Introduction
Immune System

The human body is an amazingly complex organism which can be viewed at a number of levels. Cells are the basic structural and functional units of biological organisms. All together, humans have around $10^{14}$ cells. The immune system is a system of biological structures and processes within an organism that protects against disease. Structurally, the immune system is a collection of cells, molecules, tissue, organs and circulatory systems (Janeway CA Jr, 2001; Segerstrom and Miller, 2004). Immune system cells are produced and mature in specialised areas of the body called primary lymphoid organs such as the thymus or bone marrow. They are transported through the cardiovascular and lymphatic circulatory systems to peripheral tissues or specialised secondary lymphoid organs such as the lymph nodes or spleen. To function properly, an immune system must detect a wide variety of agents, from viruses to parasitic worms, and distinguish them from the organism's own healthy tissue.

Innate and adaptive immunity

The immune system is often divided into two distinct yet interrelated subsystems: the innate immune system and adaptive immune system. The main characteristics of adaptive immunity are specific recognition of pathogen leading to the generation of pathogen specific long-term memory (Janeway CA Jr, 2001; Segerstrom and Miller, 2004). The adaptive immune system allows for a stronger immune response along with immunological memory. The adaptive immune response is antigen-specific and can differentiate between self and non-self. Antigen specificity allows for the generation of responses that are tailored to specific pathogens or pathogen-infected cells. Adaptive immune system responses is maintained in the body by "memory cells". Memory cells are retained within the body for a long time and infection by the same pathogen triggers a robust and quick immune response. The cells of the adaptive immune system are special types of leukocytes, called lymphocytes. B cells and T cells are the major types of lymphocytes and are derived from hematopoietic stem cells in the bone marrow (Kalia et al., 2006). B cells are involved in the humoral immune response, whereas T cells are involved in cell-mediated immune response. Either B cells and/or T cells have receptor molecules that recognize specific targets. T cells recognize a "non-self" target, such as a pathogen, only after antigens are presented by MHC molecules via APC cells. There are two major subtypes of T cells: the killer and helper T cell. Killer T cells recognize antigens coupled to Class I MHC molecules, while helper T cells recognize antigens coupled
to Class II MHC molecules. However, B cell contains antigen-specific receptor (BCR) on its cell surface, and recognizes whole pathogens without any need for antigen processing. The principal functions of B-cells are to make antibodies against antigens, to perform the role of antigen-presenting cells (APCs), and to develop into memory B cells after activation by antigen interaction. Each lineage of B cell expresses a different antibody, so the complete set of B cell antigen receptors represent all the antibodies that the body can manufacture (Figure 1). However, adaptive immune is the second line of defence against any invading pathogen and has certain limitations, but, first line of defence is through innate immune system. The innate immune system is characterised as having three roles: host defence in the early stages of infection through nonspecific recognition of a pathogen, induction of the adaptive immune response, and determination of the type of adaptive response (Janeway, 1998). Microorganisms or toxins that successfully enter an organism encounter the cells and mechanisms of the innate immune system. The innate response is usually triggered when microbes are identified by pathogen-associated molecular patterns (PAMPs), which recognize components that are conserved among broad groups of microorganisms (Bochner and Schleimer, 2001). Innate immune defenses are non-specific, meaning these systems respond to pathogens in a generic way (Medzhitov and Janeway, 2002). This system does not confer long-lasting immunity against a pathogen. Innate immune system consists of white blood cells (WBC) or leukocytes. Leukocytes are different not tightly associated with a particular organ or tissue; so, they can act as single cell organisms. Leukocytes move freely, interact and capture cellular debris, foreign particles, or invading microorganisms. Innate immune leukocytes cannot divide or reproduce on their own, but are produced by hematopoietic stem cells present in the bone marrow (Ogawa, 1993). Leucocytes can be divided into: mast cells, Natural Killer cells (NK cells), basophils and eosinophils, while the phagocytic cells contain neutrophils, dendritic cells and macrophages (Figure 1).
Macrophages:

Macrophages are the major differentiating cells of the mononuclear phagocyte system, which comprises bone marrow monoblast and promonoblast, peripheral monocytes and tissue macrophages. Macrophages (Greek: big eaters, from makros "large" + phagein "eat"), and was discovered by a Russian bacteriologist, Ilya Mechnikov, in 1884. Cells of mononuclear phagocyte system originate from pluripotent stemm cell during haemotoeosis and differentiate under the influence of specific signals (Artis et al., 2003) through several intermediary stages to mature macrophages. Macrophages also play a significant role in regulating immune response by engulfing pathogens and acting as APC (Unanue and Allen, 1987). Their widespread dispersal during early and adult life, versatile biosynthetic activities of well defined products (Takemura and Werb, 1984), possession of specific surface receptors and remarkable responsiveness to local environmental influences (Mantovani et al., 2004) these cells with the ability to interact with many cells and molecules of the body in health and disease. Mature macrophages are larger with more granular cytoplasm (Figure 2). The nucleus of macrophage varies in size and shape, the chromatin is usually margined and a nucleolus of conventional structure. However, human macrophages are about 15-22 μm in diameter. Lysosomes are found in large number in all types of macrophages within the body. Biochemically lysosomes contains hydrolytic enzymes which are maintained within lysosomes at acidic pH. This is also a cell survival strategy, since if lysosomes rupture abnormally they will not be able to digest the cell from within, since pH of cell in normally neutral.
Development differentiation and distribution of macrophages

In the adult, proliferating mononuclear phagocytes are found only in the bone marrow where macrophage precursors undergo several cycles of proliferation and differentiation (approx. 8-10 days). The most immature cells of the mononuclear phagocyte cell line is monoblast. Division of monoblast ultimately gives rise to monocytes. Monocytes then migrate from the blood vessels into the tissues or body cavities. Adhesion molecules on monocytes and on endothelial cells, together with chemotactic factors, are involved in the migration. After entering tissues, terminal differentiation takes place where monocytes transform into macrophages. The efficient production of monocytes – macrophages requires the operation of growth factors known as colony-stimulating factors (CSFs). The two principal CSFs are macrophage CSF (M-CSF), which is macrophage lineage specific and granulocyte-macrophage CSF (GM-CSF), which acts on both macrophage and granulocyte lineages. Monocyte production and differentiation is also promoted by interleukin-3 (IL-3), a factor that increases monocytopoiesis and a distinct inducer of macrophage differentiation. Monocytopoiesis is inhibited by prostaglandin E2 (PGE₂), tumour necrosis factor (TNF)-α, and a monocyte production inhibitor. As products of macrophage, PGE₂ and TNF-α can provide negative feedback. Mature macrophages once formed, are long-lived and are continuously replaced by fresh cells from blood and bone marrow (Figure 3). Little is known about the ultimate fate of macrophage. Dead or effete alveolar macrophages could be moved up the respiratory tree by ciliary action and macrophages in the intestinal wall could migrate
through the gut epithelium into the lumen. In other sides they could be disposed of by young macrophages in a scavenging role. Two general classes of mononuclear phagocytes have been recognized, resident or fixed and wandering or mobile. The mobile cells include the circulating monocytes in the peripheral blood and the free macrophages of different organs. Fixed cells, the permanent residents are present in most tissues of the adult, especially in haematopoietic and lymphoid organs like bone marrow, liver (Kupffer cells), spleen and lymph nodes.

![Figure 3. Development of Macrophages](image)

**Phagocytosis**

Macrophages have evolved a variety of strategies to internalize particles and solutes, including pinocytosis, receptor-mediated endocytosis and phagocytosis (Allen and Aderem, 1996; Rabinovitch, 1995; Silverstein, 1995). Pinocytosis and receptor-mediated endocytosis share a clathrin-based mechanism and usually occur independently of actin polymerization. By contrast, phagocytosis, the uptake of large particles into cells, occurs by an actin-dependent mechanism and is usually independent of clathrin. Thus phagocytosis by macrophages is critical for the uptake and degradation of infectious agents and senescent cells; and it participates in development, tissue remodeling, the immune response and inflammation.
Phagocytosis is extremely complex and no single model can fully account for the diverse structures and outcomes associated with particle internalization. The fact that most particles are recognized by more than one receptor, and that these receptors are capable of cross-talk and synergy, further complicates our understanding. Despite the complexity associated with different phagocytic mechanisms, the process can be considered to have four stages: (i) movement of the macrophage toward the particle (e.g. the microorganism or the cell) to be ingested, usually in response to chemotactic stimuli, (ii) attachment of the particle to the surface, (iii) ingestion, (iv) intracellular disposal (Figure 4).

(i) Chemotaxis

Chemotactic stimuli to which macrophages respond include the split product of the fifth component of complement (C5a), chemotactic lymphokines (e.g. macrophage chemotactic factor and macrophage procoagulant-inducing factor), leukotriene B4, formylated peptides (e.g. formyl-methionine-leucine-phenylalanine), thrombin and fibrogen cleavage products. In each case the chemotactic factor binds to a receptor on the macrophage surface from which signals are transmitted to the cell’s interior through changes in the metabolism of phosphoinositides, the intracellular calcium concentration, and the activity of protein kinase C. Movement is preceded by polarization of the cell so that it adopts a wedge shape with a broad leading edge. Movement towards the chemotactic stimulus requires of course, the expenditure of energy.
(ii) Attachment

Attachment is commonly mediated by antibodies directed against surface antigens of the particle (e.g. the cell or the bacterium) being ingested. Particles may also be found by way of complement receptors (especially CR1) and in the absence of antibodies or complement, by lectin like receptors reacting with surface sugars on the particle. Attachment requires no expenditure of energy by the macrophage.

(iii) Ingestion

The process of ingestion involves the enclosure of the particle by the macrophage cell membrane, forming a vesicle – the phagosome – which buds off into the interior of the cell. During ingestion, polymerization of actin takes place at the site of ingestion followed by the internalization of the particle via an actin based mechanism. Ingestion requires the expenditure of energy to move the particle/phagosome by means of the cell’s microfilaments (i.e. the cell’s muscles).

(iv) Intracellular Fate

After internalization actin is shed from the phagosome and phagosome enters the endocytic processing pathway. In this pathway a phagosome moves toward the cell interior, where it fuses with a lysosome to form a phagolysosome. Lysosome contain hydrogen peroxide, oxygen free radicals, peroxidase, lysozyme and various hydrolytic enzymes, which digest the ingested material. This might ensure complete destruction of the ingested material but undigestible material can be excreted from the cell (i.e. exocytosis) or can persist as microscopically or ultrastructurally visible debris (referred to as residual bodies, myelin figures etc.).

MACROPHAGES IN IMMUNE DEFENCE

The macrophages for the most part become and remain highly efficient phagocytes and play an important role in pathogen recognition and clearance as well as removal of senescent and dying cells. Macrophages produce a range of secretory products, can degrade antigens as well as can act as APC. Once activated, the macrophage plays a major role in clearing the invading pathogens. Macrophages are extremely heterogenous in their gene expression and cellular activities, in all tissues thus regulating both innate and acquired immunity.
Activation of macrophages

Activation of macrophages in general applies to the changes in its cellular environment that allows them to destroy intracellular microorganisms, tumor cells or cellular debris. Macrophages can also be stimulated to exhibit increases in one or more of several activities, for example, the secretion of important products (e.g. IL-1), the capacity for phagocytosis or the capacity to adhere to glass or plastic. Macrophages are involved in many different processes such as tissue remodelling during embryogenesis, wound repair, removal of damaged or senescent cells subsequent to injury or infection, haematopoiesis and homeostasis. Macrophages can kill microorganisms or tumor cells in a direct manner, involving the release of products such as oxygen radicals, nitric oxide and tumour necrosis factor that are harmful to microorganisms or cancer cells. On the other hand, they also play an indirect role in these anti-microbial or anti-tumour activities by secretion of cytokines or by antigen processing and presentation. Macrophage participation in host defence against tumour cells requires activation signals. Activation of macrophages proceeds via different stages accompanied by gradual changes in macrophage properties and can be characterized as unstimulated, primed and fully activated macrophages (Rabinovitch, 1995). Each stage is accompanied by specific expression of membranous and secreted proteins, which can be down or up-regulated when macrophages develop into another activation state. For example, when macrophages act as APC MHC class II molecules are highly expressed on primed macrophages. Hence antigen presentation is maximal in the primed state (Gordon, 2003). Since macrophage activation is regulated by both inductive and suppressive signals, activated macrophages can switch from the activated state to the responsive state. In experimental animals monocytes or young macrophages are much more readily stimulated than old or resident macrophages. Immunologically primed macrophages differ from resident macrophages. Monocyte recruitment is enhanced and yields macrophages macrophages with pro-inflammatory and cytotoxic properties. Macrophages can secrete a surprisingly large range of biologically important products. These include multiple enzymes, cytokines, growth factors, coagulation factors, metabolites of arachidonic acid, enzyme inhibitors, components of the complement cascade, numerous proteins, lipids and small molecules. Some products, such as lysozymes are secreted continuously by macrophages whereas others are secreted only in response to external stimuli. Macrophage products may have contrasting, antagonistic or synergistic effects.
Th1/Th2 paradigm in macrophage response

T-helper cells have been categorized with regard to their cytokine profile. Th1 cytokines (IL-12, IFN-γ) support cell-mediated immune responses whereas Th2 cytokines support humoral immune responses. As far as macrophages are concerned, Th1 cytokines support activation, whereas Th2 cytokines are, in general, suppressive in nature. Studies with macrophages have indicated that treatment with different cytokines yield different results, via activation and suppression of different subset of gens. While IL-10 exerts anti-inflammatory response IFN-γ can counteract this and help in anti-microbial activities. IL-4 and IL-13 exert distinctive effects on MHC class II and mannose receptor expression, which have been termed as alternate activation. IL-12 is a cytokine produced by APC cells, such as macrophages, monocytes and dendritic cells and can activate immune system by either stimulation of cytolysis activity and/or induction of IFN-γ production by T cells and NK cells. (Gordon, 2003). Other proinflammatory cytokines produced by macrophages and other target cells are IL-1, IL-6, IL-8 and TNFα. These cytokines also appear to play an essential role in the innate responses and macrophage activation during infection. IL-10 is an anti-inflammatory cytokine produced by macrophages as well as T cells, which blocks generation of Nitric oxide and other pro-inflammatory mediators thus helping in maintain the autocrine activity of macrophages (Cenci et al., 1993; Cunha et al., 1992).

GENERATION OF ROS AND RNI

Immune cells for controlling infection exploit a variety of systems for e.g generation of reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI). ROS and RNI, generated under physiological conditions, are capable of damaging DNA bases and lipids and disrupting the activity of important cellular proteins containing Fe-S clusters, transition metals, hemes, thiols, sulphydryl or tyrosyl groups (Nathan and Shiloh, 2000) (Figure 5). Macrophages have evolved specialized systems that allow them to be potent generators of both as needed, due in part to their ability to induce expression of nitric oxide synthase 2 (iNOS) and assemble components of NADPH oxidase.
There are three categories of oxidants generated by the NADPH oxidase and iNOS.

First, NADPH oxidase generates ROS through the reduction of molecular oxygen, which generates superoxide (O$_2^-$), hydrogen peroxide (H$_2$O$_2$) and hydroxyl radical (OH) (Nathan and Shiloh, 2000). The H$_2$O$_2$ generated is converted into H$_2$O and O$_2$ in a reaction catalyzed by catalase present in cytosol. An alternative pathway of converting H$_2$O$_2$ into H$_2$O involves the enzyme glutathione peroxidase (GSH-PO). Oxidised glutathione (GSSG) is then converted back to GSH in a reaction catalyzed by glutathione reductase. H$_2$O$_2$ is toxic to microbes, but to increase it toxicity it forms HOCl and HOBr with the help of eukaryotic myeloperoxidases (MPO) (Foote et al., 1983; Klebanoff, 1968). When phagosome containing microorganisms fuses with lysosomes or granules, MPO is released into the vacuole. MPO reacts there with a halide and H$_2$O$_2$ to form a microbicidal system which is highly destructible and acts on a variety of cellular components and DNA (Dukan and Touati, 1996; Imlay and Linn, 1988). iNOS uses L-arginine as a substrate to generate nitric oxide (NO) in a stepwise reaction (Figure 5) (Nathan and Shiloh, 2000). Furthermore, peroxynitrite (ONOO-) is generated by reaction of ROS with NO and is one of the most potent oxidant molecule (Pacelli et al., 1995; Szabo, 1996).
**Sequence of reactions is as follows:**

\[
\text{NO} + \text{O}_2 \rightarrow \text{ONOO} \\
\text{ONOO}^- \rightarrow \text{OH}^- + \text{NO}_2^-
\]

Although the generation of ROS and RNI is beneficial to the host in defence (Fang, 1997), unregulated production of these agents can also lead to extensive host cell damage via cellular dysfunction, DNA breakage and apoptosis (Nathan and Shiloh, 2000).

**NITRIC OXIDE**

Nitric oxide (NO) was recognized in as a biologically active molecule present in mammalian tissues, was thought to be a regulator of blood pressure (Furchgott and Vanhoutte, 1989) and having antitumor activities (Hibbs et al., 1987). Three different isoforms of Nitric oxide Synthase are present in the body namely: endothelial NOS (eNOS), present in endothelial cells, neuronal NOS (nNOS), in neural tissue and inducible NOS (iNOS), in macrophages (Hiki et al., 1991). iNOS leads to elaboration of high quantities of NO (Stuehr and Nathan, 1989). As the name suggests iNOS is not produced constitutively but is induced by either infection, tumors of stimulation by LPS (Ding et al., 1988). Nitric Oxide is an uncharged molecule composed of seven electrons from nitrogen and eight electrons from oxygen. The majority of biological molecules contain bonds filled with two electrons, which makes them unreactive with nitric oxide. Nitric oxide only reacts rapidly with a selective range of molecules that have orbitals with unpaired electrons. It is an oversimplification to limit ourselves to just three reactions, but the key point is that nitric oxide does not react rapidly with most biological molecules at the dilute concentrations produced in vivo.

**Control of iNOS/NO production by macrophages**

NO production was first established in humans. Macrophage antitumour activity as well as its role in vasoregulation, platelet aggregation and neurotransmission braked the notion that host defense is primarily via specific immune responses, emphasizing the fact that most nucleated cells can defend themselves with molecules that act on a variety of pathogens. The inducible form produces nitric oxide at 1000-fold greater levels than eNOS and exerts its biological effects independent of the guanylate cyclase/cGMP system. The biological effects of iNOS activation include cytotoxic/cytostatic activity via inhibition of ribonucleotide
reductase activity (Kwon et al., 1991), mitochondria respiration (Moncada et al., 1991) and induction of apoptosis (Cui et al., 1994). The cytotoxic effects of nitric oxide are due in part to its reaction with superoxide anion forming peroxynitrite with subsequent formation of nitrogen dioxide, hydroxyl radical and peroxynitrous acid that have potent oxidant activities (Szabo, 1996). Under normal physiological conditions, the constitutive forms of NOS are expressed and active, whereas iNOS activity is induced by pro-inflammatory stimuli such as LPS, TNF-α and IFN-γ.

Role of iNOS in macrophage response against infectious agents was best described by (Fang, 1997; Nathan and Shiloh, 2000). NO produced by macrophages kills the microbes by directly acting on it or forming peroxynitrite, which is one of the most potent oxidant (Fang, 1997). These reactive intermediates were shown to cause a variety of DNA alterations, protein modifications and inactivation of metal containing enzymes.

Overproduction of NO can have detrimental effect of the organism, which can suffer from septic shock and ultimately death (Szabo, 1996). NO overproduction is known to cause a number of inflammatory conditions including rheumatoid arthritis, Crohn’s disease and asthma (Moncada et al., 1991). Because of its role as both an immune mediator and an effector molecule, NO is a two edged sword that can have detrimental of beneficial role (Kolb and Kolb-Bachofen, 1998; Nathan and Shiloh, 2000).

iNOS is stimulated by numerous cytokines and microbial products. The effective agents and combinations depend on cell type and species. TNF as a cytokine is particularly important because ingestion of most microbes elicits autocrine production of an action of both TNF and IFN-α/β (Green et al., 1994; Riches and Underwood, 1991). Another important example of synergy is that between IFN-γ and LPS (Xie et al., 1992); LPS also induces macrophages to make TNF and IFN-α/β. Several readily encountered, noncytokine, nonmicrobial agents of diverse composition, such as ozone and asbestos, induce iNOS or augment its induction (Xie et al., 1994). It is not known whether these work indirectly by eliciting cytokines. iNOS can be regulated at either transcriptional or post-transcriptional levels: In iNOS promoter only one other site have been reported: a canonical hypoxia-responsive element (HRE), lying between positions –227 and –209, conferred responsiveness to picolinic acid and hypoxia (Melillo et al., 1995). Pharmacologic levels of glucocorticoids suppress induction of iNOS. Multiple mechanisms have been proposed, involving a combination of both transcriptional and post transcriptional effects (Kunz et al., 1996). IL-4 exerted a delayed suppressive effect on transcription of NOS2 (Kolb and Kolb-Bachofen,
IL-10’s suppressive effect was exerted indirectly, via its suppression of TNF production and ranged from minimal to significant, depending on the roles of autocrine TNF in the culture conditions under study (Oswald et al., 1992). Post-transcriptional regulation of iNOS is mainly controlled by TGF-β1 (Vodovotz and Bogdan, 1994). Pharmologically administered TGF-β1 in low doses suppressed the expression of iNOS in LPS-treated rats (Letterio and Roberts, 1998). Heme depletion may account for the loss of NOS2 enzyme activity in macrophages that contained normal amounts NOS2 protein after prolonged culture following a single application of IFN-γ and LPS (Vodovotz and Bogdan, 1994). The distribution between cytosolic and vesicular compartments of iNOS has presumably functional significance, but this remains to be demonstrated.

**Figure 6.** Transcriptional regulation of iNOS

### Signal Transduction Pathways in Macrophage

**The mitogen-activated protein kinase (MAPK) pathway**

MAP kinases are Ser-Thr kinases that have been shown to activate a number of transcription factors, including activator protein-1 (AP-1), activating transcription factor (ATF)-2, cAMP-responsive element binding protein, NF-κB, and certain members of the Ets family (Gupta et al., 1995; Janssen-Heininger, 1999 #57; Meyer, 1996 #58). MAPKs consists of three members: extracellular signal-regulated kinases (ERKs), p38 MAPKs, and c-
Jun NH2-terminal kinases (JNKs) or stress-activated protein kinases (SAPKs) (Chan and Riches, 2001). MAPKs are activated by a family of dual-specificity kinases [MAPK kinases or (MKKs)] that phosphorylate MAPKs on specific Thr and Tyr residues: MKK4 and MKK7 activate the JNK/ SAPKs, MKK3 and MKK6 activate the p38 MAPK, and MAPK/ERK kinase (MEK)-1 and -2 activate the ERKs (Derijard et al., 1995; Lin et al., 1995; Sanchez et al., 1994). Moreover MAPK are also activated by Protein kinase C (PKC) kinase C (PKC), leading to the release of Ca\(^{2+}\) ions which are then sequestered by integrins. These active integrins may then activate focal adhesion kinases (FAK), which constitutes a major initiating pathway to activating MAPKs. Alternatively, integrins may immediately be activated by their associations with the extracellular matrix, which then activate FAK in a similar fashion. The dominance of ionic over mechanical activation is still unknown, and a combination of these mechanisms may contribute to the initiation of MAPKs. Furthermore ERK1/2 and p38 MAPK signalling pathway have been found to play a key role in the transcriptional and post-transcriptional regulation of iNOS in glial cells treated with LPS in the presence or absence of IFN-\(\gamma\) (Bhat et al., 1998). In murine macrophages, p38 has been shown to be involved in LPS mediated NF-\(\kappa\)B activation and subsequent iNOS expression and NO release (Chen and Wang, 1999).

**Figure 7.** Simplified representation of activation of JNK, ERK1/2 and p38 MAPK.
Regulation of MAP kinases by MAP kinase phosphatases

Activation of MAPKs can be brought down by dephosphorylation of threonine and/or tyrosine residues. The dephosphorylation could be achieved by serine/threonine phosphatases, tyrosine phosphatases and dual-specificity phosphatases (Figure 8). The serine/threonine phosphatases which dephosphorylate MAPKs include PP2A and PP2C (Alessi et al., 1995). The tyrosine phosphatases which dephosphorylate MAPKs include three MAPK-specific tyrosine phosphatases, STEP, HePTP and PTP-SL (Pulido et al., 1998; Saxena et al., 1999). One new protein family named MAP kinase phosphatases (MKP) or dual specificity phosphatases (DUSP) have been defined as negative regulators of MAP kinases (Camps et al., 2000; Theodosiou and Ashworth, 2002). They are identified by their common features: a C-terminal catalytic domain containing a highly conserved signature motif HCXXXXXR and two Cdc25-like domains. They inactivate MAP kinases through dephosphorylation of threonine and/or tyrosine residues within the signature sequences –Thr-X-Tyr located in the activation loop of MAP kinases. Thirteen MKPs have been identified so far with differential substrate specificities, distinct subcellular localizations and modes of regulation. For instance, in vitro studies indicated that VHR and MKP3 are highly specific for ERK; MKP1, DUSP2, MKP5, MKP7 and VH5 have preference for p38/JNK; MKP2 and MKP4 showed similar preference to the three MAPKs. However, their in vivo substrate specificities could be different from their in vitro substrate preference. For example, in vitro studies indicated that MKP5 substrate preference is as p38~JNK>>ERK (Tanoue et al., 1999; Theodosiou et al., 1999). Intricate balance between kinases and phosphatases is a prerequisite for optimal macrophage response against invading pathogens.

**Figure 8.** Depiction of dephosphorylation of MAPK by MAPK phosphatase
The NF-κB pathway (Activation and Regulation)

NF-κB, family consists of five members (p50), (p52), Rel A (p65), Rel B, and c-Rel. These proteins pair to form homo- and heterodimers that are sequestered in the cytoplasm by the inhibitor-kappa B (IκB) proteins (Delhase and Karin, 1999). Interestingly, the p50 and p52 genes produce proteins of 105 and 100 kDa (p105 and p100), respectively, which are cleaved to give rise to p50 and p52. Phosphorylation and degradation IκB is a prerequisite, to allow nuclear localization of NF-κB dimmers. A variety of signaling pathways lead to the phosphorylation and degradation of IκB proteins. Though numerous stimuli which lead to the activation of NF-κB exist, some of the best characterized are microbial products. Thus, the binding of molecules such as lipopolysaccharide (LPS), bacterial DNA, peptidoglycans, and parasite mucins to Toll-like receptors results in the activation of NF-κB, which is important in the initiation of innate responses (Imler and Hoffmann, 2000; MacDonald and Pettersson, 2000; Ropert, 2001 #75; Zhang, 2001 #76) (Figure 9). In addition, signaling through many cytokine receptors and the T- and B-cell receptors as well as costimulatory molecules results in the activation of NF-κB (Aune et al., 1999; Liou et al., 1999). These diverse signaling pathways converge at the level of phosphorylation of IκB and its degradation to allow nuclear localization of NF-κB. Post-translational modifications achieved by both phosphorylation and acetylation of p65 subunit play particularly important roles in the activation of NF-κB in addition to the activation of classical pathway. MSK1, a downstream kinase of both ERK1/2 and p38, can phosphorylate p65 subunit at Ser276 residue and acts as a post-translational modifier that integrates the upstream MAPK signaling cascades with NF-κB transactivation (Vermeulen et al., 2003). NF-κB once activated is involved in the regulation of numerous genes involved in immune function as generation of Th1 cytokines, receptors (CD25) and (intercellular adhesion molecule 1 [ICAM-1], vascular cell adhesion molecule [VCAM]), as well as proteins involved in cell proliferation and survival (Collins et al., 1995; Gerondakis et al., 1998; Hofer et al., 2001; Sica et al., 1997).
Stimulus

Production of cytokines

Figure 9. NF-κB activation and production of pro-inflammatory cytokines

PKC pathway

Signal transduction pathway are an important mediator via which immune cells respond to various stimuli, including microbial antigens, mitogens and cytokines. Balancing of signalling pathways is very important for the host; as inadequate reactions to antigens can lead to disease while reactions with self-antigens can lead to autoimmune diseases. PKC family are important intermediates of immune signalling, with gene knock out studies revealing non-redundant role of PKC isoenzymes in different immune cells. PKC isoenzymes were identified as a cyclic nucleotide-independent protein kinase that phosphorylated histone and protamine in bovine cerebellum. Mammalian PKC can be broadly grouped under three categories which are conventional [PKC: α, βI, βII and γ], novel (nPKC: δ, ε, η and θ) and atypical [PKC: ζ, PKMζ catalytic fragment of PKCζ, and PKCl] (Zeng et al., 2012). PKC isoenzymes are phosphorylated shortly after translation, but due to presence of
pseudosubstrate domain in catalytic site they remain non-functional. In resting cells PKC are predominantly localized into the cytosol. Activation is achieved by hydrolysis of phosphatidylinositol 4,5-bisphosphate (PIP2), generating DAG and myo-inositol 1,4,5-trisphosphate (IP3). DAG binds to the Catalytic domain and increases the affinity of PKC for membrane phospholipids (Zeng et al., 2012). As a result there is conformational changes in PKC which are associated with phospholipid binding and displacement of pseudosubstrate region from catalytic domain of PKC thus enabling their activation. Recent studies have implicated a role of PKCe as an important mediator of macrophage function. Macrophage-specific inhibition of PKCe has been linked to an inhibition of IL-4-induced NO production (Sands et al., 1994), whereas expression of PKCe was sufficient to induce NO synthesis in RAW264.7 macrophage cell line (Diaz-Guerra et al., 1996). PKCs demonstrate relatively broad in vitro substrate specificity, yet clearly have distinct in vivo functions. The diverse and distinct roles of individual PKCs are, at least in part, attributed to differences in their structural features and the mechanisms that modulate their activation (Figure 10).

Figure 10. Activation of PKC pathway resulting in MAPK activation
Leishmaniasis: the disease

Leishmaniasis constitutes a diverse collection of diseases ranging in severity from a spontaneously healing skin ulcer to fatal visceral disease. According to the World Health Organization (WHO), this is one of the six major human health problems. The disease is caused by a group of parasites constituted by a total of about 21 different species under the genera *Leishmania*.

The leishmaniases are a complex set of diseases caused by the intracellular protozoan *Leishmania*, and is considered by the World Health Organization (WHO) as one of the 6 major problems in the world. They are widely spread and their disease burden is high, with 350 million people considered at risk. The disease is caused by a group of parasites constituted by a total of about 21 different species under the genera *Leishmania*, which are transmitted by about 30 species of female phlebotomus sandflies. There are an estimated 1.5–2 million new cases per year, up to 500,000 of which are visceral and 1,500,000 are (muco-)cutaneous. Whereas cutaneous leishmaniasis (CL) has a tendency to spontaneously self-heal with resulting scars, visceral leishmaniasis (VL), the most dangerous of leishmaniasis, is fatal when left untreated, causing a global annual mortality estimated at 59,000 (35,000 men and 24,000 women); in some areas, however the case fatality rate is three times higher in women than in men.

Visceral Leishmaniasi

Visceral leishmaniasis (VL), also known as Kala-azar or black fever, is the most severe form of leishmaniasis, a disease, caused by parasites of the *Leishmania donovani* complex, *L. donovani, L. infantum* and *L. archibaldi* in the old world (Asia, Europe and Africa) and *L. chagasi* in the new world (South America). It is the second-largest parasitic killer in the world (after malaria), responsible for an estimated 60,000 who die from the disease each year out of half-million infections worldwide (Figure 11). After recovery, about 5% of the patients develop post Kala-azar dermal leishmaniasis (PKDL) within a year. PKDL ultimately results in the appearance of depigmented patches of skin which progress to scattered nodular lesions over the body. *L. donovani* parasites infect macrophages of liver, spleen and bone marrow that are preferentially parasitized supporting intracellular replication. The etiological agents belong to the *Leishmania donovani* complex, *L. donovani, L. infantum* and *L. archibaldi* in the old world (Asia, Europe and Africa) and *L. chagasi* in the new world (South America). The majority of the disease is asymptomatic with the parasites being eliminated by an effective cell-mediated immune response. A few
people develop progressive visceral leishmaniasis; also known as Kala-azar, which is characterized by fever, weight loss, hypergammaglobulinemia, hepatomegaly and massive splenomegaly.

Figure 11. Distribution of Visceral Leishmaniasis across the world

History and epidemiology

*Leishmania donovani*, the causative agent of VL or kala-azar was first isolated by Scottish doctor William Leishman and Irish physician Charls Donovan, working independently of each other and the species was named for both of them *Leishmania donovani*. Visceral leishmaniasis is the most acute form of the disease caused by *L. donovani*. The disease is endemic in West Bengal, where it was first discovered, but is most deadly in North and East Africa. It also appears in Western Asia and Southern Europe, and *L. chagasi*, a slightly different strain of the pathogen, is responsible for leishmaniasis in the new world. The geographical distribution of the disease clusters around areas of drought, famine, and high population density. In Africa infection centers are mostly in Sudan, Kenya, Somalia, parts of the Sudan, in particular, the Upper Nile region.
**Leishmania donovani: Lifecycle**

*Leishmania donovani* is a unicellular lower eukaryotic haemoflagellate with a digenetic lifecycle. Leishmaniasis is transmitted by the bite of the female phlebotomine sandfly. After bloodmeal, the vector receives a number of amastigotes from the vertebrate host, which transforms into procyclic promastigotes in the mid gut of the insect. Procyclic promastigotes amplify themselves and migrate to the probosis where they differentiate into infective metacyclic promastigotes. The sandflies inject metacyclic promastigotes, along with saliva, during blood meals. Metacyclic promastigotes that reach the puncture wound are phagocytized by activated macrophages and transform into amastigotes. Amastigotes multiply in infected cells and affect different tissues (Figure 12).

![Figure 12. Schematic diagram depicting lifecycle of *Leishmania sp.*](image-url)
Immunosuppressive role of *Leishmania*

**Microbicidal Free Radical Production**

Two types of microbicidal molecules are recognized for their efficacy against *Leishmania*: NO (Liew et al., 1990) and ROS (Murray and Nathan, 1999). NO is critical for parasite clearance, since mice lacking inducible nitric oxide synthase (iNOS) (also called NOS2) are unable to control infection. IL-10 and/or TGF-β result in inhibition of NO production via inactivation of MAPK pathway. ROS generation is also inhibited by *L. donovani* infection (Olivier et al., 1992b). Inhibition appears to be dependent on the surface molecules LPG and gp63 (Sacks and Sher, 2002; Sorensen et al., 1994). Contrary to NO deficiency mice deficient in ROS production can control the infection after an initial period of increased susceptibility, substantiating the fact that ROS may play a less important role in parasite clearance (Murray and Nathan, 1999).

**Induction of Immunosuppressive Molecules**

*Leishmania* parasite can trigger production of many immunosuppressive molecules as IL-10 and TGF-β, which affect numerous cell types, thus distorting the normal immune response against the parasite. Production of TGF-β correlates with decrease iNOS expression and reduced NK cell activity in lymph nodes (Scharton-Kersten et al., 1995; Stenger et al., 1994). Moreover, this interaction has also been proposed to be triggered by phosphatidylserine motifs on the amastigote surface (de Freitas Balanco et al., 2001). IL-10 production may be responsible for the suppression of macrophage microbicidal activity involving NO, production of several cytokines (IL-1, IL-12, and TNF), and expression of costimulatory molecules such as B7-1/2 (Cunningham, 2002). Its importance *in vivo* is illustrated by the observation that transgenic mice constitutively expressing IL-10 are unable to control *Leishmania* infection (Kane and Mosser, 2000). Prostaglandin E2 (PGE2) seems to be generated by *Leishmania*-infected macrophages and to favor parasite survival and progression (Farrell and Kirkpatrick, 1987; Reiner and Malemud, 1984). Prostaglandin has been reported to cause inhibition of macrophage proliferation and to suppress production of TNF-α, IL-1, and reactive oxygen intermediates (Belley and Chadee, 1995). So, *Leishmania* parasites are capable of modulating numerous macrophage functions in order to promote survival within the host. While the parasite surface coat is responsible for triggering many of these effects, the fate of intracellular signalling pathway after infection is discussed below.
Repression of Cytokine Production

*Leishmania* prevents the activation of an effective immune response by inhibiting production of a number of cytokines, particularly those involved in the inflammatory response (IL-1 and TNF-α) or in T-lymphocyte activation (IL-12). LPS-induced IL-1β secretion has been reported to be inhibited in *L. donovani*-infected (Reiner et al., 1990) and LPG exposed (Frankenburg et al., 1990) macrophages. TNF-α production is also repressed in infected macrophages treated with LPS (Descoteaux and Matlashewski, 1989). The cytokine IL-12 plays a critical role in the regulation of cellular immune responses. It is essential for T-lymphocyte activation and subsequent IFN-γ secretion leading to macrophage activation and production of microbicidal molecules. *Leishmania* has developed the ability to inhibit IL-12 production, this has been shown for promastigotes of *L. donovani* and *L. major* (Carrera et al., 1996), *L. mexicana* amastigotes (Weinheber et al., 1998). IL-12 inhibition has been also reported to occur in *L. major*-infected mice (Belkaid et al., 1998). Macrophage complement receptors and Fcg receptor, which are known to interact with *Leishmania* during phagocytosis, have been shown to repress IL-12 (Sutterwala et al., 1997). Recently it was shown that the abilities of *L. mexicana* amastigotes to degrade NF-κB and to repress IL-12 are both dependent on cysteine peptidase B activity (Cameron, 2004 #108).

Host signalling alteration: role of *Leishmania* parasites

Ligand binding to the cell surface receptor transmutes a stimulus into the inside of cytoplasm with the help of second messengers. These second messengers are often protein kinases, which then phosphorylate other kinases to continue a cascade that ultimately results in the activation of effector molecules, such as transcription factors or actin filaments, and causing a change in the cell’s behavior. Activation of a given kinase cascade will often result in the activation of its opposing phosphatases, in a classic example of negative feedback. Many prokaryotic and eukaryotic pathogens, including *Leishmania*, have evolved various strategies to exploit host cell signaling regulatory mechanisms by distorting this balance between positive and negative influences (Figure 13).

Dysregulation of PKC pathway

LPG molecules present on *Leishmania* parasites have known to chelate Ca²⁺ thus contributing to the fact that there is rapid elevation in intracellular Ca²⁺ levels (Eilam et al., 1985). This property in part can be attributed to downregulation of classical PKC isoforms (PKC-α and β), which require Ca²⁺, along with increase in levels of novel PKC isoforms.
(PKC-ε and ζ) (Kar et al., 2010). Inhibition of ROS production may also be due to a significant reduction of the inositol-1,4,5-triphosphate (IP3) (Olivier et al., 1992b). This may be due to the action of the elevated Ca\(^{2+}\) concentration itself could activate a host IP\(_3\) phosphatase as part of a calcium-dependent cascade (Eilam et al., 1985).

**MAP Kinase Family**

*Leishmania* infection leads to inhibition of MAP kinases even after stimulation by LPS or phorbol myristate acetate (Martiny et al., 1999; Nandan et al., 1999). *L. donovani* amastigotes was shown to demonstrate that ERK1/2 MAP kinase inactivation was accompanied by the inhibition of the transcription factor Elk-1 and c-fos expression (Nandan et al., 1999). *Leishmania* parasites were able to inhibit induction of MAPK’s, which is a major pathway for the control of infection, in response to a variety of agonists, thus helping the parasite to grow and survive within the host (Martiny et al., 1999; Nandan et al., 1999). Studies revealed that ERK1/2 and p38 MAPK play a key role in regulation of iNOS (Ajizian et al, 1999). Activation of p38 by anisomycin enhanced macrophage-dependent leishmanicidal effects whereas its inhibition by *Leishmania* co-relates well with impaired generation of iNOS and production of NO (Junghae and Raynes, 2002). Activation of MAPK signaling pathways by various stimuli induced NF-κB activation either through the phosphorylation of its inhibitor, IκB or by direct post-transcriptional modification of its p65 subunit (Goebeler et al., 2001). Reduced phosphorylation of ERK after infection with *L. donovani* resulted in increased synthesis of ceramide apparently via activation of an endogenous phosphatase, with resultant repression of NF-κB and API, reduced NO generation, and enhanced parasite survival (Ghosh et al., 2001; Ghosh et al., 2002). p38 MAP kinase inactivation contributed to dephosphorylation and thus deactivation of p38 MAPK after stimulation with anti-CD40 antibody during *L. major* infection (Awasthi et al., 2003). These studies are supported by the observation that all three MAP kinases subfamilies (ERK1/2, p38, and JNK) are not induced when *L. donovani* is phagocytosed by naive macrophages (Prive and Descoteaux, 2000).

**Protein Phosphatases**

*Leishmania* also has the ability to activate certain molecules that will help in its survival as phosphatases. Phosphatases can be classified based on their effector functions as PTPs, DSPs, and STPs. The PTP superfamily is divided further into transmembrane receptor and nonreceptor tyrosine phosphatases (Barr et al., 2009). During resting phase there is
perfect balance between PTP and PTK within the macrophages. Phosphatases act as a negative regulator of signaling pathways which is exploited by *Leishmania* parasite to live and thrive within the macrophages. SHP-1 (Src homology 2 domain-containing tyrosine phosphatase, also called SHPTP-1, HCP, and PTP1C) is an important PTP that is known to be upregulated during *Leishmania* infection. SHP-1 interacts strongly with MAP kinases upon *L. donovani* infection (Nandan et al., 1999). Recent studies indicated that MKP1/3 and PP2A decrease the activation of ERK and p38 (Kar et al., 2010).

![Figure 13. Subversion of various macrophage signalling by *Leishmania* parasite](image)

**Present therapeutic approaches against visceral leishmaniasis**

VL is transmitted through hematophagous sandflies and is caused by *Leishmania donovani* in the Indian subcontinent, Asia, and Africa, *L. infantum* in the Mediterranean basin, and *L. chagasi* in South America. Leishmaniasis transmitted in or near houses can be prevented with insecticides. Since there is no antileishmanial vaccine in clinical use, control of VL relies almost exclusively on chemotherapy. For almost seven decades pentavalent antimonials constituted the standard antileishmanial treatment worldwide, however the last 15 years their clinical value was jeopardized due to the widespread emergence of resistance to these agents (Sundar et al., 2007). The last decade novel formulations of conventional antileishmanials as well as new drugs, including the oral agent miltefosine, became available or are under investigation. In practice, however, their wide use in poor countries is hampered...
mainly due to high costs and also due to concerns of toxicity and emergence of resistance (Sundar et al., 2007). In response to concerns about preserving the currently available antileishmanials, especially in regions with anthroponotic parasite transmission, there is growing interest on combination regimens. The available drugs though highly effective suffer from the disadvantages:

- parenteral mode of administration
- long duration of therapy
- effectiveness in some settings
- bothersome toxic effects
- relapse after chemotherapy

Although pentavalent antimonials Sb(V) are in clinical use for several decades, and thiol metabolism is critical in their mechanism of action. Trypanothione is the major thiol in leishmanias. SbIII inhibits trypanothione reductase in vitro, inducing the loss of intracellular thiols and a lethal imbalance in thiol homeostasis, leading to accumulation of reactive oxygen species (Singh, 2006; Wyllie et al., 2008). In order for antimonials to exert their action, an almost intact immune system of the host is required. Initially antimonials were given at lower dose, however after the treatment failures the doses were (Sundar et al., 2000). However, the dose escalation policy did not prevent further emergence of resistance, but rather selected resistant parasites. During the last decade, antimonial resistance and therapeutic failures reached epidemic dimensions. Since Sb (V) is the mainstay therapy against VL in India and at present ~65% of population is either resistant to the therapy, or relapse after the treatment.

Conventional amphotericin B or its liposomal formation has been used as a secondline treatment for VL since the 1960s. This drug exhibits an excellent antileishmanial activity. Unresponsiveness and relapses occur rarely, however it must be mentioned here that some cases of Amphotericin B resistance has already been found in India. Moreover amphotericin B acts independent of the immune response and thus, infection may relapse once the drug is discontinued (Murray, 2000). Miltefosine (hexadecylphosphocholine) is the first orally administered drug for VL and the latest to enter the market. This agent is associated with high efficacy rates, including cases unresponsive to antimonials (Dube et al., 2005; Ritmeijer et al., 2006). Major concerns for the wide use of miltefosine include its teratogenic potential and its long half-life (approximately 150 hours) which may facilitate the emergence of resistance. Miltefosine is strictly forbidden in women of child-bearing age who may become pregnant up to two months following drug discontinuation. Moreover Miltefosine is still in phase IV
clinical trials. However, target specific and efficient leishmanicidal drugs are yet to be discovered and there is an emerging need of targets against which new generation drugs could be designed.

**Combinatorial therapy**

In a recent study conducted among VL patients, it was found that combination of sodium stibogluconate and paromomycin administered for 17 days was associated with higher cure and survival rates compared to sodium stibogluconate monotherapy administered for 30 days (Melaku et al., 2007). Combinations of miltefosine with amphotericin B, paromomycin or pentavalent antimonials have been evaluated in an in vivo model and revealed that the combinations of miltefosine with amphotericin B or paromomycin were much more efficacious than either agent alone (Seifert and Croft, 2006). These drugs might prove to be potential candidates to combine since, they have been in clinical use.

Agents which directly stimulate the host macrophages to kill intracellular amastigotes are termed as immunoenhancers. This involves the expression of basic Th1-cell antileishmanial immune response, thereby producing a broader range of effector mechanisms including macrophage activation (marked by IFN-γ production). An ideal approach would be to stimulate NO and Th1 cytokines, thus inducing the immune system to kill the parasite, this has been already proved in our laboratory (Das et al., 2001; Ukil et al., 2005).

**Immunomodulators: therapeutic marvels**

Immunomodulators of natural, synthetic and recombinant origin pose a number of advantages as they are safe, cheap, effective and readily available and act through modulation of immune system in treating diverse viral, bacterial, parasitic and fungal diseases. Awareness of power of pharmacological approach to research problems has initiated the usefulness of immune modulating agents in deciphering the cellular immune system. The immune system can be manipulated specifically by vaccination or nonspecifically by immunomodulation (Masihi, 2000). Immunomodulators include both immunostimulatory and immunosuppressive agents. While immunosuppressive agents decrease the inflammation in host, providing beneficial effects against auto-immune diseases, inflammatory bowel diseases etc., on the contrary immunostimulatory agents are capable of enhancing host defense mechanisms to provide protection against infections. The mode of action includes augmentation of the anti-infectious immunity by the cells of the immune system thus
restoring the effector immune response. Immunomodulators are becoming a viable adjunct to established therapeutic modalities offering a novel approach for the treatment of infectious and malignant conditions in the coming decades.

**Cytokine Immunomodulators**

**Interferons**

Interferons play an important role in host defense against infectious pathogens and in regulation immune responses. Natural interferon-α is used for the treatment of a rare form of cancer, hairy cell leukemia, while recombinant interferon-α-2a is licensed for treatment of chronic active Hepatitis B and for Hepatitis C virus infections. Interferon-α is also approved for treating by human papiloma virus (HPV) and for Kaposi sarcoma in patients with HIV infection. In patients having viral encephalitis, herpes zoster, multiple sclerosis and varicella, Interferon-β obtained from human FS-4 fibroblast cell lines is used. Numerous experimental studies have demonstrated an important role for interferon-γ in protection against *Listeria monocytogenes* (Nakane et al., 1988) *Francisella tularensis* (Leiby et al., 1992), *Chlamydia trachomatis* (Williams et al., 1988) and primary *Shigella flexneri* infections (Way et al., 1998).

**Tumor necrosis factor (TNF)**

*K. pneumoniae, S. pneumoniae, Legionella pneumophila* bacterial infections were cured after administration of TNF (Parant et al., 1992), salmonellosis. Tumor necrosis factor (TNF) has been shown to exert antiviral activity against ecephalomyocarditis virus or vesicular stomatitis virus (Mestan et al., 1986), and respiratory syncytial virus (Merolla et al., 1995).

**Colony stimulating factors**

The colony stimulating factors (CSF) are intimately involved in production and differentiation of stem cells in the bone marrow to phagocytic cells. Resistance against infections by *P. aeruginosa* (Mooney et al., 1988), *S. aures, Serratia marcescens* (Matsumoto et al., 1987) and deseminated *C. albicans* (Netea et al., 2003) was conferred by G-CSF. Resistance against cesicular stomatitis virus and herpes simplex virus was shown after treatment with M-CSF (Lee and Warren, 1987). Human macrophages treated with GM-CSF
exhibited significant inhibition of growth of *M. avium* complex (Suzuki et al., 1994). Moreover it has been shown to have activity against *C. albicans* (Marodi et al., 1998) and to clear the infection of *Salmonella typhimurium* (Morrissey and Charrier, 1991).

**Natural and Synthetic Immunomodulators**

**Isoprinosines:**

They have been found to be useful in treatment of several viral diseases such as herpes simplex, herpes zoster, influenza, rhinovirus infections and multicentre double blind clinical trials of isoprinosine in asymptomatic HIV-positive patients showed a delay in the progression of HIV infection to AIDS in some trails (De Simone et al., 1991).

**Thymic hormones and peptide analogs**

Acute respiratory infections have been reduced after treatment with thymus extract (thymostimulin) (De Mattia et al., 1993). HIV patients showed substantial increase in the number of CD4+ cells after treatment with a combination of thymosin-α1, interferon-α and AZT (Garaci et al., 1994). Clinical trials have shown that combination of thymosin and antiviral drug could significantly improve treatment of viral hepatic disease (Mutchnick et al., 1994). Normalization of faulty response to IL-2, induction of delayed type hypersensitivity and proliferation of lymphocytes has been shown by Methyl inosine monophosphate (MIMP) (Hadden et al., 1992).

**Glucans**

Lentinan, a 1-3-β-glucan, is licensed as an adjunctive for antitumour therapy in Japan. Prevention against *Listeria* and relapse of *M. tuberculosis* can be done Lentinan, a 1-3-β-glucan (Mashihi, 2000). Poly-(1-6)-β-glucotriosyl-(1-3)-β-glucopyranoseglucan (PGG-glucan) reduces serious postoperative infections or death after high-risk non-colorectal operations (Dellinger et al., 1999). Yeast glucan could enhance resistance against herpes simplex virus types I and II, and murine hepatitis virus; induced non specific resistance against *Klebsiella pneumoniae* infection and protect patients from sepsis, bacteremia and peritonitis resulting from acidity of *Escherichia coli*, *Staphylococcus aureus* and *Pseudomonas aeruginosa* infections; and prolonged survival against parasitic infections by...
Plasmodium berghei and Leishmania donovani and exert antifungal activity against Candida, Cryptococcus and Sporotichum (Masihi, 2000).

**Fucoidan : a natural polysaccharide immonomodulator**

As the first isolation of fucoidan acronym (fucoidin) was described in 1918 (Kylin, 1918). However since only 70% of the information available is in English which makes this research field makes it very difficult to obtain all possible information. The nomenclature of fucoidan has evolved through several steps. In 1918 Kylin named the sulphated polysaccharide ‘fucoidin’. However, this name was changed by McNeely (Berteau and Mulloy, 2003) in 1959 to ‘fucoidan’ to follow the usual polysaccharide nomenclature. Fucoidans are mainly found in the cell wall matrix of brown sea algae (Descamps et al., 2006). In recent years many different algae have been analysed for their content of fucoidans including Fucus vesiculosus (Beress et al., 1993), Ascophyllum nodosum (Nakayasu et al., 2009), Fucus serratus (Bilan et al., 2006) and Fucus distichus (Bilan et al., 2004) etc. However the most studied fucoidan is obtained from Fucus vesiculosus (Hyun et al., 2009; Kwak et al., 2010; Min et al., 2012; Rhee and Lee, 2011) and its structure is depicted in (Figure 14). Fucoidan present in algal cell wall may help in maintaining the cell wall structure and protecting the algae against higher amounts of UV light (Mabeau et al., 1990).

![Figure 14. Structure of fucoidan from Fucus vesiculosus](image-url)
Characterisation

Structure of fucoidan was first deciphered from *Fucus vesiculosus*, but it was not before 1993 that its structure successfully elucidated this structure and described it as a polysaccharide consisting mainly of α-1,3-L-fucose (Patankar et al., 1993). The elucidation of the structure is not concluded yet, since the size is big enough and NMR spectroscopy can not be done, moreover the use of chemicals disrupt the structure and thus the algae cannot be grouped by their fucoidan structure. Other common sugars in fucoidan apart from L-fucose include galactose, mannose, xylose and uronic acids (Duarte et al., 2001). The molecular weights of fucoidans depends also on the source from which they are obtained. The molecular weights of fucoidans obtained from some algae are described below (Table 1).

<table>
<thead>
<tr>
<th>Species Name</th>
<th>Molecular Weight</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Ascophyllum nodosum</em></td>
<td>13 kDa</td>
</tr>
<tr>
<td><em>Fucus vesiculosus</em></td>
<td>100-180 kDa</td>
</tr>
<tr>
<td><em>Laminaria japonica</em></td>
<td>189 kDa</td>
</tr>
</tbody>
</table>

Fucoidan have been attributed to several different bioactivities. These include anti-tumoral (Takeda et al., 2012), anti-coagulant (Kosteljanetz et al., 1979), anti-viral (Queiroz et al., 2008; Sinha et al., 2010) and immuno-stimulatory (He et al., 2009; Kim and Joo, 2008) activities.

Immunomodulation by fucoidan

Fucoidan from *Fucus vesiculosus* as a ligand of Scavenger receptor-A (SR-A), induces protein kinase C (PKC) activity, and specifically stimulates the activity of the ERK, JNK, p38 MAPK leading to inflammatory cytokine secretion and urokinase-type plasminogen activator expression (Hsu et al., 2001; Hsu et al., 1998; Nakamura et al., 2006). It has also been demonstrated that fucoidan modulates the function of immune cells including macrophages, natural killer (NK) cells, lymphocytes, and neutrophils (Ale et al., 2011; Kuznetsova et al., 2003; Nakamura et al., 2006; Yang et al., 2008) [3–6]. In macrophages, fucoidan induces the production of tumor necrosis factor (TNF)-α and interleukin (IL)-1β, which are inflammatory cytokines. Fucoidan also shows mitogenic activity for splenic
lymphocytes and cytotoxic activity for NK cells. Recent studies indicate that fucoidan upregulates the production of nitric oxide (NO) in RAW 264.7 macrophages and dendritic cells from by modulating the transcription and expression of nitric oxide synthase (Jin et al., 2009; Nakamura et al., 2006). Some studies also suggest that activation of p38, ERK and JNK MAPK and subsequent NO production by fucoidan is mediated through TLR4, CD14 and SRA (Yu et al., 2012). Fucoidan mediated killing of malarial parasite can be endowed to its property of stimulating the immune system and in turn increasing the number of cytotoxic T-cell which is essential for elimination of Plasmodium. It has been already established that fucoidan (Fucus vesiculosus) can help in maintaining type 1 immune response, which is already known to kill Leishmania parasite via immune stimulation. Leishmania parasites have the capacity to reside and grow within the host macrophages through dysregulation of MAPK/NF-κB pathway ultimately resulting in inhibition of ROS and NO. Therefore, the present study was conducted in order to understand the immunotherapeutic role of fucoidan as an optimum immunomodulator candidate against both antimony-susceptible and-resistant Visceral Leishmainiasis.