CHAPTER IX

General Discussion

Leishmania is an obligatory intracellular parasite having a digenetic life cycle. The parasite alternates between promastigotes that reside within the midgut of sandfly vector and intracellular amastigotes in the phagolysomes of the vertebrate host macrophages. *Leishmania* promastigotes are introduced into the blood circulation of the host by insect vector. Macrophages are the main host cells targeted by *Leishmania* organisms, and infection of macrophages involves specific attachment of the promastigotes to the host macrophage receptor, followed by phagocytosis and intracellular replication (Chang, 1983). Two promastigotes glycoconjugates, a 63-kDa glycoprotein (gp63) and lipophosphoglycan (LPG) have been implicated in the initial attachment of promastigotes to macrophages (Russell and Talamas-Rohana, 1989; Russell and Wright, 1988).

Various methods have been proposed for studying host-parasite interaction. Most common and manual method used in the laboratories is Giemsa staining of the infected macrophages and counting the parasite under the microscope. Firefly luciferase, a widely used reporter for rapid detection and quantitation of host-parasite interaction has been developed and was found to be advantageous over manual and tedious Giemsa staining. Genetic reporters are used commonly in cell biology to study gene expression and other cellular events coupled to gene expression, such as receptor activity, intracellular signal transduction, mRNA processing, protein folding and protein-protein interactions (Alam and Cook, 1990; Wood, 1991). In this assay, reporter activity is available immediately upon translation since the protein does not require post-translational processing (Ow et al., 1986; de Wet et al., 1987). It is very sensitive because its light production has the highest quantum efficiency known for any chemiluminescent reaction (Wood, 1990) and no background luminescence is found in the host cells or the assay chemistry. Moreover, the assay is rapid, requiring only a few seconds per sample. In this study, *Leishmania* were

electroporated with pGL-αNEOαLUC vector, containing the neomycin phosphotransferase gene (NEO) and two α-tubulin intergenic regions and transfectants were selected for resistance to G418.

The growth profile of *L. donovani* promastigotes *in vitro* transfected with LUC (luciferase) gene containing, episomal vector pGL-αNEOαLUC, at different time intervals was examined. Correlation between the number of *L. donovani* promastigotes and luciferase activity indicated the sensitivity of this assay.

Resistance of leishmanial isolates to pentavalent antimonial compound (Sb^V-Cl_2) *in vitro* by luciferase assay was also measured. It was observed that strains 41-R, CK2-R and AG83-W were resistant to pentavalent antimonial compound (Sb^V-Cl_2). Clinical isolate NS2-R strain, which was supposedly resistant, was found to be sensitive in these studies. On the other hand AG83-W that had been passaged in the laboratory for several years was found to be resistant to antimony. It is well known that several passages of a strain in the laboratory result in the loss of virulence of the parasites. It is possible that strain AG83 has undergone some changes at the genetic level in the laboratory and these changes need to be looked into. These changes may have resulted in the development of resistance to antimony.

The unique properties of the firefly luciferase and the cloning of the gene for this enzyme have spawned a number of novel applications of this protein, as a tool in molecular and cell biology. This novel method would further aid in developing tools for a new and effective therapeutic approach against protozoan parasites.

The mechanism of signal transduction as a result of parasite interacting with the host is a very important area to be studied and this would basically decide if the parasite is going to survive in the host macrophages or not. One of the key determinants of parasite

infectivity and survival in the host macrophages is a novel glycoconjugate, lipophosphoglycan (LPG) (Turco and Descoteaux, 1992; Turco, 1990). This is the major macromolecule on the surface of *Leishmania.* It is the major macromolecule on the surface glycocalyx that coats the promastigote cell surface (Turco and Descoteaux, 1992). LPG consists of a polymer of phosphorylated disaccharide repeat units attached by a polysaccharide core to a novel lipid anchor. The promastigotes plasma membrane contains about 1.25 million copies/cell of LPG, accounting for at least 25% of its total cell wall (McConville and Blackwell, 1991). Lipophosphoglycan (LPG) is thought to play an important role in the biology of the parasite due to its surface location, its developmental regulation during the life cycle and the reduced virulence of the LPG-deficient organisms (Elhay et al., 1990; McConville et al., 1992). Evidence that LPG is a virulence factor, was found as the surface of infected macrophages had LPG. The PG may be derived from *Leishmania* surface being sloughed off or directly transferred to the host cell during parasite attachment and internalization and it was found that promastigotes in culture differentiate from a non-infectious "procyclic" form in early lag phase growth into an infective "metacyclic" form in stationary phase. Both stages have LPG, but the number of repeat unit's doubles in stationary phase cells.

The *Leishmania* are easily propagated, each stage of parasite-phagocyte interactions can be easily studied *in vitro*, and excellent animal model systems of infection are available, including inbred strains of mice that vary dramatically in their susceptibility to infection. Tremendous progress has been made in the past decade in defining the immunogenetics of leishmaniasis using murine models. Inbred strains of mice vary dramatically in their susceptibility to infection with various *Leishmania* species. These murine models have proven particularly useful in defining the genetic determinants of

suspectibility to infection (Bradley et al., 1979; Behin et al., 1979; Gorczynski, 1982; Mock et al., 1985; Blackwell, 1982; Crocker et al., 1984; Nickol and Bonventre, 1985; Plant et al., 1982; Cox, 1981; Blackwell, Roberts and Alexander, 1985). Bradley and co-workers were the first to observe that some inbred strains of mice such as BALB/c or C57BL/10 were highly susceptible to infection with *L. donovani* whereas others like C3H/HeJ or C.D2 were resistant (Bradley et al., 1979; Blackwell, 1982; Nickol and Bonventre, 1985). Within a given mouse strain, there was little variation in susceptibility. However, the course of visceral leishmaniasis in susceptible mice differs from that in humans in that mice do not usually die of *L. donovani* infection, even though they develop high parasite burdens. Bradley and co-workers found that suspectibility in mice was mediated by a single gene, termed *Lsh*, located on chromosome 1. The regulator(s) of resistance to two other intracellular pathogens, *Salmonella typhimurium* (*Ity*) and *Mycobacterium bovis* (*Bcg*) are seemingly identical to *Lsh* (Plant et al., 1982). The *Leishmania*-resistant allele (Lsh^r) behaves as an incomplete dominant, and the *Leishmania*-sensitive allele (Lsh^s) as a recessive. *In vitro* and *in vivo* studies suggest that the *Lsh* gene product is expressed at the level of the liver macrophage (Kupffer cell) (Crocker, Blackwell and Bradley, 1984). C.D2 expresses Lsh^r phenotype, which exhibits innate resistance against *Salmonella enterica serover typhimurium*, *Mycobacterium bovis*, and *L. donovani* (*Ity/Bcg/Lsh* gene) (Mock et al., 1994) and BALB/c expresses Lsh^s phenotype. Lsh^s mice support the growth and division of amastigotes, whereas Lsh^r mice limit parasite proliferation after a brief period of multiplication. The expression is T cell independent. It results in upregulation of MHC II expression and increase in responsiveness to LPS and IFN-γ, increase in production of TNF-α and IL-1 and increased production of nitric oxide (NO) in macrophages when stimulated with IFN-γ or LPS. The candidate gene for the natural resistance *Ity/Bcg/Lsh*

was cloned and designated Nramp (Natural resistance-associated macrophage protein), which was found to function as a transporter of divalent cations (Jabado et al., 2000; Vidal et al., 1995; Wardrop and Richardson, 2000).

The outcome of *Leishmania* infection depends not only on the species initiating disease but also on the immunological competence of the individual to combat parasite growth. Studies in mice and man have shown that multiple genetic loci influence the success of infection, affecting both acquired and innate immune responses against the parasite. The suspecibility or resistance to the disease is determined by ratio of Th1 or Th2 cells. Where susceptibility is associated with expansion of subsets of $CD4^+$ cells expressing Th2 phenotype and producing IL-4, IL-5, IL-6 and IL-10, the resistance is associated with expansion of Th1 cells producing IL-12, IL-2, IFN-γ and TNF-β (Kemp et al., 1996; Miralles et al., 1994). Recent studies suggest that both $CD4^+$ and $CD8^+$ T cells are required for immunity against the parasite (Liew and O'Donnel, 1993). Once the balance between Th1 or Th2 becomes skewed, it results in progression of disease. The mechanism of signal transduction that leads to this polarized response is not known.

In the present study signal transduction mechanism in macrophages infected with *Leishmania donovani* has been explored. *Leishmania donovani* is an obligate intracellular parasite that survives inside the macrophages of the vertebrate host, resulting in visceral leishmaniasis in humans. Macrophages have to become activated in order to kill infectious organisms indicating the need for signal transduction pathways that can transmit the information from the cell membrane to the nucleus. Since, *L. donovani* is known to survive within the macrophages, this could be due to the impairment of several macrophage accessory functions responsible for microbicidal action. For instance *L. donovani* infected macrophages are known to have an impaired oxidative burst, which is a primary defense

mechanism of the cell during *L. donovani* invasion (Buchmuller-Roiller and Mauel, 1987). Infected macrophages are impaired in their ability to produce gene products like IL-1β or MHC, which are essential for induction of T cell dependent immune responses (Reiner, 1987; Reiner, Ng and McMaster, 1987; Reiner et al., 1988). Impairment of signal transduction pathways in *L. donovani* infected macrophages is yet another mechanism by which parasite evades intracellular killing (Descoteaux et al., 1991; Moore, Labrecque and Matlashewski, 1993). Thus, the signal transduction pathway represents an attractive target for the parasite to result in inactivation of macrophage functions. Several early genes like c-fos, c-jun and c-myc protooncogenes are involved in the general process of cell activation (Hamilton and Adams, 1987). The c-fos protooncogene is among the immediate early genes that are expressed immediately after macrophage activation with LPS (Introna et al., 1986). Ornithine decarboxylase (ODC) is the first and rate-limiting enzyme in the biosynthesis of polyamines; which is implicated to have a role in a variety of metabolic processes (Pegg and McCann, 1982; Tabor and Tabor, 1984). The accumulation of ODC mRNA in mammalian cells is associated with the stimulation of a variety of physiological responses indicating that the ODC gene belongs to a group of early genes like c-fos, c-myc and c-jun protooncogenes (Kaczmarek and Kaminska, 1989). Earlier work has shown that bacterial lipopolysaccharide (LPS), an activator of macrophage effector functions (Higuchi et al., 1988), induces ODC mRNA accumulation in human and mouse monocytes and macrophages (Messina et al., 1990).

A major surface glycoconjugate of *Leishmania* species, lipophosphoglycan (LPG) is involved in macrophage-parasite interaction (Turco and Descoteaux, 1992) and internalization of the promastigotes (Descoteaux et al., 1991) as well as in the protection against microbicidal activities displayed by macrophages. One of the most important

features of LPG may be its ability to inhibit the activation of PKC as shown earlier in *in vivo* systems (McNeely and Turco, 1987). Since PKC is the key enzyme of the cell machinery, which conveys signals from the surface of the cell to the nucleus (Nishizuka, 1986), inhibition of PKC is indeed a crucial breach in the defence mechanism of the cell.

In this study we explored the effect of LPG on signal transduction pathways in a J774A.1 murine macrophage cell line. *L. donovani*, major surface molecule lipophosphoglycan (LPG) and bacterial lipopolysacchride (LPS) were found to stimulate ODC activity in macrophages in a dose and time dependent manner, confirming the possible role of ODC as an early marker of macrophage activation. LPG stimulated the rapid increase in ODC activity within 60 min after exposure, suggesting that the interaction of LPG with its receptor stimulated a specific signal transduction pathway. However, LPG induced ODC activity was a transient event and activity appeared to be coupled to the activation of PKC in macrophages as activators of PKC caused a rapid increase in the ODC activity. The ODC gene, like the c-fos gene, is among the early genes that are expressed immediately after macrophage activation with LPS and thus represents a useful marker to examine signal transduction in macrophages (Hamilton and Adams, 1987).

LPG stimulated ODC through protein kinase signal transduction as judged by the use of a broad-spectrum inhibitor of protein kinase, staurosporine. The use of OKA along with staurosporine showed that a protein serine-threonine kinase might not be the relevant target for staurosporine within macrophages. Another possibility could be that the pretreatment with OKA may not have been sufficient to cause changes in the degree of protein phosphorylation. Specific inhibitors of individual protein kinases may help to sort out which of the many protein kinases present in the macrophage is the relevant target for staurosporine.

Depletion of PKC by PMA treatment greatly reduced ODC activity in response to LPG. This demonstrated the involvement of PKC in macrophages exposed to LPG. Role of PKC in activation of ODC by LPG in macrophages was confirmed by using H7, an inhibitor of PKC. LPG did not inhibit induction of ODC activity by dibutyryl cyclic AMP (dBcAMP). This result ruled out the role of PKA in LPG stimulation of ODC activity. This is consistent with the previous finding that LPG has no effect on PKA activity *in vitro* (McNeely and Turco, 1990). Since the ODC activity is stimulated immediately after macrophage activation with LPG, it represents a useful marker to examine signal transduction in macrophages.

Thus, the present study shows that the ODC gene like the c-fos gene is among the immediate early genes that are expressed immediately after macrophage activation with LPG, it therefore represents a useful marker to examine signal transduction in macrophages.

Lipophosphoglycan, the surface glycoconjuagate of the protozoan parasite *Leishmania*, is also known to regulate the production of several key immune effector molecules including nitric oxide (NO) and proinflammatory cytokines in macrophages. In macrophages the mitogen-activated protein kinases (MAPK) cascades, NF-κB, activating protein 1 (AP-1) and IFN regulatory factors (IRFs) play an important role in induction of these macrophage effectors function. The MAP kinases are an important group of serine/threonine signaling kinases that modulate the phosphorylation and activation of transcription factors, thus linking transmembrane signaling with gene induction events in the nucleus. The MAPK cascades are activated by various cellular stresses and growth factors, and are involved in various biological responses such as cytokine production, differentiation, proliferation and cell death (Ichijo, 1990; Davis, 2000). In mammals,

MAPK cascades are composed of three distinct signaling modules, JNK, p38 MAPK and extracellular signal-regulated kinase (ERK). Upon cytokine or growth factor stimulation, or in response to various stresses, MAPKKKs are rapidly activated by oligomerization or undefined mechanisms, and phosphorylate down stream MAP kinases, called MAPK kinases (MAPKKs) (Ichijo, 1990; Davis, 2000). Then, activated MAPKKs finally phosphorylate and activate MAPKs, which in turn phosphorylate specific targets and activate their transcriptional activities.

Most signal transduction pathways in response to extracellular stimuli ultimately impinge on the gene expression via the regulation of the transcription factors. Thus activated protein-1 (AP-1), NF-κB and IFN regulatory factors (IRFs) are important pleiotropic transcription factors that regulate a variety of gene expression.

Nuclear factor- κB (NF-κB) is a collective name for dimeric transcription factors comprising members of the Rel family of DNA-binding protein that recognize a common sequence motif 5'-GGG(A/G)NN(T/C)(T/C)CC-3', where N is any base, which regulate the expression of numerous cellular and viral genes that participate in inflammatory, immune and acute-phase responses. NF-κB exist in the cytoplasm in an inactive form associated with an inhibitor subunit, IκB, which on phosphorylation results in active DNA binding forms of NF-κB. The active forms of NF-κB are dimeric complexes composed of various combinations of members constituting *rel* family. Activation of NF-κB correlates with the increased activation of the MAPK/JNK pathway.

AP-1 is a sequence-specific transcriptional activator composed of members of the Jun and Fos families (Angel and Karin, 1991), which belong to the bZIP group of DNA binding proteins, associate to form a variety of homo- and heterodimers that bind to a common site and behave as "immediate-early" genes. The activities of both pre-existing

and newly synthesized AP-1 components are modulated through their phosphorylation, by three different types of MAPKs, the ERKs, the JNKs and FRKs in response to a diverse array of extracellular stimuli. ERKs phosphorylate TCF/Elk-1 and induce c-Fos synthesis, on the other hand, JNKs phosphorylates the stimulatory sites of c-Jun. The newly synthesized c-Fos and c-Jun protein combine to form stable AP-1 heterodimers.

The IRF families of transcription factors are cellular DNA binding proteins that act as activators or repressors of promoters containing variations on the IRF binding sequence. IRF-1 (interferon-regulatory factor-1), a member of the JAK-STAT pathway, regulates expression of MHC class I in different tissues and also binds to IFN-stimulated regulatory elements (ISREs), which are found in promoters of IFN-inducible genes, such as inducible nitric oxide synthase (iNOS). Similarly, IFN regulatory factors (IRFs) also appear to play a key role in the induction of both macrophages effectors like NO and IL-12 via the IFN-stimulating response element in iNOS and the nuclear complex termed F1, which includes multiple nuclear factors (IRF-1, c-Rel, and GLp109) and acts at the Ets site in the IL-12 promoter. However, little is known about the early signaling events underlying the up-regulation of iNOS or IL-12 (p40) expression by LPS in macrophages.

LPG stimulated the simultaneous activation of all three classes of MAP kinases, extracellular signal-related kinases (ERKs), the c-Jun amino-terminal kinase (JNK) and the p38 MAP kinase with differential kinetics in J774A.1 macrophage cell line. LPG stimulated phosphorylation of p38, which was maximal at 10 min and sustained till 60 min. LPG strongly stimulated JNK MAP kinase following a lag period of 30 min before reaching peak with maximum activity at 60 min. LPG-stimulation of ERK peaked at 5 minutes and was considerably reduced at 60 min.

L. donovani and its surface molecule lipophosphoglycan resulted in a dose and time dependent induction of AP-1, NF-κB and IFN regulatory factor (IRF) DNA-binding activity. Dose dependent increase in AP-1 DNA-binding activity was observed with both low and high-virulent strains of *L. donovani*. The effect of viability of the parasite on NF-κB activation and IκB-α degradation in macrophages was also observed with both live and heat-killed parasite. Pretreatment of macrophages with cytochalasin B (10 μM), an actin-depolymerizing drug that inhibits phagocytosis, indicated that the activation of AP-1, NF-κB and IFN regulatory factor (IRF) do not require infectivity of *L. donovani* in the cells and its cellular uptake.

To demonstrate that the DNA oligomer-protein complexes formed in the electrophoretic mobility shift assay (EMSA) represent specific molecular interactions, unlabeled probe competition experiment was performed. The corresponding unlabeled probe strongly competes for complex formation. For AP-1, NF-κB, IFN regulatory factors (IRFs) consensus oligomer, a predominant AP-1, NF-κB, IFN regulatory factors (IRFs) consensus probe-binding complex was present in untreated nuclei. This data indicates that the proteins activated by LPG and detected by EMSA were highly specific for AP-1, NF-κB and IFN regulatory factors (IRFs).

The super-shift assays were performed using antibodies against specific AP-1, NF-κB and IFN regulatory factors (IRFs) subunits. The nuclear extracts from the *L. donovani* treated J774A.1 cells were incubated with specific polyclonal antibodies to c-Jun and c-Fos for AP-1, p50, p65 or c-rel for NF-κB and IRF-1 for IRF before addition of respective labeled oligonucleotide. To determine the specificity of the complex formed in EMSA, supershift assay were performed using antibody (Abs) against the AP-1 dimer. It was only

the c-Fos antibody that reacted with AP-1 and not the c-Jun as observed in the supershift assay. Supershift assay with different antibodies against different subunits of NF-κB indicated that both p65 and c-rel antibodies reacted with NF-κB complex. However, no supershift was noticed with p50 antibody. These results suggest that both p65 and c-rel subunits constitute the *L. donovani* activated NF-κB-DNA complex. Whereas, in case of IRF, the identity of the *L. donovani*, LPG and LPS-induced IRF-like complexes is unclear since the antibody supershift studies showed that they do not contain IRF-1. Earlier studies have ruled out a role of any of the well-characterized factor (IRF-1, IRF-2, ICSBP (IFN consensus sequence-binding protein), or ICSAT) in LPS-stimulated IRF-like complex.

Using inhibitors of MAP kinase signaling pathway, it was observed that the activation of AP-1 and NF-κB transcription factors was markedly affected by pretreatment of macrophages, with either PD98059 (20 μM), a specific inhibitor of ERK1 and ERK2 pathway or SB203580 (5 μM), a specific inhibitor of p38 pathway. Preincubation of cells with PD98059 (20 μM) and SB203580 (5 μM), followed by treatment with *L. donovani*, did not affect the binding activity of IRF suggesting that both p38 and ERK MAP kinase activation are not involved in the IRF activation. At these concentrations the inhibitors did not cause any cellular damage.

It has been reported earlier that NF-κB is recognized as a redox-sensitive transcription factor, and has been implicated in the cellular response to oxidative stress. Effect of antioxidants on *L. donovani* stimulated NF-κB-DNA binding activity was studied. Macrophages were pretreated with antioxidants like BHA (5 μg/ml), BHT (5 μg/ml) or GSH (1 mM, 2 mM and 4 mM) for 1 h and then infected with *L. donovani* for 1 h and NF-κB-DNA binding activity induced by *L. donovani* was determined. BHA (5 μg/ml), BHT (5

μg/ml) and GSH (1 mM, 2 mM and 4 mM) resulted in inhibition of NF-κB activation, whereas no significant difference was noticed with mannitol (25 mM).

An understanding of the role of the different signaling cascades, their individual members, their interacting partners and importantly their cross talk will pave the way for the design of new preventive and therapeutic modalities.

Survival of *Leishmania* parasite in macrophages has also been shown to be associated with the ability of the LPG to regulate production of nitric oxide (NO) (Proudfoot et al., 1996) and IL-12. NO, which mediates many of the inflammatory responses of macrophages following infection by pathogens is generated following the up-regulation of expression of inducible NO synthase (iNOS). Macrophages express iNOS following activation by a variety of immunological stimuli such as interferon γ (IFN-γ), tumor necrosis factor α (TNF-α) and bacterial lipopolysaccharide (LPS) (Hibbs et al., 1988; Nathan and Xie, 1994). LPS has been shown to stimulate a variety of signal transduction elements such as Src-related protein tyrosine kinases, phospholipases, protein kinase C, raf, mitogen-activated protein kinases in a variety of cellular systems. An earlier study reports that MAPK- and NF-κB-dependent induction of NO generation in the case of turberculosis (Chan et al., 1995). It is well established that NF-κB is one of the transcriptional requirement for induction of NO synthesis. The promoter of the murine gene encoding for iNOS was found at 55 bp upstream of the TATA box of NF-κB binding site. Involvement of signal transduction elements in regulating nitric oxide production during *Leishmania* infection, by using specific inhibitors for the relevant kinase pathway was worked out. The kinases were found to be involved upstream of the macrophage effector molecules. Thus cytokines, nitric oxide production might all be controlled by the direct

action of the parasite through the induction of the endogenous signaling pathway leading to the activation/inactivation of transcription factors.

Dose and time dependent increase in nitrite levels were observed, when macrophages were stimulated with LPG (10 μg/ml) for 24 h. To study the role of ERK1/2 and p38 MAPK in the regulation of LPG induced nitrite levels, PD98059 (20 μM) and SB203580 (5 μM), a selective inhibitor of the ERK1/2 kinase and p38 MAPK respectively were used. These results indicate that both PD98059 (20 μM) and SB203580 (5 μM) resulted in inhibition of nitrite levels of LPG treated groups.

The effect of well-characterized signaling pathways in the macrophages; protein kinase C (PKC)-mediated response on LPG induced nitrite levels was also examined. Macrophages were pretreated with staurosporine (2 μM), a broad-spectrum inhibitor of protein kinase. Pre-treatment of macrophages with staurosporine (2 μM) for 15 min followed by treatment with LPG (10 μg/ml) for 24 h resulted in the inhibition of nitrite level over LPG treated groups.

The effect of both H7 and PMA, a non-specific and specific general kinase inhibitor that inhibits PKC and W7, a specific inhibitor of calmodulin protein kinase (CaM-PK) on LPG induced nitrite levels was also studied. Macrophages were pretreated with H7 (50 nM) or PMA (100 nM) or W7 (100 mM) for stipulated time and then stimulated with LPG (10 μg/ml) for 24 h. This demonstrated the involvement of PKC- and CaM-PK dependent response on LPG stimulated nitrite levels in macrophages.

The effect of LPG in *Leishmania* is quite analogous to that of LPS, which has also been known to regulate NO synthesis. Both LPG and LPS may be used by the pathogens to increase their survival in the non-immune host by inhibiting the subsequent activation of macrophages by IFN-γ for NO synthesis. This is consistent with an earlier report that a

mutant strain of *L. major* deficient in LPG expression was avirulent and that its survival in macrophages was prolonged when LPG was passively inserted into the membrane of live promastigotes (Handman et al., 1986). However, in immunized hosts where IFN-γ is already present in considerable levels, or can be rapidly elevated to a high level, the presence of LPS/LPG would lead to enhanced NO synthesis and the accelerated destruction of the pathogens. This synergistic effect would therefore contribute toward the resistance of the immune individuals to the infections.

It has been reported earlier (Ghosh et al., 2002) that ceramide induces the suppression of ERK activation, AP-1 and NF-κB activity and NO generation leading to intracellular survival of the parasite in BALB/c susceptible mice. However in C.D2 resistant mice, protection against *Leishmania* pathogenesis in *Leishmania*-resistant strain involved upregulation by ceramide of ERK, AP-1 and NF-κB activity and NO genearation. The present study showing activation of protein kinases, ERK1/2, JNK and p38, NF-κB, AP-1 and IRF in J774A.1 cells is analogous to the macrophages from the resistant mouse model.

Thus, the present study shows the role of *Leishmania* lipophosphoglycan (LPG) in signal transduction events in macrophages. Selective impairment of signal transduction may therefore represent a new strategy for identifying targets for treating *Leishmania* infection.