells.

The role of CD8\textsuperscript{+} T cells in maintaining the long lasting memory response against *Mycobacterium tuberculosis* has been demonstrated\textsuperscript{187}. Though CD4\textsuperscript{+} T cells are generally accepted to be responsible for the determination of resistance to infection in experimental murine cutaneous leishmaniasis\textsuperscript{139,141}, a contribution of CD8\textsuperscript{+} lymphocytes in immunity can be demonstrated under certain well-defined conditions. Immune BALB/c mice rechallenged with live parasites have shown protective phenotype associated with IFN-γ production from CD8\textsuperscript{+} T cells\textsuperscript{188,189}. MHC class II deficient mice of resistant background which were resistant for *L. major* infection otherwise showed fatal disease compared to wild type\textsuperscript{190} whereas MHC class I deficient mice were showing the curative phenotype\textsuperscript{177} ruling out the protective role CD8\textsuperscript{+} T cells during primary infections. Consistent with these observations, another study showed that CD8 or β2 microglobulin deficient mice were resistant for the *Leishmania* infection and maintained the long term Th1 immunity in these mice\textsuperscript{190}. It has also been shown that low parasite dose and inoculation into a dermal site during the parasite transmission played a decisive role in disease protection of C57BL/6 mice associated with CD8\textsuperscript{+}
T cells accumulations in the skin whereas CD8 deficient mice abrogated the protection\textsuperscript{191}. Further, β2 microglobulin and perforin deficient mice primed with a leishmanial antigen were not able to control a challenge infection after vaccination\textsuperscript{192} suggesting the role of CD8\textsuperscript{+} T cell in leishmaniasis. Taken together these observations suggest that the role of CD8\textsuperscript{+} T cells is still not defined in \textit{Leishmania} infection. It may perhaps be possible that the requirement of CD4 helps in the generation of long lasting memory against \textit{Leishmania} parasite by CD8\textsuperscript{+} T cells depending upon the early priming of CD4\textsuperscript{+} T cells and the leishmanial antigen.

The role of IL-12 in Th1 development has clearly been demonstrated \textit{in vitro} by using transgenic TCR CD 4\textsuperscript{+} T cells\textsuperscript{153-156}. As noted previously, however, \textit{Leishmania} promastigotes evade IL-12 induction during invasion of macrophages from both resistant and susceptible host\textsuperscript{193}. Neutralization of IL-12 during infection makes resistant mice susceptible for \textit{Leishmania} infection\textsuperscript{194}. Another report suggested that reconstitution of IL-12p40/-/- mice with exogenous IL-12 initiates the Th1 response and protect the mice\textsuperscript{195}. Moreover, IL-12 gene deficient mice of resistant background were susceptible for \textit{L. donovani} infection\textsuperscript{196}. These observations suggested the critical and decisive role of endogenous IL-12 in leishmaniasis\textsuperscript{197}. The capacity of exogenous IL-12 to heal infected BALB/c mice correlated with powerful effect of IL-12 in suppressing IL-4 transcription and protein production. Further, the lack of Th1 immune response in BALB/c mice was due to unresponsiveness of these T cells for IL-12\textsuperscript{198} possibly through the inhibitory effect of IL-4 on IL-12 receptor and responsiveness\textsuperscript{199}. It is now known that T-bet regulates Th1\textsuperscript{200} while GATA-3 regulates Th2 development\textsuperscript{201} of naive T cells. These factors regulate each other expression reciprocally depending upon the priming condition. It may perhaps be possible that these transcription factors may play a regulatory role either in resistance or susceptibility for \textit{Leishmania} infection and need to be characterized further.

\textbf{Drug therapy and immuno modulation}

The drugs currently recommended for the treatment of leishmaniasis include the pentavalent antimonials sodium stibogluconate (pentostam) and meglumine antimoniate, amphotericin B and its lipid formulation, and pentamidine. The antimonials were first used in 1945 and remain effective treatments for some forms of the leishmaniasis but the requirement for up to 28 days of parenteral administration, the variable efficacy against VL and CL, and the emergence of significant drug resistance are factors limiting the usefulness of antimonials\textsuperscript{202}. Drug resistance has great public health importance for anthropononotic \textit{L. donovani} and \textit{L. tropica} rather than other forms of leishmaniasis, which are zoonotic. Up to 60 per cent cases in Bihar State, India do not respond to pentavalent antimonials\textsuperscript{203}.

Chemotherapeutic cure of leishmaniasis is largely dependent upon the development of an effective immune response that activates macrophages to produce toxic nitrogen and oxygen intermediates to kill the amastigotes. This process is suppressed by the infection itself which down regulates the requisite signaling between macrophage and T cells, for example, the production of IL-12\textsuperscript{193} or the antigen presentation of major histocompatibility complex (MHC) and co-stimulatory molecules at the macrophage surface\textsuperscript{136}. It has been reported that IFN-γ enhanced the efficacy of antimonials in the treatment of VL and CL\textsuperscript{202}. The substituted benzaldehyde tucaresol restores the signaling and stimulates the T cells toward Th1 differentiation, and showed the protection in mouse VL model\textsuperscript{204}. A report showing that anisomycin restores the CD40 induced p38 MAPK phosphorylation in \textit{L. major}-infected macrophages\textsuperscript{98} suggested the potential drug candidate of host, modulated by the parasite for its own survival and bypass the drug resistance.

\textbf{Conclusions and further possibilities}

IL-10 is secreted mainly by the macrophages, dendritic cells and T lymphocytes whereas IL-4, Th2 cytokine are produced chiefly by lymphocytes but not by the macrophages. Initially IL-4 has been characterized as a critical factor that dictates susceptibility for leishmaniasis by promoting the Th2 differentiation in susceptible BALB/c mice and helps in disease progression. CD4\textsuperscript{+} T cell populations were found to be a crucial factor either in disease progression through IL-4 or in disease prevention through IFN-γ. Macrophages are proposed primary host cells for \textit{Leishmania} but the role of these cells has not been well characterized neither in disease prevention nor in progression independent of
T cell. The effector functions of macrophages for *Leishmania* have always been described in a T-dependent manner. The fate of infected macrophages in pre-T cell phase is not well known. Since T cells come later during infection, it is possible that parasite modulates its host in terms of signaling or antigen presentation for its own benefit and induces factors that provide disease progressive environment and prime T cells for Th2 differentiation. It is also possible that parasites starts modulating the macrophages at the time of entry and later on modulated parasitized macrophages interact with T cells and may induce IL-4 and disease inducing factors from T cells that help in disease progression and parasite survival in susceptible host. The above discussion suggesting the delayed or later role of T cells that may be a part of the same series that starts from the macrophages. It is now known that IL-10 plays a role in disease progression but whether with IL-4 or prior to IL-4 phase is not known. It is also known that *Leishmania* parasitized macrophages produce IL-10 but not IL-4 suggesting the role of IL-10 prior to IL-4 in disease progression or in susceptibility of the host. This suggests the crucial role of IL-10 in disease initiation independent of T cells and in disease progression later on in combination with IL-4. It is also obvious that parasites modulate the macrophages in terms of their antigen presenting capacity and signaling capability, for example, CD40 signalling and imbalance in kinase or phosphatase activities, prior to T cell phase. Once the CD40 signalling pathway is skewed by the parasite towards the pro-parasitic pathway in macrophages, interaction between CD40 ligand on activated T cells and CD40 on these macrophages may not be able to revert the signalling towards the anti-parasitic pathway and thus, such macrophage-T cell interaction may not be host-protective at all.

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