Chapter 1

Introduction
Introduction

1.1 *Leishmania* and Leishmaniasis

Infectious diseases are caused by pathogenic microorganism such as bacteria, viruses, parasites and fungi etc. and can be spread directly or indirectly from one person to other person. These diseases can be called as “neglected” in the absence of effective, affordable or ease of treatment. This concept of “neglected disease” has been originated from the requirements in the development of new drugs to combat from the infectious diseases which have been ignored by public and private sector such as cholera, Buruli ulcer, Lymphatic filariasis, chagas disease and African trypanosomiasis etc. Approximately 1 billion people suffer from one or more “neglected tropical diseases” (NTDs) primarily to the poor population living in tropical and subtropical regions (WHO Fact Sheet, 2008). Neglected tropical diseases typically affect the poorest in communities, usually the most marginalized and those least able to demand services. Neglected tropical diseases forms a group, with more than 70% of affected countries in low-income or lower middle-income economies. Therefore these NTDs are thought to be synonym of poverty and disadvantages. Although diverse in symptoms, they have common feature to share that allow them to persist and cluster in geographical areas and frequently overlap. Individuals are often afflicted with more than one parasite or infection. Neglected tropical diseases are a devastating obstacle to human settlement and socioeconomic development of already impoverished countries. These diseases have consequences for affected individuals, families and entire communities in terms of burden of disease, quality of life, loss of productivity and aggravation of poverty. Still these NTDs are ignored at all level public private bodies and pharmaceutical industry (Yamey G., 2002).

Among the classical neglected diseases, leishmaniasis has a major impact on the mortality, morbidity and its geographical distribution and remains one of the major burdens on human health in developing countries (Hotez et al., 2004), therefore leishmaniasis is ranked as one of the fourteen “most neglected tropical diseases” (Yamey G. and Torreele E, 2002; WHO Fact Sheet 2008).
1.1.1 *Leishmania* and Leishmaniasis

*Leishmania* are the protozoan parasites that are causative agents of a variety of diseases in humans and animals. The etiological agents causing leishmaniasis was first observed by Sir William Leishman in 1900, and was independently confirmed by Charles Donovan in 1903. The term "Leishmania", was first introduced by Sir Ronald Ross in 1903. The spectrum of disease caused by the different species of *Leishmania* is collectively known as Leishmaniasis (Lainson and Shaw, 1973; Bray, 1972). Different species of Leishmania cause different clinical manifestations that differ in severity. General status of health and genetic makeup of the host also contribute in the severity and clinical manifestation of disease (Blackwell et al, 1985; Chang et al, 1999). At least 20 species of *Leishmania* infect humans and are summarize in Table 1.1.

<table>
<thead>
<tr>
<th>Old World species</th>
<th>New World species</th>
<th>Disease type</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>L. major complex</em></td>
<td><em>L. mexicana complex</em></td>
<td>Cutaneous</td>
</tr>
<tr>
<td><em>L. (L.) major</em></td>
<td><em>L. (L.) mexicana</em></td>
<td></td>
</tr>
<tr>
<td><em>L. (L.) tropica</em></td>
<td><em>L. (L.) amazonensis</em></td>
<td></td>
</tr>
<tr>
<td><em>L. (L.) aethiopica</em></td>
<td><em>L. (L.) pifanoi</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>L. (L.) venezuelensis</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td><em>L. (Viannia) subgenus</em></td>
<td></td>
</tr>
<tr>
<td><em>L. (V.) braziliensis</em></td>
<td><em>L. (V.) braziliensis</em></td>
<td>Mucocutaneous</td>
</tr>
<tr>
<td><em>L. (V.) panamensis</em></td>
<td><em>L. (V.) peruviana</em></td>
<td>Diffuse cutaneous</td>
</tr>
<tr>
<td><em>L. (V.) guyanensis</em></td>
<td><em>L. (V.) lansoni</em></td>
<td></td>
</tr>
<tr>
<td><em>L. (V.) peruviana</em></td>
<td><em>L. (V.) amazonensis</em></td>
<td></td>
</tr>
<tr>
<td><em>L. (L.) aethiopica</em></td>
<td><em>L. (L.) pifanoi</em></td>
<td></td>
</tr>
<tr>
<td><em>L. donovani complex</em></td>
<td></td>
<td>Visceral</td>
</tr>
<tr>
<td><em>L. (L.) donovani</em></td>
<td></td>
<td></td>
</tr>
<tr>
<td><em>L. (L.) infantum</em></td>
<td><em>L. (L.) chagasi</em></td>
<td></td>
</tr>
</tbody>
</table>

Table 1.1 Different *Leishmania* spp those are infective to humans. The main species complexes and subgenus are shown in bold. * These species are also associated with cutaneous leishmaniasis. (Modified from Smith et al., 2007)

The spectrum of clinical manifestations by these species of *Leishmania* are characterized broadly into three type: ulcerative skin lesions (cutaneous leishmaniasis [CL]); destructive mucosal inflammation (muco-cutaneous leishmaniasis [MCL]); and disseminated visceral infection (visceral leishmaniasis [VL]).
1. **Cutaneous Leishmaniasis (CL):** is characterized by the development of a lesion on the skin in the exposed parts of the body such as the face, earlobes, arms and legs (Evans, 1993). The lesion starts as a nodule at the site of inoculation. A crust develops centrally which subsequently falls off exposing an ulcerous necrotic lesion. A dry lesion heals gradually leaving a depressed scar with altered pigmentation (WHO expert committee report, 1984). Satellite nodules tend to arise along the edges of the healing lesion. In some cases, multiple necrotic lesions may appear and spread throughout the skin resulting in severe ulceration and leaving the patient badly disfigured. Such infections do not heal spontaneously (Evans, 1993). In many cases of severe cutaneous Leishmaniasis, the infection may spread along the lymphatic system affecting the general physiology of the whole body. In some cases, untreated and apparently healed cutaneous infection may resurface as muco-cutaneous invasions (Evans, 1993).

2. **Muco cutaneous Leishmaniasis (MCL):** develops as a primary cutaneous lesion but involves a slow metastatic spread of infection to the oral, nasal and pharyngeal mucosa (Evans, 1993). Severe ulceration and erosion leads to progressive destruction of the soft tissue and cartilage of the mouth, nasal cavity and pharynx. MCL lesions do not heal spontaneously and may result in severe mutilation and death (Evans, 1993; Chang and Bray, 1985).

3. **Visceral Leishmaniasis (VL):** is characterized by bouts of fever, malaise and substantial weight loss (el Hag et al, 1994). The common clinical signs are splenomegaly, heptomegaly, lymphatic adenopathy, wasting and pallor of mucous membranes. VL is accompanied by reticulo-endothelial hyperplasia affecting the spleen, liver, mucosa of the small intestine, bone marrow, lymph nodes and other lymphatic tissues (el Hag et al, 1994; Gaafar et al, 1996). Macrophages in all these tissues are severely parasitized. Hematopoiesis is repressed and life span of blood cells is reduced leading to severe anemia (Eleshoura, 1994). Diarrhoea may occur due to intestinal parasitism and ulceration (WHO committee report, 1991, Fahal et al, 1995). In advanced stages VL is complicated by serious secondary bacterial infections such as pneumonia, dysentery and pulmonary tuberculosis (WHO committee report, 1991), which
are often the cause of death in VL patients. Other complications include hemolytic anemia, renal damage and severe mucosal hemorrhage.

The three clinical forms of leishmaniasis are believed to result from the different affinities of the various *Leishmania* species for macrophages located in different parts of the body of the host (Colmenares et al, 2002). Temperature may be one of the factors involved in this tropism (Callahan et al, 1996). The *Leishmania* species that produce CL and MCL (e.g *L. major*, *L. mexicana*) grow better at slightly cooler temperatures (35°C) found in the skin and mucous layers. In contrast, *Leishmania donovani* that causes VL prefers the slightly higher temperatures (39°C) found inside organs (Callahan et al, 1996).

1.1.2 Prevalence of Leishmaniasis

Currently, leishmaniasis is prevalent in four continents and is considered to be endemic in 88 countries. It is estimated that 350 million people are exposed to the risk of infection by different species of the parasite. It is believed that, at present, there are 17 million people affected by leishmaniasis worldwide (WHO report, 2008). The annual incidence of new cases is estimated to be about 1.5-2 million, of which there are 500,000 new cases of visceral leishmaniasis occurring every year. 90% of all visceral leishmaniasis cases occur in five countries Bangladesh, Brazil, India, Nepal and Sudan. In India, the disease has been spreading very rapidly in the past few years and has assumed alarming proportions. Already there have been two large epidemics during the last decade claiming the lives of thousands of people. 40% of all the world's cases of VL are found in northern Bihar alone (WHO report, 2001). Although initially, Leishmaniasis in India was restricted to four districts in north Bihar but in recent years it has spread to almost the whole of Bihar and sporadic cases are being reported from West Bengal, Uttar Pradesh and Andhra Pradesh. A few cases of CL have also been reported from the northwestern regions of India. The geographical distribution of the visceral form of leishmaniasis is illustrated in Fig 1.1.
Leishmaniasis being one of the opportunistic infections can also attack immuno-compromised hosts such as those infected with the human immunodeficiency (HIV) virus or those individuals taking immuno-suppressive drugs, such as in cancer therapy (da Costa, 2000; Ferreira and Borges, 2002; Choi and Lerner, 2002). Most of the cases of co-infection with HIV reported so far occur with visceral leishmaniasis (Alvar et al, 1989; Rosenthal et al, 1995; Cabie et al, 1992). The spread and overlap of both leishmaniasis and HIV infections in the major foci of leishmaniasis (India, Brazil, and Eastern Africa) make VL-HIV co-infection a serious worldwide concern.

1.1.3 Vectors and Reservoirs

Parasites like *Leishmania* have to go through vector-borne transmission in order to successfully spread from one host to another. The vector is usually a hematophagous arthropod that serves as an intermediary between successive vertebrate hosts. It is dependent on the presence of vector species occurs in tropical, subtropical region. Among the ~400 species of sand flies, only ~ 50 are associated with the transmission of *Leishmania* parasites (Kamhawi, 2006). Most *Leishmania* species are suprephyllarian parasite i. e. their development is confined to digestive
tract of sand fly. However, the vector is not simply a ‘flying syringe’ but plays an important role in the development of the parasite (Sacks and Kamhawi, 2001). The natural vectors of all *Leishmania* species are sandflies belonging to the family *Psychodidae* and sub-family *Phlebotominae*. *Lutzomyia* is the main genus causing leishmaniasis in America (new world) while *Sergentomyia* and *Phlebotomus* are the main genera responsible for transmitting disease in Africa, Europe and Asia (old world) (Killick-Kendrick, 1990; Killick-Kendrick, 1999). Adult sand flies are about 2-3 mm in length and are characterized by the typical angle of their wings to the abdomen, the presence of hair on their body and wings and hopping motion due to very short stretches of flight (Sacks, 2001; Dillon and Lane; 1993). Their natural source of food is sugars present in the sap of plants. The disease is usually transmitted when the female sandfly takes a blood meal from an infected host. The protein content in the blood is required for the development of its eggs (Daba et al, 1997a; Daba et al, 1997b; Killick-Kendrick, 1990; Killick-Kendrick, 1999).

Leishmaniasis can be a zoonosis where domestic or wild animal serve as a reservoir or an anthropoponosis where humans serve as reservoirs. The most important reservoir hosts that harbor *Leishmania* are dogs, foxes, jackals, gerbils, rodents, sloths and opossums (Ashford, 2000). In these animals, infection with *Leishmania* is chronic and asymptomatic and does not lead to any significant morbidity. However, cutaneous Leishmaniasis caused by *L. tropica* in Mediterranean urban areas and visceral Leishmaniasis caused by *L. donovani*, in India, are transmitted by sandflies directly from human to human (Killick-Kendrick et al, 1994). The geographical distribution of Leishmaniasis is limited by the distribution of the competent species of the sandfly vector and on the availability of appropriate reservoir hosts. Amongst the vectors, there is extreme specificity for the *Leishmania* species they can harbor and transmit (Kamhawi et al, 2000).

1.1.4 Life cycle of *Leishmania*

All parasites of the genus *Leishmania* have a digenetic lifecycle during which the parasites differentiate into two distinct morphological forms. In the alimentary tract of the insect vectors, the parasite exists extracellularly as a flagellated, motile promastigote form (Sacks and Kamhawi, 2001). Promastigotes have a spindle
shaped cell body with a single anterior flagellum. They are 15-20 μm in length and 1.5-3.5 μm in diameter in the widest part (Killick-Kendrick et al, 1977; Glew et al, 1988). In the vertebrate host the parasite resides within the phagolysosomes of the cells of the mononuclear phagocyte lineage as non-motile, intracellular amastigote form. The amastigotes are spherical or oval in shape with a diameter of approximately 2-3 μm and possess a truncated flagellum that rarely extends beyond the flagellar pocket (Chang, 1979; Alexander and Russell, 1992).

A typical life cycle of *Leishmania* parasite is shown in **Fig 1.2**. Infection is started when sand fly ingest blood meal that contains macrophages infected with amastigotes and passed into the abdominal cavity (midgut) enclosed in a peritrophic membrane (PM). Within the PM, the amastigotes transform into promastigotes in about 12-18 hours. These freshly transformed promastigotes are termed as procyclics and are short, ovoid and only slightly motile. Procyclic promastigotes are non-infective and multiply very rapidly by binary fission. By the 7th day, the PM ruptures and the promastigotes start migrating towards the anterior gut and mouthparts. The rapidly dividing procyclics transform into long, slender and highly motile nectomonads, which remain attached to the vector gut epithelial microvilli with the help of their flagella (Sacks, 1989; Alexander and Russell, 1999). The nectomonads stop dividing and transform into short, slender and highly active metacyclic form, with flagella twice the length of the cell (Sacks, 1989; Sacks and Perkins, 1984). Metacyclic promastigotes are highly virulent; they migrate into and block the pharynx, mouth and proboscis of the sandfly. The sequential development of the promastigotes from an actively dividing, non-infective stage to a non-dividing, highly infectious stage in the sandfly gut is known as metacyclogenesis (Sacks and Perkins, 1984). Similar developmental transition has been observed in case of promastigotes growing axenically in tissue culture (Sacks and Perkins, 1984). When the sandfly subsequently bites another host, the metacyclic promastigotes are regurgitated at the wound site (Alexander and Russell, 1999). Phagolysosomes of the macrophages are the final destination of the promastigote. Earlier it was thought that macrophages are the first cells that encounters with the promastigotes. Promastigotes reaching the dermis at the wound site rapidly adhere to resident recruited macrophages (Alexander and Russell, 1992).
Figure 1.2 Typical *Leishmania* lifecycle. The promastigote form of *Leishmania* is transmitted into the skin by female sand fly. Once transmitted, the parasites are internalized by dendritic cells and macrophages in the dermis where they lose their flagella, transforming into the amastigote form. The amastigotes multiply, destroy the host cell and infect other phagocytic cells. These infected cells will be taken up by sand fly while taking blood meal from infected mammalian host. In the mid gut these amastigotes transform into less infective procyclic promastigotes. (Modified from Chappuis et al., 2007)

The binding of the parasite to the macrophage is mediated through a number of receptors of which complement receptor I (CRI) and complement receptor III (CRIII) are the most important (Brittingham and Mosser, 1997). Components of the complement system that bind to various molecules on the parasite membrane help in docking the parasite to the receptors on the macrophage cell surface (Brittingham and Mosser, 1997; Alexander and Russell, 1999). Now a day’s, evidences are
increasing that PMNs are the first cells that first encounter the promastigotes as they enters into host. In both type of cells adhesion of the parasite is followed by rapid internalization. Phagocytosis by the host cell is the basic mechanism involved in the uptake of the parasites (Antoine et al, 1998). Immediately following phagocytosis, Leishmania are located in endocytic compartments limited by membrane derived from the plasmalemma of the macrophage (Antoine et al, 1998). These phagosomes undergo maturation and subsequently fuse with the lysosome, giving rise to the parasitophorous vacuoles (PV) (Lang et al, 1994a; 1994b). Within the PV, the promastigote converts into the amastigote form in about 2-5 days after the infectious bite. Leishmania amastigotes remain tightly bound to the PV membrane via the posterior pole (Alexander and Russell, 1992). Amastigotes are acidophilic, surviving in the PV lumen at a pH of 4.5-5.0 (Burchmore and Barrett, 2001; Zilberstein and Shapira, 1994; Mukkada et al, 1985). Amastigotes express stage specific proton pumps and metabolite transporters with optimum activity in acidic pH in their plasma membrane, which are involved in the capture of metabolites (Zilberstein and Shapira, 1994).

In addition, the surface of the amastigote cell is covered by a glycocalyx composed of large quantities of glycoinositol phospholipids and glycosphingolipids in the plasma membrane (McConville and Blackwell, 1991), which protect them from the acidic media and digestion by lysosomal enzymes e.g. acidic hydrolases (McConville and Ralton, 1997). Amastigotes replicate within the PV and are released following lysis of the infected macrophage. These free amastigotes then invade other neighbouring macrophages (Alexander and Russell, 1992). Infected macrophages are eventually taken up by the sandfly thereby completing the life cycle of the parasite (Fig 1.2).

1.1.5 Leishmania genome

Old World Leishmania (L. donovani and L. major groups) have 36 chromosome pairs (0.28 to 2.8 Mb)(Wincker et al., 1996), whereas New World species have 34 or 35, with chromosomes 8th & 29th and 20th & 36th fused in the L. mexicana group and 20th & 34th in the L. braziliensis group (Britto et al., 1998). The Leishmania genome network (LGN) was set up in 1994 with the support of the
World Health Organization (WHO). The reference strain *Leishmania major* MHOM/IL/81/Friedlin (LmjF) was selected for genome mapping and sequencing studies (Ravel et al., 1998). The genome sequence of *Leishmania major* (the causative agent of cutaneous leishmaniasis) (Ivens et al., 2005) was released along with the genome sequence of two other kinetoplastid parasitic protozoan, *Trypanosoma brucei* (the causative agent of African trypanosomiasis) (Berriman et al., 2005) and *Trypanosoma cruzi* (the causative agent of Chagas disease) (El-Syed et al., 2005). The sequencing of *L. infantum* clone JPCM5 (MCAN/ES/98/LLM-877) and *L. braziliensis* clone MHOM/BR/75M2904 are also completed and sequencing of *L. mexicana* clone MHOM/GT/2001/U1103 is already started (http://www.genedb.org). Sequencing projects of tritryps revealed that they have ~6200 trypanosomatid specific genes. Most of the *Leishmania* genes have ortholog in the other *T. brucei* and *T. cruzi*. Still 910 genes in *L. major* don’t have their ortholog in tritryps and supposed to be "*Leishmania*-restricted" genes. These genes are randomly distributed in the genome and most of them (~ 68%) have unknown functions (Ivens et al., 2005). A comparative study of three genome of *Leishmania* spp revealed conserved gene content, synteny and architecture of genome but also found ~ 200 disparities in the gene or pseudo gene content. There are ~78 genes that are limited to individual species. Comparison of three genomes is summarized in Table 1.2 and Distribution of total 8187 genes in different *Leishmania* species are shown in Venn diagram (Fig 1.3). Pathologies of *L. infantum* and *L. donovani* are very similar therefore genome sequences and organization could also be similar.
<table>
<thead>
<tr>
<th></th>
<th>Leishmania major</th>
<th>Leishmania infantum</th>
<th>Leishmania braziliensis</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total size (Mb)</td>
<td>32.8</td>
<td>32.1</td>
<td>32.0</td>
</tr>
<tr>
<td>Contigs</td>
<td>36</td>
<td>562</td>
<td>1.041</td>
</tr>
<tr>
<td>No. of chromosomes</td>
<td>36</td>
<td>36</td>
<td>35</td>
</tr>
<tr>
<td>Chromosome size range</td>
<td>0.3–2.8</td>
<td>0.3–2.8</td>
<td>0.3–2.8</td>
</tr>
<tr>
<td>(Mb)</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overall G+C content %</td>
<td>59.7</td>
<td>59.3</td>
<td>60.4</td>
</tr>
<tr>
<td>No. of genes</td>
<td>8,298</td>
<td>8,154</td>
<td>8,153</td>
</tr>
<tr>
<td>No. of pseudogenes</td>
<td>97</td>
<td>41</td>
<td>161</td>
</tr>
<tr>
<td>Average gene size (bp)</td>
<td>1,894</td>
<td>1,868</td>
<td>1,873</td>
</tr>
<tr>
<td>Gene density (per Mb)</td>
<td>252</td>
<td>235</td>
<td>258</td>
</tr>
<tr>
<td>Coding percentage</td>
<td>48.0</td>
<td>44.0</td>
<td>48.5</td>
</tr>
<tr>
<td>Coding G+C content %</td>
<td>62.5</td>
<td>62.4</td>
<td>60.4</td>
</tr>
<tr>
<td>No. of DGCs</td>
<td>133</td>
<td>133</td>
<td>n/a</td>
</tr>
<tr>
<td>Average DGC length (kb)</td>
<td>240.61</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>No. of tRNAs</td>
<td>83</td>
<td>62</td>
<td>66</td>
</tr>
<tr>
<td>No. of snoRNAs</td>
<td>693</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>No. of snRNAs</td>
<td>6</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>No. of rRNAs</td>
<td>63</td>
<td>n/a</td>
<td>n/a</td>
</tr>
<tr>
<td>Average intergenic size</td>
<td>1.939</td>
<td>2.049</td>
<td>1.976</td>
</tr>
</tbody>
</table>

Table 1.2 Comparison and summary of the *L. major*, *L. infantum* and *L. braziliensis* genomes. DGCs, Directional gene clusters; tRNA, transfer RNA; snoRNA, small nucleolar RNA; snRNA, small nuclear RNA; rRNA ribosomal RNA (Modified from Smith et al., 2007)

**Figure 1.3 Leishmania species-specific gene.** The Venn diagram shows the distribution of total 8,187 genes in three *Leishmania spp.* (Modified from Smith et al., 2007)
Although disease phenotype differs broadly in different *Leishmania* spp, very few species-specific genes were identified that might contribute in differential pathogenesis and tissue tropism by differential regulation of these genes (Peacock et al., 2007). A study showed that only 0.2%-5.0% genes have stage specific expression in *L. mexicana* (Holzer et al. 2006). Small number of species-specific genes and constitutive expression of *Leishmania* genome indicate that neither gene content nor gene expression is responsible for disease differences but translational control and protein stability might be more important for these differences. In *Leishmania infantum*, mRNA abundance determined by microarray analysis showed a very weak correlation with protein abundance determined by ICAT analysis (McNicoll et al. 2006).

### 1.1.6 Genomic organization

*Leishmania* exhibits many peculiar features in their genome organization and gene expression. In *Leishmania*, genes are organized in a large clusters compromising up to hundreds of genes, in the same 5'-3' direction along the chromosome DNA strand called Directional gene clusters (DGCs) (Myler et al., 1999; Worthey et al., 2003). The *L. major genome* is categorized into 133 DGCs of tens to hundreds of protein coding genes with unrelated predicted function. The cluster size can be up to 1260 kb and separated by divergent or convergent strand switch regions of 0.9-14 kb (Ivens et al., 2005). None of the known sequences responsible for initiation and regulation of eukaryotic gene expression have been observed in *Leishmania* (Cruz and Tossi, 1996). Generally transcription undergoes polycistronic transcription which initiates in both direction from a divergent strand switch region (Worthey et al., 2003; Martinez-Calvillo et al., 2003; Martinez-Calvillo et al., 2004) and ends with convergent strand switch regions which often include tRNA, rRNA and/or snRNA genes (Martinez-Calvillo et al., 2004). At telomeres transcription progress towards the telomere or DGCs are very short with one to three genes (Ivens et al., 2005). A pfam search for the domains homologues to the eukaryotic transcriptional regulators revealed very few potential molecules than in the other eukaryotes.
1.1.7 Transcription in *Leishmania*

Genes in Tritryps are transcribed as polycistronic precursors of mature mRNAs (Flinn et al, 1992). Mature RNA is produced by 5' trans-splicing and 3' polyadenylation that occurs co-transcriptionally (Clayton, 2002; Palenchar and Bellofatto, 2006). Two small stretches of pyrimidine rich sequences is found in the flanking region of each protein-coding gene in *Leishmania* that contain the signals for trans-splicing and poly-adenylation (LeBowitz et al, 1993). While trans-splicing leads to the addition of 5' mini-exon sequences, poly-adenylation involves the addition of a 3' poly-adenine sequence to the mRNA. Trans-splicing involves the addition of a non-coding 39 nucleotide sequence known as the "splice leader" or "mini-exon" to the 5' end of every mRNA at an AG dinucleotide preceded by a polypyrimidine tract (Cruz and Tosi, 1996). All mature nuclear transcripts in *Leishmania* possess this common 5' segment. This splice leader sequence is transcribed separately from a gene cluster (Fernandes et al, 1994; Antoniazi et al, 2000). The mechanism by which this trans-splicing of genes occurs in *Leishmania* is very much similar to the cis-splicing process in eukaryotes and requires small nuclear RNAs as co-factors (Miller et al, 1988; Xu et al, 2000; Roberts et al, 1998; Liang et al, 2002). No conserved poly(A) signals have been described in trypanosomatid, trans-splicing of the downstream gene is coupled with polyadenylation of the gene immediately upstream in the cluster. Till date only four RNA are matured by cis-splicing (Mair et al., 2000; Ivens et al., 2005). Signal for these processes are separated by a region with a length ranging from 200-500 nts (LeBowitz et al, 1993; Lamontagne and Papadopoulou, 1999; Xu et al, 2000). Presence of DGCs, constitutive expression of genes in *Leishmania* can explain this weak correlation and importance of the post translational regulation of protein expression level.

1.1.8 Regulation of transcription

Unlike eukaryotes no α-amanitin-sensitive RNA polymerase II promoter regions have been identified in *Leishmania* so far (Vanhamme and Pays, 1995; Teixeira, 1998). There is only one promoter-like sequence identified so far located upstream of the 18S rRNA gene (Yan et al, 1999; Gay et al, 1996). Thus, the control of transcription initiation might not play a primary regulatory role in *Leishmania*.
gene expression (Cruz and Tossi, 1996). Therefore differential stage-specific RNA expression is regulated at post transcriptional level and leads to differential mRNA stability (Charest et al., 1996; Boucher et al., 2002). In Leishmania, transcriptional control of gene expression is mediated through sequences within 3'UTRs of genes (Clayton, 2002; Kelly et al., 2001; McNicoll et al., 2006; Palenchar and Bellofatto, 2006; Murray et al., 2007). As a consequence of these various post-transcriptional regulatory events, transcripts show differences in their steady state levels despite the lack of a transcriptional control.

1.1.9 Translation, protein modification and degradation in Leishmania

Components of translational machinery, present in Leishmania genome, are conserved to eukaryotic translational components. There is paralogous expansion of number the genes was observed (Ivens et al., 2005). This expanded numbers of potential translational factors suggest a high degree of specialization. Protein modifications consist of phosphorylation, glycosylation and lipidation as in eukaryotes for stabilization or activation of proteins. Already several modifications are reported in Leishmania such as GPI-anchor addition, acylation, N-myristoylation, and prenylation. All of these are associated with membrane attachment and protein-protein interaction hence plays an important role in Leishmania biology.

Trypanosomatids are the only eukaryotes which has both a proteasome (Wang et al., 2003) and a eubacterial HsLUV complex (Couvreur et al., 2002). Genome analysis revealed these parasites have many ubiquitin-conjugating enzymes. This means that nonlysosomal cytosolic protein degradation system also exist in Leishmania. Presence of two ATG4 cystein proteases and their substrate ATG4 and other ATG genes indicate that autophagy operates in Leishmania organelle and protein turnover. No homologues of mammalian peptidase inhibitors are found in Leishmania but they encode inhibitors of cysteine proteases (ICP) that mammals don't have (Ivens et al., 2005).

1.1.10 Diagnosis of Leishmaniasis

The detection of leishmanial parasites by microscopy or culture in different tissue samples is considered the gold standard for the diagnosis of leishmaniasis. Diagnosis of visceral leishmaniasis involves acquisition of splenic aspirates followed
by microscopic examination of the sample to identify presence of the intracellular amastigote form of parasite and/or subsequent propagation of promastigote stage. Various serological tests such as indirect immunofluorescent antibody test (IFAT), direct agglutination test (DAT), enzyme-linked immunosorbent assay (ELISA) using rK39 antigen are designed. Specific antibodies can also be used to further confirm the presence of an ongoing infection (Chappuis et al., 2007). Recent developments in the molecular diagnostic technology have lead to the advent of PCR based detection of parasite in the patient (Maurya et al., 2005; Cruz et al., 2006). Quantitative Real-time PCR (QRT-PCR) was recently applied to the diagnosis and monitoring of Leishmania infections. Its main advantages include a reduction in the time needed for the assay and the possibility to quantify the parasitic load as low as 0.001 parasite/ml of the clinical sample (Tupperwar et al., 2008; Mary et al., 2006).

In spite of the recent progress in diagnostic procedures, a major proportion of cases in endemic regions still go unreported due to lack of knowledge, poverty and unavailability of proper health care facilities in rural areas.

1.1.12 Treatment of leishmaniasis

Treatment of leishmaniasis is relies on specific anti-leishmanial drugs. But these drugs are inaccessible because of their price, lack of registration to the government authorities, toxicity or ineffectiveness or because they have not yet been tested in these patients (Alvar et al., 2006). Current regimens for the treatment of leishmaniasis use pentavalent antimonials and aromatic diamines as primary therapy (Chappuis et al., 2007). These drugs are highly toxic and produce serious side-effects such as, nausea, vomiting, diarrhea, anorexia, convulsions, cardiac toxicity, hepatotoxicity and hematological changes. Amphotericin B (AMPB) is the most widely used second line of treatment. Recently developed lipid associated formulations have been found to have improved therapeutic index and reduced toxicity. Liposomal amphotericin B is considered to be the best existing drug available now and used as the first line drug in Europe and USA (Chappuis et al., 2007). Meltifosine is the only medicine that can be administered orally (Jha et al, 1999). Recently combination therapy is the suggested to increase treatment efficacy, prevent the development of drug resistance, reduce treatment duration and perhaps
decrease treatment cost (Bryceson, 2001). The association of sodium stibogluconate and paromomycin was found to be safest and effective in early trials (Seaman et al., 1990).

1.1.12 Vaccines

A well known fact that healing and recovery of leishmaniasis protects individuals from re-infection indicates that it should be possible to develop a suitable vaccine against leishmaniasis. Vaccines of potential interest are live leishmania (leishmanization) (Khamesipour et al., 2005), killed and attenuated *Leishmania*, mutants of virulence factors such as *lpg1*, *lpg2* and *Hsp100*⁻, recombinant antigens such as, LACK, flagellar pocket antigen, cysteine protease, GP63, HSP80 and DNA vaccines such as GP63, GP46, LACK, A2 (TDR news, 2007). Many of these candidate vaccines have shown to be effective in preliminary experiments in the laboratory and their potential to be developed as therapeutics are currently being explored (Desjeux, 2004).

Though the treatment of leishmaniasis has made a significant progress and promising new drugs have come to the forefront, yet the success of anti-leishmanial chemotherapy is still limited. All the studies about leishmaniasis in progress are directly or indirectly aim to obtain new drugs to be used in treatment with low or no toxic effects compared to that of current treatment. The use of the extensive knowledge we now have gathered for leishmania biology into effective treatment and generating effective vaccine and their rigorous field validation are essential steps to remove leishmaniasis from the list of the most neglected diseases.
1.2 Concepts of Virulence

1.2.1 Basic definition of Virulence

Virulence is a difficult word to define since early days of biology. However from the past time many definitions of virulence have been applied. Most popular words like “virulence” and “virulent” have been derived from the Latin word “virulentus,” meaning “full of poison” that itself derives from “virus” or related to a Sanskrit word “visham” that means “poison” and “lentus” (“fullness”) (Wains, 1958). In the ancient time virulence describe as a microbial characteristic that is the ability to deliver poison and thereby causing the disease. Even the Koch’s postulate and the germ theory of disease also describe the microbes are the responsible for the any pathogenesis.

Early views or definitions of the virulence are pathogen centered and on the assumption that this is an intrinsic property of a microorganism such as its degree of pathogenicity; its capacity to overcome host defenses; the severity of the disease that it caused; the percentage of death an infection with it induced; its invasive power; its infectivity or the damage it induced; and its capacity to grow and multiply in a host (Casadevall and Pirofski, 1999). In 1913, Smith emphasized the importance of the host but still he suggested that virulence is primarily microbial property that determined its virulence (Smith, 1913). In 1914, Zinsser described that virulence has two attributes: one passive that is responsible for the persistence in the host and consisted of microbial characteristics and the other an aggressive that included toxins (Casadevall A and Pirofski L, 1999). Virulence was also defined as the inverse of resistance (immunity) because of the relative contribution of microbial virulence and host resistance (Falk, 1928). Most recent concepts of microbial pathogenesis are emerged primarily from the studies of the bacterial virulence. Presence of pathogenicity islands in the bacteria also support the microbe oriented view of virulence. It suggests that acquisitions of such DNA fragments are sufficient for a bacterium to become virulent (Hacker et al, 1997). Microbial pathogenesis was also described as “a capacity for the antigenic variations which allows the selection of characteristics which helps in escaping the host immune defense (Deitsch et al, 1997).
As the evidences increases towards the role of host factors in the host-parasite interaction and its outcome in the form of disease, it was difficult to accept that virulence is an exclusive microbial property. Thus virulence should be considered as “a host-centered measure of a phenomenon that is neither host nor parasite but of the host-parasite complex” (Poulin R and Combes C, 1999). Because the outcome of virulence is only seen in the susceptible host therefore it should not be consider as an independent variable. Since it depends on the availability of a susceptible host and the context and nature of host-microbe interaction, currently virulence is considered to be the relative capability of a microbe to cause the disease (Casadevall and Pirofski, 2001). In addition to host involvement, qualities of the aggressiveness, invasiveness, and infectivity and transmission abilities are other independent factors that added more complexity into notion of virulence. Therefore virulence should be considered as a dynamic and complex phenomenon that includes both the host and microbial factors. The ability of a microorganism to cause disease in an animal model system is the basis of the measurement of virulence. Virulence has been often considered as a property of a parasite but define as a property of host-parasite interaction. Still it is measured on the basis of its effect on host fitness. This is one of the basic problems in understanding virulence. The feature of one organism is measured by its effect on another organism.

In other words through virulence, pathogens exploit their host for their multiplication and transmission. Thus according to the modern definition “virulence is the ability of a pathogen to multiply and cause damage to the host” (Casadevall and Pirofski, 2001) or the host damage (also can be referred as clinical symptoms). Most of the microbiologists use this definition of virulence but ecologist and evolutionary biologist have different view in this definition. According to them virulence is microbe-induced effects on host fitness (Poulin and Combes, 1999; Read, 1994). Recently Brown et al refines this definition as the damage of the host during infection with a pathogen (Brown et al., 2006). This definition provides the explanation of the situation where some times pathogen themselves do not cause the damage it’s the host response that cause major part of damage.
1.2.2 Definition of Pathogen

Brown et al define pathogen as an organism capable of colonizing a host where interaction results in disease (Brown et al., 2006). But this definition doesn't fit on pathogens that don't cause disease in all hosts. These pathogens can be referred as opportunistic pathogens. They cause disease only in immunocompromised host or there pathogenesis is facilitated by breaching of an epithelial barrier. Some pathogens are accidental, they adapted to one host but disease occurs when they infect a host that the pathogen does not generally encounter (Shuman et al., 1998). Therefore Pathogen should be referred as a microbe capable of causing host damage; host damage can be a result of direct microbial action of host response (Casadevall and Pirofski, 1999).

1.2.3 The concept of virulence factor

Classical definition of virulence factors is the components of a pathogen that confers the disease causing capacity of pathogen or virulence but not the viability (Casadevall A and Pirofski L, 1999) such as capsules of the capsule of S. pneumoniae, the toxins of C. diphtheriae and Vibrio cholera, and the M protein of group A Streptococcus. Virulence factors can be involved in many functions including the capacity to facilitate pathogen attachment, invasion, or both, as well as the promotion of the growth of a microbe in a host through evasion of host detection, inhibition of phagocytosis, and regulation of the capacity for intracellular survival. These factor can work in all or none (requisite) or relative (contributory) fashion. The concept of virulence factor holds true when host has intact immunity or defense system. But in the case "opportunistic" or "accidental" pathogen the requisite or contributory virulence factors cannot be well define. Therefore, the immune status of the host, nutritional status of the host, which other microorganisms already infected etc can modify the expression of virulence factors and their ability to confer pathogenicity in the context of a given host-microbe interaction (Poulin and Combes, 1999).

Virulence factors produced by opportunistic and accidental pathogens will become outliers of the classical definition of virulence factors because they are not capable of causing the disease in all type of host. It has been suggested that factors
essential for the growth or replication of the parasite into the host should be considered as virulence factors. Therefore problematic survival criteria should omit from the definition. Hence virulence factors can be referred as "a component of a pathogen that damages the host and include the components which are essential for viability in the host" (Casadevall A and Pirofski L, 1999).

1.2.4 Virulence mechanisms in *Leishmania*

Virulence of a pathogen can be defined as its ability to cause the damage or the spectrum of clinical symptoms in human disease that result from the continuous and complex host-microbe interactions. Damage or clinical symptoms are also dependent on their respective genetic constitution and general health of the host. *Leishmania* virulence certainly is modified by the environmental and genetic factor of its mammalian host (Blackwell, 1996) and sand fly vector (Titus et al., 1998). During life cycle *Leishmania* has to survive in different hostile environments, such as:

(i) The sand fly mid gut, where it is susceptible to the insect's digestive enzymes.

(ii) The blood stream of the vertebrate host, where the organism exists transiently and is exposed to the lytic complement cascade and innate defense molecules.

(iii) The phagolysosomes of the host's macrophages, where the parasite has to survive in acidic pH and escape from the effect of hydrolytic enzymes.

To counter these drastic conditions the parasite has also adopted a number of different mechanisms. For this purpose the pathogen expresses a repertoire of "virulence factors" which not only help it to invade the host but also to overcome the innate defensive barriers and compromise the subsequent immune responses. There is also a known fact that leishmaniasis does not occur without the infection with living *Leishmania* parasite. Till date there is no evidence that *Leishmania* spp produces toxins or some other molecules that can give raise the leishmaniasis like symptoms.
Leishmania have several factors that help to establish a successful infection (Chang et al., 1990). These can be referred as invasive/evasive determinants of Leishmania infection. The primary host cell for Leishmania is macrophages but there are several other type of host cells are also seen such as dendritic cells (Qi et al., 2001) and fibroblasts (Bogdon et al., 2000). In in-vitro system infection in macrophages does not produce cytopathology or any cytolysis of the host cells. Therefore it can be postulated that pathology and symptoms of the leishmaniasis is the result of interaction of these infected macrophages with several other unknown factors of the host. It is proposed that leishmania-specific antigens (Pathoantigens) from the infected cells interacted to the immune system of host in a negative manner and these interactions may turn out into presentation of disease symptoms in the host (Chang and McGwire, 2002). Therefore virulence factors of Leishmania can be divided into two major groups (Chang 1993; Chang et al., 1999; Chang and McGwire, 2002):

1. Invasive/evasive determinants: these are the determinants which crucial for infection but are not responsible for pathology in the host (Chang et al., 1990; Alexander and Russell, 1992; Bogdan and Rollinghoff, 1999; Rittig and Bogdan, 2000). These determinants help in following events of intracellular parasitism:

a. Evasion of humoral lytic factors like complement system
b. Attachment and internalization of Leishmania parasite into macrophages
c. Survival of the parasite into macrophage
d. Differentiation of Promastigotes into amastigote
e. Replication of amastigote form into macrophage

These determinants help intracellular amastigotes maintain continuous infection by growing at a slow rate in the parasitophorous vacuoles of host macrophages. These determinants proposed to be evolutionarily selected to become immunologically 'invisible', hence facilitating pathogen invasion into the hosts by evading their immune response (Chang et al., 2003). There are several invasive/evasive molecular determinants are very well characterized and well studied. Some are lipophosphoglycan (LPG), Leishmanolysin (GP63), phosphoglycans (PG), Proteophosphoglycans (PPG), Cystein Proteases (CPs),
Glycosylphospholipid (GIPL), Glycosylphosphotidylinositol (GPI) and others. These molecules by some means help *Leishmania* in one of above events during infection.

**Lipophosphoglycan (LPG):** LPG molecules present on the surface of the *Leishmania* promastigotes binds to mannann-binding protein present in the host serum. This protein has a complement activating C1q domain (Green et al, 1994). Complement activation leads to the lysis of procyclic promastigotes. The components of the membrane attack complex are released from the metacyclic parasite surface (Puentes et al, 1990). Addition of the number of disaccharide repeats units at LPG in procyclic promastigote is the responsible for transforming into metacyclic promastigote (Sacks et al, 1995).

**Leishmania** lipophosphoglycan (LPG)

![Diagram of LPG structure](image)

**Figure 1.4 A schematic showing a generic structure of LPG in Leishmania.**

LPG also transiently inhibits the fusion of the phagosome with endosomes (Dermine et al, 2000), scavenges oxygen radicals produced during the oxidative burst (Chan et al, 1989), inhibits protein kinase C (PKC) activity (Giorgione et al, 1996), inhibits macrophage IL-12 synthesis () and suppresses macrophage nitrogen oxide synthase 2 expressions and NO production (Proudfoot et al, 1996). Loss of LPG through the alteration in LPG1 glectofurnosyl transferase gene in *L. major* results in the loss of ability to survive in the sand fly vector and to establish the infection in macrophages. This is due to the increased susceptibility to complement system and oxidative stress (Späth et al., 2000; Späth et al., 2003). However LPG synthesis is very much down regulated in amastigote form and seems to have no role in virulence for amastigote (Moody et al., 1993; Späth et al., 2000). *lpg2* mutant of *L.*
major showed 10-fold decreased survival in sand fly vector and also able to cause the infection in BALB/c mice but no pathology was observed. Interestingly these parasites were able to persist at the site of infection for a long period of time at very low level (Späth et al., 2003).

Leishmanolysin (GP63): GP63 is a zinc dependent metallo-proteinase attached to the parasite membrane by a GPI anchor (Wilson et al., 1993). Expression of GP63 is found to be up regulated during metacyclogenesis and plays a role in protecting the parasite from complement-mediated lysis (Yao et al., 2003; Beverley and Turco, 1998). GP63 is the only ecto-proteases expressed by all pathogenic Leishmania and serve as ligands for binding macrophage complement by cleaving C3b to C3bi which binds to CR1 and CR3 fibronectin receptors on the macrophage surface (Russell and Wilhelm, 1986; McGwire and Chang, 1994; Brittingham et al., 1995; Brittingham et al., 1997). GP63 also plays a role in suppressing the oxidative burst (Sorensen et al, 1994) and its proteolytic activity protects the parasite from cytolysis and degradation by lysosomal enzymes (Seay et al, 1996).

Phosphoglycans (PG): These are most abundant molecules of Leishmania surface and consist of polymer of disaccharide phosphate repeat back bones [Galα1,4Manα1-PO₄]. When they are attached to the membrane through GPI (Glycosylphosphotidylinositol) they are LPG and when they are attached through proteins they are PPGs (Proteophosphoglycans) (Ilg et al., 1999; Ilgoutz et al., 2001; Turco et al., 2001).

Cystein Proteases (CPs): Leishmania contains multiple CPs in which some are stage regulated (Sacks et al., 2000). These enzymes serve to be as degradative role for nutrient benefit of amastigotes to modulate lysosomal activity for their survival. Targeted gene deletion of CP-B gene array in L. mexicana cause the attenuation of the parasite and parasite lost the ability to infect the macrophages in-vitro and to survive in BALB/c mice in-vivo (Ilg, 2000).

Glycoconiositol phospholipids (GIPL) containing glycosphingo lipids constitutes a dense glycocalyx that remains closely associated with the parasite surface and protects it from the hostile environment of the parasitophorous vacuole (PV) (McConville and Ralton, 1997). Membrane bound and secretory acid
phosphatases produced by the parasite are non-specific monoesterases that hydrolyse a variety of phosphorylated substrates at low pH. This provides protection to the parasite from enzymatic degradation in the PV (Glew et al., 1988; Lovelace and Dwyer, 1986).

It has been shown that promastigote is involved in interacting with different receptors of macrophages hence producing different responses. But recent studies showed that neutrophils are the first host cells that interact with promastigotes because they arrive before the macrophages at the site of infection (van Zandbergen et al., 2004). Subsequently dead neutrophils will deliver the parasite into macrophages and transformed into amastigotes. It is clear that interactions between amastigote and macrophages are of important in establishing and maintaining the infection (Kima, 2007). Some study revealed that heat shock response is trigger for this transformation indicating that these signals may be originated in cytocol of macrophage or neutrophils (Barak et al., 2005). Very little is known about how these external signals are perceived by Leishmania and transmitted to downstream targets required for differentiation. Moreover till date no signal transduction pathways have been fully studied in the Leishmania.

Another important mechanism involved in the survival of the Leishmania parasite is successful subversion of the host’s immune responses by modulating the macrophage signaling into its own favor. Suppression of superoxide and NO and inhibition IL-2 production (require for protective TH1 response) are the results of leishmania infection in macrophages. On the other hands secretion of IL-10 and TGF-β is increased. It has been shown that L. donovani amastigotes are capable of suppressing class II and class I MHC expression (Reiner et al, 1987). Antigen presentation by the MHC class II molecules to T-cells is important for the activation and proliferation of the IFN-γ producing CD4+ Th1 subset required for control of a Leishmania infection (Liew and O'Donnell, 1993). Further, it has been shown that processing of exogenous antigen for presentation by MHC class II molecules is defective in Leishmania infected macrophages (Fruth et al, 1993; Prina et al, 1993). Class II MHC molecules that reach the PV are endocytosed and degraded by the parasite's cysteine proteinases (De Souza et al, 1995). Also some parasite proteins like members of cystein protease B family and EL-1α are transported to the cytosol
of the macrophage and directly interacting with signaling pathway of macrophages (Kima, 2007). It is yet unknown how this transportation occur from the lumen of phagolysosomes to the cytosol of the macrophages.

2. Pathoantigenic determinants: These are the highly conserved structural or cytoplasmic proteins which are responsible for immunopathology of leishmaniasis. There are several Leishmania antigens found to elicit antibody generation at very high titer. Kinesin, tubulin, Heat shock proteins (HSP 60, 70, 83 and STI1), histone proteins (H2A/B, H3, H4), proteins in spliceosomes, proteosomes, ribosomes and glycosomes. Since these antigens are present inside the cytoplasm of amastigote, they are very difficult to approach for any protective antibodies. Therefore leishmania can afford to have these factors. These molecules have been found to have immunogenic B-cell epitopes (Mougneau et al., 1995; Requena et al., 2000; Probst et al., 2001). The titers of these pathoantigenic determinants are higher than that of invasive/evasive determinants (Chang and McGwire, 2002). Low anitgenicity of the invasive/evasive determinates might have selected in evolutionary pressure because they can escape from neutralization by the specific antibodies and/or other lytic factor to ensure successful parasitism.

Only at the cytolysis of infected macrophages these antigens are exposed to immune system of host which is necessary for initial recognition and immunopathological response of the host in the form of disease. Epitope-mapping revealed that these pathoantigens contain Leishmania-unique epitopes which are immunologically active during leishmaniasis and only recognized by sera from patient with visceral leishmaniasis (Requena et al., 2000). A unique 117 bp repeats of leishmania kinesin-like gene is expressed by amastigote of visceral leishmaniasis but not by the cutaneous species. Antibodies for these regions (anti-rK39) are used for serodiagnostic for VL patient. The release of pathoantigens causes the immunopahtology requires parasite replication.

Induction of a protective immune response against Leishmania also depends on the production of IL-12, which helps in triggering and maintaining a predominantly Th1 response and IFN-γ production (Liew and O'Donnell, 1993). Metacyclic
*Leishmania* promastigotes are capable of inhibiting macrophage IL-12 production both *in vitro* (Carrera et al, 1996; Sartori et al, 1997; Piedrafita et al, 1999) and *in vivo* (Reiner et al, 1994). The phosphoglycan moiety of LPG can modulate IL-12 synthesis at the level of transcription (Piedrafita et al, 1999; McDowell and Sacks, 1999). The immunological responses that lead to a non-healing *Leishmania* infection are IL-4 driven Th2 response which in turn downregulates Th1 responses (Chatelain et al, 1992; Leal et al, 1993; Afonso and Scott, 1993; Satoskar and Alexander, 1995; Reiner et al, 1994). IL-4 also down regulates NO production, which is critical for the leishmanicidal activity of the macrophage (Liew and O'Donnell, 1993). The LACK (*Leishmania* homologue of receptor for activated protein C kinase) antigen induces a rapid IL-4 response early during infection (Launois et al, 1997). However, early induction of IL-4 is not sufficient for susceptibility to disease (Scott et al, 1996) and it is not yet clear what triggers a Th2 response during a *Leishmania* infection. It has been argued that parasite mediated subversion of IL-12 and absence of a Th1 response rather than just a predominant IL-4 dependent Th2 response, is important.

Therefore *Leishmania* virulence requires both invasive/evasive and pathoantigenic determinants sequentially and/or combinatorially manner. The first group of determinants help in overcomes the host barriers to establish the infection but not the disease. Infection is the prerequisite for disease manifestation. According to classical definition these determinants are only responsible for infection not for the disease manifestation therefore these determinants are considered as the indirect virulent factors. Subsequently cytolysis of some amastigote exposes pathoantigenic determinants. These were not produce the anti-leishmanial immunity but the immunopathology of leishmaniasis i.e. disease manifestation. **Fig 1.5** shows a proposed model for *Leishmania* virulence. This model shows the interaction of both determinants with immune systems of the host independently if occur sequentially and or combinatorially that leads to clinical manifestation of leishmaniasis.
Figure 1.5 A model to explain virulence in *Leishmania*. The two groups of determinants can be interacting with host immune system independently, if they progress sequentially and/or combinatorially they will produce the spectrum of subclinical and clinical manifestations as the outcome.