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# Immunology of Leishmaniasis and Future Prospective of Vaccines

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## 1. Introduction

Leishmaniasis causes human suffering on a global scale and there are more than 12 million current cases with 2 million additional cases annually. There is a serious threat to get infected cases of 350 million in endemic areas specifically in South East Asia. The epidemiological studies revealed that there are 20 protozoan parasite species of the genus *Leishmania* known to cause leishmaniasis in humans (Table 1)(WHO 2004). Leishmaniasis is prevalent in tropical and subtropical regions and endemic in more than 88 countries where annually 2 million new cases are reported. The geographic distribution of each *Leishmania* species affects the type of disease that occurs in each region of the world. Visceral leishmaniasis (VL; commonly known as kala-azar) is caused by *Leishmania donovani* in South Asia and Africa, while *Leishmania infantum* causes VL in the Mediterranean, the Middle East, Latin America and parts of Asia too (Table 2)(WHO 2010). Other mammals can also be infected with *Leishmania* spp., dogs develop canine visceral leishmaniasis (CaVL) and they serve as an important parasitic reservoir in these regions. Cutaneous leishmaniasis (CL) is caused by *L. major* in Africa, the Middle East and parts of Asia, by *Leishmania tropica* in the Middle East, the Mediterranean and parts of Asia, and by *Leishmania aethiopica* in parts of Africa. Many different species may be involved in the Americas, where CL can be found throughout South America and as far as Mexico in the north (Table 1 and 2). Infection have also been reported in Canada and the US. Australia is free of *Leishmania* spp. but infection among local animals like captive kangaroos, wallabies and other marsupials have been reported recently and there are chances of transmission of this disease to human through infected meat and also due to close proximity with these native animals (Gelanew, Kuhls et al. 2010).

<b>Old world, subgenus <i>Leishmania</i></b>	
Visceral leishmaniasis	<i>Leishmania donovani</i> , <i>L. infantum</i>
Cutaneous leishmaniasis	<i>L. major</i> , <i>L. tropica</i> , <i>L. aethiopica</i>
<b>New world, subgenus <i>Leishmania</i></b>	
Visceral leishmaniasis	<i>L. infantum</i>
Cutaneous leishmaniasis	<i>L. infantum</i> , <i>L. mexicana</i> , <i>L. pifanol</i> , <i>L. amazonensis</i>
Diffuse cutaneous leishmaniasis	<i>L. mexicana</i> , <i>L. amazonensis</i>
<b>New world, subgenus <i>Viannia</i></b>	
Cutaneous leishmaniasis	<i>L. braziliensis</i> , <i>L. guyanensis</i> , <i>L. panamensis</i> , <i>L. peruviana</i>
Mucocutaneous leishmaniasis	<i>L. braziliensis</i> , <i>L. panamensis</i>

Table 1. Main species of *Leishmania* that affect humans.

## 2. Immunology of leishmaniasis

Leishmaniasis is caused by one of several species of *Leishmania*. The clinical spectrum depends upon both the parasite species and the host's immune response. Some *Leishmania* spp. cause cutaneous, mucocutaneous or diffuse cutaneous leishmaniasis whereas others may disseminate to internal organs such as the liver, spleen and bone marrow to cause visceral leishmaniasis. The main species of *Leishmania* that affect humans are given Table 1&2.

*Leishmania* parasite exists in two different morphological forms i.e. promastigotes (flagellate form) and amastigote (aflagellated form). Promastigotes develops inside the midgut of sandfly and become infective, non-dividing metacyclic promastigotes which are located near stomodeal valve (an invagination of the foregut into midgut). During blood feeding metacyclic promastigotes are regurgitated along with immunomodulatory parasite-derived proteophosphoglycans and various salivary components. The metacyclic promastigotes are rapidly phagocytosed by one of several possible cell types that are found in the local environment. The various cell types may include neutrophils, tissue-resident macrophages or dendritic cell (DC) or monocyte derived DCs (moDCs). After establishing an intracellular niche, metacyclic promastigotes are transformed to non motile amastigote form. These amastigotes replicate within the host cells, which rupture to release too many amastigotes, allowing reinfection of phagocytes. The transmission is complete when infected phagocytes are taken up by another sandfly with the blood meal and amastigotes then convert into promastigotes in the sandfly midgut. (Fig 1: Life cycle of *Leishmania* parasite)

Disease	<i>Leishmania</i> sp.(CFSPH 2009)	Geographical burden
Cutaneous Leishmaniasis (CL)	<i>L. mexicana</i> complex (ZCL)	Argentina, Belize, Bolivia, Brazil. Colombia, Costa Rica, Ecuador, French Guiana, Guatemala, Mexico, Peru, Suriname, USA, and Venezuela
	<i>L. tropica</i> complex (ACL)	Afghanistan, Azerbaijan, India, Iran, Iraq, Israel, Morocco, Pakistan, Syria, Turkey, and Uzbekistan
	<i>L. major</i> complex (ZCL)	Afghanistan, Algeria, Azerbaijan, Burkina Faso, Cameroon, Chad, Egypt, Ethiopia, Gambia, Georgia, Ghana, Guinea Bissau, India, Iran, Iraq, Israel, Jordan, Kazakhstan, Kenya, Kuwait, Libya, Mali, Mauritania, Mongolia, Morocco, Niger, Nigeria, Oman, Pakistan, Saudi Arabia, Senegal, the Sudan, Syria, Tunisia, Turkey, Turkmenistan, Uzbekistan, and Yemen
	<i>L. aethiopica</i> complex (ZCL)	Ethiopia, Kenya, and Uganda
	<i>L. braziliensis</i> complex (ZCL)	Argentina, Belize, Bolivia, Brazil. Colombia, Costa Rica, Ecuador, French Guiana, Guatemala, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, and Venezuela
	<i>L. guyanensis</i> complex (ZCL)	Argentina, Belize, Bolivia, Brazil. Colombia, Costa Rica, Ecuador, French Guiana, Guatemala, Guyana, Honduras, Nicaragua, Panama, Peru, Suriname, and Venezuela
Mucosal/mucocutaneous Leishmaniasis (ML)	<i>L. braziliensis</i> complex	Argentina, Belize, Bolivia, Brazil. Colombia, Costa Rica, Ecuador, French Guiana, Guatemala, Honduras, Mexico, Nicaragua, Panama, Paraguay, Peru, and Venezuela
	<i>L. guyanensis</i> complex	Colombia, Costa Rica, Ecuador, Guatemala, Honduras, Nicaragua, and Panama
Visceral Leishmaniasis (VL; Kala-azar)	<i>L. donovani</i> complex (AVL, ZVL)	Afghanistan, Albania, Algeria, Argentina, Armenia, Azerbaijan, Bangladesh, Bhutan, Bolivia, Bosnia & Herzegovina, Brazil, Bulgaria, Chad, Central African Republic, China, Colombia, Croatia, Cyprus, Djibouti, Egypt, El Salvador, Eritrea, Ethiopia, France, Gambia, Georgia, Greece, Guatemala, Honduras, India, Iran, Iraq, Israel. Italy, Jordan, Kazakhstan, Kenya, Kyrgyzstan, Lebanon, Libya, Macedonia, Malta, Mauritania, Mexico, Monaco, Montenegro, Morocco, Nepal, Nicaragua, Oman, Pakistan, Paraguay, Portugal, Romania, Saudi Arabia, Senegal. Slovenia, Somalia, Spain, Sri Lanka, the Sudan, Syria, and Yemen
Post-Kala-azar Dermal Leishmaniasis (PKDL)	<i>L. donovani</i> complex	Bangladesh, China, Nepal, India, Iran, Iraq, Kenya, Pakistan, the Sudan

Table 2. Disease phenotype and geographical burden attributed to various *Leishmania* species (WHO 2010).

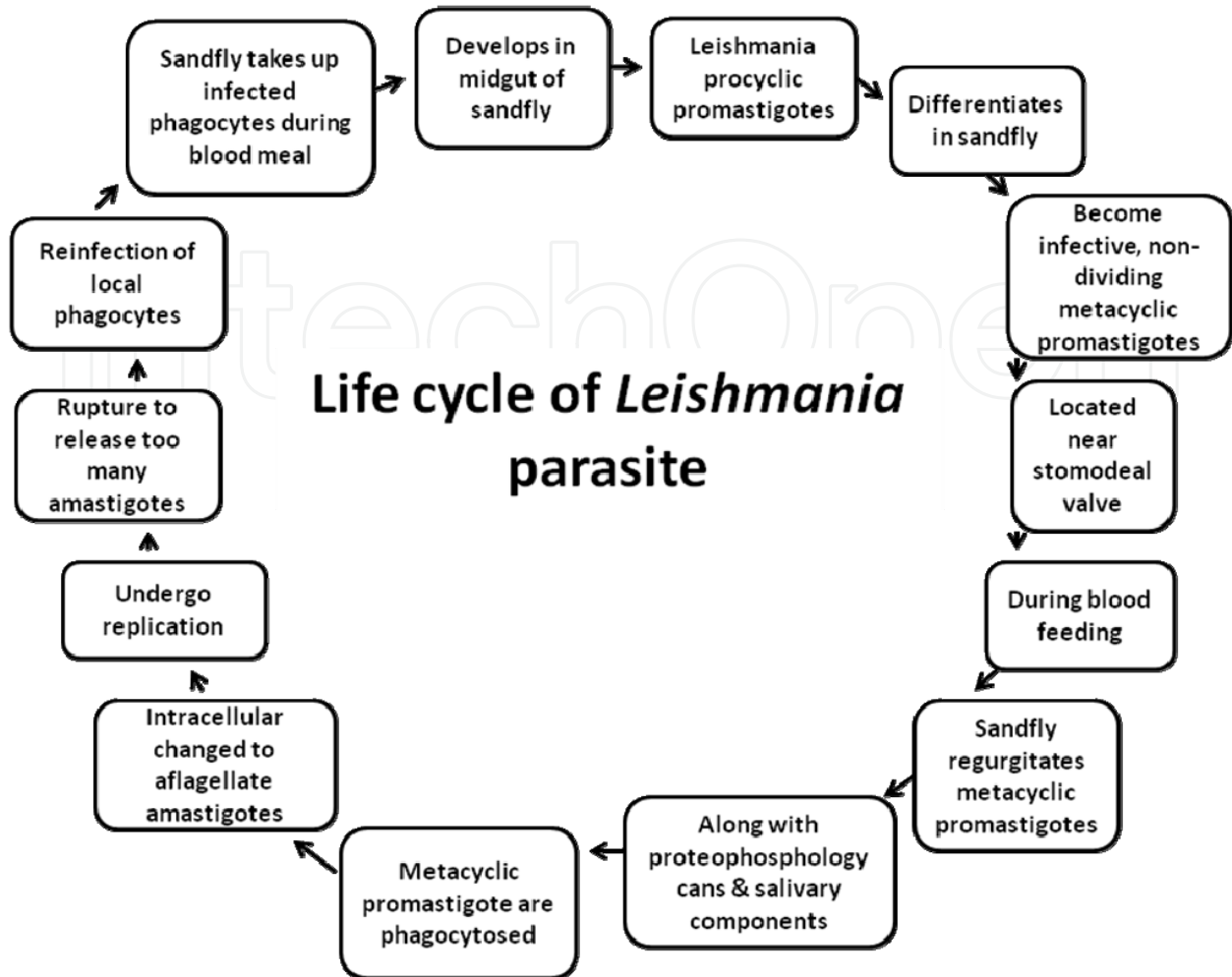


Fig. 1. Life cycle of *Leishmania* parasite.

### 3. Host cells for *Leishmania* parasites

*Leishmania* spp. is an obligate intracellular pathogen which mainly infects macrophages. Recent studies have shown that it can infect multiple cell types. Neutrophils have been regarded as Trojan Horses which help promastigotes to establish intracellular niche in macrophages without triggering their antimicrobial defences. The promastigotes are phagocytosed by neutrophils and they reside in their phagosomes. They become phagocytotic meal for the macrophages when undergo apoptosis. Since these apoptotic bodies are phagocytosed through receptor mediated pathways that fail to trigger antimicrobial defences. (Ravichandran and Lorenz 2007) The neutrophils are attracted at local site of sandfly bite due to alarmins (IL-33, IL1 $\beta$ , high mobility group protein B1-HMGB1), which are endogenous molecules that provide signal of tissue damage. (Haraldsen, Balogh et al. 2009) Mononuclear phagocytes which are infected with *Leishmania* parasites also produce various chemokines which help in recruitment of neutrophils. (Lopez Kostka, Dinges et al. 2009; Xin, Vargas-Inchaustegui et al. 2010)

*Leishmania* promastigotes have a dense covering of glycocalyx which is attached to the plasma membrane with the help of GPI (glycophosphoinositol). Lipophosphoglycan (LPG) is an important molecule which promotes the infectivity of the parasite in mammalian host.

It is a long phosphoglycan molecule having repeated sugar residues, glycan side chains and a capping oligosaccharide. It shows a great variability in its structure which helps in immune evasion. Another important surface glycoprotein is zinc metalloproteinase(GP-63) which acts as a virulence factor (Gomez, Contreras et al. 2009). *Leishmania donovani* promastigotes stimulate neutrophil extracellular traps (NETs) by a LPG independent pathway (Gabriel, McMaster et al. 2010). These NETs are filamentous DNA which are decorated with antimicrobial peptides.

Though the neutrophils play an important role but mononuclear phagocytes are equally essential for the replication and long term survival of parasites. Dermal DCs uptake the parasite within first few hours of infection by pseudopodium formation (Ng, Hsu et al. 2008). As the number of resident macrophages and dendritic cells is limited in the skin, the parasitic multiplication is accompanied by the recruitment of monocytes (precursor of DCs) (Charmoy, Brunner-Agten et al. 2010). Infected inflammatory moDCs may facilitate parasite to reach the draining lymph node. *Leishmania* parasite can hide itself in skin and lymph node fibroblasts.

In human neutrophils, phagosomes containing promastigotes fuse with myeloperoxidase(mpo) containing primary granules. It is an additional fusion of phagosome with tertiary and specific granules which lead to parasite degradation. These tertiary and specific granules are responsible for acidification and superoxide generation (Fig 2).

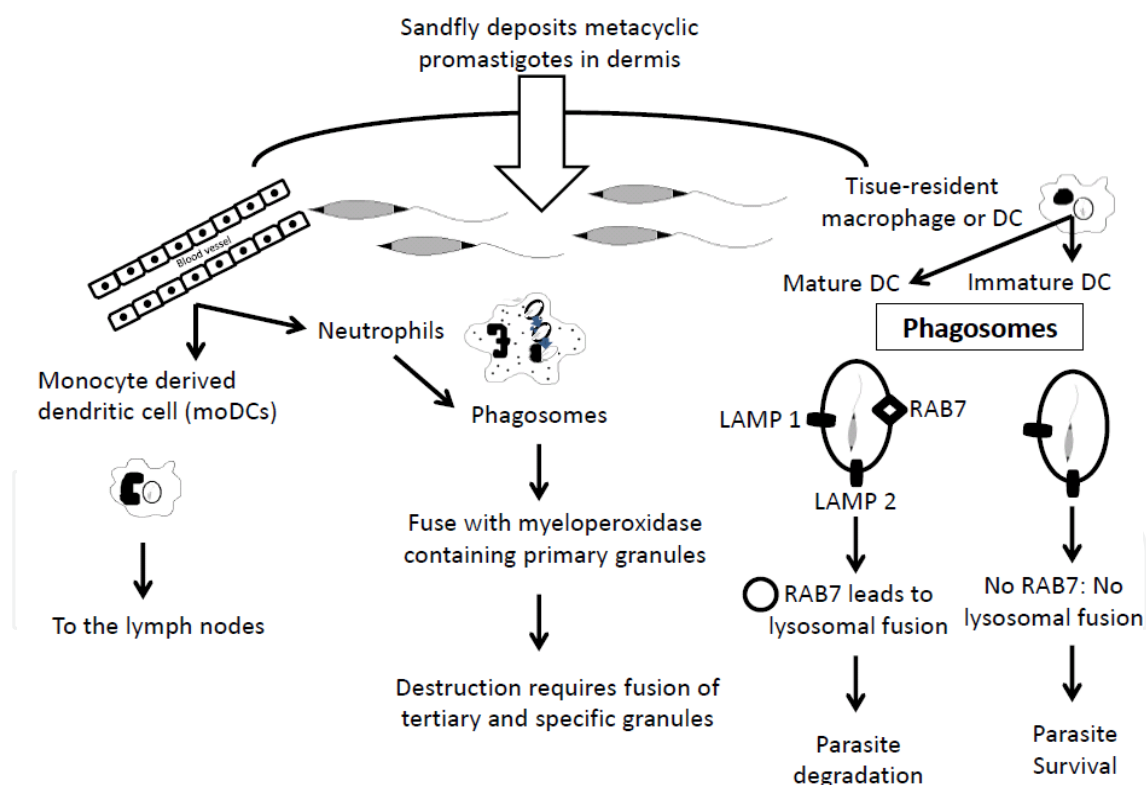


Fig. 2. Different cell types involved in Leishmaniasis and fate of phagosome. Metacyclic promastigotes are deposited in the dermis and taken up by various cells like neutrophils, monocyte derived dendritic cells, macrophages. Small GTPase RAB 7 helps in lysosomal fusion and its degradation. This fusion is inhibited in immature dendritic cells. This could be a mechanism to ensure the transport of live parasites to lymphnodes. Adapted from (Kaye and Scott 2011).

Inside macrophages parasite containing phagosomes mature to form phagolysosome but promastigotes inhibit this process. Lysosomal-associated membrane protein 1 (LAMP 1) and LAMP 2 are found in phagosomes containing *Leishmania* promastigotes in both immature DCs and mature DCs. Maturation of parasite containing phagosomes is arrested at late endosomal stage. Fusion of lysosome occurs with the help of GTPase RAB7 which is observed in mature DCs only. Thus, inhibition of RAB7 recruitment could be a mechanism used by *Leishmania* to transport the live parasites safely to lymph nodes (Lippuner, Paape et al. 2009).

LPG also provides an opportunity for the parasite to survive inside phagosomes by altering acidification (Vinet, Fukuda et al. 2009). Integration of LPG into phagosome membrane leads to extrusion of synaptotagmin V, which helps in acidification of phagosome by recruiting vesicular portion of ATPase. Thus, LPG-deficient parasites die rapidly before they fully adapted to an intracellular lifestyle.

Size of the parasite containing phagosomes also helps in parasite survival. Larger the size more is the dilutional effect on leishmanicidal factors like nitric oxide. Lysosomal size is regulated by a Beige protein; also known as lysosomal trafficking regulator (LYST). Mutations in LYST gene (Chediak-Higashi syndrome) leads to increase in size of lysosomes whereas induction of this gene (Leishmaniasis) leads to decrease in size of lysosomes. Thus, LYST behaves as an inducible innate response gene during Leishmaniasis, leading to increased susceptibility to killing by nitric oxide (Wilson, Huynh et al. 2008).

Iron has an important role in survival of *Leishmania* parasite as it is used by amastigotes (Huynh and Andrews 2008). There is an efflux pump present in phagosomal membrane which translocates  $Fe^{2+}$  and  $Mn^{2+}$  ions into the cytosol and thus limits iron availability to the parasite (Blackwell, Goswami et al. 2001). To overcome this decrease in iron availability, there occurs an upregulation of iron transporters, after its entry into macrophages. Thus intra-phagosomal competition for iron leads to activation of cytosolic iron sensors which helps in increased production of iron-binding protein transferrin and transferrin-mediated iron uptake (Das, Biswas et al. 2009).

Lipid microdomains present on macrophage surface helps the promastigotes of *Leishmania* to enter into macrophages (Fig 3). It also directs the entry of various virulence factors such as major surface protein also known as GP63 (Joshi, Rodriguez et al. 2009). These virulence factors can also be transferred to the macrophages by parasite-produced exosomes (Silverman and Reiner 2010). When promastigote enters into the phagosome, LPG inserts itself into lipid rafts and inhibits phagosome-lysosome fusion (Winberg, Holm et al. 2009). The inhibition of fusion is accompanied by accumulation of periphagosomal filamentous actin (F-actin) near lipid microdomains. Various virulence factors also use lipid microdomains to channel themselves into cytoplasm of macrophages. Altered lipid rafts may also be responsible for defective antigen presentation and CD40 signalling, MHC class II, major histocompatibility complex class II.

*Leishmania* is known to activate various inhibitor molecules that inhibit intracellular signaling pathways such as a negative regulatory molecule is the PTP SHP-1 (Src homology 2 domain containing tyrosine phosphatase)(Yi, Cleveland et al. 1992). SHP-1 is responsible for the negative regulation of many signaling pathways (Gregory and Olivier 2005). The majority of documented SHP-1 effects are the result of the inhibition by

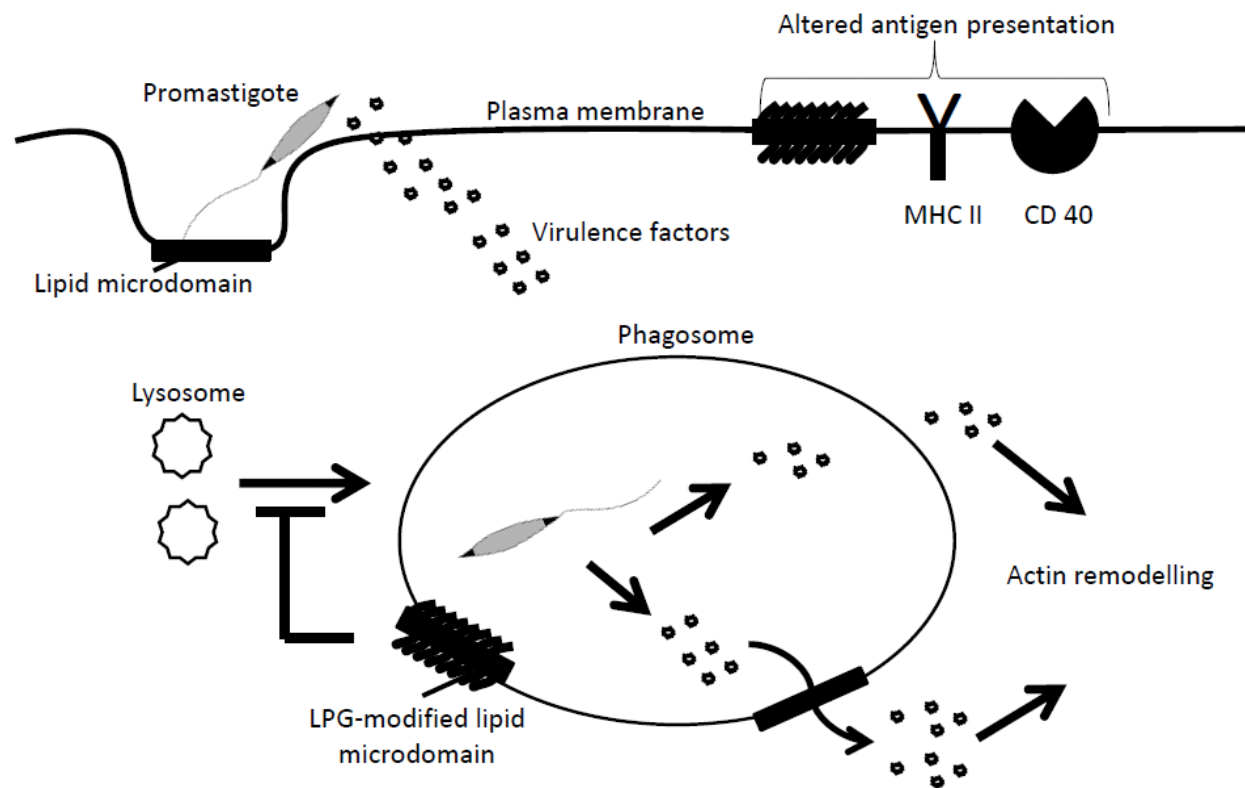


Fig. 3. Lipid microdomains, *Leishmania* parasite and macrophages. Role of lipid microdomains in transporting parasite and its virulence factors inside macrophages. Altered lipid domains by lipophosphoglycan (LPG) inhibits phagosome-lysosome fusion and also responsible for defective antigen presentation and CD40 signalling, MHC class II. Adapted from (Kaye and Scott 2011).

dephosphorylation of various kinases and their signaling pathways (Frearson and Alexander 1997). SHP-1 plays a vital role in limiting the activation of the JAK/STAT pathways following cytokine receptor stimulation. SHP-1 is known to be activated by MSP (major surface protein, GP63). *Leishmania spp.* contains multiple MSPs and can be found on the promastigote surface as well as in the parasite cytoplasm. Surface MSP is involved in parasite development within sandfly and the cytoplasmic MSP which is in preformed form is ready to use by the mammalian host (Yao, Donelson et al. 2007). This action is analogous to various effectors that are used by type III secretion system in bacteria which behaves like syringe and needle to inject various factors into cells (Winnen, Schlumberger et al. 2008). SHP-2 also known as PTPN 11 also shares many downstream targets with SHP-1 and provides anti-leishmanial immunity. The first line anti-leishmanial drug (sodium stibogluconate) also targets SHP-1 at concentrations that are used for chemotherapy in humans (Pathak and Yi 2001).

Another mechanism used by *Leishmania* parasite when inside the macrophages is by interference with host cell signalling at the level of macrophage protein C (PKC) (Olivier, Baimbridge et al. 1992). After initial contact with the target cells *Leishmania* parasite leads to leads to transient activation of MAPK and NF- $\kappa$ B. These signalling pathways lead to stimulation of cytokines and chemokines required for the efficient control of invading



pathogen. Thus, amplitude and duration of this immune response must be maintained under strict control to avoid harmful effects on host itself. Important mechanism by which cells protect themselves is by developing refractoriness state to repeated stimulation. It is well known that prolonged stimulation of toll like receptors and macrophages by microbial components such as LPS (lipopolysaccharide), lead these cells to hyporesponsiveness to the same stimulus (Ben-Othman, Guizani-Tabbane et al. 2008). This phenomenon is termed as LPS tolerance similar phenomenon of and similar hyporesponsiveness is seen in macrophages infected by *L. major* promastigotes. *Leishmania* parasite is able to induce a state of tolerance which correlates with a blockade of intracellular MAPK and/or NF- $\kappa$ B signalling pathway (Ben-Othman, Guizani-Tabbane et al. 2008).

Type 1 interferon response is usually associated with viral infections but their role in leishmaniasis is increasingly becoming important. Such response has been seen in infection with *Leishmaniasis*, which induces the expression in macrophages of PKR, a protein kinase that is activated by double stranded RNA. PKR appears to promote parasite survival through induction of the macrophage-deactivating cytokine IL-10 (Pereira, Teixeira et al. 2010).

CD4<sup>+</sup>T<sub>H</sub>1 cells are important for the control of *Leishmania* infections, owing their ability to make IFN $\gamma$ , which activates macrophages and DCs, leading to parasite death (Fig 4). CD8<sup>+</sup> T cells are known to provide immunity in visceral leishmaniasis and play an important role in resistance to reinfection (Muller, Kropf et al. 1993). CD8<sup>+</sup> T cells are not always associated with disease resolution as seen in patients infected with *L. braziliensis*. These cells are correlated with disease progression when they express the granule-associated serine protease granzyme B. The factors that determine when CD8<sup>+</sup> T cells are protective and when they promote disease remain puzzle to the investigators. Chronicity of infection with *L. donovani* appears to be caused by depletion of CD8<sup>+</sup> T cells (Joshi, Rodriguez et al. 2009). Activation of CD8<sup>+</sup> T cells depend upon dermal DCs and CD8<sup>+</sup> T cells activated during Leishmaniasis infections can provide increased resistance to previously encountered pathogens.

In spite of robust immune response, small number of parasites persist following disease resolution. The production of IL-10 dampens the immune response and allows the some parasites to escape destruction. The IL-10 is produced by a variety of cells following Leishmanial infection, such as regulatory T cells, T helper 1 cells, CD 8<sup>+</sup> T cells, B cells, natural killer cells, DCs, macrophages and neutrophils. CD8<sup>+</sup>CD40<sup>+</sup> T cells may act against regulatory T cells, limiting the production of IL-10 during the early phase of infection, but themselves become susceptible to IL-10 as the infection progresses (Belkaid, Piccirillo et al. 2002; Charmoy, Megnekou et al. 2007; Maroof, Beattie et al. 2008). Exactly how these immune mechanisms operate still remains unanswered and is an active area of research.

Dramatic remodelling occurs when leishmaniasis involve infection of lymphoid tissues like spleen and lymph nodes. Immune suppression occurs due to loss of architectural integrity. Interventions which can restore tissue microarchitecture can have important immune restorative functions.

A concept of concomitant immunity has been proposed in Leishmaniasis. It is a situation in which immunological resistance to reinfection co-exists at the same time as persistence of the original infection. The T cells which contribute to such immunity include CD4<sup>+</sup>T cells

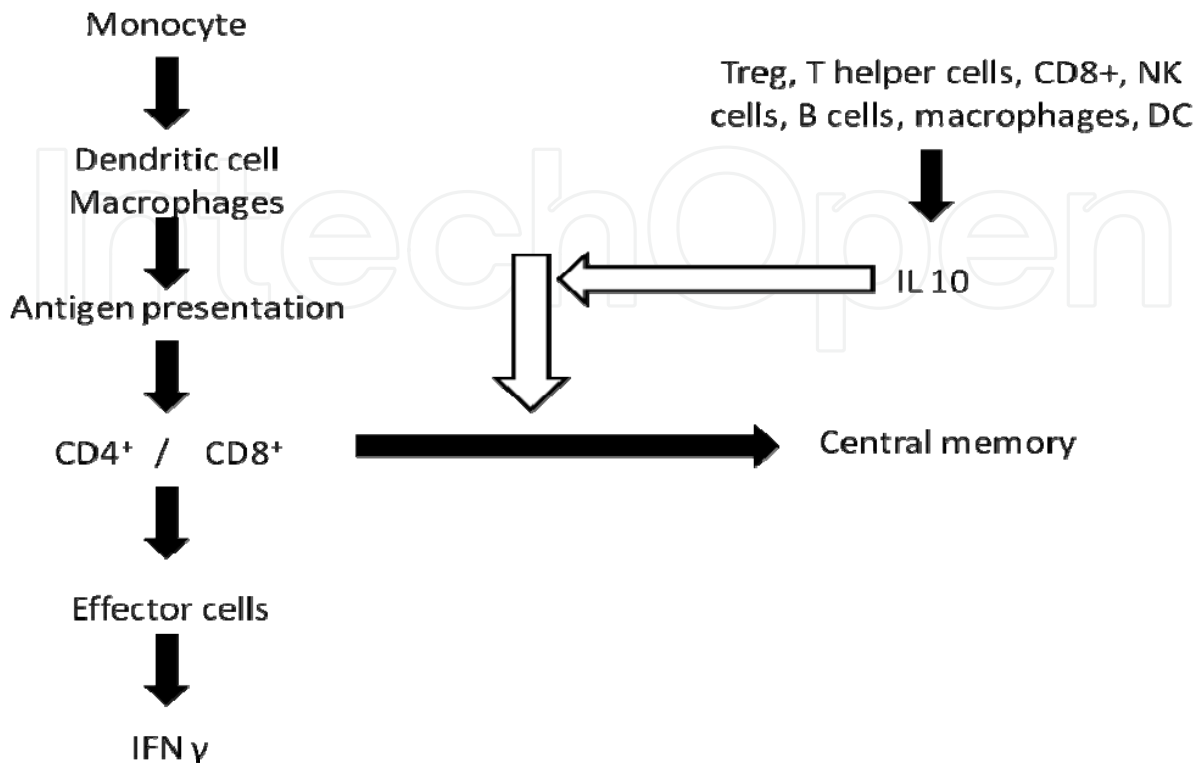


Fig. 4. Cellular components of immune response. Control of response is produced by IL 10, produced by different cell types. Effector cells produce interferon- $\gamma$  which mediate parasite killing.

with a phenotype of central memory T cells, effector T helper type 1 cells, and resting effector T helper type 1 cells. CD8<sup>+</sup> T cells are important in providing resistance to reinfection. Till date no successful vaccine has been developed but recent studies have shown that most protective CD4<sup>+</sup> T cells are those which are multifunctional, capable of producing IFN $\gamma$ , IL-2 and TNF. IL-10 appears to limit the generation of these protective T cells during vaccination (Kedzierski 2010). In future, the application of genomic approaches and study of host factors will lead to a better understanding of pathogenesis and immunology related to leishmaniasis. Further studies are required to investigate unanswered questions related to innate and T cell response in leishmaniasis.

#### 4. *Leishmania* vaccines

WHO has classified Leishmaniasis is an emerging disease. The available treatment options are various chemotherapeutic drugs which are not only costly but also have many adverse side effects. Safe and cost effective vaccine is a need of an hour. Various vaccine strategies have been tried but these are of a little hope. The classical vaccinology or first generation vaccines have been tried in the past which includes infectious material for inoculation, live attenuated parasites and killed parasites for vaccination. Leishmanization, was based on the fact that individual is refractory to reinfection after the lesions of primary illness heals. Initially, infectious lesion material was used but later it was replaced by culture of parasites

to inoculate uninfected individuals. This method was abandoned due to poor quality control, parasite persistence, emergence of HIV and ethical issues. Killed parasites replaced leishmanization, but they showed poor efficacy in clinical trials (Noazin, Modabber et al. 2008). Second generation vaccines (modern vaccinology) using subunit vaccines, DNA vaccines and recombinant vaccines are being tried but their efficacy in field trials have not been reported. The major hurdle in vaccine designing is the translation of data from animal models to human disease, and the transition of laboratory experiments to field trials. Table 3 summarizes the important vaccine candidates tested for the cure of leishmaniasis.

### **Killed vaccines**

Vaccination trials in Brazil and Ecuador with killed *Leishmania* stocks have shown to provide immunity from natural infection. Killed vaccination induced Th1 type of immune response and delayed type of hypersensitivity skin test conversion can be used as a surrogate marker for protective immune response (Olivier, Baimbridge et al. 1992; Mendonca, De Luca et al. 1995).

Convit and colleagues used a combination of killed *L. mexicana* or *L. braziliensis* promastigotes and *M. bovis* BCG to induce the immunity against South American leishmaniasis. High cure rate have been documented with the induction of Th1 type of immune response (Castes, Moros et al. 1989; Convit and Ulrich 1993). Recombinant IL-12 has been tried as an adjuvant in monkeys to provide the immunity against cutaneous leishmaniasis using killed *L. amazonensis* (Kenney, Sacks et al. 1999)

### **Live attenuated**

Live attenuated vaccines are well known for their better immunogenicity but there are chances of reverting back to virulent forms. However, recent advances in genomics have provided an opportunity to manipulate the *Leishmania* genome by eliminating the virulent genes to produce the attenuated forms. Genes required for long term survival have been manipulated to produce the short lived forms in humans. In a mouse model, *L. major* parasites lacking the gene encoding for enzyme dihydrofolate reductase-thymidylate synthetase DHFR-TS have been produced to induce the protection against infection with either *L. major* or *L. amazonensis*. Mutant lacking genes encoding for cysteine proteases *cpa* and *cpb* have also been studied. Thus, the use of attenuated organisms is very useful as it closely mimics to natural infection and can lead to similar immune responses (Titus, Gueiros-Filho et al. 1995).

### **Synthetic recombinant vaccines:**

These newer vaccines include recombinant DNA-derived antigens and peptides. The targets used as antigens may be species or life cycle stage specific. Recombinant antigens can be delivered as purified proteins, as the naked DNA encoding them, or as bacteria manufacturing the proteins of interest. These can be used as a potential vaccine candidate. Bioinformatics can be used to predict the immunogenic peptides which can be synthetically constructed. Though this approach sounds better but it suffers from many disadvantages such as the magnitude of the T-cell memory induced, the inability of all individuals in the population to respond to the peptide, and the high cost of production on large scale. Despite these limitations gp63 peptides have been successfully tested in animals (Campbell et al. 2011; Carrión J. 2011).

### Immunogens expressing Bacteria and Viruses as vaccines

Leishmaniolysin or gp63 is the first recombinant antigen to be used against as a vaccine candidate against leishmaniasis (Chang, Chaudhuri et al. 1990). The surface expressed glycoprotein leishmaniolysin (gp63) is one of the parasite receptors for host macrophages and mutants lacking this protein are avirulent. However, the T-cell responses to gp63 have been variable in animals and human studies (Olobo, Anjili et al. 1995). Parasite surface antigen have also been tested as a vaccine candidate. gp46/M2 or parasite surface antigen 2 (PSA-2) is expressed in all *Leishmania* species except *L. braziliensis*. Thus, providing an opportunity for developing pan-*Leishmania* vaccine (Handman, Symons et al. 1995). The leishmanial eukaryotic ribosomal protein (LeIF), a homologue of the ribosomal protein cIF4A, is an another important vaccine candidate as it can induce Th1-type cytokines in humans (Skeiky, Coler et al. 2002). This protein is highly conserved in evolution, but parasite specific epitopes can be used for vaccination, so that autoimmune responses can be avoided. Other vaccine candidates are amastigote specific proteins, such as A2, P4, and P8 of *L. mexicana pifanoi* (Soong, Duboise et al. 1995). Another vaccine candidate is a flagellar antigen, lcr1, from *L. donovani chagasi* (Streit, Recker et al. 2000) but its role in humans is debatable as amastigote forms have a rudimentary flagellum.

Candidate vaccine	Advantages	Disadvantages
Whole killed	Cost effective Good safety profile in South America and Sudan	Quality control, difficult to standardize, variable potency
Surface expressed glycoprotein leishmaniolysin (gp63)	Good results in animals	Poor T cell response in humans
GPI-anchored membrane protein gp46 or Parasite Surface Antigen 2 (PSA-2)	Native polypeptides derived from promastigotes provide protection in mice	Recombinant protein derived from either promastigotes or amastigotes protein showed poor efficacy
<i>Leishmania</i> homologue for receptors of activated C kinase (LACK)	Promote IL-4 secreting T cells (Th2 responses)	Fails to provide protection against visceral leishmaniasis.
Leish-111f: Single molecule constructed by fusion of three molecules: <i>L. major</i> homologue of eukaryotic thiol-specific antioxidant (TSA) <i>L. major</i> stress-inducible protein-1 (LmSTI1) <i>L. braziliensis</i> elongation and initiation factor (LeIF) Leish-110f: improved version of Leish-111f	Provides protection in mice against <i>L. major</i> and <i>L. amazonensis</i> infection Provides partial protection against visceral leishmaniasis in animal models Phase I and II clinical trials done	Failed to protect dogs against infection
Sandfly saliva components: maxadilan, 15 kDa protein, SP15, LJM19	LJM 19: protection in hamsters Dogs: IgG2 and IFN- $\gamma$	Experimental stage

Table 3. Summary of important vaccine candidates for leishmaniasis.

### DNA vaccine

Vaccinations with DNA encoding gp63 and PSA-2 have been tried. It has shown a good protection in animal models which is accompanied by Th1 immune responses (Gurunathan, Sacks et al. 1997; Walker, Scharon-Kersten et al. 1998). The genes encoding the vaccine candidate is cloned into mammalian expression vector, and the DNA is injected directly into muscle or skin. The plasmid DNA is taken up by cells and translocated to the nucleus, where it is transcribed into RNA and then translated in the cytoplasm. It has shown to induce both CD4<sup>+</sup> and CD8<sup>+</sup> T cell responses and they also ensure proper folding of proteins. Another advantage is that production on large scale is cheap and DNA is highly stable, so does not require cold chain. Research is still going on for developing a vaccine which can provide life long immunity without any side effects. Newer adjuvants are also being tried. Till date no successful vaccine has been developed but recent studies have shown that most protective CD4<sup>+</sup> T cells are those which are multifunctional, capable of producing IFN $\gamma$ , IL-2 and TNF. IL-10 appears to limit the generation of these protective T cells during vaccination (Kedzierski 2010). In future, the application of genomic approaches and study of host factors will lead to a better understanding of pathogenesis and immunology related to leishmaniasis. Further studies are required to investigate unanswered questions related to innate and T cell response in leishmaniasis.

### 5. Conclusions

Recent studies have provided new and important information on the biology of *Leishmania*. The *Leishmania* genome sequence is now available as well as new methods for its manipulation. We have learned that *Leishmania* can exchange genetic material during its journey in the sand fly, and we understand better the molecular mechanisms that allow *Leishmania* promastigotes and amastigotes to survive in their respective environments. Recent investigation have provided new insight into the role of cells of the innate immunity, such as neutrophils, monocytes, NK, and DCs, as well as 'non-immune' cells such as keratinocytes. Now we have better understand how *Leishmania* evade the mammalian immune response and avoid the development of sterilizing immunity, therefore increasing its chances to secure transmission to a new host. The identification of a greater range of antigen candidates with broad species coverage, and a greater understanding of the immunology of protective immunity, these arguments should be balanced by the need to develop a stronger base in clinical vaccinology. This end is only likely to be accomplished by an accelerated programme of well-defined clinical trials, and in this context the use of therapeutic vaccine trials as a first step has much to offer. New generation vaccines hold promises to control leishmaniasis and data suggest that prophylactic vaccination in humans and dogs could generate protection and may able to interrupt transmission, ultimately reducing disease incidence. These new generation vaccines in a therapeutic setting as an adjunct with various chemotherapies have demonstrated safety and efficacy against various manifestations of *Leishmania* infection. New generation's refined antigens and adjuvants for vaccines may provide the best range of vaccines aimed at controlling disease incidence and severity to *Leishmania* infection.

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Immunology is the branch of biomedical sciences to study of the immune system physiology both in healthy and diseased states. Some aspects of autoimmunity draws our attention to the fact that it is not always associated with pathology. For instance, autoimmune reactions are highly useful in clearing off the excess, unwanted or aged tissues from the body. Also, generation of autoimmunity occurs after the exposure to the non-self antigen that is structurally similar to the self, aided by the stimulatory molecules like the cytokines. Thus, a narrow margin differentiates immunity from auto-immunity as already discussed. Hence, finding answers for how the physiologic immunity turns to pathologic autoimmunity always remains a question of intense interest. However, this margin could be cut down only if the physiology of the immune system is better understood. The individual chapters included in this book will cover all the possible aspects of immunology and pathologies associated with it. The authors have taken strenuous effort in elaborating the concepts that are lucid and will be of reader's interest.

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