CHAPTER 1
LEISHMANIASIS:
AN OVERVIEW
Parasitic infections are a major health problem worldwide, especially in underdeveloped and developing countries. Several factors which are responsible for increasing parasitic diseases are population crowding, poor sanitation and health education, inadequate control of parasite vectors and reservoirs of infection, population migration and lastly development of resistance towards agents/drugs used for control of these diseases or vectors. In the recent past the World Health Organisation (WHO) has selected six important parasitic diseases in tropical and subtropical regions of the world for inclusion in its specific programme for research and training. It can be said that amongst the selected parasitic diseases, human leishmaniasis is second in importance only to malaria, as it possesses much greater problem both in treatment and control. Leishmaniasis is a general name given to the disease caused by infection with any member of the genus Leishmania. The current survey by WHO showed that about 12 million people are thought to be infected worldwide with more than 400,000 new cases appearing each year (WHO, 1984; Anon, 1990) and approximately 350 million people are at risk (WHO, 1990; Ashford et al., 1992)

HISTORY OF THE PARASITE:

Leishmaniasis includes a group of unicellular organism inhabiting macrophages of vertebrate hosts as an obligate parasite. They are transmitted from one host to another by a vector, sandfly (Phlebotomus spp). The natural hosts of Leishmania spp. are lizards, and other mammals, particularly canine, rodents, and primates including man.

The human leishmaniasis was first reported in 1885 by Cunningham in histological sections of oriental sore. The parasite was first named as Piroplasma donovanii, which was subsequently changed to Leishmania donovani by Ross in 1903 (Zuckerman and Lainson, 1977) after its discoverers, Leishman and Donovan who reported the organism independently, Leishman in 1900 from London and Donovan in 1903 from Madras (Peters, 1988). Wright (1903) named the causative agent of oriental sore as Helcosoma tropica (Zuckerman and Lainson, 1977). Borovsky (1988) and Wright (1903), gave an accurate description of its morphology and was named Leishmania tropica by Luhe in 1906. Nicolle (1908) established the Trypanosomatid nature of L. donovani and L. tropica, and also gave the name L. infantam to the parasite which caused infantile Kala-azar. The Russian workers Yakimoff and Schockov in 1915 described L. tropica and L. major, the causative agent of zoonotic cutaneous leishmaniasis (Zuckerman and Lainson, 1977). Leishmania braziliensis was discovered in 1913 by Vianna and Muniz and Medina discovered L. enriettii in 1948 in Panama state (Zuckerman and Lainson, 1977). The western hemisphere parasites were classified into two major groups of L. mexicana and L. braziliensis complexed (Lainson and
Visceral leishmaniasis was discovered in America in 1913 and the causative parasite was named *L. chagasi* by Margues Dacunha and Chagas in 1937 (Zuckerman and Lainson, 1977).

**GEOGRAPHICAL DISTRIBUTION:**

*Leishmania donovani* is a causative agent of Kala-azar or visceral leishmaniasis. It is widely distributed in Europe (Portugal, Spain, Italy, Malta, Greece, and Southern Russia), Africa (Morocco, Algeria, Tunisia, Libya, Abyssinia, Sudan, Northern Kenya, Nigeria) Asia (India, China, Turkistan etc.) and in South America.

*Leishmania tropica* has been reported from Africa (mainly Mediterranean sea) Europe (Spain, Italy, France, and Greece), Asia (Syria, Palestine, Armenia, Southern Russia, Iraq, Iran, Arabia, Turkistan, India, Indo-China and China), and Australia (Northern (Queensland).

*Leishmania braziliensis* (Vianna) has been reported from Brazil, Peru, Paraguay, Argentina, Uruguay, Bolivia, Venezuela, Ecuador, Colombia, Panama, Costa Rica, Mexico and South as well as Central America.

In India, the first available record of leishmaniasis was in 1862, and the disease was known as 'Jwar Vikar' in the local language of the district Jessore and took a total of 75,000 lives in three years (Sengupta, 1944). The disease is now highly endemic in the states of Bengal (Jayaraman, 1988), Orissa (Satyavati and Nando, 1987), Bihar (Thakur, 1984) and North west India (Naik et al., 1979). Few cases have also been reported from Gujarat (Gajwani et al., 1967), Kashmir (Jacob and Kalra, 1951), Himachal Pradesh (Gupta and Bhatia, 1975), Chandigarh (Naik et al., 1979), Madras (Sivaprakasam et al., 1988), Mussourie (Chand et al., 1988), Delhi and Uttar Pradesh (Kapur et al., 1979).

**TAXONOMY:**


Simplified and traditional classification of leishmaniasis based on clinical manifestations are as follows.

1. **Visceral leishmaniasis or Kala-azar:** *L. donovani*, a chronic and often fatal disease which affects the macrophages of the liver, spleen, bone marrow and lymphnode.

2. **Cutaneous leishmaniasis or Oriental sore:** *L. major, L. tropica, L. mexicana, and L. aethiopica*, which restrict their infection to dermal tissues only. This disease produces skin ulcers which leave an unsightly scar on healing. New World cutaneous leishmaniasis (NWCL) tends to be more severe and chronic than Old World cutaneous leishmaniasis (OWCL). Diffuse cutaneous
leishmaniasis (DCL) causes widespread thickening of skin.

3. Mucocutaneous leishmaniasis or Espundia: *L. braziliensis*, which replicates primarily in mucous tissues and causes gross disfigurements. This affects mucosal region of nose, ear and mouth (Marr *et al.*, 1978). The details of clinical taxonomy is summarized in Table 1. The taxonomy of genus *Leishmania* was based primarily on clinical factors, differences in developmental vectors and virulence in experimental host. Since these criteria provide insufficient basis of classification, hence, techniques like monoclonal antibody (mAb) typing, recognizing specific antigens and species specific probes (McMabon *et al.*, 1982), isoenzyme analysis (Kreutzer, 1980), DNA buoyant density (Chance *et al.*, 1974) and restriction endonucleases analysis of nuclear and mitochondrial DNA (Arnot *et al.*, 1981) have recently been used. The most common and so far most successful DNA identification method involves the use of kinetoplast DNA. It has about $10^7$ base pairs of mitochondrial DNA, including approximately 10-20000 highly reiterated mini sequences of 500-2500 base pairs, used for identification of the species. (Barker, 1987)

**MORPHOLOGY:**

The *Leishmania* species are unicellular protozoa that exist in two distinct morphological forms. The flagellated promastigotes form, resides in the midgut and hindgut of alimentary tract of their insect vector (Sandflies). The parasite exists extracellularly as the flagellated, motile promastigote (Killick-kendrick and Robertson, 1977). Promastigotes, measure 15 to 20 um in length and 2 to 3 um in width. The fully developed ones are long slender spindle shaped body. They are monoflagellated parasite having nucleus, kinetoplast and eosinophilic vacuole etc.

The nonflagellated amastigote form is spherical or ovoid 2-4 um along the longitudinal axis. It contains a central rod shaped kinetoplast and axoneme which represents the root of the nonfunctional flagellum (Zuckerman and Lainson, 1977; Chang, 1983). The different morphological form of promastigotes and their developmental sites are described in (table 2) (L.L Walter *et al.*, 1993)

**LIFE CYCLE OF LEISHMANIA PARASITE:**

The life cycle of *Leishmania* parasite consists of two different stages. In the first stage, the parasite lives as an extracellular flagellated promastigote form in the hindgut and midgut of alimentary tract of its insect vector, while in the second stage, as amastigote form residing in the cells of mononuclear phagocytic cells of the reticuloendothelial system of the mammalian host. The disease occurs when the insect vector (Phlebotomus argentipes) bites a healthy human host and injects the extracellular promastigotes into the blood stream. There, they encounter complement,
TABLE 1. TAXONOMY OF *LEISHMANIA* SPECIES KNOWN TO INFECT MAN

<table>
<thead>
<tr>
<th>Species</th>
<th>Disease</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Leishmania donovani</em></td>
<td></td>
</tr>
<tr>
<td><em>L.d. donovani</em></td>
<td>Visceral (Kala-azar)</td>
</tr>
<tr>
<td><em>L.d. infantum</em></td>
<td>Infantile Visceral</td>
</tr>
<tr>
<td><em>L.d. chagasi</em></td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>Leishmania major</em></td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>Leishmania tropica</em></td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>Leishmania aethiopica</em></td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>Leishmania mexicana</em></td>
<td></td>
</tr>
<tr>
<td><em>L.m. mexicana</em></td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>L.m. amazonensis</em></td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>L.m. pifanoi</em></td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>L.m. garnhami</em></td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>Leishmania braziliensis</em></td>
<td></td>
</tr>
<tr>
<td><em>L.m. venezuelensis</em></td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>L.b. braziliensis</em></td>
<td>Mucocutaneous</td>
</tr>
<tr>
<td><em>L.b. guyanensis</em></td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>L.b. panamanensis</em></td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>Leishmania peruvana</em></td>
<td>Cutaneous</td>
</tr>
</tbody>
</table>

* Some consider these to be separate species.
Table 2. Proposed designation of *Leishmania* forms in the sand fly gut and developmental sites.

<table>
<thead>
<tr>
<th>Designation/Name</th>
<th>Location in gut</th>
</tr>
</thead>
<tbody>
<tr>
<td>AM Amastigote</td>
<td>Bloodmeal</td>
</tr>
<tr>
<td>P1 First stumpy promastigote</td>
<td>Bloodmeal</td>
</tr>
<tr>
<td>P2 Second stumpy promastigote</td>
<td>Bloodmeal</td>
</tr>
<tr>
<td>P3 Third stumpy promastigote</td>
<td>Bloodmeal</td>
</tr>
<tr>
<td><strong>Free-swimming phase</strong></td>
<td></td>
</tr>
<tr>
<td>N1 Elongate nectomonad promastigote</td>
<td>Bloodmeal, midgut, hindgut</td>
</tr>
<tr>
<td>N2 Spatulate nectomonad promastigote</td>
<td>Bloodmeal, hindgut</td>
</tr>
<tr>
<td>N3 Short nectomonad promastigote</td>
<td>Midgut, foregut?</td>
</tr>
<tr>
<td>N4 Nectomonad paramastigote</td>
<td>Midgut, foregut?</td>
</tr>
<tr>
<td>N5 Metacyclic nectomonad promastigote</td>
<td>Midgut, foregut</td>
</tr>
<tr>
<td><strong>Attached phase</strong></td>
<td></td>
</tr>
<tr>
<td>H0 Pear-shaped haptomonad promastigote</td>
<td>Hindgut, foregut</td>
</tr>
<tr>
<td>H1 Elongate haptomonad promastigote</td>
<td>Hindgut, stomodeal valve?</td>
</tr>
<tr>
<td>H2 Spatulate haptomonad promastigote</td>
<td>Hindgut</td>
</tr>
<tr>
<td>H3 Short haptomonad promastigote</td>
<td>Stomodeal valve</td>
</tr>
<tr>
<td>H4 Haptomonad paramastigote</td>
<td>Hindgut, foregut</td>
</tr>
</tbody>
</table>

* The bloodmeal is surrounded by the peritrophic membrane.
* Parasites with a free flagellum in the lumen of the midgut, hindgut or foregut.
* In *Ph. papatasi*, this form of *Le. panamensis* develops inside the retained peritrophic sac.
* Parasites colonize the cuticular lining of the hindgut or foregut and are attached by a modified flagellum.
antibodies and phagocytic cells, all of which can kill promastigote (Hoover et al., 1985; Pearson et al., 1983). As many as 80% of the promastigotes are effectively killed by such mechanisms (Lewis and Peters, 1977). Shortly thereafter, the survivors can be found within the phagocytic cells, both neutrophils and mononuclear phagocytes. Different factors contribute to the attachment and uptake of the parasites into appropriate host cells. Long term survival is possible only in cells of macrophage family.

When infected sandfly takes blood meal, the infective promastigotes form present in its pharynx enter into the blood stream of the vertebrate host. Once this is inside the blood stream of the vertebrate host, promastigotes are phagocytosed by the mononuclear phagocytic cells of the host and transform into amastigotes which then begin replicating by binary fission within phagolysosomes. The host cells lyse, releasing free amastigotes. These amastigotes then infect the other cells (spleen, liver, bone marrow and lymph nodes) of reticuloendo-thelial system (Chatterjee et al., 1957; Ghosh et al., 1987).

The amastigotes are ingested by the sandfly during a blood meal from an infected vertebrate host. The parasite migrates to the midgut of the sandfly, where the amastigotes transform into promastigotes within 3-4 days. The flagellated parasite then gradually migrates forward to the pharynx by the fourth and fifth days. The transformation of amastigote to promastigote takes place in approximately one week (Figure -1). The promastigotes attach itself to the epithelial cell lining of the midgut of the sandfly and multiply. These are avirulent promastigotes. When promastigotes eventually cease dividing, they detach from the epithelial cells and migrate to the mouth part of the insect and these are termed as metacyclic promastigotes which are virulent form of the parasite (Sacks, 1988). The promastigotes need a basic environment (pH 7.0 to 7.5) with low temperature (24 ± 2°C) for growth. In contrast, the amastigotes perform their metabolic process, survival and multiplication at (pH 5.0 to 5.5) and high temperature of 37°C in 5% CO₂ (Chang et al., 1985).

HOST PARASITE INTERACTIONS:

There are several factors which regulate the attachment of promastigotes to the phagocytic cells. Initially, infection is dependent on the ability of promastigotes and later on amastigotes. Some problems are involved during interaction, like, homogeneity of promastigotes population (Sacks and Perkins, 1984; 1985), differentiation and activation of host cell receptors (Tait and Sacks, 1988), parasite differentiation from non-infective to infective stage in the alimentary canal of the midgut of sandfly (Sacks, 1990). In spite of these problems, parasites do succeed in developing infection in host cells. The Leishmania promastigotes bind and are internalize by all phagocytes, but they survive only in macrophages and less mature monocytes. Interaction of Leishmania promastigotes with macrophages is a receptor mediated event. The promastigotes bind
Figure 1: Life cycle of *Leishmania donovani* showing reticuloendothelial system, distribution of *Leishmania* in Kala-azar and sandfly cycle (Chatterjee, 1957).
directly to macrophage receptors or through the intervention of the host factors, i.e., complement receptors (CR1/CR 3), mannose-fucose receptor (MFR) and fibronectin receptor (FnR) (Mosser and Edelson, 1984; 1985; 1987; Russell et al., 1989). Promastigotes may utilize several different macrophage receptors depending on the parasite species and developmental stage and on the presence or absence of serum. In the presence of serum, however, the promastigotes surface is opsonized with complement component such as C3b and iC3b, and promastigotes uptake occurs via the complement receptors CR1 or CR3 (da Silva et al., 1989; Mosser et al., 1992). The host parasite attachment in the presence of serum factors of the mammalian host, may also involve, natural antibodies, oxidative metabolites, and digestive fluids or secretory products of salivary glands of the sandfly vectors.

It has been demonstrated that surface molecules of parasite like 63kDa glycoprotein (gp63) and lipophosphoglycan (LPG), bind directly to a number of macrophage receptors in the absence of serum (Handman and Goding 1985; Chang and Chang, 1986; Russell and Wilhelm, 1986) (Fig. 2). Purified gp63 has been shown to bind to the macrophage receptors as its binding to macrophage was blocked with anti-CR3 monoclonal antibodies (Russell and Wright, 1988). Thus, the binding of gp63 is mediated by CR3 receptor of the macrophages (Russell, 1990). The binding of LPG to macrophages was first demonstrated by Handman and Goding, 1985. Differential inhibition of individual members of the macrophage receptor indicated that CR3 and \( \text{P}^{150,95} \) were primarily responsible for LPG binding (Talamas-Rohana et al., 1990). LPG undergoes extensive elongation during differentiation of procyclic promastigotes to the metacyclic promastigotes. This alteration leads to increased complement resistance of Leishmania parasites and a thick coat is formed on the parasite surface (7nm in log phase-17nm in stationary phase) (Sacks and da silva, 1987, Pimenta et al., 1989; da Silva et al., 1989). The MFR of macrophage may also binds to the LPG and help in parasite phagocytosis. It has been observed that lectins like receptors are present in the midgut of sandfly that act as hemagglutinins and are inhibited by only three sugars (galactosamine, glucosamine, mannosamine). The phosphorylated tetrasaccharides, effectively inhibit (>70%) the binding of procyclic promastigotes to P. papatasi (Pimenta et al., 1992). LPG has been shown to be a ligand to the sandfly midgut epithelium as only L. major strain having complete procyclic LPG was found to be attached to the midgut of their natural vector, P. Papatasi, while Leishmania of other species like, L. donovani, L. tropica and L. amazonensis failed to attach to the epithelium cell lining of midgut of P. papatasi. Contrary of this, P. argentipes a natural vector of L. donovani supported the growth of L. donovani, L. major, L. tropica and L. amazonensis promastigotes in the epithelium of sandfly midgut. Furthermore, L. donovani mutant deficient in LPG failed to attach to the midgut of this sandfly. The multiple \( \beta \) linked galactose residue of L. major LPG was found to be responsible for the attachment to the epithelial cell line of midgut of P.
Figure 2: Receptor ligand interactions between *Leishmania* major promastigotes and human macrophages. The two major cell surface glycoconjugates, LPG and gp63 are depicted as controlling the attachment of different developmental stages of promastigotes on which the expression of the LPG is developmentally modified. Multiple interactions might be required for subsequent events associated with receptor activation and internalization (Tait and Sacks, 1988).
*Papataki* and was found to be species specific. Contrast to this the *P. argentipes* midgut possesses a receptor for relatively conserved oligosaccharides or 'lectin like' molecules of procyclic LPG of different species of *Leishmania* parasites. These observation suggesting that LPG is a ligand to the sandfly midgut epithelium and plays an important role in host parasite interaction (Pimenta *et al.*, 1994; Volf *et al.*, 1994; Sacks and Turco, 1995). Acid phosphatase is also a cell surface molecule and reported to be secreted in the culture medium, (Dwyer and Gottlieb, 1985; Glew *et al.*, 1988). Two form of acid phosphatase are present, a membrane bound, tartrate resistant and other a secretory acid phosphatase. They are antigenically distinct and exist as multiple isoenzymes present in both stages of most *Leishmania* spp. It has been shown that acid phosphatasases dephosphorylate certain phospholipids and phosphoproteins which may provide the parasite a source of energy as inorganic phosphate for growth. *Leishmania* spp. possess a superoxide dismutase which converts \( \text{O}_2^- \) to \( \text{H}_2\text{O}_2 \), which can diffuse into the extracellular spaces and kill *Leishmania* parasites. Promastigotes are generally more sensitive than amastigotes to \( \text{H}_2\text{O}_2 \) and \( \text{O}_2^- \) (Murray, 1981; Pearson *et al.*, 1983). Thus, this ectoenzyme is thought to protect *Leishmania* spp. by interfering with regulatory mechanism of the macrophages that produces microbicidal free radicals (Glew *et al.*, 1988).

The mechanism of host resistance and susceptibility are dependent on the \( T_H1/T_H2 \) type response and macrophage-cytokine interactions. The resistance correlates with the \( T_H1 \) cell response, while susceptibility correlates with a \( T_H2 \) cell response (Maingon *et al.*, 1995; Kemp *et al.*, 1996). Production of IFN-\( \gamma \) by \( T_H1 \) cells has been found to be essential for macrophage activation, microbial clearance, healing and protective immunity in *Leishmania* infection (Belosevic *et al.*, 1989). Conversely, nonhealing response in susceptible mice have been related to the expression of the \( T_H2 \) subset and the production of cytokines such as IL-4 and IL-10 (Heinzl *et al.*, 1989; Locksley *et al.*, 1991). IL-12 and IFN-\( \gamma \) are known to be associated with the healing process and also play an important role in immunity against human visceral leishmaniasis (Ghalib *et al.*, 1995). The production of both IL-12 and IFN-\( \gamma \) is inhibited by IL-10. It has also been demonstrated that the disruption of IL-4 gene can confer protective immunity by \( T_H1 \) 1 type immune response, as determined by positive DTH and IFN-\( \gamma \) production (Satoskar *et al.*, 1995). In addition, production of nitric oxide has been found to be most likely an effector mechanism for parasite killing (Bhakuni *et al.*, 1996; Stefani *et al.*, 1994; Bogdan *et al.*, 1993).

**PATHOGENESIS AND SYMPTOMS:**

*Leishmania* is an obligate intracellular parasite that cause a broad spectrum of diseases in man and other animals, ranging from self-healing skin ulcers to a fatal visceral infection.
The *Leishmania* parasite after entering the host, invades the cells of the reticuloendothelial system where it resides and multiplies. Thus, the parasites are distributed all over the body and are particularly found in tissues rich in reticuloendothelial cells. They multiply in the reticuloendothelial cells, specially the macrophages in spleen, liver, lymph nodes and bone marrow, which become heavily parasitized. The initial infection and the appearance of the clinical manifestations are generally observed from three weeks to six months (Thakur, 1984), but the incubation period may extend up to 2-9 years (Faust and Russell, 1964). Early stage of visceral leishmaniasis is characterized by malaise, headache, and fever occurring at irregular intervals later becoming regular and accompanied by chills and sweating. Chronic visceral leishmaniasis produces marked enlargement of liver and spleen (hepatosplenomegaly). The spleen is more enlarged as compared to liver. The other symptoms are high fever, pyrexia, lymphadenopathy, anaemia, the skin over the body becomes dry, rough and harsh and is often pigmented (darkened) (Sen Gupta et al., 1956; Khalid et al., 1990). The hair tends to be brittle and fall off. There is a reversal of normal albumin-globulin ratio.

In the active stage of disease, the peripheral blood (T/B cell) ratio is also reversed (Rezai et al., 1977). Kala-azar patients are also found to have a defective cellular immunity, as detected through skin test (Manson Bahr, 1961). Lesions in the bronchi and lungs, intestinal ulcers and hemorrhages in the internal organs are also reported to occur simultaneously. In the later stage of disease the skin usually becomes black in colour and there is continuous high fever, justifying the name of the disease 'Kala-azar' (Black sickness). If patients are untreated, 75–95% die within a period of two years. Death in Kala-azar is always due to secondary infections, such as amoebic or bacillary dysentery, pneumonia, pulmonary tuberculosis, cancerum oris and other septic infection (Nag and Ghosh, 1955). Due to the suppressed immune system tuberculosis and AIDS are opportunistic infection in Kala-azar patients (Fuzibet et al., 1988, TDR News No. 36, 1991).

In about 20% of Indian Kala-azar patients, there are non-ulcerative nodular cutaneous lesions known as post Kala-azar dermal leishmaniasis (PKDL), generally occurring one or two years after the completion of sodium stibogluconate treatment. PKDL lesions have also been found in some spontaneously cured Kala-azar cases (Rees and Kagar, 1987; Chessborough, 1988).

**DIAGNOSIS OF KALA-AZAR:**

Clinical diagnosis of the kala azar on the basis of signs and symptoms (prolonged fever, hepatosplenomegaly, abdominal distension, pallor, anorexia, anaemia, leucopaenia, thrombocytopenia and hyperglobulinemia, etc.) is very difficult because these symptoms may also be present due to other diseases. Therefore, parasitological confirmation before treatment is essential. Parasites may be demonstrated in the aspirates of spleen, bone marrow and lymph nodes. The demonstration of the *Leishmania* parasites by (direct evidence method) blood smears or blood
culture and biopsy of the parasitized tissues is possible and biopsy of bone marrow and spleen are still used today. Although spleen aspiration is a high risk technique and there is no consensus on its use, however, because of its superior sensitivity it is the preferred method. The bone marrow puncture, which is widely practiced, is less hazardous and easy to perform than spleen aspirates, is more painful and labour extensive. Bone marrow aspirated samples are cultured in vitro for propagation of the parasite and also smeared on microslides for microscopic examination. Blood smears are generally negative because of low levels of circulating parasites. Blood culture method is time-consuming, and less sensitive. Therefore, it is neither preferred nor used now a days.

A number of serological, immunological and DNA based techniques are now being applied for the diagnosis of Kala-azar. The most widely applied immunological test in leishmaniasis has been the leishmanin or Montenegro's skin test. Which is usually positive in CL and MCL. It is negative in acute VL and most cases of PKDL but becomes positive after recovery. It is also negative in DCL. The leishmanin reaction is not species specific (Show and Lainson, 1967; 1974; 1975; Southgate and Manson Bahr, 1967). The skin test could be used for evolution for the effective immunization with killed Leishmania promastigotes (Mayrink, et al., 1978) or leishmanization applied in the control of cutaneous leishmaniasis (Nadim et al., 1983). Some serological tests based on specific antibody or antigens present in the serum, such as ELISA, have been developed. This is widely used and is most suitable and sensitive technique for detection of Kala-azar. ELISA has the advantage of being useful for both antigen and antibody detection. The Dot-blot ELISA test is similar to ELISA but the antigens are used on paper or plastic support to detect antibody which is then visualized using antiglobulins labelled with enzymes (Voller, 1993).

Diagnosis method based on parasite DNA are very useful but still face a number of limitations as it requires specific DNA probe and sophisticated instrument facilities. Recently a more sophisticated polymerase chain reaction (PCR) technique, is being used for detection of Kala-azar (Salkl et al., 1988; Audya et al., 1992). In PCR, a specific target DNA fragment is enzymatically amplified from total DNA or RNA of given organism using a thermostable DNA polymerase and oligonucleotide primers flanking the target site. The specificity of the primers normally ensures that other DNA sequences are not amplified during amplification. The main advantage of PCR is greater sensitivity for diagnosis of Kala-azar, very low level of parasitemia can be detected by this. At present PCR has no field application since the technique requires high skill and also sophisticated equipments.

**IMMUNITY TO THE VISCERAL LEISHMANIASIS:**

In human infections, the specific immune response to the pathogen may be of vital importance for host defense. An inappropriate response may not only result in lack of
protection, it may even contribute to the pathology of disease. After infection with *Leishmania* parasites, humans often develop immunity to reinfection. The protective immunity against both visceral (Skov and Twohy, 1974 a;b) and cutaneous leishmaniasis (Preston and Dumonde 1976) is a T-cell mediated process. Generally the killing of amastigotes inside the macrophages requires the release of T-cell factors that lead to activation of the macrophages and produce more of the lethal substances which eventually kill the parasites. If T-cell activation and lymphokine production does not occur, then the macrophages will not be able to kill the parasites (Leiw, 1990). T-lymphocytes can recognise antigens associated with class I and class II MHC molecules i.e. CD8 and CD4 T-cells respectively, on the surface of accessory or antigen presenting cells (APCs). Leishmanial antigen has been demonstrated on the surface of infected macrophages (Farah et al., 1975; Berman and Dwyer, 1981; Handman and Hocking, 1982), but it has not been associated with the concomitant expression of class II molecules.

The production of co-stimulatory factors, including IL-1, IL-2, IFN-γ by the accessory cells promote antigen specific lymphocyte activation (Unanue, 1984; Kaye, 1987; Unanue and Allen, 1987; Weaver and Unanue, 1990; Kaye et al., 1991). An adherent cell populations (macrophages) have been found to be associated with immunosuppression in leishmaniasis (Scott and Farrell, 1981; Reiner and Malemude 1984; 1985; Murray et al., 1986). Impairment of the immune response has been found to be associated with inability of macrophages to produce IL-2 upon specific or mitogenic stimulation (Reiner and Finke, 1983; Cillari et al., 1986; Murray et al., 1987).

An antigen specific unresponsiveness is common during active visceral leishmaniasis. It is associated with reduced IFN-γ and IL-2 production (Carvalho et al., 1984a; Kaye et al., 1991). However, the absence of these co-stimulatory factors in chronic infected mice with *L. donovani* resulted in the failure of providing the necessary signals to activate IFN-γ producing T-cells. So far, IL-1 has been identified as co-stimulatory factor for activation of Th2 cells, but not Th1 cells which induced Th2 cell proliferation and disease exacerbation. (Kurt Jones et al., 1987). Freund's complete adjuvant is a potent stimulator of Th1 cells (Gun and maurer, 1989), which can promote a protective response against PT3, a T-cells epitope derived from the primary structure of *L. major* gp63 (Jardim et al., 1990). PT3 is found to activate IL-2 and IFN-γ producing T-cells in presence of adjuvant, while PT3 alone is able to induce Th2 cell and these cells produced more IL-1. Therefore, disease exacerbation and protection depend on the expression of Th2 and Th1 cells, respectively (Fig. 3).

**Humoral Immunity:**

Immune responses, including antibody, complement and phagocytes, participates in the destruction of promastigotes that enter the body. The local production of antibody at the site
Figure 3: The development of antigen-specific responses by T-cell subset in humans infected with *Leishmania* depends upon balance of TH₁ and TH₂ cells which are responsible for disease resistance and disease exacerbation. In addition, cytokines produced locally by non specific cells may provide an environment that favours development of either TH₁ or TH₂ responses (Kemp et al., 1996).
of infection have been shown by presence of plasma cells in leishmanial lesions (Stahur et al., 1970). A few studies had revealed the production of IgG1, IgG3, IgE and IgA during the course of infection of leishmaniasis (EL-Amin et al., 1986; Lunch et al., 1986). Clinically, non-healing visceral leishmaniasis have been associated with high immunoglobulin levels and negative DTH, while healing or cured individuals display strong DTH and low levels of antibody (Turk and Bryceson, 1971). Antibodies may also influence the course of infection by directly affecting parasite-macrophage interactions. The potential protective functions of antibodies have also been demonstrated in vivo (Anderson et al., 1983). A monoclonal antibody against a surface glycoprotein (M-2) of L. mexicana has been shown to be protective, if inoculated into the footpads of BALB/c mice along with parasites. Vaccination with M-2 (glycoprotein) plus adjuvant also induce protection, which is associated with increased antibody levels (Champsi and McMahon-Pratt, 1988, Reiner and Locksley, 1995; Liew and O'Donnell, 1993).

Cellular immune response:

Not only humoral immunity plays a role in Leishmania infection but cell mediated immunity also plays a major role in protection. The more protective immune response for the resolution of Leishmania infection is the cellular reactions (Pearson et al., 1983; Liew, 1986; 1989). Control of the infection is influenced by the induction of immune responses in various T-cell subpopulations. There may be preferential induction of protective T-cell reactions. The protective T-cell secretes γ-interferon (IFN-γ), which is a potent activator of macrophage microbicidal activity (Liew, 1989). Although interferon acts by stimulating macrophages to activate antimicrobial activity, but other lymphokines are also necessary for the efficacy of interferon (Davis et al., 1988). During early infection, all types of lymphokines are produced, but as infection progresses, either the protective or the disease-promoting responses take precedence (Scott et al., 1988). Recently it has been demonstrated that a balance between Th1 and Th2 cells is responsible for disease exacerbation or resistance (Kemp et al., 1996) (Figure 3).

(I) CD8⁺ T cells mediated immunity:

The T-cells conferring protection or counter protection primarily belong to the CD4⁻ T-cell subset (Mitchell et al., 1981; Liew et al., 1982; Louis et al., 1982; Gorczynski, 1985). CD8⁺ T-cell plays a protective role in leishmaniasis. Administration of anti CD4⁺ monoclonal antibodies increases resistance to L. major (Titus et al., 1985; Hill et al., 1989) while administration of anti CD8⁺ monoclonal antibodies exacerbated infection in vivo condition in mice (Farrell et al., 1989). A protective role of CD8⁺ T-cell subset during L. donovani infection is found which is associated with the inhibition of parasite growth in hepatic nodules (Mc Elrath et al., 1988). However, CD8⁺ T-cells can produce IFN-γ upon specific stimulation (Kaufmann 1988; Salgame et al., 1992), and
activate macrophages to kill *Leishmania* parasites (Smith *et al.*, 1991).

(II) CD4+ T-cells mediated Immunity:

The resistance and susceptibility of the parasites are mediated by at least two different CD4+ T-cell subsets (Scott, 1989; Liew, 1989). These are the T_H1 and T_H2 CD4+ T-cell subsets which are morphologically identical, but functionally distinguishable, on the basis of cytokines production. (Mosmann *et al.*, 1986; Cherwinski *et al.*, 1987). T_H1 cells produce IFN-γ and IL-2, and mediate DTH and IgG2a antibody production, while T_H2 cells produces IL-4 and IL-5, and promote IgE and IgG1 production but do not mediate DTH. Several other cytokines including IL-3 and Granulocyte-macrophage colony-stimulating factor (GMCSF) are produced in varying amounts by both cell types. Based on the various studies on the *Leishmania* resistant and susceptible mice it has been suggested that the T_H1 cells have a protective role and T_H2 cells are counter protective. The protective T-cell lines and clones secreted IFN-γ and IL-2, while the counter protective T-cell lines and clones secreted IL-4 and IL-5. Healing and resistance are invariably associated with IFN-γ and IL-2 secretion, and susceptibility and non-healing with IL-4 and IL-5 production. The IL-3, which is produced by both T_H1 and T_H2 cells, has been shown to be counter protective (Scott *et al.*, 1989; Muller and Louis, 1989; Alexander and Russell, 1992; Rollinghoff, 1990).

(III) Lymphokines/cytokines mediated immunity:

Killing of *Leishmania* parasites by IFN-γ and TNF-α in activated macrophages have been demonstrated. TNF-α and IFN-γ act synergistically to activate macrophages leading to the killing of *Leishmania* parasites (Liew *et al.*, 1990; Paul, 1993). The leishmanial protection mediated by IFN-γ has been shown to be inhibited by IL-3 (Liew *et al.*, 1989). Pretreatment of macrophage with IL-4 inhibits macrophage activation and parasite killing mediated by IFN-γ, while treatment of infected macrophages suggested that IL-4 enhances IFN-γ mediated leishmanicidal activity (Phillipes *et al.*, 1990). Thus, interplay between IL-4 and IFN-γ in inducing macrophage for leishmanicidal activity is very complex. IL-4 act synergistically with IFN-γ to promote macrophage activation resulting in release of TNF-α, which activates the leishmanicidal activity of macrophages (Solbach *et al.*, 1991).

Recent studies have suggested that IFN-γ has a capacity to enhance host defense in euthmic animals (Murray, 1988; 1994). Antileishmanial activity in vitro or in vivo in euthmic mice can be induced by regulated T-cells or can be modulated by IFN-γ (Murray *et al.*, 1993; 1995; Murray, 1995; Tumang *et al.*, 1994; Liew *et al.*, 1990). IFN-γ can directly activate macrophages in vivo regardless of the host's complement of T-cells and indicates that CD4+ or CD8+ T-cells can act with IFN-γ to activate intracellular microbial activity. Based on these findings it was suggested that for most T-cell-deficient patients, including AIDS patients, with preserved CD8+ cells, an adjunctive
therapy with IFN-γ should be explored for opportunistic intracellular infection.

In addition to IFN-γ, in vivo responsiveness to exogenous administration of other cytokines like, TNF-α, IL-1, IL-2, IL-7, IL-12 and GM-CSF also appears to require participation of host T-cells for optimal induction of antitumor or non-viral antimicrobial activity (Murray et al., 1993; 1995; Murray and Hariprasad, 1995; Brunda et al., 1993; Hock et al., 1991; North et al., 1988; Aukerman et al., 1990).

IL-10, a TH2 type associated cytokine, inhibits antigen-specific cellular responses in active visceral leishmaniasis. It has been shown to induce many inhibitory effects on IFN-γ production and function on the macrophage capacity of antigen presentation and cytotoxicity (Moore et al., 1990; Bogdan et al., 1991; De Waal Malefyt et al., 1991). IL-10 was shown to inhibit human lymphocyte IFN-γ production, by suppressing NK-cell stimulating factor.

IL-12 is a pluripotent cytokine that interacts with NK and T-cells to play a central role in the initiation and maintenance of Th1 responses and IFN-γ production and is associated with cure of L. major infections (Heinzel et al., 1993; Scott, 1993; Sypek et al., 1993; Chan et al., 1991). The production of IFN-γ by Th1 cells appears to be essential for macrophage activation, microbial clearance, healing and protective immunity in leishmanial infection (Belosevic et al., 1989). The production of IL-12p40 by PBMC from treated patients has been reported to be markedly reduced by addition of anti-IFN-γ (Kubin et al., 1994). The production of these two cytokines after successful treatment was found to be interdependent. This provides evidence that IL-12 and IFN-γ are associated with healing process and could play an important role in immunity against human visceral leishmaniasis (Finkelman et al., 1994; Zhang et al., 1994; Ghalib et al., 1995; Murray et al., 1995; Kemp et al., 1996, Tremblay et al. 1996, Milon et al, 1995, Solbach and Laskay, 1995) Figure 4.

(iv) Reactive Oxygen Species:

The toxic metabolites of oxygen, superoxide (O₂·), singlet oxygen (O₃), the hydroxyl radical(OH) and most especially hydrogen peroxide (H₂O₂) have been thought to be responsible for macrophage leishmanicidal activity (Murray, 1981; Pearson et al., 1982; da Silva et al., 1989). Evidence for this viewpoint arose because of studies that demonstrated that amastigotes of L. donovani and metacyclic promastigotes of L. major survived better than, and triggered the macrophage respiratory burst only weakly compared with, log phase promastigotes. This ability was also attributable to amastigotes having higher levels of glutathione peroxidase, superoxide and catalase than promastigotes (Murray, 1982; Pearson et al., 1983). Intracellular amastigotes are also capable of down-regulating the macrophage oxygen-dependent microbicidal potential (Buchmüller-Rouiller and Mauel. 1987). Nevertheless, there has been increasing evidence from several studies
Figure 4: How the immune system fights *Leishmania* parasites:

1. *Leishmania* parasites proliferate inside the organelles of cells, where they are beyond the reach of antibodies. Macrophages ingest *Leishmania* organisms and hold them in vesicles called endosomes. Many organisms trapped in endosomes are later destroyed by enzymes from lysosomes. *Leishmania* parasites, however, survive and multiply in the endosomes. (Paul, 1993).

2. Class II MHC molecules produced by the infected macrophage bind to peptides from the parasite. The MHC molecule carries the peptide to the cell surface.

3. CD4+ T cells that have complementary receptor molecules are activated by peptides class II complex and by the B7 molecule on the surface of the macrophage.

4. The activated T cells secrete certain cytokines that bind to receptors on the macrophage. In response, the macrophage produces TNF, NO and other factors that kill the parasites.
that oxygen-independent mechanisms are capable of killing *Leishmania*; not only are *L. donovani*, *L. mexicana*, and *L. major* resistant to oxygen radicals (Pearson *et al.*, 1982; Mallinson and Coombs, 1989b), but they can be killed by macrophages deficient in the production of oxygen metabolites (Murray and Cartelli, 1983; Scott *et al.*, 1985).

Recently a new mechanism of macrophage anti-leishmanial killing has been characterized as a novel metabolic pathway synthesizing nitric oxides (nitric oxide (NO), nitrite oxide (NO$_2^-$) and nitrate oxide (NO$_3^-$)) from L-arginine with L-citrulline as a co-product (Green *et al.*, 1990; Liew *et al.*, 1990a,b).

Nitric oxide is derived from the guanidinonitrogen of L-arginine and molecular oxygen in a reaction catalyzed by the enzyme nitric oxide synthase (NOS) (Palmer *et al.*, 1987; Marletta *et al.*, 1988; Hibbs *et al.*, 1988). Nitric oxide has been shown to play an important role in the killing of the protozoan parasites of *Leishmania* species (Liew and Cox, 1991; Mauel *et al.*, 1991; Roach *et al.*, 1991; Bogdan *et al.*, 1993; Paul *et al.*, 1993; Stefani *et al.*, 1994). Macrophage NO mediated killing of *L. major* has been shown to be induced by TNF-α acting synergistically with IFN-γ (Liew *et al.*, 1990a). This biochemical pathways, as well as anti *Leishmania* activity, is inhibited in the presence of D-arginine and N-monomethy L-arginine (Liew *et al.*, 1990b; James and Hibbs 1990).

**TREATMENT OF LEISHMANIASIS:**

The World Health Organization (WHO) estimates 12 million cases of leishmaniasis worldwide, with annual figures of one million for visceral leishmaniasis (VL) and three million for cutaneous leishmaniasis (CL) (Anon, 1990). Cutaneous leishmaniasis is a self healing and relatively benign disease but visceral leishmaniasis or kala-azar, is a wide spread, severe and often fatal disease in the absence of specific chemotherapy. Several chemotherapeutic agents against human visceral leishmaniasis are available.

**CHEMOTHERAPY OF LEISHMANIASIS:**

Antimonials Drugs:

The antimonial drugs are most suitable for chemotherapy of leishmaniasis. These are known to be tissue specific in their action. They accumulate most rapidly in liver where their efficacy is higher as compared to that in spleen (Collins *et al.*, 1992). The antimonial drugs are divided into two main groups: trivalent and pentavalent antimonials. Trivalent antimonial (urea stabamine) is now rarely used for the treatment of leishmaniasis. Pentavalent antimonials are as follows:
Pentavalent antimonials:
The important pentavalent drugs are:
(I) Sodium stibogluconate (Pentostam)
(II) Meglumine antimonate (Glucantime)
(III) Neostibosan (Ethyl stibamine)
(IV) Urea stibamine (Corbostibamine, stiborea) and derivatives of stibanic acid such as stibacetin.

Among these, sodium stibogluconate and meglumine antimonate are drugs of choice till date. Sodium stibogluconate (Pentostam Sb\textsuperscript{+}) developed by Wellcome foundation, UK, in 1925 was first used in China with great success. Pentostam is more potent and well tolerated antimonial drug (Berman, 1988; Thakur et al., 1988), which is used for visceral leishmaniasis (Black et al., 1977; Chulay et al., 1988; Thakur et al., 1988; 1990). The recommended dose of Sb\textsuperscript{+} is 10-20 mg/kg/day (im or iv) for 20-40 days depending on the geographical distribution and, body surface area of patients and also to reduce unresponsiveness to the drug (Thakur et al., 1988; 1989; Olliaro and Bryceson, 1993; Chulay et al., 1983; Chance, 1995).

The drawback of Pentavalent antimonial is their rapid excretion by the kidneys, which is about 60-80% within six hrs of administration. This leads to acute toxicity, perhaps due to the production of trivalent metabolites, which increases in proportion with dose and duration (Bryceson, 1987). Besides incomplete cure, the usual side effects are vomiting, diarrhoea, abdominal pain, anorexia, malaise, itching, fever, dizziness, headache, cardiotoxicity and nephrotoxicity with pentostam have also been reported (Chulay et al., 1985; Ree et al., 1985; Karen et al., 1987).

In cutaneous leishmaniasis (CL), the parasites are relatively insensitive to antimonials, as the concentration of the drug tends to be more in liver and spleen (Bryceson, 1987), than reaching in desired concentration to skin (Thakur and Kumar, 1990; Herwaldt and Berman, 1992). Mucocutaneous leishmaniasis (MCL) is relatively poor responder to antimonials, possibly due to the site of infection, poor immune response and partial drug resistance to antimonials (Grogl, 1989).

Meglumine antimoniate (Glucantime\textsuperscript{b}) is equally well accepted for American leishmaniasis (Chulay et al., 1988; McGreyv and Morsden, 1986). The recommended dose and schedule is summarized in table 3.

Aromatic Diamidines:

The search for alternatives to antimony therapy, led to the discovery of some aromatic diamidines, such as stilbamidine (Ashley et al., 1942), hydroxystilbamidine (Kagan et al., 1987), and pentamidine (Ashley et al., 1946; Ashley and Harris, 1946; Fastier, 1962). In 1944, stilbamidine was used for treatment of kala-azar in Mediterranean region. It has several side effects, viz. lowering of blood pressure, respiratory disorder, neurotoxicity and trigeminal neuropathy.
<table>
<thead>
<tr>
<th>Drug of Choice</th>
<th>Toxicity</th>
<th>Status in Clinical Medicine</th>
<th>Schedule in Clinical Medicine</th>
<th>Dose Schedule</th>
<th>Notes</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**Table 3: Drugs in Use Against Visceral Leishmaniasis in Humans**
(Napier et al., 1942). Hydroxystilbamidine being stable and less toxic was preferred over stilbamidine (Sen Gupta, 1950; Snape, 1952).

**Pentamidine:**

Pentamidine is the drug of choice for treating Kala-azar patients, who have failed to respond to antimony therapy (Jha 1983). Pantamidine is less toxic than stilbamidine and can be administered intramuscularly. It is more effective against visceral leishmaniasis than cutaneous leishmaniasis and mucocutaneous leishmaniasis (Steck, 1974; Iyer, 1985). The recommended dose, mode of action and various side effects are summarized in table 3.

**Antifungal antibiotics:**

(I) Amphotericin-B:

Amphotericin B desoxycholate is a polyene antibiotic. It is effective against cutaneous and mucocutaneous leishmaniasis, (Sampio et al., 1985). Amphotericin B is 400 times as potent as sodium stibogluconate in infected hamsters and monkeys. Amphotericin B is formulated as a colloidal suspension which is administered as a slow intravenous infusion. Its usefulness is limited by adverse reactions including anaphylaxis, thrombocytopenia, flushing, generalized pain, convulsions, chills, fever, phlebitis, anaemia, anorexia, decreased renal tubular and glomerular function and hypokalaemia in about one-third of patients treated (Bryceson, 1978). It has therefore, never been considered a satisfactory first line drug for leishmaniasis and there is little evidence of its use in visceral leishmaniasis (Thakur, 1991; Mishra et al., 1992). Amphotericin B tried at very low dose administration on alternate days, with a shorter schedule was found to be more effective with moderate toxicity (Mishra et al., 1994; Thakur et al., 1994; 1996).

Amphotericin B is still of considerable interest because of its mode of action. It binds to sterols in the plasma membrane, forming pores and leakage of ions. It binds preferentially to 24 substituted sterols such as ergosterol, which is the major cell membrane sterol of *Leishmania* parasite but not of mammalian cell membranes (Barman, 1991). To a lesser extent it binds to cholesterol in human membranes, and this leads to its toxicity. (Lianos-cuentas et al., 1991). The major advancement has been the new formulations of amphotericin B with lipids (Pearson, et al, 1996).

Liposomal amphotericin B (AmBisome, Vestar, San, Dimas, CA, USA) was developed by incorporating amphotericin B into liposomes made of phosphatidylcholine, cholesterol, and disteroyl phosphatidyl glycerol. Pharmacokinetic data, indicates that AmBisome (Liposomal amphotericin B) is well suited for the treatment of visceral leishmaniasis. It has been used successfully in patient with visceral leishmaniasis unresponsive to standard treatment (Davidson et al., 1994; 1996, Seamon et al 1995). Amphocil is a mixture of amphotericinB with cholesterol sulphate (a 1:1 molar ratio).
has shown to be effective against visceral leishmaniasis (Dietze et al., 1993; 1995).

(ii) Ketoconazole:

This drug has been used to treat cutaneous and visceral leishmaniasis (Berman, 1988; Sunder et al., 1990; Wali et al., 1990). Like pentamidine and amphotericin B, Ketoconazole was also found effective in antimony resistant cases (Berman, 1982; Scott et al., 1992; Wali et al., 1990; Sunder et al., 1990). The major side effects of the drug are vomiting, nausea and hepatic toxicity. Itraconazole is safer than ketoconazole and has a more favorable pharmacokinetic profile. Being retained in skin for up to two weeks.

Other Antileishmanial Drugs

(i) Levamisole:

In the chronic forms of L. tropica infection, good therapeutic response by levamisole in clinical trial has been reported by Butler, (1978) and also against L. mexicana in mice (Grimaldi et al., 1980; Rezai et al., 1988).

(ii) Aminosidine:

Aminosidine (Paromomycin) is an antibiotic of amino-glycoside family. It is available as an injectable formulation given either by intramuscular injection or intravenous infusion. Aminosidine is highly effective against visceralizing species and Old World cutaneous leishmaniasis, but susceptibility varies in different leishmanial species of New World cutaneous leishmaniasis. Aminosidine is found to be highly active against antimony resistant strains. It is nearly six fold and more than 600 fold active than sodium stibogluconate against L. mexicana and L. major, respectively. Aminosidine is a safe, well tolerated effective 'first line' alternative to pentavalent antimony for the treatment of native and unresponsive visceral leishmaniasis (Olliaro and Bryceson, 1993).

Although chemotherapy has been available for many years but it is not sufficient to cure the patients of leishmaniasis. The drugs are fairly toxic, duration of treatment is undefined, mechanism of action is poorly understood and their success rate is variable and particularly poor in immunodepressed individuals most notably in patients with acquired immunodeficiency syndrome (AIDS). Visceral leishmaniasis (VL) has now been established as an HIV-associated infection. The immunopathological picture in VL/HIV infections is different. Patients with VL and HIV infections have defecting cell mediated immunity. They need prolonged treatment with antimonials and are liable to relapse. The recent epidemics of VL, parasite resistance to antimony, and the poor response in patients suffering from HIV infection has increased the urgency of new therapy or
The success of any chemotherapeutic regimen is often dependent on the potential or latent immunological response by the infected host. Successful chemotherapy of leishmaniasis in human results in the generation of antigen-specific T-cells and delayed type hypersensitivity (DTH). When the patients have defective immune response, chemotherapy tends to be ineffective (Rizzi et al., 1988).

The macrophage is an essential cell for *Leishmania donovani*, because it is the only cell in which the *Leishmania* species replicate during mammalian infection. Therefore, an alternative approach to therapy of visceral leishmaniasis would be to take the advantage of the host macrophages. Macrophages have been implicated in host defense against tumors and various intracellular and extracellular pathogens. The effective control of *Leishmania* infection in both mice and human has been shown to occur through cytokine induced macrophage activation (Green et al., 1991; Murray, 1990; 1994a; 1994b).

Two new immunotherapeutic approaches in VL have been explored treatment with IFN-γ alone or treatment with IFN-γ plus conventional antileishmanial drugs. The appeal of the latter regimen stems from its potential to simultaneously attack intracellular *L. donovani* by different mechanisms: one utilizing direct toxicity to the protozoan with the parasitized macrophage (antimony) and the other involving effects on the macrophage itself (Murray, 1990; 1994). The studies have demonstrated that IFN-γ enhances both *in vitro* and *in vivo* antileishmanial effects of sodium stibogluconate (Murray et al., 1988). Recently a successful trials of combined therapy of IFN-γ and antimony has been carried out in Indian kala-azar patients (Sunder et al., 1994; Shyam et al., 1995). It was observed that patients who were earlier unresponsive to antimonials were found to be cured with this immunochemotherapeutic regimen. Successful clinical studies with GM-CSF have also been reported, although the efficacy of this cytokine is mostly related to the reversal of neutropenia (Badaro et al., 1994; 1995).

**IMMUNOTHERAPY:**

Immunotherapy is comparatively better than chemotherapy. It directly induces cell mediated immune response. Various immunopotentiator, including *Bacillus calmette-Guerin* (BCG) (Fortier et al., 1987), BCG plus promastigotes (castes et al., 1989), levamisole (Rezai et al., 1988), cyclosporin A (Bogdan et al., 1989), *C. parvum* (Hill, 1987), and ghican (Cook et al., 1980) have been used to modify the course of *Leishmania* infection. These immunopotentiators have an ability...
to activate macrophages non-specifically. The complex immunopotentiators like BCG and C. 
parvum have been excluded from general human usage because of their undesirable side effects. The 
recombinant cytokines are more effective, more controlled and have negligible side effects against 
Leishmania infection. Several cytokines have been tested in models for intervention of visceral 
leishmaniasis, including IFN-γ (Murray et al., 1988), IL-1 (Curry and Kaye, 1992), IL-2 (Murray 
et al., 1993), TNF-α (Tumang et al., 1994), GM-CSF (Murray et al., 1995) and IL-12 (Murray and 
Hariprasad, 1995). Success has been variable and precise mode of action is difficult to determine 
in some cases. The effective cytokine therapy requires the presence of T-cells or other cytokines. 
The complexity of experimental cytokine therapy is particularly evident from recent studies on IL- 
12. The IL-12 can significantly reduce liver parasite burden and recipients of IL-12 indicate the 
presence of CD4+ and CD8+ T-cells, NK-cells and endogenous IL-2, TNFα and IFN-γ (Murray and 
Hariprasad, 1995). Therefore, number of workers have suggested that immunological intervention 
of disease in combination with chemotherapy is much better than chemotherapy or immunotherapy 
alone.

VACCINATION:

Vaccination against leishmaniasis has a long history (Alexander, 1988b). From 
as early as the 19th century, and as recently as 1990, living organisms have been used for 
vaccination (Peters et al., 1990). Vaccination with L. arabica against L. major infection observed 
exacerbated lesion growth. Experimental studies using subcutaneous vaccination with heat-killed 
or radio-attenuated parasites have also often resulted in disease exacerbation following challenge 
of infective parasites (Liew et al., 1985b). Thus, these studies were not useful as a vaccine in 
humans. After several years, Scientists prompted to try and use killed parasites, with or without 
adjuvant, as vaccines, or as an immunotherapeutic agents. These studies have shown some light in 
the progress of development of vaccine, which is not sufficient and still lot is to be done. In order 
to limit the development of a candidate vaccine, it should consists immunologically characterized, 
purified antigen or its derivatives. Studies have shown that apart from parasite membrane antigens, 
soluble non-membrane antigens can also induce protection (Handman and Mitchell, 1985, Russell 
and Alexander, 1988, Scott et al., 1989). There are two types of vaccines, (I) 'first generation' 
vaccines are composed of heat killed or radio-attenuated parasites with or without adjuvant and (ii) 
the 'second generation' vaccines have different recombinant molecules, either parasite fractions or 
genetically constructed by removing virulent genes or bacteria carrying and expressing leishmanial 
genres which are avirulent. At present, the "first generation" vaccines are at various stages of Phase 
I (safety), II (reactivity) or III (efficacy) trials in humans. In contrast, 'second generation' vaccines 
are only in the preclinical state (Modabber, 1989; 1995; Kaye et al., 1995) (Table 4).
### TABLE 4
Potential leishmaniasis vaccines currently being developed

<table>
<thead>
<tr>
<th>Candidate vaccine</th>
<th>Current development stage</th>
<th>Reference</th>
</tr>
</thead>
</table>
| **LIVE PARASITE WITH OR WITHOUT BCG**<br>
| L. major ± BCG* | | Bahar et al. (1993) |
| **Mixed Leishmania strains**<br>
  L. amazonensis | Efficacy<br>Preparation, Phase I–II | See Antunes et al. (1981), K. Marzochi (unpubl. obs.) |
| **LIVE VACCINES**<br>
  Recombinant *Leishmania* (DHFR/TS–) | Pre-clinical development<br>Development and animal testing | Cruz et al. (1991), R. G. Titus et al. (1995) |
| Recombinant bacteria<br>
  *Salmonella–gp63* | In-vivo immunization (mice, dogs) | Yang et al. (1990) |
| BCG–gp63 | Preparation and mouse vaccination | Connell et al. (1993) |
| Recombinant virus | Preparation and animal testing | McMahon-Pratt et al. (1993) |
| Vaccinia–gp46 | Primate studies | G. Grimaldi (unpubl. obs.) |
| **DEFINED SUBUNITS**<br>
  r–gp63*<br>
  LeIF | Pre-clinical development<br>Animal studies | Russo et al. (1991), Skeisky et al. (1994) |
| LACK | Mice and in-vitro human cells | Mougneau et al. (1999), Rasafiti et al. (unpubl. obs.) |
| **OTHERS**<br>
  dp70-72 | Gene cloned and protein expressed | Rachamin et al. (1992) |
| Synthetic peptides | Animal studies | Jardim et al. (1990) |

Field efficacy trials are underway in Iran, Pakistan and Sudan.
The 'second generation' vaccine candidates can be divided into three categories: (I) live vaccine (ii) defined subunits and (iii) crude fractions. It is possible to produce stable mutants which, at least, would cause an abortive infection, if inoculated into human (Cruz et al., 1991). An other attempt to develop an oral vaccine against leishmaniasis, a Salmonella typhimurium that expresses the L. major, promastigote surface protein gp63 has been constructed. It is found that the construct is stable, capable of expressing the protein and could induce T\(_h\)1 type response in mice (Yang et al., 1990). Another important development is the construction of recombinant BCG carrying gp63, which has shown to protect mice against L. maxicana infection (Connel et al., 1993).

Defined recombinant subunit and synthetic vaccines have been shown to induce protection in experimental animals. Various recombinant proteins or synthetic peptides, like r-gp63 (gp63 expressed in E. coli) or various synthetic peptides of gp63 have represented T-cell epitopes and have also induced partial protection (Russo et al., 1991; Jardim et al., 1991). Another recombinant protein of L. braziliensis (LeIF), stimulates human T\(_h\)1-type cells and induces IL-12 production by normal peripheral monocytes, in vitro. A protein of L. major, Leishmania homologue of receptors for activated C kinase (LACK) which could activate protein kinase C, was used as protective T-cell clone against infection (Rivas et al., 1991; Mougneau et al., 1994). Russell and Alexander (1988) reconstituted crude Ag or purified Ag (gp63) into liposomes and then inoculated into CBA/Ca or BALB/c mice, both by subcutaneous and intraperitoneal routes. They observed complete protection in CBA/Ca mice, when administered by both routes and poor protection in BALB/c mice.

Handman and Mitchell (1985) reported successful vaccination of BALB/c mice with purified L. major LPG. Interestingly, soluble LPG that lacked the phosphatidylinositol anchor induced a disease exacerbation. Similar protection against L. mexicana in CBA/Ca mice is also reported with homologous LPG, which is constituted into liposomes (Russell and Alexander, 1988). Purified LPG from L. major reconstituted into liposomes with or without C. Parvum when inoculated into BALB/c mice prior to infection resulted in survival of animals (McConville et al., 1987). The protective efficacy of LPG has been speculated to be due to some peptides/proteins associated with LPG. There are several proteins which are tightly associated with LPG, such as the B protein and KMP-11. These proteins are highly immunogenic in nature which lack NH\(_2\) terminal signal peptides (Jardim et al., 1991; 1995; Flinn et al., 1994). These peptides are responsible for the T-cell response to LPG (Jardim et al., 1991; Moll et al., 1989). Jaffe and colleagues have isolated and purified a protein which is associated from L. donovani LPG molecules. This protein protect mice against cutaneous as well as, visceral leishmaniasis (Rachamim and Jaffe, 1993).

Although recent vaccine literature is available in abundance and many antigens have been studied, the advantages and disadvantages of each approach for vaccine development have been discussed above but molecular vaccine development are open to question.