Introduction
During host-pathogen interaction many tiers of immune defenses harmoniously interplay to clear the body of microorganisms and their products, which are sensed as foreign by the host, thus protect host from infection. Immune system of vertebrates is based upon two types of immunity: innate and adaptive. The innate immune system consists of dendritic cells (DCs), macrophages, and neutrophils, is genetically systematized to identify invariant features of invading microbes. In contrary, the adaptive immune system includes T and B lymphocytes, exploits antigen receptors generated by de novo pathway for pathogen clearance in each organism. So this type of immune response is highly specific (Iwasaki & Medzhitov, 2010). Moreover, the innate immune system not only serves as bodies primary defense system but it also induces the adaptive immune response in mammals through the elaboration of cytokines and co-stimulatory molecules, thus helps to modulate the intensity of the immune response. The major component of innate immune system is macrophages. Macrophages are source of different microbicidal effector molecules as well as capable of phagocytosing pathogens and degrading them within the phagolysosome (Mogensen, 2009). Because of these combined attributes, macrophages appear to be well armed to combat microbial infection through a combination of cytokine release and direct microbicidal attack.

**A brief outline of macrophage behaviour:**

Macrophages recognized by Metchnikoff, presence in all tissues and as circulating cells called blood monocytes, which are around 20% of the peripheral blood mononuclear cell (PBMC) fraction, considerably less numerous than the other major phagocyte population (Dale et al, 2008). In tissues, macrophages are very populous and they live in a specific anatomical niche in respect to other tissue cell types. Macrophages specifically have a closed relationship with epithelial and endothelial cells. In simple epithelia, and throughout the capillary and lymphatic circulation, tissue macrophages spread along basement membranes; in stratified and pseudostratified epithelia such as skin, trachea and cervix, they are integrated within the epithelium. Sinusoidal macrophages, such as those of liver, spleen and some endocrine organs have direct contact with the blood. But the separation by endothelium does not prevent pericapillary macrophages from extending processes into the lumen and sampling the blood contents. The ability of macrophages to extend processes across epithelia and into lymphatic vessels has also been recognized. Macrophages not only serve as cells of the immune system,
they also show central function in many other features of embryonic development, homeostasis and wound repair. Langerhans cells, the macrophages of the epidermis, form the centre of epidermal proliferative units and control the proliferation and differentiation of keratinocytes. Macrophage depletion in the CSF-1-deficient op/op mouse (osteopetrosis (op) mutation) affected somatic growth, development of the pancreas and nervous system, and male and female fertility (Gow et al, 2010; Pollard, 2009). Resident macrophages become redesigned to perform particular functions in different organs; for this brain macrophages (microglia) are very different from alveolar macrophages of the lung, kupffer cells of the liver, or the largest tissue macrophage population, those lining the wall of the gut.

**Morphology**

In general monocytes are round, 15-22 μm in diameter, with a single nucleus that may be visible in blood films staining as round, oval, reniform or folded. Mature macrophages can be differentiated by their larger size with more granular cytoplasm (Figure 1).

![Figure 1. Morphology of macrophage](image)

The nucleus of macrophage differs in size and shape with a marginated chromatin and a nucleolus of conventional structure. The cells are surrounded by plasma membrane, consists of two electron dense layers which is about 80Å° in thickness and separated by a relatively clear layer. Peritoneal macrophage possesses numerous microvilli or filopodia, whereas they may be sparse or absent from macrophage in other sites, e.g. pulmonary alveoli and lymph node follicles (van Furth et al, 1972). Transmission electron microscopy pictures of macrophages usually reveal the presence of large number of lysosomes, a prominent golgi apparatus, mitochondria, a varying amount of rough endoplasmic reticulam, centrosome and (depending on the source and
condition of the cells) inclusions derived from phagocytosed material (Gopal et al, 2006). The presence of abundant lysosomes is characteristic of mature macrophages throughout the body. A single unit of membrane separates lysosomes from rest of the cellular contents. The number of lysosomes per cell varies in a macrophage population and within a single macrophage they vary in morphology. The characteristic biochemical feature of lysosomes is their content of Different hydrolytic enzymes gives lysosomes unique characteristic feature with their acid pH optima.

**Development and Differentiation**

Committed cells within the mononuclear phagocyte lineage progress through a series of well specified morphologically-distinct stages; a common myeloid progenitor shared with granulocytes giving rise to monoblasts, promonocytes and then monocytes which migrate into tissues (Hume, 2000). The production of mononuclear phagocytes from progenitor cells is directed by colony-stimulating factors, to some extent lineage restricted and stratified in their actions. They are comprised of macrophage colony-stimulating factor (CSF-1), granulocyte macrophage colony-stimulating factor (GM-CSF) and fms-like tyrosine kinase 3 ligand (Flt3-ligand). These growth factors command the common myeloid progenitor to adopt a macrophage fate (Stanley, 2009). In the adult, proliferating mononuclear phagocytes are obtained only in the bone marrow where macrophage precursors go through several cycles of proliferation and differentiation (approx. 8-10 days). The process of the formation and development of mature blood cells arising from the survival, proliferation, lineage-commitment and differentiation of early progenitors is termed as haematopoiesis. It serves a mechanism by which totipotent stem cells differentiate to all the cells present in blood giving a mechanism of normal cellular turnover. Early in the development of a blood cell, pluripotent stem cells with self-renewing capacity exist inside defined haematopoietic tissues such as bone marrow and spleen red pulp. These cells are in a quiescent state until they receive signals targeting for the up regulation of haematopoietic events (Figure 2). Of these, the colony-stimulating factors have proven crucial for the development of cells of different blood lineages. In bone marrow during adult life or in fetal liver, IL-1, IL-3 and/or IL-6 induces stem cell division that provides a new stem cell and a pluripotent myeloid cell also termed as granulocyte-erythrocyte-megakaryocyte macrophage colony-forming unit (GEMM-CFU). In the presence of IL-1 and/or IL-3, this precursor is committed to perform as a progenitor of both macrophages and granulocytes known as the granulocyte macrophage colony-forming unit (GM-CFU) which is also committed to the
macrophage colony-forming unit (M-CFU) by the macrophage colony stimulating factor (M-CSF) the granulocyte-macrophage colony stimulating factor (GM-CSF) and interleukin IL-3. Subsequently, from the M-CFU (macrophage colony forming unit) there is deviation either to the osteoclast, myeloid dendritic cells or to the macrophage lineage (Valledor et al, 1998). Differentiation along the macrophage lineage assumes that the macrophage colony forming unit (M-CFU) differentiates in the presence of the M-CSF to monoblast, promonocyte, monocyte and differentiated macrophages. Monoblastis the most immature cells of the mononuclear phagocyte cell line. Division of monoblast ultimately arises to monocytes. Monocytes reside in the bone marrow for only a short time (less than 24 h) and then enter the circulation, where the half-life of monocyte is of the order of 3 days (Whitelaw, 1966). Monocytes then move from the blood vessels into the tissues or body cavities.

Migration of monocytes takes place with the help of adhesion molecules on monocytes and on endothelial cells, together with chemotactic factors. After entering tissues, terminal differentiation occurs where monocytes transform into macrophages (Figure 2). The efficient production of monocytes – macrophages requires the operation of growth factors termed as colony-stimulating factors (CSFs). The two major CSFs are macrophage CSF (M-CSF), which is macrophage lineage specific, and granulocyte macrophage-CSF (GM-CSF), which acts upon
both macrophage and granulocyte lineages. Monocyte production and differentiation is also promoted by interleukin-3 (IL-3), a factor that induces monocytopoiesis and is a distinct inducer of macrophage differentiation. Monocytopoiesis is prevented by prostaglandin E2 (PGE2), tumour necrosis factor (TNF)-α, and a monocyte production inhibitor. As products of macrophage, PGE2 and TNF-α can cause negative feedback. Mature macrophages once formed, are long-lived and are rapidly replaced by fresh cells from blood and bone marrow.

Distribution

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. In their normal state, these macrophages may be localized as temporary residents in connective tissue (histiocytes), lung (alveolar macrophage) and serous cavities. 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).
**Spectrum of macrophage defences against intracellular parasite infection:**

Acquiring an in depth knowledge on macrophage biology in the context of a whole living organism provides unique possibilities to penetrate the contribution of this extremely dynamic cell subset in the reaction to infections, and has uncovered the relevance of cellular and molecular processes that are fundamental to the cell-mediated innate immune response. Macrophages are highly specialized in removal of a range of microorganisms including that of bacteria, viruses, fungi, and protozoa, thus it represent as a major defence system against invading pathogen. They have the excellent ability to recognize and ingest foreign antigens through receptors on the surface of their cell membranes; these antigens are then demolished by lysosomes. Residing in the peripheral lymphoid tissues macrophages serve as the major scavengers of the blood, clearing it of abnormal or old cells and cellular debris as well as pathogenic organisms.

Macrophages also serve a vital role by processing antigens and presenting them to Tcells, activating the specific immune response (figure.4).

They also release many substances that participate in inflammation, including chemokines and cytokines, lytic enzymes, oxygen radicals, coagulation factors, growth factors (Parameswaran & Patial, 2010). Apart from secretion of a repertoire of cytokines/chemokines, macrophages also respond to these products in an autocrine/paracrine manner, thus strengthening the inflammatory response. As macrophages are important regulator of host immune system, these cells have been connected with a number of disease processes including rheumatoid arthritis, autoimmune and primary immunodeficiency diseases, Alzheimer’s, wound healing processes, and atherosclerosis, as well as in tumour biology. Due to the involvement in a large spectrum of physiological and pathophysiological processes, mechanisms by which macrophages respond to extracellular stimuli have been greatly inspected.
Macrophages have exceptional flexibility that enable them to efficiently respond to environmental signals and change their phenotype, and their physiology can be markedly altered by both innate and adaptive immune responses (Mosser & Edwards, 2008). The work of Mackaness in the 1970s clearly indicated that changes in the physiology of macrophages in response to some environmental signals can provide them with enhanced antimicrobial activity. However, macrophages do not always face environmental signals that increase macrophage immune function. In fact, both innate and adaptive immune responses are responsible factors that can generate macrophages which are more susceptible to pathogenic infections and less equipped to produce cytokines that enhance the immune response.

1. Recognition of pathogenic molecule
The innate immune system serves as the first line of defense against invading microbial pathogens and macrophage being a major part of this immune response system, are provided with certain ‘sensors’ which help to identify the pathogen. These are large family of pattern recognition receptors (PRRs), which detect distinct evolutionarily conserved structures on
pathogens, termed pathogen-associated molecular patterns (PAMPs). Among the PRRs, the Toll-like receptors have been studied most broadly. TLRs are a family of evolutionarily conserved transmembrane glycoproteins and constructs body’s first line of defence against invading pathogens (Miggin & O'Neill, 2006). Upon PAMP engagement, PRRs trigger intracellular signaling cascades ultimately resulting in the expression of a variety of proinflammatory molecules, which together orchestrate the early host response to infection, and also is a prerequisite for the subsequent activation and formation of adaptive immunity. This system is tightly regulated by a number of endogenous molecules that limit the magnitude and duration of the inflammatory response. Moreover, pathogenic microbes have developed sophisticated molecular strategies to subvert host defenses by interfering with molecules involved in inflammatory signaling.

**The TLR signalling pathway**

Toll-Like Receptors (TLRs) play a critical role in the early innate immune response to invading pathogens by recognizing microorganism and are participating in sensing endogenous danger signals.

Toll like receptors are evolutionarily conserved receptors, homologues of the Drosophila Toll protein. These receptors sense highly conserved structural motifs known as pathogen-associated microbial patterns (PAMPs), entirely expressed by microbial pathogens or danger-associated molecular patterns (DAMPs). PAMPs include various bacterial cell wall components such as peptidoglycan (PGN), lipopolysaccharide (LPS) and lipopeptides, as well as bacterial DNA, flagellin and viral double-stranded RNA. DAMPs include intracellular proteins such as heat shock proteins as well as protein fragments from the extracellular matrix. Upon stimulation by interacting with PAMP or DAMP, TLR initiates signaling cascade culminating in the activation of transcription factors, such as AP-1, NF-κB and interferon regulatory factors (IRFs)(Miggin & O'Neill, 2006). Ultimate production of TLR signaling pathway is the variety of cellular responses including the production of interferons (IFNs), pro-inflammatory cytokines and effector cytokines that direct the adaptive immune response.

**Structure of TLR receptors:**

TLRs are type I transmembrane proteins specified by an extracellular domain containing leucine-
rich repeats (LRRs) and a cytoplasmic tail that possesses a conserved region known to be the Toll/IL-1 receptor (TIR) domain. The cytoplasmic tail of TLRs and IL-1Rs composed of a conserved region of 200 amino acids, which is known as the Toll/IL-1R (TIR) domain (Slack et al., 2000). Within the TIR domain, the regions of homology contain three conserved boxes, which are vital for signalling (Figure 5).

![Figure 5. Structure of TLR](image)

The TIR domains has generally 20–30% amino acid sequence conservation, however these domains vary in size. From the crystal structures of the TIR domains of human TLR1 and TLR2 it has been found that, this specific type of TLR receptors contain a central five-stranded parallel sheet, which is surrounded by five helices on each side (Xu et al., 2000). These two secondary structural elements are attached by loops: for example, the BB loop connects the strand -B and the helix -B. The conserved boxes 1 and 2 and the BB loop are adjacent and display most of their side chains for interaction with adaptor molecules. They are evolutionarily conserved to sense pathogen associated molecular patterns (PAMPs), from viruses, bacteria, fungi and parasites, and they exhibit substantial target specificity (Figure 5). Cognate ligand binding to TLRs leads to recruitment of myeloid differentiation primary response gene 88 (MyD88), interleukin receptor-associated kinase 1/4 (IRAK1/4), TNF receptor-associated factor 6 (TRAF6), ubiquitin-
conjugating enzyme 13 (Ubc13), and cellular inhibitor of apoptosis 1/2 (cIAP1/2) (Dong et al, 2006). Here lies a complex signaling event that is precisely timed and critically regulated by ubiquitination events. This is followed by subsequent release and translocation of the entire complex to the cytosol resulting in activation of TGF-β-associated kinase 1 (TAK1) and downstream mitogen activated protein kinases (MAPKs) ultimately resulting in IKK/NF-κB activation. This leads to an increase in inflammatory cytokine gene expression, facilitating enhancement of pro-inflammatory responses (Figure 6).

**Components of TLR signalling pathway:**

**MyD88.** The TIR domain-containing adaptor MyD88 is essential for the inflammatory responses regulated by all the TLR family members. MyD88 knockout mice reacted neutrally on exposure of TLR4 ligand LPS in terms of macrophage production of inflammatory mediators, B cell proliferation, or endotoxin shock (Kawai et al, 1999). The cellular responses to the TLR2 ligands peptidoglycan and lipoproteins were destroyed in MyD88 knockout mice. MyD88 functions like...
an adaptor molecule in the recruitment of IRAK to the IL-1R complex following stimulation with IL-1. The association between MyD88 and IRAK is occurred via DD–DD interaction. After recruitment to the receptor complex, MyD88 forms homodimers through DD–DD and TIR-domain–TIR domain interactions. Therefore, MyD88 works as an adaptor connecting TLRs/IL-1Rs with downstream signaling molecules that have DDs (Muzio et al., 1997; Wesche et al., 1997).

**IRAK.** IRAK was originally recognized as a serine/threonine kinase associated with the IL-1 receptor, which also harbors the TIR domain (Cao et al., 1996). Four members of the IRAK family, showing distinct gene expression patterns, have been identified till date: IRAK-1, IRAK-2, IRAK-M, and IRAK-4. IRAK proteins contain an N-terminal death domain, which is responsible for interaction with MyD88, and a central kinase domain. The importance of the IRAK family members in TLR-mediated signaling pathways was first deciphered in IRAK-1 knockout mice, which exhibited defective LPS-induced responses (Swantek et al, 2000). However, this impairment was only partial. On the contrary, IRAK-4 knockout mice showed almost complete impairment in the response to microbial components that activate TLR2, TLR3, TLR4, and TLR9 (Suzuki et al, 2002). A biochemical study revealed that IRAK-4 acts upstream of, and phosphorylates, IRAK-1 upon stimulation (Li et al, 2002). Thus, IRAK-4 plays as a central mediator of TLR signaling by activating IRAK-1. In sharp contrast to mice lacking IRAK-1 and IRAK-4, IRAK-M knockout mice showed increased production of inflammatory cytokines in response to the TLR ligands and exaggerated inflammatory response to bacterial infection, indicating that IRAK-M plays a negative inhibitory role in the TLR signaling pathway (Kobayashi et al, 2002)

**TRAF6.** TRAF6 is a member of the tumor necrosis factor receptor (TNFR)-associated factor (TRAF) family that regulates cytokine signaling pathways (Arch et al, 1998). TRAF proteins consist of two C-terminal TRAF domains (TRAF-N and TRAF-C), which regulate the interaction with TRAF proteins and other signaling molecules, N-terminal RING finger, and zinc finger domains. Among the TRAF family members, TRAF6 is known to be involved in the TLR signaling pathway in addition to signaling pathways via the OPGL receptor and CD40 (Lomaga et al, 1999). Upon stimulation of TLRs, TRAF6 is recruited to the receptor complex, and activated by IRAK-1 that binds to the TRAF domain of TRAF6. After then, the IRAK-1/TRAF6
complex separates from the receptor and associates with TGF--activated kinase 1 (TAK1) and TAK1-binding proteins, TAB1 and TAB2, at the membrane portion. IRAK-1 stays in the membrane and is degraded, whereas the complex of TRAF6, TAK1, TAB1, and TAB2 translocate into the cytoplasm, where it makes a large complex with other proteins, such as the E2 ligases Ubc13 and Uev1A (Deng et al, 2000). The Ubc13 and Uev1A complex has been shown to catalyse the synthesis of a Lys 63-linked polyubiquitin chain of TRAF6 and thereby induce TRAF6-mediated activation of TAK1 and finally of NF-κB (Wang et al, 2001).

**TRAF3.** TRAF3 also belongs to TRAF family protein. TRAF3, an ubiquitin ligase that interacts with both MyD88 and TRIF, regulated the production of interferon and proinflammatory cytokines in different ways. Degradative ubiquitination of TRAF3 during MyD88-dependent TLR signaling is prerequisite for the activation of mitogen-activated protein kinases (MAPKs) and production of inflammatory cytokines.

**NF-κB.** The NF-κB family of transcription factors is comprised of five members — p65 (RELA), REL-B, cytoplasmic (c) REL, p50 and p52 — which act as homo- and heterodimers. The inhibitor of NF-κB (IκB) family, usually sequester NF-κB in the cytoplasm as an inactive form. Activation of NF-κB is occurred through the phosphorylation and proteolysis of the IκB proteins and the concomitant release and nuclear translocation of the NF-κB factors. This acute activation process is regulated by the IκB kinase (IKK) complex, which is composed of two catalytic subunits — IKK-α and IKK-β (also known as IKK1 and IKK2) — and a regulatory subunit, IKK-γ (also known as NF-κB essential modulator, NEMO) (Karin and BenNeriah, 2000). Upon activation by upstream signals, IKK phosphorylates the IκBs, leading to their polyubiquitination mediated proteasomal degradation.

**Regulation of TLR signalling pathway**

The inflammatory cytokines produced as a result of TLR signalling, when released in excess, induce serious systemic disorders that are associated with a high mortality rate — such as endotoxic shock, which can be induced by the TLR4 ligand LPS. It is therefore not surprising that organisms have evolved mechanisms for modulating their TLR-mediated responses (FIG. 7). The molecules thought to negatively regulate TLR signalling are discussed briefly here; these
include IRAK-M, SOCS1 (suppressor of cytokine signalling 1), MyD88 short (MyD88s), SIGIRR (single immunoglobulin IL-1R-related molecule) and ST2.

**IRAK-M.** Unlike the other IRAKS, which are ubiquitously expressed, the expression of IRAK-M is restricted to monocytes and macrophages and increases following stimulation with TLR ligands. IRAK-M also lacks kinase activity. In response to TLR ligands, IRAK-M-deficient mice show increased production of inflammatory cytokines and defective induction of LPS tolerance. Biochemical analysis has revealed that IRAK-M prevents the dissociation of the IRAK1–IRAK4 complex from MyD88, thereby preventing the formation of the IRAK1–TRAF6 complex. These findings indicate that IRAK-M negatively regulates TLR-signalling pathways.

**SOCS1.** is a member of the SOCS family of proteins, which are induced by cytokines and negatively regulate cytokine-signalling pathway. LPS and CpG-containing DNA have been shown to induce the expression of SOCS1 in macrophages and SOCS1-deficient mice have been
shown to be hypersensitive to LPS-induced endotoxic shock (that is, to show increased production of inflammatory cytokines). Furthermore, LPS tolerance was not induced in SOCS1-deficient mice and the ectopic introduction of SOCS1 into macrophages inhibited LPS-induced NF-κB activation. These findings indicate that SOCS1 directly downmodulates TLR-signalling pathways. Although SOCS1 has been shown to associate with IRAK1, the precise mechanism by which SOCS1 inhibits TLR signalling remains unclear.

**MyD88s** is an alternatively spliced variant of MyD88 that lacks the intermediary domain, is induced in monocytes following stimulation with LPS. Unlike MyD88, MyD88s does not bind IRAK4, and overexpression of MyD88s does not induce IRAK1 phosphorylation. Therefore, MyD88s inhibits LPS-induced NF-κB activation because of its inability to bind to IRAK4 and promote IRAK1 phosphorylation.

In addition to these cytoplasmic molecules, the negative effects of which are induced by TLR signalling, membrane-bound molecules that contain a TIR domain — such as SIGIRR and ST2 — have recently been shown to be involved in the negative regulation of TLR signalling.

**SIGIRR-deficient** mice were found to be highly sensitive to LPS-induced endotoxic shock. Following TLR stimulation, SIGIRR has also been shown to interact transiently with TLR4, IRAK1 and TRAF6, thereby negatively regulating TLR-signalling pathways (Wald J. et al., 2003).

Similarly, **ST2-deficient** mice showed increased production of inflammatory cytokines in response to LPS; moreover, they also showed defective induction of LPS tolerance. Overexpression of ST2 was found to inhibit NF-κB activation, because ST2 associated with, and probably sequestered, MyD88 and TIRAP. Therefore, TIR-domain-containing orphan receptors, such as SIGIRR and ST2, are implicated in the negative regulation of TLR signalling (Brint, E. K. et al., 2004)

**TRAF** family members, comprising TRAF1–6, are proposed to be the adaptor molecules linking upstream receptor signals to gene activation. The TRAF proteins are characterized by the presence of a novel TRAF domain at the C terminus, which consists of a coiled-coil domain, followed by a conserved TRAF-C domain. The TRAF domain plays an important role by
mediating self-association and upstream interactions with receptors and other signaling proteins. Initially, TRAF6 was thought to be the sole member of this family to be involved in TLR signaling. TRAF6, an ubiquitin ligase, can ubiquitinate itself on Lys-63 (K63)-linked polyubiquitin chains. Ubiquitination targets proteins for proteolytic degradation or activation, depending on the lysine that is modified. For example, polyubiquitination on Lys-48 targets proteins for adenosine 5-triphosphate-dependent proteolysis by the 26S proteasome. In contrast, polyubiquitination on Lys-63 modulates protein function in the absence of degradation. As TRAF6 is not degraded following ubiquitination, its signaling must be negatively regulated in some manner. It has been shown that the de-ubiquitination enzyme A20 terminates TLR signaling by removing ubiquitin moieties from TRAF6 (Boone et al., 2004). A20-deficient macrophages showed enhanced NF-κB activity in response to TLR2, TLR4, and TLR9 ligands.

TRAF3 is an E3 ubiquitin ligase that regulates TLR pathway through distinct protein ubiquitination at specific residues. In contrast to other TRAF members, TRAF3 is particularly known as a negative regulator of NF-B signaling in response to TLR ligation in bone marrow-derived macrophages (BMDMs), plasmacytoid dendritic cells (pDCs), and murine embryonic fibroblasts (MEFs). TRAF3-null cells demonstrate prolonged activation of NF-B and subsequent release of proinflammatory cytokines (Hacker, H. et al., 2006; Oganesyan, G. et al, 2006). Moreover, TRAF3- null mice have been shown to illustrate postnatal lethality with hyper-inflammatory phenotype before death on d 10.

2. Alteration of macrophage populations in response to environmental stimuli: cytokines or chemokines production

Macrophages can respond to endogenous stimuli that are rapidly synthesized following injury or infection. Innate immune cells specifically produced these early stimuli which can exert a marked, though usually transient, effect on the physiology of macrophages. Macrophages can also stimulated by signals that are produced by antigen-specific immune cells (Figure.8). These signals act in a more focused way and are prolonged than innate immune stimuli. Normally they give rise to longer-term alterations in macrophages. To complicate matters, macrophages themselves can generate several factors that influence their own physiology. In many ways, the response of macrophages to stress, tissue damage or other homeostatic processes can predict how these cells will respond during the onset of an adaptive immune response.
Classically activated macrophages
The term classically activated has been used to classify the effector macrophages that are generated during cell-mediated immune responses. In the original characterization of activated macrophages, the combination of two signals, interferon-γ (IFNγ) and tumour-necrosis factor (TNF), give rise to a macrophage population that had enhanced microbicidal or tumoricidal capacity and released high levels of pro-inflammatory cytokines and mediators (Mackaness, 1977). IFNγ can be synthesized by innate or adaptive immune cells; natural killer (NK) cells are a major innate early source of this cytokine. NK cells respond to stress and infections by generating IFNγ, which can activates macrophages to secrete pro-inflammatory cytokines, produce higher amounts of superoxide anions and oxygen and nitrogen radicals to increase their killing ability (Dale et al., 2008). Therefore, innate immune mediators allow macrophages to provide better resistance against infection. An adaptive immune response is usually necessary to maintain classically activated macrophages and confer stable host defence against many intracellular microorganisms. This is typically provided by the sustained production of IFNγ by

Figure: 8. Macrophage population
T helper 1 (TH1) cells. These T cells are antigen specific, but the microbicidal macrophages that they induce can kill indiscriminately. In addition to MyD88, some TLR ligands can also activate TIR-domain-containing adaptor protein inducing IFNβ (TRIF)-dependent pathways, which signal through IFN-regulatory factor 3 (IRF3) and result in IFNβ production (Yamamoto et al., 2003). This endogenously produced IFNβ can replace the IFNγ that is produced by NK cells and T cells and activate classically activated macrophages. Therefore, the original two-signal requirement for the activation of this macrophage population can be overcome by certain TLR agonists that induce both TNF and IFNβ. The pro-inflammatory cytokines that are produced by classically activated macrophages are an important component of host defence, but they can cause extensive damage to the host. For example, IL-1, IL-6 and IL-23 are produced by classically activated macrophages and have been associated with the development and expansion of TH17 cells (Langrish et al., 2005). These cells produce IL-17, a cytokine that is associated with high levels of polymorphonuclear leukocyte (PMN) recruitment to tissues, which can contribute to inflammatory autoimmune pathologies Conversely, the clearance of apoptotic PMNs by macrophages during inflammation can lead to an inhibition of inflammation, owing in part to the production of transforming growth factor-β (TGFβ). The role of classically activated macrophages in host defence to intracellular pathogens has been well documented (Dale et al., 2008). Indeed, mice lacking IFNγ expression are more susceptible to various bacterial, protozoal or viral infections, as are humans with genetic mutations in these signalling pathways. However, there are some specific examples of the killing of intracellular microorganisms by classically activated macrophages that reveal the complex interplay between host and pathogen. *Leishmania* spp. are intracellular parasites that replicate primarily in tissue-resident macrophages. The stimulation of these cells with IFNγ and TNF before infection yields a population of macrophages that efficiently kills the parasite. However, stimulation of macrophages with IFNγ alone results in less efficient clearance of the parasite because *Leishmania* spp. are eukaryotic organisms that do not express TLR ligands and therefore do not trigger detectable TNF production. Stimulation of macrophages with exogenous TNF or with a TLR ligand, such as lipopolysaccharide (LPS), results in complete clearance of the parasite, thereby confirming the importance of TLR activation or TNF production in the development of classically activated macrophages. In simplified molecular terms, the panoply of genes that is triggered during the activation of classically activated macrophages is induced by a combination of transcription
factors. These include signal transducer and activator of transcription (STAT) molecules, which are activated following IFN\(\gamma\) receptor ligation, and nuclear factor-\(\kappa\)B (NF\(\kappa\)B) and mitogen activated protein kinases (MAPKs), which are activated in response to TLR or TNF receptor ligation (O’Shea et al., 2008). In summary, classically activated macrophages are products of a cell-mediated immune response. They can also be transiently generated in response to innate stimuli following stress or viral infections. Some pathogens have developed the ability to interfere with IFN\(\gamma\) signalling and prevent efficient macrophage activation. These classically activated macrophages are vital components of host defence, but their activation must be tightly controlled because the cytokines and mediators that they produce can lead to host-tissue damage. Indeed, classically activated macrophages are key mediators of the immune pathology that occurs during several autoimmune diseases, including rheumatoid arthritis and inflammatory bowel disease.

**Wound-healing macrophages**

Like classically activated macrophages, wound-healing macrophages can be generated in response to innate or adaptive signals. IL-4 is thought to be one of the first innate signals to be secreted during tissue injury (Loke et al., 2007). This early IL-4 production rapidly transforms resident macrophages into a population of cells that are programmed to direct wound healing; Adaptive immune responses can also trigger the production of IL-4, and it is thought that this is the primary pathway for the generation of wound healing macrophages. Induction of TH2-type immune responses occurs primarily in response to disturbances at mucosal surfaces and they are particularly important in the lung and intestines (Reese et al., 2007). The signature cytokines of a TH2-type immune response are IL-4 and IL-13. Macrophages treated in vitro with IL-4 and/or IL-13 fail to present antigen to T cells, produce minimal amounts of pro-inflammatory cytokines and are less efficient than classically activated macrophages at producing toxic oxygen and nitrogen radicals, and at killing intracellular pathogens (Edwards et al., 2006). However, these cells secrete components of the extracellular matrix and therefore their primary function seems to be related to wound healing. These macrophages can also exert indirect regulatory effects on the immune response because the polyamines they produce can influence the production of cytokines and suppress the clonal expansion of neighbouring lymphocytes (Cordeiro-da-Silva et al., 2004). The role of these macrophages in host defence and adaptive immunity remains somewhat enigmatic. Several studies have shown that alternatively activated macrophages
produce large amounts of chitinase and chitinase-like molecules, including YM1 and YM2 (Raes et al., 2002) acidic mammalian chitinase (AMcase) and stabilin-interacting chitinase-like protein (Kzhyshkowska et al., 2006). Wound-healing macrophages can be detrimental to the host when their matrix-enhancing activity is dysregulated, similarly to the dysregulated activity of classically activated macrophages in autoimmunity. Accumulating evidence indicates that IL-4- or IL-13-treated macrophages are more susceptible to some intracellular infections.

**Regulatory macrophages**

Similarly to the two populations of macrophages described above, regulatory macrophages can arise after innate or adaptive immune responses. Although stress responses are not typically regarded as a part of innate immunity, the hypothalamic–pituitary–adrenal (HPA) axis can exhibit marked effects on macrophages. Glucocorticoids are secreted by adrenal cells in response to stress and can prevent macrophage mediated host defence and inflammatory functions by inhibiting the transcription of pro-inflammatory cytokine genes and decreasing mRNA stability (Sternberg, 2006), arising a population of regulatory macrophages. The production of the regulatory cytokine TGFβ by macrophages following the phagocytosis of apoptotic cells in the presence of pro-inflammatory stimuli can also contribute to the immune-regulatory function of these macrophages (Fadok et al., 1998). Regulatory macrophages can also arise during the later stages of adaptive immune responses, the primary role of which seems to be to dampen the immune response and limit inflammation (Mosser, 2003). Although there can be subtle differences among the regulatory macrophage subpopulations that are generated by different stimuli, some characteristics are common to all. For example, one characteristic that seems to be shared by most of these regulatory cells is the need for two stimuli to induce their anti-inflammatory activity. The first signal (for example, immune complexes, prostaglandins, and adenosine or apoptotic cells) generally has little or no stimulatory function on its own. However, when combined with a second stimulus, such as a TLR ligand, the two signals reprogramme macrophages to produce IL-10 (Edwards et al., 2006), the production of which is the most important and reliable characteristic of regulatory macrophages. In addition to IL-10 production, these regulatory macrophages also downregulate IL-12 production (Gerber and Mosser, 2001); therefore, the ratio of IL-10 to IL-12 could be used to define regulatory macrophages. Because IL-10 can inhibit the production and activity of various pro-inflammatory cytokines, these regulatory macrophages are potent inhibitors of inflammation, despite the fact that they retain the...
ability to produce many pro-inflammatory cytokines. This indicates that the presence of regulatory macrophages could negatively correlate with vaccine protection, which requires the induction of pro-inflammatory cytokines. Consequently, one potential therapeutic intervention could be to manipulate macrophage populations during vaccination, for example, to minimize the induction of regulatory macrophages. Unlike wound-healing macrophages, these regulatory macrophages do not contribute to the production of the extracellular matrix, and many of these regulatory cells express high levels of co-stimulatory molecules (cD80 and cD86) and therefore can present antigens to T cells (Edwards et al., 2006). So, there are clear functional, as well as biochemical differences between regulatory and wound-healing macrophages. Regulatory macrophages can also be exploited by parasitic, bacterial and viral pathogens. In many cases these pathogens mimic some of the stimuli mentioned above. For example, the amastigote stage of intracellular protozoan *Leishmania* spp. binds host IgG and engages the macrophage Fc receptor for IgG (FcγR) on entry into these cells. This engagement, and the activation of downstream signalling pathways, induces the development of regulatory macrophages that can be permissive to intracellular growth (Miles et al., 2005). Infection with *Coxiella burnetti* results in host-cell apoptosis and the uptake of apoptotic cells by macrophages renders these cells permissive to intracellular bacterial growth (Benoit et al., 2008). Therefore, there is a growing list of pathogens that either interfere with macrophage activation or specifically induce the development of regulatory macrophages. In either case, the result is the same: defective pathogen killing and enhanced survival and spread of these microorganisms.

In summary, both innate and adaptive signals can influence macrophage physiology, and these alterations allow macrophages to participate in homeostastic processes, such as tissue remodelling and wound healing, as well as in host defence. However, each of these alterations can have potentially dangerous consequences if not appropriately regulated. For example, classically activated macrophages can cause damage to host tissues, predispose surrounding tissue to neoplastic transformation and influence glucose metabolism by promoting insulin resistance. Macrophages that are normally involved in wound healing can promote fibrosis, exacerbate allergic responses and be exploited by pathogens for intracellular survival. Regulatory macrophages can contribute to the progression of neoplasia and the high levels of IL-10 that these cells produce can predispose the host to infection.
3. Phagocytosis for pathogen killing

Phagocytosis of pathogens by macrophages triggers the innate immune response, which in turn orchestrates the adaptive response. In order to differentiate between infectious agents and self, macrophages have evolved a restricted number of phagocytic receptors, like the mannose receptor, that recognize conserved motifs on pathogens.

There are different strategies for parasite internalization, evolved by macrophages. They are pinocytosis, receptor-mediated endocytosis and phagocytosis (Swanson and Baer, 1995). Pinocytosis usually refers to the uptake of extracellular fluid and its contents and it involves the development of invaginations by the cell membrane, which closes and breaks off to make fluid-filled vacuoles in the cytoplasm. Pinocytosis and receptor mediated endocytosis are mediated by a clathrin-based mechanism and no actin polymerization is occurred during these processes (Figure 9A.).

![Figure: 9A. Pinocytosis](image)

On the other hand, phagocytosis is the clathrin independent process, evolved for the uptake of large particles (> 0.5\(\mu\)m) into cells; occurs by an actin-dependent mechanism. Macrophages and neutrophils are specialized cells where the metazoan phagocytosis occurs. Generally, lower organisms use phagocytosis primarily for the acquisition of nutrients. Phagocytosis in metazoa
has been evolved into an extraordinarily complex process composed of a variety of critical biological phenomena. Not only phagocytosis by macrophages is critical for the uptake and degradation of infectious agents and senescent cells; but it is also needed for development, tissue remodeling, the immune response and inflammation. In a simplified mode, the process can be considered to have four defined 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 9B.).

(i) Chemotaxis

Macrophages sense a wide variety of chemoattractants that can in principle lead their migratory path within tissues. Macrophages can respond to a number Chemotactic stimuli like, the split product of the fifth component of complement (C5a), chemotactic lymphokines (e.g. macrophage chemotactic factor and macrophage procoagulant-inducing factor), leukotrine B4, formylated peptides (e.g. formylmethionine-leucine-phenylalanine), thrombin and fibrogenclevage products. Upon binding of chemotactic factor to a receptor on the macrophage surface signaling cascades start on and gradually transmitted to the cell’s interior (i.e. signal-transduction) with changes in the metabolism of phosphoinositides, the intracellular calcium concentration, and the activity of protein kinase C. It is an energy consuming process.

![Figure:9B. Phases of phagocytosis](image-url)
(ii) Attachment
Attachment on the macrophage cell surface takes place by recognition of antigen presence on engulfing particle (e.g. the cell or the bacterium) by the specific antibodies. Complement receptors (especially CR1) also involved in recognition of particles. In the absence of antibodies or complement, lectin-like receptors react with surface sugars on the particle. It is an energy independent process.

(iii) Ingestion
The process of ingestion is mediated by the formation of a vesicle surrounding the particle, and then the vesicle buds off into the interior of the cell. Polymerization of actin at the site of ingestion facilitates the internalization of the particle via an actin based mechanism. Ingestion needs the consumption of energy to move the particle/phagosome by means of the cell’s microfilaments.

(iv) Intracellular Fate
Actin is removed from the phagosome following internalization of vesicle and immediately after phagosome enters the endocytic processing pathway. This pathway is marked by movement of phagosome toward the cell interior, where it fuses with a lysosome to form a phagolysosome. Lysosomes contain a rich pool of hydrogen peroxide, oxygen free radicals, peroxidase, lysozyme and various hydrolytic enzymes, which digest the ingested material (Melo and Dvorak, 2012). Indigestible material can be excreted from the cell by the process of exocytosis, or exist like microscopically or ultrastructurally visible debris (residual bodies, myelin figures etc.).

4. Generation of free radicals for enhancing microbicidal effect
Oxygen-derived free radicals are important in both innate and acquired immunity. Neutrophil and macrophage phagocytosis triggers various cellular processes including the "respiratory burst" whereby increased cellular oxygen uptake causes the production of the potent oxidant bactericidal agents, hypochlorous acid and hydroxyl radical. Furthermore, nitric oxide, a gaseous radical produced by macrophages, reacts with superoxide to form peroxynitrite, also a potent bactericidal agent. Conversely, oxidative stress may be deleterious in acquired immunity by activation of NF-κB, which directs gene expression involving various cytokines, chemokines, and cell adhesion molecules, among others. However, antioxidant supplementation essentially
reverses several age-associated immune deficiencies, causing increased levels of interleukin-2, elevated numbers of total lymphocytes and T-cell subsets, enhanced mitogen responsiveness, increased killer cell activity, augmented antibody response to antigen stimulation, decreased lipid peroxidation, and decreased prostaglandin synthesis.

II. Major types of free radicals and their derivatives produced in macrophages

Reactive Oxygen Species
The superoxide anion is formed by the univalent reduction of triplet-state molecular oxygen \( \left( {^3}{O}_2 \right) \). This process is mediated by enzymes such as NAD(P)H oxidases and xanthine oxidase or non-enzymically by redox-reactive compounds such as the semi-ubiquinone compound of the mitochondrial electron transport chain (Figure.10). SODs convert superoxide enzymically into hydrogen peroxide (Deby et al., 1990). In biological tissues superoxide can also be converted non-enzymically into the non-radical species hydrogen peroxide and singlet oxygen \( ({}^1{O}_2) \) (Steinbeck et al., 1993). In the presence of reduced transition metals (e.g., ferrous or cuprous ions), hydrogen peroxide can be converted into the highly reactive hydroxyl radical (·OH). Alternatively, hydrogen peroxide may be converted into water by the enzymes catalase or glutathione peroxidase. In the glutathione peroxidase reaction glutathione is oxidized to glutathione disulfide, which can be converted back to glutathione by glutathione reductase in an NADPH-consuming process.

![Figure 10. Pathways of ROS production and clearance](image-url)
Because superoxide and NO are readily converted by enzymes or nonenzymic chemical reactions into reactive nonradical species such as singlet oxygen ($^{1}\text{O}_2$), hydrogen peroxide, or peroxynitrite (ONOO$^-$), i.e., species which can in turn give rise to new radicals, the regulatory effects of these nonradical species have also been included in this review. Most of the regulatory effects are indeed not directly mediated by superoxide but rather by its reactive oxygen species (ROS) derivatives. Frequently, different reactive species coexist in the reactive environment and make it difficult to identify unequivocally which agent is responsible for a given biological effect.

**Reactive nitrogen Species**

The NO radical (NO·) is produced in higher organisms by the oxidation of one of the terminal guanido-nitrogen atoms of L-arginine (Pahlavani et al., 1998). This process is catalyzed by the enzyme NOS. Depending on the microenvironment, NO can be converted to various other reactive nitrogen species (RNS) such as nitrosonium cation (NO$^+$), nitroxyl anion (NO$^-$) or peroxynitrite (ONOO$^-$) (Stamler et al., 1992). Some of the physiological effects may be mediated through the intermediate formation of S-nitroso-cysteine or S-nitroso-glutathione (Goulet et al., 1994).

5. **Macrophage death pathways: a strong barrier for intracellular pathogens**

Macrophage-pathogen interaction is a complicated procedure and the consequence of this tag-of-war for both sides is to live or die. There are different strategies taken by macrophages to defeat interacting pathogens but when macrophages fail to eliminate infecting pathogens, programmed cell death is often triggered as a last resort to resolve the infection. Three major modes of cell death may happen in response to infection: apoptosis, necrosis, and pyroptosis. Apoptosis is characterised by retention of membrane integrity in addition with the formation of apoptotic bodies and is generally regarded as being immunologically silent. Apoptotic cells are engulfed by neighbouring phagocytes followed by pathogen degradation and starting of adaptive immunity. On the contrary, necrosis and pyroptosis initiate fast inflammatory responses, as necrotic and pyroptotic cells secrete pro-inflammatory cytokines and release cytoplasmic contents extracellularly. In order to make their intracellular niche safe, many pathogens prevent host cell death. A tricky way to inhibit apoptosis is interference with caspase signaling cascades. Inhibition of effector caspases 3 and 8 has been found during infection with *T. gondii* (Keller et al., 2006) and *T. cruzi* (Hashimoto et al., 2005). Activation of caspase death cascades can also be
prevented by blocking cytochrome c release from mitochondria, a strategy used by *T. gondii* (Goebel et al., 2001) and *Leishmania* (Akarid et al., 2004). Activation of the PI3K/Akt axis is another pro-survival signalling cascade exploited by *L. major, L. amazonensis* (Ruhland et al., 2007) and *T. gondii* (Kim et al., 2006). Activation of NF-kB is exploited by *Ehrlichia chaffeensis* (Zhang et al., 2004), *L. monocytogenes* (Mansell et al., 2000) and *L. pneumophila* (Bu-Zant et al., 2005; Laguna et al., 2006). Activation of these pathways results in the upregulation of pro-survival molecules of the Bcl-2 family, inhibition of cytochrome C release, and activation of inhibitor of apoptosis proteins (IAPs). Additional strategies to prevent apoptosis is expression of orthologs of host anti-apoptotic molecules, such as macrophage migration inhibitory factor by *L. major* (Kamir et al., 2008), or upregulation of anti-apoptotic heat shock proteins by *T. gondii* (Hwang et al., 2010). Conversely, several pathogens trigger cell death in order to promote dissemination, or to circumvent systemic immunity or both. Initiation of macrophage apoptosis has been shown for *T. cruzi* (Kyei et al., 2006), *L. major, Leishmania aethiopica*, and *Leishmania tropica* (Harrison et al., 2004), and *Yersinia pseudotuberculosis* (Ohlson et al., 2008). The fact that, both *T. cruzi* and *Leishmania spp.* have been shown to be capable of either preventing or inducing apoptosis at first blush appears paradoxical. It seems plausible, however, that these juxtaposed behaviours may reflect different stages of infection. Thus, prevention of apoptosis early after infection may allow for preservation of the intracellular niche and replication, whereas during late stage infection, induction of apoptosis facilitates pathogen release, dissemination, and silent uptake by other macrophages. It has further been shown that *T. gondii* (Bannai et al., 2008), *Leishmania donovani* [Das et al., 1999] induce apoptosis of non-infected bystander cells to deplete monocytes, macrophages, and CD4+ and/or CD8+ T cells.

**Leishmaniasis: Epidemiology and Immunobiology**

According to WHO, the disease is considered endemic in over 80 countries and about 2 million new cases occur every year, of which approximately 50% of these new cases are children (Bhattacharya et al., 2006). More than 12 million people are believed to be presently infected and a population of over 350 million people are at risk and around 7000 death annually are due to the disease (Bernetal., 2008). All types of Leishmaniasis are transmitted by the female phlebotomine sand flies, which infect a range of mammalians including humans, rodents, and
canids (Ashford, 1996). During the irrelatively simple life cycle, *Leishmania* parasites alternate between two distinct developmental stages: the flagellated, motile “promastigote” form residing in the mid gut of sandfly vectors, and the non-motile “amastigote” form that reside within phagolysosomal vesicles of the vertebrate host macrophages. Several clinical syndromes were subsumed under the term leishmaniasis: most notably visceral, cutaneous and mucosal leishmaniasis, which result from replication of the parasite in the mononuclear phagocytes in viscera, dermis and nasopharyngeal mucosa respectively. All three forms collectively pose great threat to human health, particularly in developing countries.

**Clinical syndromes**
Leishmanial disease generally causes three main human syndromes, and some lesser prevalent clinical entities. The outcome of each is dependent on the species of infecting parasite and the genetic susceptibility of the host.

**Cutaneous disease**
Cutaneous leishmaniasis (CL) is the least severe form of disease and several species such as *Leishmania major, Leishmania tropica, Leishmania braziliensis* are the causative agents of this disease. This disease is generally found in various regions of Central and South America. Cutaneous disease occurs as singular ulcerative or nodular lesions at or near the site of insect exposure. These are usually seen in uncovered areas of the body such as the face, forearms and lower legs and evolve over weeks to months (Figure.11A).

**Mucocutaneous disease**
Mucocutaneous leishmaniasis (MCL) is occasioned by *L. braziliensis* which can be due to extension of, or parasite metastasis from, local skin disease into the mucocutaneous tissue. MCL can exist months to years after resolution of primary lesions. This is often a horribly disfiguring infection causing from the chronic local destruction of tissue of the nose, mouth oro- and naso-pharynx and eyelids and can spread to affect respiratory function and hamper nutrition (Figure.11B).
Visceral disease

Visceral leishmaniasis (VL, also known as kala-azar) occurs from the infection of phagocytes within the reticuloendothelial system due to metastasis of parasites and parasite-infected macrophages from the initial site of cutaneous infection. In the Old world, VL is caused by *Leishmania donovani* (in regions of India, Pakistan, China and Africa) and *Leishmania infantum* (in the Mediterranean region). In the New World, VL is also caused by *L. infantum* (also known as *Leishmania chagasi* or *L. infantum chagasi*), which is obtained primarily in Brazil. Visceral disease has been reported in the Middle East caused by viscerotropic strains of *L. tropica*, which has been classically considered as an agent of CL. The proliferation of parasites in macrophages in the liver, spleen and bone marrow of patients with VL arises to progressive hepatosplenomegaly and bone marrow suppression (Figure.11C). If left untreated, patients develop pancytopenia and immune suppression and are prone to super-infections with other microbes. Without therapy patients with VL will finally succumb their disease. Individuals co-infected with HIV have a particular susceptibility to developing atypical presentations and increased severity, of VL and the development of VL in HIV patients is an AIDS-defining illness. This is probably due to the dysregulation effects of both agents on the immune system of the host.
Characteristic features:
Parasite always exhibits the greatest ingenuity when it is time to infect their host, to survive and propagate, and the parasite *Leishmania* proves itself clearly champion in this matter. *Leishmania* is a genus of Trypanosomatid protozoan parasites that are transmitted by the bite of phlebotomine Sandflies, results in a range of diseases (collectively known as leishmaniases) that affect over 150 million people worldwide. The flagellated, motile forms of *Leishmania* species are called promastigotes. They are found within the sandfly and progress through various morphologically distinct stages of differentiation to ultimately become the non-dividing, infectious 'metacyclic' promastigotes that are transmitted during a sandfly bite. Amastigotes do not have an exteriorized flagellum and live as intracellular parasites in a variety of mammalian cells, most notably within professional phagocytes such as macrophages (Figure 12).

Life cycle:
*Leishmania* have two main lifecycle stages: the motile flagellated promastigote, which is present in the sandfly vector, and intracellular non-flagellated amastigote, which is present within mammalian host cells (Figure 1). *Leishmania* are parasites of professional phagocytes (macrophages and dendritic cells) which initiate infection through receptor-mediated binding of infective promastigotes delivered into host tissue during the feeding of infected sandflies.

Parasites housed in parasitophorous vacuoles fuse with lysosomes to form phagolysosomes wherein promastigotes transform into and replicate as amastigotes. Eventually, the parasite
burden increases physically disrupting infected host macrophages delivering extracellular amastigotes into surrounding tissue where they are engulfed by uninfected macrophages. Parasites and infected macrophages can metastasize within the skin and visceral organs. Host control of infection is a complex interplay of innate and adaptive immune factors which are incompletely understood.

Epidemiology
Leishmaniasis currently threatens 350 million men, women and children around the world. It is estimated that 12 million people currently suffer from the disease. It is considered endemic in 88 countries ranging across all four continents. Leishmaniasis is endemic from northern Argentina to southern Texas (not in Uruguay, Chile or Canada), in southern Europe, Asia (except south-east Asia), the middle-east, and Africa. Within Africa, cases are most common in the eastern and northern regions, with sporadic incidence else. It is found in both rural and urban regions. Each

Figure: 13. Life cycle of *Leishmania*
different geographical area presents diversity in acquisition of infection, clinical presentation, method of diagnosis and response to therapy. 90% of visceral leishmaniasis cases occur in Bangladesh, Brazil, India, Nepal and Sudan. 90% of mucocutaneous leishmaniasis occurs in Bolivia, Brazil, and Peru. 90% of cutaneous leishmaniasis cases occur in Afghanistan, Brazil, Iran, Peru, Saudi Arabia and Syria. As notification of Leishmaniasis is only obligatory in 33 of the 88 countries, a significant number of cases are never reported (World Health Organisation, 2002). Deadly epidemics of visceral leishmaniasis sporadically flare up, often coinciding with environmental changes such as disforestation etc. Leishmaniasis has also emerged in new regions and settings as an AIDS-associated opportunistic infection. Thus this disease puts even more economic and physical strain on nations that are hardest hit by the burden of HIV related morbidity and mortality.

![Figure: Worldwide distribution of leishmaniasis](image_url)

**Microscopic diagnosis and treatments:**
Parasites are usually identified to the genus *Leishmania* by light microscopy. The amastigote form is usually detected microscopically after Giemsa staining. Blood examinations are the easiest to perform, but frequently there are very few circulating parasitized cells in buffy coat films. Several treatments including systemic antimonials, liposomal amphotericin B and miltefosine are currently available for leishmaniasis. However, these chemotherapeutic interventions are toxic and have poor patient compliance because many of them require daily systemic administration for periods ranging from 3 to 5 weeks. Furthermore, the emergence of
drug-resistant strains is rapidly increasing worldwide and these treatments fail to induce a sterile cure because they do not eliminate persistent parasites from the host. Therefore, there is a continued need for new therapies against leishmaniasis that are safe, effective in inducing long-term cure and that are easier to administer.

Evasion of Host Defence by *Leishmania donovani*: exploitation of host cell-signaling machinery

Lifestyle of *Leishmania*, in its mammalian host, is that of an obligate intracellular pathogen infecting the hematopoietic cells of the monocyte/macrophage lineage, which it enters by phagocytosis. Since this cell type is restricted for the eradication of invading pathogens and priming of the host immune response, it is inevitable that *Leishmania* has to evolve a range of sophisticated mechanisms to subvert normal macrophage function. This facilitates the parasite to bypass the innate immune response and to divide within the phagolysosome of the infected macrophage, from where it can spread and propagate the disease within the host.

Initial Interaction and Phagocytosis

To protect themselves from complement mediated lysis after immediate entry into mammalian host, *Leishmania* parasites must first evade complement-mediated lysis until they are phagocytosed by a macrophage. *L. major* procyclic promastigotes cannot combat complement action, whereas the metacyclic form, which is specified for transmission to the host, can fully evade complement-driven lysis. This difference in complement resistance depends upon structure of LPG, the dominant surface molecule of promastigotes on the parasite surface. The surface glycoprotein gp63, a protease, has also been reported to screen *L. amazonensis* and *L. major* against cellular lysis by converting C3b into C3bi, facilitating parasite opsonization and internalization. Parasite surface molecules also play a major part during attachment to the macrophage.

Inhibition of macrophages functions

Sequestering itself inside the cells of the host enables *Leishmania* to avoid many of the immune responses that would otherwise be directed against it. However, it is also necessary to tamper numerous macrophage functions, particularly those related to immune surveillance and macrophage activation, at either the protein or gene expression level.
**Microbicidal free radical production**

Two types of microbicidal molecules have been reported for their efficacy against *Leishmania*: NO and ROI (Liew et al., 1990; Murray et al., 1982). NO is important for parasite clearance, since mice lacking inducible nitric oxide synthase (iNOS) (also called NOS2) fail to control infection, and macrophages derived from these mice are unable to eliminate promastigotes in culture. Infected macrophages or macrophages incubated with purified LPG or GIPL *Leishmania* surface molecules lose their ability to induce iNOS or to generate NO in response to gamma interferon (IFN-γ) and/or lipopolysaccharide (LPS). However, it seems that IFN- and LPG can synergize to generate NO when administered simultaneously to naive macrophages (Proudfoot et al., 1996; Proudfoot et al., 1995). This suggests that contact between the parasite and the macrophage prevents the macrophage from responding to subsequent exposure to IFN- produced by lymphoid cells. Inhibition of NO production may result from the production of interleukin-10 (IL-10) and/or transforming growth factor (TGF-β), inactivation of the JAK/STAT pathway, activation of phosphotyrosine phosphatases, and/or ceramide production, as discussed below. In contrast to mice deficient for NO production, mice deficient for the generation of ROI can ultimately control the infection, after an initial period of increased susceptibility (Murray, 1999), indicating that ROI play a less important role in parasite clearance. However, ROI generation is also inhibited by *L. donovani* infection (Olivier, 1992). Inhibition appears to be dependent on the surface molecules LPG and gp63 (Descoteaux et al., 1999) and has been shown to involve abnormal PKC activity.

**Repression of Cytokine Production**

*Leishmania* subvert the immune machinery of macrophages by preventing the synthesis of a number of cytokines production, particularly those involved in the inflammatory response (IL-1 and tumor necrosis factor alpha [TNF-α] or in T-lymphocyte activation (IL-12), thus allowing the intracellular persistence of the pathogen. It has been reported that *L. donovani* infection as well as LPG treatment successfully prevents LPS-induced IL-1 secretion (Reiner et al., 1987; Frankenberg et al., 1990). LPG acts through promoter repression sequence in order to supress IL-1 transcription. Interestingly although, *L. major* induce IL-1transcription, this is not reflected in increased secretion, indicating secretion of various immunosuppressive signaling molecules, such as arachidonic acid metabolites and the cytokines TGF-β and IL-10(Hawn et al., 2002).
These directly and indirectly affect numerous different cell types, thus damaging the normal immune response and helping parasite survival. Several *Leishmania* species have been reported to induce TGF-β production in vitro and in vivo (Bogdan et al., 1998). Augmentation of TGF-β secretion correlated with reduced iNOS expression and retarded NK cell activity in lymph nodes, having consistency with the idea that TGF-β suppresses macrophage microbicidal action and the production of IFN-γ by NK cells, although the exact role of NK cells during leishmaniasis is somewhat debatable (Scharton and Scott, 1993). A recent study showed that *L. chagasi* induces TGF-β production immediately after entry into human macrophages, and this may permit the local inhibition of immune responses. Interestingly, at least for the case of *L. chagasi*, the increased production causes to be a result not of induced gene expression but of cleavage of pro-TGF-β by amastigote cysteine proteases to produce active TGF-β (Gantt et al., 2003). Interaction between the macrophage and phosphatidylserine motifs on the amastigote surface has also been proposed to trigger this induction. IL-10 is another anti-inflammatory cytokine produced by *Leishmania*-infected macrophages in vitro, apparently via interaction with the Fc receptor (Sutterwala et al., 1998). It may be the causative agent of the suppression of macrophage microbicidal activity involving NO, production of several cytokines (IL-1, IL-12, and TNF-α), and expression of costimulatory molecules such as B7-1/2 (Cunningham et al., 2002). Its importance in vivo is explained by the observation that transgenic mice constitutively expressing IL-10 are unable to control *Leishmania* infection (Kane et al., 2000). As for TGF-β, IL-10 is apparently induced following recognition of amastigote surface phosphatidylserine residues by the macrophage. Prostaglandin E2 (PGE2) seems to be generated by *Leishmania*-infected macrophages and is involved in parasite survival and progression (Farrell et al., 1987). This arachidonic acid metabolite has been reported to cause inhibition of macrophage proliferation and to suppress production of TNF-α, IL-1, and reactive oxygen intermediates. A recent study reports that PGE2 induction in *L. donovani*-infected macrophages depends upon PKC activation and cyclooxygenase-2 expression (Matte et al., 2001). Interestingly, increased visceralization of *L. donovani* has been correlated with increased PGE2 production in the lymph nodes in malnourished mice with (Anstead et al., 2001). It is therefore clear that *Leishmania* parasites are capable of modulating numerous macrophage functions in order to suppress inflammatory cytokines secretion, thus making a protective niche.
Inhibition of apoptosis

One of the vital mechanisms by which host cells defend themselves against intracellular pathogens is the induction of apoptosis (Griffin and Hardwick, 1999). On the contrary, pathogens relentlessly try to defeat the host defence systems and evolved a number of ways to inhibit host cell apoptosis which allows them more time to replicate (Goebel et al., 2001). Various studies have documented the molecular mechanisms in viruses (Cahir-McFarland et al., 2000), bacteria (Alli et al., 2000) and protozoan parasites (Heussler et al., 2001) that tamper with the host defensive apoptotic machinery. *Leishmania*, being an obligate intracellular parasite, makes host cells resistant to a variety of pro-apoptotic signals in murine and human cell lines (Moore et al., 1994; Lisi et al., 2005). Apoptotic processes take place through different pathways, but all ultimately activate execution caspases-3 (-6, -7), triggering disassembly of the nucleus and other organelles and encapsulation of the products in membrane-bound apoptotic bodies. The extrinsic pathway is triggered by binding of cell surface receptors, like CD95; Tumor Necrosis Factor Receptor superfamily member 6 or CD120 by FasL (Fas ligand, TNF superfamily member 6) or TNF-α (Tumor necrosis factor-α), followed by caspase-8 activation. The intrinsic pathway is marked by DNA damage, release of cytochrome c and other mitochondrial components into the cytosol, due to loss of integrity of the mitochondrial outer membrane. Mitochondria permeabilization can be inhibited by some Bcl-2 (B-cell leukemia, chronic lymphatic, type 2) family proteins, which prevent apoptosis. Activation of the caspase cascade, observed in apoptotic process, causes cleavage of a variety of target proteins with a structural or regulatory function, including poly (ADP-ribose) polymerase (PARP), nuclear lamins, and protein kinase C (PKC), leading to disassembly of the cell (Goebel et al., 2001; Kaufmann et al., 1993). PKC isozymes play an important role in controlling cell survival and cell death during intracellular microbial infection (Olivier et al., 1992). In this regard, *Leishmania* has received a great deal of attention because it impairs PKC-dependent processes in infected macrophages (Descoteaux et al., 1992) by synthesizing lipophosphoglycan (LPG), the most abundant of several glicosyl phosphatidylinositol (GPI)-anchored molecules, which are inhibitory toward PKC expression (Descoteaux et al., 1992). Mitochondria permeabilization can be prevented by some Bcl-2 (B-cell leukemia, chronic lymphatic, type 2) family proteins which inhibit apoptosis. It is not surprising that intracellular pathogens subvert these pathways to ensure their own survival in the infected cell. It has been reported that *Leishmania donovani* inhibits apoptosis of its host cell. *L.*
*donovani* infection protects bone marrow-derived macrophages (BMDM) from growth factor withdrawal induced apoptosis (Moore et al., 1994). As treatment of macrophages with lipophosphoglycan of the parasite also induced this effect, it was suggested that lipophosphoglycan might be involved in the signaling events leading to inhibition of apoptosis. In this context, it is interesting to note that *L. donovani* infection leads to an inhibition of protein kinase C-mediated c-fos gene expression in macrophages. Since the transcription factor c-fos triggers the expression of pro-apoptotic molecules, impairment of its expression might prevent apoptosis.

**Encounter with Toll-Like Receptor Signaling cascades**

TLR family members play a crucial role in connecting the innate immune response to the adaptive one by recognizing pathogen-associated molecular patterns (PAMPs). In the aspect of the ongoing host-pathogen arms race, the detection of parasite PAMPs by TLRs has two main inferences: first, the ability of cells of the immune system to recognize parasites and eradicate them when favourable conditions are present. Second, the ability of parasites to oppose TLR detection by tampering TLR signaling keeping immune cells in an inactive state and rendering them obstinate to subsequent TLR stimulation. GPI-anchored proteins is one of the main parasite derived molecules involved in TLR binding and activation. LPG of *Leishmania* is such a GPI-anchored protein, which can be sensed by TLRs (Becker et al., 2003; de Veer et al., 2003). It has been elucidated that both active macrophages and NK cells can detect LPG of *L. major* followed by ligand binding. In contrary, non-GPI-related ligands are less numerous but represent an important group of parasite-related molecules detected by TLRs (Flandin et al., 2006). TLR3 was recently revealed to be induced in IFN-γ-primed macrophages and to participate in their Leishmanicidal activity. The silencing of TLR3 led to diminish NO and TNF-α production in IFN-γ-primed macrophages in response to *L. donovani* infection subsequently increased parasite survival. Though TLRs can efficiently detect parasite PAMPs the fact that many successful infections are mediated through silent entry to target cells suggests that parasites must have evasion strategies to block TLR signaling and functions. Some of these mechanisms have been already described, while others are needed to be deciphered. *Leishmania* modulate signaling pathways to alter critical macrophage functions to the advantage of the parasite. Challenges still remain in understanding Leishmanial biology, the host responses to the parasites and how to use such knowledge to develop new ways of combating the infection. However, advances over the
past several years provide a roadmap for future discovery. In the present study, we tried to elucidate the cellular and molecular mechanisms applied by the parasite to modulate the defensive signaling mechanisms of the host during various stages of infection i.e. before their entry into macrophages, during their internalization, and sustained suppression of immune response after successful internalization.