REVIEW OF LITERATURE
Over a century ago, Robert Koch identified *Mycobacterium tuberculosis* as the causative agent of tuberculosis in humans and was the first person to realize that the efficacy of treatment of tuberculosis was largely dependent on the patient's immune response. He also reported the therapy of tuberculosis with soluble extracts of *M. tuberculosis* in the guinea pig model. Later clinical trials demonstrated the ineffectiveness of his therapy in human beings. Since then, a victory over tuberculosis has erroneously been claimed several times but we are still unable to eliminate or even control the menace of tuberculosis.

Historically, prevalence of tuberculosis has been notably higher in urban than in rural communities because of greater crowding, poorer sanitation and hygiene associated with city lives (hence greater opportunity for transmission of the bacillus). The disease reached near epidemic proportions in the 18th and 19th century in Europe and North America. Then with the improved health and sanitation facilities and overall development in early 20th century the incidence of tuberculosis showed a marked decline in the western world. It reemerged as a major public health problem with the spread of AIDS in the late 20th century. Despite the availability of effective drugs, the incidence of tuberculosis is increasing in most of the developing as well as industrialized countries today. *M. tuberculosis* is a multifaceted pathogen capable of causing an acute disease process as well as an asymptomatic latent infection. In most of the cases latent bacteria persist in the body for years, even decades, before resulting in reactivation of tuberculosis (Fig. I).

The magnitude of tuberculosis problem is staggering. As bacterial pathogens go, *M. tuberculosis* has an enviable penetration of its host population. 1.7 billion people (one-third of the world population) are estimated to harbour latent *M. tuberculosis* infection. There are 10 million active cases of tuberculosis every year, with 3 million annual deaths (WHO report, 2000). Infection does not usually lead to active disease, rather certain changes in the existing milieu like HIV infection, malnutrition, alcohol or drug abuse and
Figure 1. Main features of tuberculosis: from infection to host defence.

There are three potential outcomes of infection of the human host in *Mycobacterium tuberculosis*. a | The frequency of abortive infection resulting in spontaneous healing is unknown, but is assumed to be minute. b | In the immunocompromised host, disease can develop directly after infection. c | In most cases, mycobacteria are initially contained and disease develops later as a result of reactivation. The granuloma is the site of infection, persistence, pathology and protection. Effector T cells (including conventional CD4+ and CD8+ T cells, and unconventional T cells, such as T cells, and double-negative or CD4/CD8 single-positive T cells that recognize antigen in the context of CD1) and macrophages participate in the control of tuberculosis. Interferon-γ (IFN-γ) and tumour-necrosis factor-α (TNF-α), produced by T cells, are important macrophage activators. Macrophage activation permits phagosomal maturation and the production of antimicrobial molecules such as reactive nitrogen intermediates (RNI) and reactive oxygen intermediates (ROI).
immunosuppressive drug therapy may alter the course of latent tuberculosis infection and are believed to be the prime factors behind reactivation (Parish et al 1998). With rates of both HIV and tuberculosis increasing worldwide, the problem of HIV being superimposed upon latent tuberculosis is growing. Epidemiological studies of latent tuberculosis patients show that HIV infection increases the risk of reactivation from 2-23% per lifetime to 5-10% per year (Chassison and Benson 1995).

The epidemiological picture of tuberculosis in India is complex with wide variations in the annual risk of infection and prevalence of disease. The National Sample Survey and the Tuberculosis Research Center surveys have clearly brought out the age distribution of the disease and prevalence of the disease is found to be highest among younger age groups (10-44 years of age), similar to the situation in most developing countries. In India, the rate of infection among all age groups is estimated to be around 50% (54% in males and 46% in females). The concentration of disease among younger age groups makes tuberculosis a major socio-economic burden in India. (Prabhakar 1996). According to a recent study using enumeration of T cells specific for RD-1 encoded antigens, there is a high prevalence (80%) of latent *M. tuberculosis* infection in healthy urban Indians (Lalvani et al 2001).

Although these numbers are very high and human suffering caused by this disease is immense, yet there is cause for some optimism, as in most of the exposed individuals the immune system is capable of preventing the development of the disease. The immune response against the infection is generally successful in containing, though not eliminating, the pathogen.
Clinical Tuberculosis:

Tuberculosis can involve any organ system of the body, but pulmonary disease is the most common manifestation of tuberculosis, accounting for 80% of tuberculosis cases. In general, pulmonary tuberculosis is the only form considered to be infectious. Extrapulmonary disease can occur alone or with pulmonary involvement and the most common sites of this form of tuberculosis are lymph nodes, bones, meninges and genitourinary tract.

Pulmonary Tuberculosis:

Pulmonary tuberculosis is characterized by cough, sometimes with sputum, accompanied by weakness, fatigue, chest pain, weight loss, fever and night sweats. *M. tuberculosis* infections are acquired through inhalation of infective bacilli. Once in the lung, the bacteria are taken up by alveolar macrophages, *M. tuberculosis* uses the phagosomal compartment of macrophages as a preferred habitat and persists in macrophages within granuloma. (Shwander et al 1996). The granuloma consists of macrophages, giant cells, T cells, B cells and fibroblasts (Fig.II). During reactivation the semisolid caseous centre of granulomas begins to soften and liquefy, providing a rich and oxygenated environment for extracellular mycobacterial replication, which is the active and contagious form of the disease. Growing lesions may erode adjacent airways, releasing liquified necrotic material, resulting in the formation of cavities. An open caviated lesion can leak infectious material directly into bronchus, resulting in the continuous discharge of bacilli into sputum and is transmitted as an aerosol generated by coughing. A person with active disease infects upto 15 people annually (Kaufmann 2001) and this vicious cycle continues.
The infectious bacilli are inhaled as droplets from the atmosphere. In the lung, the bacteria are phagocytosed by alveolar macrophages and induce a localized proinflammatory response that leads to recruitment of mononuclear cells from neighbouring blood vessels. These cells are the building blocks for the granuloma, or tubercle, that defines the disease. The granuloma consists of a kernel of infected macrophages, surrounded by foamy giant cells and macrophages with a mantle of lymphocytes delineating the periphery of the structure. This tissue response typifies the 'containment' phase of the infection, during which there are no overt signs of disease and the host does not transmit the infection to others. Containment fails after a change in the immune status of the host, which is usually a consequence of old age, malnutrition, or HIV-co-infection. Under such circumstances, the centre of the granuloma undergoes caseation and spills viable, infectious bacilli into the airways. This leads to development of a productive cough that facilitates aerosol spread of infectious bacilli.
Extrapulmonary Tuberculosis:

A lymphohematogenous spread of tubercle bacilli occurs during primary infection due to lack of specific immunity and later the hematogenous phase may progress into disseminated tuberculosis. Extrapulmonary tuberculosis involves relatively inaccessible sites, and because of the nature of sites involved, fewer bacilli can cause much greater damage (Weir and Thoronton 1985). This form of tuberculosis presents more of a diagnostic and therapeutic problem than pulmonary tuberculosis and invasive procedures are generally required to establish a diagnosis. Extrapulmonary tuberculosis may involve nearly any organ or structure in the body and produce signs and symptoms related to the specific sites as well as systemic illness.

Problem of latent tuberculosis:

Latent tuberculosis is a clinical condition that occurs when the immune response against *M. tuberculosis* forces the bacteria into a quiescent stage. In contrast to the patients with active tuberculosis the individuals with latent tuberculosis do not transmit the disease. Following the inhalation of the virulent microorganism the bacilli enter and proliferate intracellularly within macrophage (Fenton and Vermeulen 1996). In most of the cases patients remain asymptomatic in the initial infection. Then T cells are recruited, following which a secondary immune response is mounted and the infection is controlled but the bacteria is not completely eradicated. It has been suggested that latent tuberculosis is induced and maintained by a Th1 kind of response involving cytokines like TNFα. In this case the Mycobacteria persist intracellularly in lung tissue with or without histological evidence of a local immune response (Arriaga et al 2002).
Regarding the physiological state of the microorganism during latency there are two alternative hypotheses. The pathogen may survive in a spore-like, metabolically inactive form awaiting a signal to resume division or in a metabolically active state of stationary or unusually slow growing pathogen. Evidence exists for both the hypotheses. The spore-like state could be associated with non acid-fast forms and the hypothesis is supported by the presence of homologs of sporulation regulatory genes in *M. tuberculosis* (De Maio et al 1996). Alternatively, there is data suggesting that latent bacilli could be metabolically active. Chemoprophylaxis trials have demonstrated that treatment, with antimicrobacterial drugs, of the asymptomatic persons have significantly reduced the risk of developing reactivation of tuberculosis (Comstock et al 1979). Since the susceptibility to antibiotics requires some level of metabolism, these results support the existence of latent Mycobacteria in a metabolically active state.

*Mycobacterium tuberculosis:*

*Mycobacterium tuberculosis* is a slim, rod-shaped, acid-fast, strictly aerobic bacillus. It is non-spore forming, non-motile, gram-positive with unusually slow growth rates (cell cycle takes 12-20 hours) relative to most bacteria (Wayne 1976). *M. tuberculosis* grows best at body temperatures but is extremely hardy; it can survive for long periods of time in a state of dryness. This is due to its unique cell wall structure, which contributes to the unusually low permeability and resistance to therapeutic agents, as well as for the generally slow rate of growth (Brennan et al. 1995). The lipid content of the cell wall is remarkably high (60 % in contrast to 20 % in Gram negative organisms, and 1-4 % in other Gram positives).
Cell envelope:

The popular model of the mycobacterial cell wall suggests complex mycobacterial lipids on the outermost lipid leaflet of the envelope (Brennan et al 1994). A mycolyl arabinogalactan-peptidoglycan (mAGP) complex covers a typical bacterial cytoplasmic membrane, with mycolyl esters (α-alkyl-, β-hydroxy-, C70 – C90 mycolic acids) extending outward, forming a thick inner lipid leaflet. An outer lipid leaflet, which is composed of a mixture of complex waxes, triacylglycerols and bioactive glycolipids, is hydrophobically associated with the inner mycolyl leaflet. Mycobacterial lipoglycans, LAM and related lipomannan (LM) and phosphomannosides (PIM) are also associated with the cell wall. A lipid moiety anchors these lipoglycans to the cytoplasmic membrane or the outer lipid leaflet. The nature of the outermost surface of the cell wall remains unresolved. Most agree that the glycosylated groups of the complex lipids are exposed on the outermost surface of the cell wall (Brennan et al 1994; Minnikin 1991), while others assert that a dense capsule like matrix of free carbohydrates (glucans, mannans and arabinomannans) and secreted proteins conceals these lipids (Daffe and Etienne 1999). This controversy can be clarified by the findings of Ortalo et al which suggested that the molecular composition of the envelope varies among mycobacterial species and culture conditions (Ortalo et al 1996).

Mycobacterial lipids exert immunosuppressive and inflammatory/granulomagenic effects. These properties, at first glance, appear to be contrary to one another; however, both types of responses contribute to the pathogenesis of mycobacterial infections. Inflammatory cytokines, including TNFα, IL-1β and multiple chemokines, are induced by LAM (Barnes et al 1992; Roach et al 1993) and GPL (Barrow 1997). Trehalose dimycolate (TDM) induces the formation of granulomas in vivo (Baba et al 1997). Immunosuppressive effects
include suppression of IFNγ-mediated activation (Sibley et al 1988; Ting et al 1999) and suppression of lymphoproliferative responses (Barrow et al 1993; Moreno et al 1988). Free lipids such as disaccharide trehalose (cord factor), mycosides, phospholipids and sulpholipids are also associated with the cell wall skeleton and may be important for the virulence of some strains of *M. tuberculosis* (Bersa and Chatterjee 1994).

**Genome:**

Complete genome sequence of H37Rv strain of *M. tuberculosis* has been reported by Stewart Cole and colleagues (Cole et al 1998). 4.4 Mb *M. tuberculosis* genome is the fourteenth complete bacterial sequence reported since that of *Haemophilus influenzae* in 1995 (Fleischmann et al 1995) and is the second largest after that of *Escherichia coli* (Blattner et al 1997). *M. tuberculosis* genome has a G+C content of 65.6% and 3,924 open reading frames have been identified, accounting for ~91% of the potential coding capacity. There are 13 RNA polymerase Sigma factors, 30 two-component regulators, 14 protein kinases or phosphatases and more than 140 transcriptional regulators, all pointing to an extensive regulatory apparatus, which is expected, considering the ability of the bacillus to successfully adapt to diverse environmental and metabolic challenges.

As per the genomic sequence, *M. tuberculosis* has the potential to synthesize all the essential amino acids, vitamins and enzyme co-factors with ~200 enzymes for general metabolism predicted from the sequence. In addition there are around 225 enzymes dedicated to lipid metabolism. Many potential drug resistance genes are also encoded in the genome including hydrolytic and drug-modifying enzymes such as β-lactamases, drug-efflux systems and numerous ABC transporters which explains why *M. tuberculosis* is naturally resistant to many antibiotics, making treatment difficult (Cole and Telenti 1995).
Table 1

Broad Classification of *M. tuberculosis* genes:

<table>
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<tr>
<th>Class</th>
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<th>Gene number</th>
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<th>Total length (kb)</th>
<th>% total coding</th>
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<tr>
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<td>22</td>
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<td>8</td>
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<td>15.3</td>
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Basic biology of *M. tuberculosis* infection:

*M. tuberculosis* enters the human host predominantly in the form of aerosol. The establishment of an infection depends on the initial interactions between *M. tuberculosis* and the alveolar macrophages which are dictated by surface characteristics of each. Intracellular pathogens bind specific host cell receptors to facilitate adherence as well as entry, the latter involves a complex signaling events between pathogens and host. The process of phagocytosis which is responsible for internalizing pathogens, has three major properties: (1) during phagocytosis particulate matters are internalized, (2) the process is initiated by the interaction of specialized plasma membrane receptors with specific molecules, ligands, located on the surface of particles, and (3) ligand-receptor complex triggers the local reorganization of the submembranous actin-based cytoskeleton that provides a driving force in the engulfment of particles. In higher organisms phagocytosis is attributed to selected cells, named ‘professional’ phagocytes to which macrophages, neutrophils and monocytes belong (Rabinovitch 1995).

**Route of entry:**

The receptors on the phagocytes can be classified into two groups, the first group of receptors bind integral surface components of particles. This group includes, mannose receptors and β-glucan receptors, which recognize surface components of microorganisms (Ezekowitz et al 1990 and Czop and Kay 1991). To this group also belong several macrophage receptors such as CD14, scavenger receptor A, CD36, and CD68 (microsialin), which are involved in phagocytosis of apoptotic cells (Savill 1998). In contrast, receptors of the second group recognize molecules of host proteins, named
opsonins, which coat the particles. The major opsonins found in serum are immunoglobulin G (IgG) antibodies and complement fragments C3b. IgG, when coating particles, interact with their surface by means of F(ab)\(_2\) domains, leaving unoccupied the Fc domain, which is recognized by Fc\(_\gamma\) receptors (Fc\(_\gamma\)Rs).

*In vitro* studies with mononuclear phagocytes have established that *M. tuberculosis* adheres nonopsonically as well as through opsonins to a selected set of receptors that include complement receptors types 1, 3 and 4 (CR1, CR3 and CR4) (Schlesinger et al 1990 and Hu et al 2000), the mannose receptor (MR) (Schlesinger et al 1996), the surfactant protein A receptor or SPA-R (Downing et al 1995), CD14 (Peterson et al 1995) and a group of pattern recognition receptors (PRRs) known as Toll like receptors or TLRs (Underhill et al 1999).

**Complement receptors:**

Phagocyte complement receptors occur in two distinct structural forms. CR1 is a monomeric transmembrane protein that binds C3b and C4b but not iC3b (Isibashi and Arai 1990 and Ernst 1998). On the other hand, CR3 and CR4 are heterodimers that contain identical \(\beta\) subunits (CD18 or \(\beta_2\) integrin) and distinct \(\alpha\) subunits (CD11a or \(\alpha_\lambda\), CD11b or \(\alpha_m\) and CD11c or \(\alpha_x\)). CR3 and CR4 bind C3bi, and CR3 also contain glycan-binding site (Thornton et al 1996). During maturation of blood monocytes to alveolar macrophages, expression of CR3 decreases and that of CR4 increases (Hirsch et al 1994). Macrophages can secrete complement proteins capable of opsonizing phagocytic particle (Schlesinger 1998). Complement activation by alternate pathway may be important in tuberculosis pathogenesis. Bacteria encounter complement proteins in the alveolus of the lung, C3 breaks down into C3b and C3bi. Pathogenic Mycobacteria uniquely recruit C2a to form a
C3 convertase on its surface, resulting in cleavage and deposition of C3b, thereby short circuiting the complement cascade. Although C3b binds CR1 and not CR3, the presence of Factor I (synthesized in the lung) would cleave C3b to C3bi and enable binding to CR3 (Sutterwala et al 1996).

*M. tuberculosis* can bind to CR3 at two distinct sites of the receptors. Opsonized *M. tuberculosis* binds CR3 at its C3bi binding domain and nonopsonized *M. tuberculosis* uses its endogenous capsular polysaccharides to interact with βglucan binding site near the C terminus of CD11b (Cywes et al 1996). Further studies have revealed that distinct strains and substrains of *M. tuberculosis* vary in their mode of interaction with CR3 (Cywes et al 1997).

**Mannose receptor:**

The macrophage mannose receptor (MR) is a monomeric transmembrane protein, with an extracellular domain containing eight carbohydrate recognition domains characteristic of C type or Calcium dependent lectins (Taylor and Drickamer 1993). This receptor recognizes glycosylated molecules with terminal mannose, fucose, N-acetyl glucosamine or LAM moieties and efficiently internalizes soluble and particulate ligand through endocytotic and phagocytotic pathways respectively (Schlesinger et al 1994). In a recent study it has been observed that mannose receptor is unable to distinguish between pathogenic and non-pathogenic Mycobacteria and MR dependent phagocytosis does not lead to free oxygen radical generation in human monocyte derived macrophages (MDMs), thus providing a safe passage for mycobacterial entry into the human MDMs (Astarie-Dequeker et al 1999). It has been suggested that the engagement of MR by ManLAM delivered a negative signal which interfered with the IL12 induction in human dendritic
cells (Nigou et al 2001). Expression of mannose receptor is highest on resident unactivated macrophages, which may be ideal to promote entry of *M. tuberculosis* in macrophages present in the uninflamed lung. The expression of MR is downregulated by IFNγ, therefore, their role in ingestion of *M. tuberculosis* during early infection and in immunocompromised hosts is more important than in the case of established granulomas (Schreiber et al 1993). In addition to a role for mannose receptors in the phagocytosis of whole bacteria, these receptors can mediate delivery of LAM to endocytic compartments that contain CD1b, thereby facilitating presentation of mycolic acid and lipoglycan antigens to CD4+CD8- T cells or CD8+ T cells (Prigozy et al 1997).

**SPA receptors:**

Specific receptors for surfactant protein A (SpA) are present on macrophages. SpA like MBP (Mannose Binding Proteins) is a member of collectin family of proteins. Opsonization with SpA enhances the binding of *M. bovis* BCG to mononuclear phagocytes, as well as alveolar macrophages (Downing et al 1995). The *M. tuberculosis* surface ligand for SpA is unknown but is likely to be similar or identical to that of MBPs viz. Phospatidyl inositol mannosides or related molecules (Ernst et al 1995).

**CD14:**

CD14, a phospatidyl inositol glycan-linked membrane protein, is best known and characterised as the high affinity receptor for LPS of grain-negative bacteria. However, CD14 also binds LAM of *M. tuberculosis* (H37Ra), and this binding induces macrophages to secrete IL-8 (Pugin et al 1994). Cells have been shown to utilize CD14 to recognize
whole *M. tuberculosis* (H37Rv) (Peterson et al 1995 and Reiling et al 2001). This recognition is followed by internalization of the bacteria.

**The Toll-Like Receptors (TLRs):**

The strategy of innate immune recognition is based on detection of constitutive conserved moieties expressed by the pathogens, called pathogen associated molecular patterns (PAMPs). Accordingly, the receptors that recognize PAMPs are known as pattern recognition receptors (PRR). The principal functions of PRRs include opsonization, activation of complement cascades, phagocytosis and induction of pro-inflammatory signalling pathways (Medzhitov and Janeway 1997).

The proteins involved in directing Drosophila embryonic dorso-ventral polarity (Toll/Cactus/Dorsal) bear striking functional and structural similarities with those responsible for the cytokine-induced activation cascade (IL-1 receptor/I-kB/NF-kB), a critical pathway in infectious and inflammatory disease processes. Medzhitov et al (Medzhitov et al 1997) confirmed the presence of a human homolog of the Drosophila Toll receptors. These human receptors were termed as Toll like receptors (TLRs).

This human TLR is a transmembrane protein with a leucine-rich repeat in the extracellular domain. Its cytoplasmic domain is homologous to that of the human IL-1 receptor. The TLR-dependent activation of the NF-kB pathway is mediated via the adaptor protein MyD88 (Medzhitov et al 1998), which is also required for signal transduction through IL-1R (Hultmark 1994).

The number of members of the human and mouse TLR family has expanded considerably. Till date about 10 of them are known. The interactions between *M. tuberculosis* and TLRs appear to be very complex, as distinct mycobacterial components may interact with
different members of the TLR family (Means et all 1999). *In vitro* studies using human cell lines and murine macrophages have provided evidence that *M. tuberculosis* can immunologically activate cells via either TLR2 or TLR4 in a CD14-independent manner (Means et al 1999). Interestingly, while TLR2 expression confers responsiveness to LAM derived from rapidly growing Mycobacteria (AraLAM), TLR4 does not. It appears that *M. tuberculosis* can signal via both human TLR2 and TLR4 in a ligand-specific manner. Inhibition of TLR signaling pathway blocks *M. tuberculosis* induced TNFα production and apoptosis but has no effect on Nitric Oxide (NO) production by infected macrophages (Means et al 2001).

In the LPS studies, *in vitro* evidence indicates that while this microbial product can signal through human TLR2 (Yang et al 1998), it can interact only with murine TLR4, but not murine TLR2 (Underhill et al 1999). TLR2 is also known to recognize a broad range of structurally unrelated ligands in combination with TLR1 and TLR6 (Fig III ). TLR3 is involved in recognition of double stranded DNA, TLR5 is specific for bacterial flagellin and TLR9 is known to be a receptor of unmethylated CpG motifs, which are abundant in bacterial DNA (Medzhitov 2001).

**Fcγ receptors:**

It has been demonstrated that intracellular trafficking of *M. tuberculosis* (H37 Rv) opsonized with immune serum is distinct from that of nonopsonized bacteria. Immunoglobulin G (IgG)-coated Mycobacteria were ingested by macrophages in vesicles that readily fused with ferritin-loaded lysosomes, whereas nonopsonized Mycobacteria resided in phagosomes that did not acquire ferritin from labeled lysosomes (Armstrong...
Toll-like receptors (TLRs) recognize a variety of pathogen-associated molecular patterns (PAMPs). Recognition of lipopolysaccharide (LPS) by TLR4 is aided by two accessory proteins: CD14 and MD-2. TLR2 recognizes a broad range of structurally unrelated ligands and functions in combination with several (but not all) other TLRs, including TLR1 and TLR6. TLR3 is involved in recognition of double-stranded (dsRNA). TLR5 is specific for bacterial flagellin, whereas TLR9 is a receptor for unmethylated CpG motifs, which are abundant in bacterial DNA. G+, Gram-positive; G-, Gram negative; GPI, glycosphingolipid; RSV, respiratory syncytial virus.
and Hart 1971). This implies that the entry through Fcγ may specify a distinct intracellular trafficking pathway for virulent *M. tuberculosis*.

**Signaling Pathways in Phagocytosis:**

Receptor clustering upon particle binding is thought to be the first step of a signaling pathway that targets the adjacent submembranous cytoskeleton. Reciprocally, actin-driven engulfment of the particle requires the progressive recruitment of receptors at the site of particle binding. It is commonly accepted that the receptors work in a ‘zipper’ manner, linking subsequent ligand molecules distributed over the surface of the particle (Griffin et al 1975). These interactions strengthen the binding of the particle and simultaneously amplify the phagocytic signal. As a result, actin microfilaments are polymerized, filling pseudopods of phagocytic cups that first embrace bound particles and later fuse into nascent phagosomes (Sheterline et al 1984 and Greenberg et al 1991). Phagocytosis is inhibited by cytochalasin B, an actin filament depolymerizing agent (Greenberg et al 1991). During phagosome maturation, actin undergoes depolymerization, enabling fusion of the phagosomes with lysosomal and endosomal compartments (Bengtsson et al 1993).

**Tyrosine phosphorylation:**

Tyrosine phosphorylation plays a key role during phagocytosis mediated by FcγRs. Two cytoplasmic tyrosine residues of FcγRs that undergo phosphorylation are arranged into short domain named immunoreceptor tyrosine-based activation motif or ITAM (Samelson and Klausner 1992). The central role of ITAM in signal transmission is believed to rely on the sequential interactions of ITAM with tyrosine kinases of Src and Syk/Zap-70 families.
(Greenberg 1995). Upon receptor ligation, kinases of the Src family are first activated, five of which (Fgr, Fyn, Hck, Lyn, Src) are identified in ‘professional’ phagocytes (Bolen 1991). Hck is associated with secretory lysosomes in neutrophils and macrophages that fuse with phagosomes. *M. tuberculosis* infected cells used to prevent phagolysosome biogenesis by not recruiting Hck (Astarie-Dequeker 2002).

**Role of protein kinase C and serine/threonine phosphorylation:**

The protein kinase C (PKC) family is composed of serine/threonine kinases involved in the transduction of phagocytic signals generated by various receptors, including FcγRs, CR3, and the mannose receptor. During phagocytosis mediated by these receptors in macrophages, PKC was shown to be accumulated around phagosomes (Allen and Aderem 1995 and 1996).

**Role of calcium ions in transduction of the phagocytic signal:**

Phagocytosis mediated by FcγRs, CR3, and mannose receptors is accompanied by the transient rise in the cytosolic free calcium concentration ([Ca\(^{2+}\)]\(_i\)) in mouse macrophages. the [Ca\(^{2+}\)]\(_i\) elevation was necessary for phagosome-lysosome fusion (Lew et al 1985 and Bengtsson et al 1993). For maximal phagocytic activity, both the release of Ca\(^{2+}\) from intracellular stores and a Ca\(^{2+}\) influx from extracellular fluid were required (Kobayashi et al 1995). *M. tuberculosis* inhibits CR mediated Ca\(^{2+}\) signalling and contributes to inhibition of phagolysosome fusion and promotion of intracellular mycobacterial survival (Malik et al 2000).
Early inflammatory responses:

The early induced but non-adaptive responses are important because they can repel a pathogen or, more often, hold it in check until an adaptive immune response can be mounted. These often occur rapidly as they do not require clonal expansion. Whenever an infection has been detected in the body the prime function of the innate immune system is to recruit more phagocytic cells and effector molecules to the site of infection through the release of a battery of cytokines and chemokines.

The initial events during a primary infection with *M. tuberculosis* are poorly understood and there are few models to evaluate the sequence of events that follow the first contact of the host with the Mycobacteria. The injection of *M. bovis* BCG into mouse pleural cavity induces an intense biphasic inflammatory reaction that peaks at 24 hours and 15 days. At 4 hours an influx of neutrophils occurs that reaches maximum at 24 hours. At this time an intense influx of eosinophils and mononuclear cells is also observed. Later leukocyte influx observed at 15 days essentially brings in mononuclear cells and some neutrophils (Menezes-de-Lima-Junior et al 1997).

Neutrophils are the predominant leukocytes to arrive at sites of acute inflammation. Neutrophil migration to peritoneal cavity of rabbits was observed after infection with BCG (Appelberg 1992). In a recent study it has been shown that a 19 kD lipoprotein from *M. tuberculosis* promotes neutrophil priming and activation (Neufert et al 2001). Whereas after 4 to 6 weeks of infection, numbers of lung leukocytes had been doubled but the proportions of lymphocytes (about 70%), macrophages (about 18%) and granulocytes (about 12%) remained essentially unaltered in a murine model of infection with *M. bovis* BCG. Both T and B cells contributed to the increase in cell recoveries from infected
tracheal lymph nodes, the T/B ratio declined significantly but CD4/CD8 ratio remained the same (Saxena et al 2002).

Eosinophilia is observed sometimes in bronchoalveolar lavage of tuberculosis patients (Vijayan et al. 1992, Nakamura et al. 1993), and it has been shown that Lipoxygenase products, PAF-acether and IL-5 are involved in eosinophil accumulation within the mouse pleural cavity after *M. bovis* BCG infection (Menezes-de Lima-Júnior et al 1997).

TNFα acts on endothelial cells to enhance their interaction with neutrophils and eosinophils. The dose-response relationship and kinetics of TNFα stimulated endothelial cell adhesiveness for neutrophils is similar to that for eosinophils (Nourshargh 1993). However, in the experiments with BCG induced-pleurisy, TNFα seems to have a more important effect on neutrophil migration (Menezes-de-Lima-Junior et al. 1997). It was demonstrated that TNFα produced by macrophages in response to PPD (the soluble antigen released from *M. tuberculosis*) could regulate NO production by these cells (Saito & Nakano 1996). This regulation between NO and TNFα can be a putative mechanism modulating the neutrophil migration induced by BCG in mouse pleural cavity (Downey 1994 and Springer 1994).

**Involvement of adhesion molecules:**

During an inflammatory response, the induction of adhesion molecules on the endothelial cells of local blood vessels, as well as induced changes in the adhesion molecules expressed on leukocytes, recruit large number of circulating leukocytes into the site of infection (Ebnet et al 1996). The migration of leukocytes out of blood vessels, a process known as extravasation is thought to occur in four steps (Fig IV).
Figure IV. The phenomenon of extravasation.

The migration of leukocytes out of blood vessels, a process known as extravasation, involves a sequence of discrete events involving different families of cell adhesion molecules. Firstly, leukocytes migrate to the wall of postcapillary venules, “margination”. Then “roll” along the endothelial cells in a process mediated by the selectin family of adhesion molecules. Thereafter the leukocytes must firmly adhere to the vessel wall to migrate to the site of tissue injury, a step mediated by another family of adhesion molecules the integrins. Finally, the leukocytes squeeze between the endothelial cells and migrate to the site of infection under the influence of chemoattractant cytokines.
1. The first step is mediated by selectins. The adhesion molecule, E and P selectin normally remain in vesicles inside the endothelial cells. As soon as the inflammatory modulators send the signal these vesicles get fused to the membrane and the selectins start appearing on the endothelial cell surface. These selectins recognize certain leukocyte glycoproteins which accounts for ‘rolling’ of leukocytes along the endothelium.

2. LFA 1 (CD11a/CD18) and Mac 1 (CD11b/CD18) are leukocyte integrins which normally adhere weakly with ICAM1 expressed on endothelial surface. However IL8 triggers a conformational change on LFA1 and Mac 1 on rolling leukocyte surface, which greatly increases its capacity to adhere to endothelial.

3. PECAM or CD31, expressed both on leukocytes and at the intracellular junctions of endothelial cells, is involved in extravasation response. These interactions enable the phagocyte to squeeze between the endothelial cells. Phagocytes then disintegrate the proteins of basement membrane with the aid of different proteolytic enzymes and enter the site of infection.

4. Finally the extravasated leukocytes migrate through tissues under influence of cytokines and chemokines.

An important early event in the recruitment of leukocytes from the microcirculation to tissues is their interaction with vascular endothelial cells. In the initial phase, leukocytes migrate to the wall of postcapillary venules and roll along the endothelial cells in a process mediated by the selectin family of adhesion molecules (Ley et al. 1995). Thereafter the leukocytes must firmly adhere to the vessel wall to migrate to the site of tissue injury, a step mediated by another family of adhesion molecules, the integrins. The role of adhesion molecules during mycobacterial infection is not clear. The leukocyte integrin, CD11b/CD18 complex seems to have a role in neutrophil and mononuclear cell
accumulation. L-selectin appears to be responsible for the neutrophil and eosinophil migration induced by BCG (Hellewell et al 1994).

Modulation of ICAM1 expression due to *M. tuberculosis* infection:

There are some contradictory reports about the changes in the expression of ICAM 1 upon mycobacterial infection. Monocytes bind ICAM 1 through its Mac 1 binding sites. This receptor ligand interaction is very important for the migration of monocytes into the tissue such as the infected lung (Sligh et. al 1993). It has been hypothesized that it would amplify and enhance the immune and inflammatory responses to *M. tuberculosis*. THP 1, a human monocyte cell line, increases surface expression of ICAM 1 in a sustained and selective manner upon stimulation with *M. tuberculosis* (Lopez-Ramirez et al 1994). Several cell wall components of Mycobacteria were also capable of eliciting this response. In addition, they have also shown that the enhanced expression of ICAM 1 in response to *M. tuberculosis* was mediated in a paracrine or autocrine manner predominantly via TNFα. On the other hand it has been showed, that following infection with *M. avium*, human blood monocytes displayed reduced levels of CD54, CD58 and CD86 molecules, and failed to respond to the regulatory signals from IFNγ to upregulate CD80 expression. This data supports the proposition that mycobacterium impedes the immune response by reducing the costimulatory activity of its host cell, the macrophage (Mohagheghpour et al 1997). Substantial proof of the role of ICAM 1 has come from gene disruption of the ICAM 1 molecule. The Mac 1 binding site was conformationally changed so that monocytes could no longer bind and the LFA binding site was intact; hence lymphocytes could still use ICAM 1 to cross the infected tissue. When infected with a low dose of aerosol of *M. tuberculosis*, these mice developed normal T cell responses but no Delayed
Type Hypersensitivity (DTH) and no appreciable granuloma formation during the first 90 days of infection (Johnson et al 1998). Thus granuloma formation was not required as long as an adequate T cell response occurred and these T cells were able to migrate to the site of infection and activate resident macrophages. However, soon after this time point the mice began to die, all succumbing in less than 140 days (Saunders et al 1999). So, while an antigen-specific T cell response protected mice against initial infection by activating infected resident macrophages, T cell activation alone was insufficient to protect mice against chronic infection in the absence of granuloma formation. The importance of interactions between the adhesion molecules during *M. tuberculosis* infection has been further emphasized in a recent study of the development of disease in susceptible mouse strains (Turner et al 2001). In this study it was shown that the CBA/J mouse possessed the capacity to generate antigen-specific T cells but failed to upregulate the expression of two adhesion molecules, CD 11a and ICAM 1 on the surface of circulating T cells. This inability to alter the expression of these two molecules clearly diminished the ability of the T lymphocytes to enter the lung. Therefore, the upregulation of CD11a and CD54 in response to *M. tuberculosis* challenge may be a useful parameter of protection against tuberculosis.

**Modulation of IFNγ signaling upon *M. tuberculosis* infection:**

In mycobacterial infections, an essential subset of T helper cells is characterized by IFNγ secretions and is termed as Th1 cells. One of the most important consequences of Th1 cell activation is IFNγ mediated nitric oxide production by macrophages. Mice lacking the gene encoding IFNγ or IFNR or the enzyme responsible for the nitric oxide
production were found to be highly susceptible to \textit{M. tuberculosis} infection (Ding et al 1988 and Cooper et al 1997). Studies of patients with tuberculosis has demonstrated the presence of IFN\(\gamma\) in pleural fluid, lung fluid and lymph nodes (Barnes et. al 1993 and Lin et. al 1996) thus suggesting a defect in response to IFN\(\gamma\) rather than inhibition of its production which allows the disease to progress. IFN\(\gamma\) activates human macrophages \textit{in vitro} to control the growth of certain intercellular pathogens, but is unable to activate human macrophages to restrict virulent \textit{M. tuberculosis} (Rook et al 1986). This suggests that \textit{M. tuberculosis} might interfere with cellular signal transduction pathways that are activated by IFN\(\gamma\) thereby avoiding being killed by macrophage. It has been found that \textit{M. tuberculosis} (Erdman) infection inhibits the JAK STAT signaling pathway by disrupting the essential interaction of STAT1 with transcriptional coactivators CBP and p300 in human monocyte derived macrophages (Ting et al 1999). However, in case of \textit{M. avium} infection, a novel mechanism of inhibiting the expression of IFN\(\gamma\) inducible genes by downregulation of IFNR on mouse macrophage cell line, RAW 264.7, has been suggested (Hussain et al 1999), with the extent of inhibition varying from gene to gene.

\textbf{Antimycobacterial Functions of Macrophages:}

It is well established that murine macrophages possess antimycobacterial functions \textit{in vitro} (Mackaness 1969). Compared to the murine system, much less is known about the activation of antimycobacterial activity in human macrophages. However, the precise mechanisms by which these cells mediate killing or inhibition of bacterial pathogens are not clearly understood.
Phagolysosome Fusion:

It is well established that phagosomes, the product of the endocytic pathway initiated by phagocytosis of large particles including microbes, can fuse with lysosomes (Kornfeld 1987). The lysosome is a complex vacuolar organelle of the late endocytic pathway. Phagocytosed microorganisms are subject to degradation by intralysosomal acidic hydrolases upon phagolysosome fusion. These enzymes function optimally at acidic pH, the acidic environment being maintained by membrane ATP-dependent proton pumps, the vacuolar HC-ATPases (Mellman et al 1986 and Ohkuma and Pool 1978).

It has been hypothesized that prevention of phagolysosomal fusion is a mechanism by which *M. tuberculosis* survives inside macrophages. This hypothesis was based on studies examining the interaction of *M. tuberculosis* and mouse macrophages by electron microscopy and labeling of lysosomes with electron-opaque ferritin (Armstrong and D’Arcy Hart 1971). An important inference was that lack of fusion with lysosomes was observed only with phagosomes containing viable bacilli.

The exclusion of vacuolar ATPase proton pumps from phagosomes containing live *M. tuberculosis* or *M. avium* (Crowle et al 1991 and Xu et al 1994) provides a mechanism for the relative lack of acidification of mycobacterial phagosomes. While it remains to be proven whether alkalinization of mycobacterial vacuoles attenuates phagolysosomal fusion, increase in intralysosomal pH is likely to help Mycobacteria evade the adverse effect of an otherwise acidic environment. It was surprising that phagosomes harboring live *M. avium* or *M. tuberculosis* acquire LAMP-1 (Fig V), the late endosomal/lysosomal marker (Crowle et al 1991, Xu et al 1994 and Clemens and Horwitz 1995). Russell proposed that this apparent paradox is explainable if mycobacterial phagosomes
Various surface receptors participate in the early encounter between *M. tuberculosis* and macrophages. Cholesterol serves as a docking site that facilitates interactions between mycobacteria and surface receptors. Once engulfed, *M. tuberculosis* ends up in a phagosome, the maturation of which is arrested at an early stage. The early phagosome-harbouring mycobacteria characteristically retains TACO, which apparently prevents its further maturation. *M. tuberculosis* inhibits phagosomal acidification (which occurs by means of a V–H⁺ ATPase) and prevents fusion with the endosomal pathway. The arrest of phagosomal maturation is, however, incomplete and some phagosomes mature to form phagolysosomes. Although phagosome and endosome maturation form a continuum, distinct steps can be distinguished by means of different markers and tracers, some of which are shown. CR, complement receptor; FeR, receptor for the constant fragment of immunoglobulin; LAMP1, lysosomal-associated membrane protein 1; LBPA, lysobiphosphatic acid; MR, mannose receptor; Rab7, member of the small GTPase family; SPR, surfactant protein receptor; TACO, tryptophane, aspartate-containing coat protein; TLR, Toll-like receptor; V–H⁺ATPase, vacuolar ATP-dependent proton pump.

Figure V. The intracellular lifestyle of *Mycobacterium tuberculosis.*
selectively block fusion with specific subsets of a heterogeneous population of endosomal vacuoles (Russell 1995).

Analysis of the biochemical composition of mycobacterial phagosomes from labeled BCG-infected macrophages has identified a 50-kDa host cell polypeptide, TACO (tryptophan aspartate-containing coat), specific for phagosomes containing live bacilli. By 2 hours post-infection with BCG, was almost completely relocalized (from the cortical distribution) to the BCG-containing phagosomal membrane, and it remained associated for a prolonged period of time. By retaining TACO and thus intercepting the fusion of phagosome with lysosome, Mycobacteria evade potent lysosomal antimicrobial functions of macrophages (Ferrari et al 1999).

**Free Radical-Based Antimycobacterial Mechanisms; Reactive Oxygen Intermediates (ROI) and the Nitrogen Oxides:**

High output NO production by immunologically activated macrophages is a major antimicrobial mechanism (Fang 1997, MacMicking et al 1997 and Chan and Flynn 1999). These phagocytes, upon activation by appropriate agents such as IFNγ and TNFα, generate NO and related RNI (Reactive Nitrogen Intermediate) via iNOS2 (inducible Nitric oxide synthase) using L-arginine as the substrate (Fig I). Regardless of the route of infection, NOS2(-/-) mice were much more susceptible than wild type mice. Mycobacteria replicated to much higher levels in the organs of NOS2(-/-) mice than in those of wild type mice (Scanga et al 2001). The significance of these toxic nitrogen oxides in host defence against *M. tuberculosis* has been well documented, both *in vitro* and *in vivo*, particularly in the murine system (Shiloh and Nathan 2000).
Activated murine macrophages and dendritic cells metabolize arginine by two alternative pathways involving either inducible NO synthase or arginase (Munder et al 1998). Inducible NO synthase catalyzes the conversion of L-arginine into NO and citrulline whereas, arginase hydrolizes arginine to ornithine and urea. The balance between these two enzymes is regulated by Th 1 and Th 2 cells via their secreted cytokines: Th 1 cells induce NO synthase while Th 2 induce arginase 1. Moreover induction of any of these enzymes is accompanied by the suppression of the other, indicative of two competitive states in murine macrophages (Corraliza et al 1995 and Modolell 1995). It has been suggested that arginase 1 induction in macrophage is used by the intracellular parasite *Leishmenia major* to survive inside the host. In *Schistosoma mansoni* infected mouse model differential activation of arginase 1/NO synthase was found to be a critical determinant in the pathogenesis of the granuloma formation. *Heliobacter pylorus* secretes its own arginase to evade the immune response by downregulating host NO production through substrate depletion (Gobert et al 2001). Though arginase 1 seems to play a very important role in the survival of the pathogen, but its role in mycobacterial infection is still not known (Hesse at al 2001). *M. tuberculosis* mounts a stubborn defense against ROI and RNI components of the immune response. A class of thioredoxin-like molecules that enables an antioxidant defense was found in virulent Mycobacteria (Bryk et al 2002).

While the role of macrophage NOS2 in host defence against *M. tuberculosis* is well established, the significance of toxic oxygen species in the control of tuberculosis remains controversial. Despite the demonstration that H₂O₂ generated by cytokine-activated macrophages was mycobacteriocidal (Walker and Lowrie 1981), the ability of ROI to kill *M. tuberculosis* remains to be confirmed (Chan et al 1992). Indeed, Mycobacteria are capable of evading the toxic effect of ROI by various means (Chan and Kaufmann 1994). For example, mycobacterial components lipoarabinomannan (LAM) and
phenolicglycolipid I (PGL-1) are potent oxygen radical scavengers (Chan et al 1989 and 1991).

**Induction of Cytokines by *M. tuberculosis***:

The immune response to all pathogens is dependent on cytokines, which regulate all the cells of the immune system (Fig VI). The inflammatory response to *M. tuberculosis* is no exception and in fact strongly induces cytokines during infection.

**Interleukin-12**:

Immunologic control of *M. tuberculosis* infection is based on Th1 response. IL-12 is induced following phagocytosis of *M. tuberculosis* bacilli by macrophages and dendritic cells (Ladel et al 1997 and Henderson et al 1997), which drives development of a Th1 response with production of IFNγ. Convincing evidence of the importance of IL-12 in resistance to tuberculosis was provided by IL-12p40−/− mice. These mice were quite susceptible to infection and had a greatly increased bacterial burden, as well as decreased survival time probably due to the substantially reduced IFNγ production (Cooper 1997). In contrast, mice lacking p35 sub unit of IL-12 exhibited a moderate ability to control bacterial growth and survived infection longer (Cooper 2002).

**IFNγ**:

IFNγ is a key cytokine in control of *M. tuberculosis* infection. This cytokine is produced by both CD4+ and CD8+ T cells in tuberculosis (Orme 1992 and 1993, Lalvani 1998 and
Figure VI | Role of TLRs in the control of adaptive immunity.

TLRs sense the presence of infection through recognition of PAMPs (pathogen-associated molecular patterns). Recognition of PAMPs by Toll-like receptors (TLRs) expressed on antigen-presenting cells (APC), such as dendritic cells, upregulates cell-surface expression of co-stimulatory (CD80 and CD86) molecules and major histocompatibility complex class II (MHC II) molecules. TLRs also induce expression of cytokines, such as interleukin-12 (IL)-12, and chemokines and their receptors, and trigger many other events associated with dendritic cell maturation. Induction of CD80/86 on APCs by TLRs leads to the activation of T cells specific for pathogens that trigger TLR signalling. IL-12 induced by TLRs also contributes to the differentiation of activated T cells into T helper (TH)1 effector cells. It is not yet known whether TLRs have any role in the induction of TH2 responses. PRR, pattern-recognition receptor.
Serbina and Flynn 1999) as well as by NK cells. A recent report suggests NK and NKT cells contribute to baseline IFNγ secretion in control lungs, expansion in the IFNγ producing T cell population was essentially responsible for the augmented response seen in lungs of BCG-infected mice 5-6 weeks after infection (Saxena et al 2002). Macrophage activation is defective in the IFNγ knockout mice. Although IFNγ production alone is insufficient to control *M. tuberculosis* infection, it is required for the protective response to this pathogen (Fig I).

**Tumor Necrosis Factor α:**

TNFα is believed to play multiple roles in immune and pathologic responses in tuberculosis. *M. tuberculosis* induces TNFα secretion by macrophages, dendritic cells, and T cells (Barnes et al 1993, Ladel et al 1997, Henderson et al 1997 and Serbina and Flynn 1999). This cytokine is required for control of acute *M. tuberculosis* infection. Infection of TNF(-/-) mice with *M. tuberculosis* resulted in an initial delay in chemokine induction and cellular recruitment to form granulomas. Subsequently, the loosely associated lymphocytes and macrophages failed to prevent progressive infection. Therefore, TNFα not only orchestrates early induction of chemokines and initial leukocyte recruitment, but has an additional role in the aggregation of leukocytes into functional granulomas capable of controlling virulent mycobacterial infection (Roach et al 2002). Besides, TNFα in synergy with IFN induces iNOS2 expression (Flesch and Kaufmann 1990 and Chan et al 1992).
Interleukin-10:

In contrast to TNFα, IL-10 is generally considered to be primarily anti-inflammatory. This cytokine, produced by macrophages and T cells during *M. tuberculosis* infection, possesses macrophage deactivating properties, including downregulation of IL-12 production, which in turn decreases IFNγ production by T cells. Macrophages from tuberculosis patients are suppressive for T cell proliferation *in vitro*, and inhibition of IL-10 partially reversed this suppression (Gong et al 1996). IL-10 directly inhibits T cell responses, as well as inhibiting APC function of cells infected with Mycobacteria (Rojas et al 1999).

Cellular Immune Responses during *M. tuberculosis* Infection:

*M. tuberculosis* live within cells, usually macrophages; thus T cell effector mechanisms, rather than antibody, are required to control or eliminate the bacteria. Historically, research is focused on the CD4+ T cell response to tuberculosis, but recently there has been an increased interest in the roles of CD8+ T cells in the immune response to this pathogen. In the mouse model, within a week of infection with virulent *M. tuberculosis*, the number of activated CD4+ and CD8+ T cells in the lung-draining lymph nodes increases (Feng et al 1999 and Serbina et al 2000). Between two to four weeks after infection, both CD4+ and CD8+ T cells migrate to the lungs and the tuberculous granuloma similarly contain both CD4+ and CD8+ T cells (Flynn et al 1992) that participate in the continuous battle to contain the infection within the granuloma and prevent reactivation. Whereas CD4+ T cells formed organized aggregates, CD8+ T cells were fewer and more
scattered and tended to be more prominent toward the periphery of the granulomas (Gonzalez-Juarrero et al 2001).

CD4+ T Cells:

*M. tuberculosis* resides primarily in a vacuole within the macrophage, and thus, MHC class II presentation of mycobacterial antigens to CD4+ T cells is an obvious outcome of infection. Murine studies have shown by antibody depletion of CD4+ T cells (Muller 1987), adoptive transfer (Orme and Collins 1983), or the use of gene-disrupted mice (Tascon et al 1998 and Caruso et al 1999) that the CD4+ T cell subset is required for control of infection (Fig VII). In humans, the tragedy of HIV has demonstrated that the loss of CD4+ T cells greatly increases susceptibility to both acute and reactivation tuberculosis. In a recent study the early influx of IFNγ producing CD4+ T cells at the site of infection has been suggested to be a very important factor in developing resistance against tuberculosis in mouse model (Chackerian et al 2001).

*M. tuberculosis* infected macrophages appear to be diminished in their ability to present antigens to CD4+ T cells, which would contribute to the inability of the host to eliminate a persistent infection. One mechanism by which *M. tuberculosis* infection might inhibit recognition of macrophages by CD4+ T cells is by downregulation of cell surface expression of MHC class II molecules (Hmama et al 1998). The effect was dependent on multiplicity of infection (MOI) and dead bacilli were much less effective than live bacilli. However, IFNγ mediated induction of MHC class II and class II transactivator (CIITA) gene expression were not inhibited by *M. tuberculosis* infection. Instead, post-Golgi transit of nascent MHC class II molecules into the endocytic pathway for antigen loading was impaired, resulting in intracellular sequestration and diminished cell surface expression.
In infected macrophages, Mycobacterium tuberculosis preferentially resides in the phagosome of macrophages, where mycobacterial peptides have ready access to the major histocompatibility complex class II (MHC II) molecules that are shuttled to the cell surface and stimulate CD4+ T cells. CD1 molecules also have access to mycobacterial glycolipids because they have contact with the phagosomal continuum at different stages of its maturation — glycolipids seem to separate from the mycobacteria and are incorporated into vesicles, which are shuttled throughout the cells and also seem to transfer antigen to bystander cells. CD1 molecules present mycobacterial glycolipids to various CD1-restricted T lymphocytes (CD4+, CD8+ or DN). Some mycobacterial proteins may enter the cytosol and be introduced into MHC-class-I processing pathways, including cytosolic proteasomes and TAP. Mycobacterial peptides and glycolipids may be transferred in vesicles along a novel pathway from infected macrophages to bystander dendritic cells. This could improve antigen presentation through MHC-class-I and CD1 pathways. Formation of these vesicles is probably stimulated during apoptosis of infected cells.
Cell surface expression of MHC II was found to decrease only slightly upon infection with H37Ra at a very high multiplicity of infection (MOI), but mRNA levels for I-A<sup>k</sup> gene were lowered by more than 75% (Noss et al 2000).

**CD8<sup>+</sup> T Cells:**

Although *M. tuberculosis* bacilli have been observed in the cytoplasm (McDonough et al 1993), most researchers believe that these organisms usually reside within a vacuole. Since MHC class I presentation is most efficient with cytoplasmic antigens, a possible role for CD8<sup>+</sup> T cells in the immune response to *M. tuberculosis* received little attention for many years. However, in recent years, CD8<sup>+</sup> T cells specific for mycobacterial antigens have been isolated from infected hosts or generated by immunization (Lalvani et al 1998, Stenger et al 1997, Zhu et al 1997 and Tan et al 1997). Recent studies in mice have demonstrated that CD8<sup>+</sup> T cells migrate to the lungs with kinetics similar to CD4<sup>+</sup> T cells following *M. tuberculosis* infection. These cells are capable of producing IFNγ and lysing infected macrophages (Serbina and Flynn 1999 and Feng et al 1999). Infiltration of CD8<sup>+</sup>T cells into infected lungs and IFNγ production by them in CD4<sup>+</sup> and wild-type mice was similar. In contrast, cytotoxic activity of CD8<sup>+</sup> T cells from lungs of *M. tuberculosis* infected mice was impaired in CD4<sup>+</sup> mice (Serbina et al 2002). CD8<sup>+</sup> T cells in mycobacterial infections are both classically and non-classically restricted.

**MHC Class I–Restricted CD8<sup>+</sup> T Cells:**

In general, access of antigen to the cytoplasm for processing and transport to the lumen of the endoplasmic reticulum by TAP molecules is necessary for loading and presentation of
epitopes by MHC class I molecules. Bacilli in macrophages have been found outside the phagosome four to five days after infection (McDonough et al 1993), but presentation of mycobacterial antigen by infected macrophages to CD8\(^+\) T cells can occur as early as 12 hours after infection, although recognition of the target improves over time (Serbina et al 2000). Two reports provide evidence for a Mycobacteria-induced pore or break in the vesicular membrane surrounding the bacilli that might allow mycobacterial antigen to enter the cytoplasm of the infected cell. *M. tuberculosis* infection of macrophages facilitated MHC class I presentation of soluble ovalbumin in a TAP-dependent manner (Fig VII), indicating that ovalbumin taken up into phagosomes along with *M. tuberculosis* gained access to the cytoplasm (Mazzaccaro et al 1996). Heat-killed or fixed *M. tuberculosis* did not facilitate presentation of ovalbumin, suggesting that *M. tuberculosis* actively generated the phagosome pore. In a model system using live cells and real time confocal microscopy, BCG infection of macrophages facilitated transport of molecules up to 70-kDa from the cytoplasm into the phagosomes containing the bacteria (Teitelbaum et al 1999). This supports the presence of a presumably bi-directional pore induced by mycobacterial infection. The bacterium may use this pore to obtain nutrients or introduce toxic molecules into the cytoplasm. A consequence may be the introduction of a wide variety of mycobacterial antigens into the cytoplasm for processing and presentation by MHC class I molecules.

**Nonclassically Restricted CD8\(^+\) T Cells:**

CD1 molecules are nonpolymorphic antigen presenting molecules; Group I CD1 molecules include CD1a, b, and c, while Group II includes CD1d molecules. In contrast to the peptide epitopes presented by MHC class Ia molecules, CD1 present lipids or
glycolipids to T cells CD1-restricted T cells are often CD4+ or CD8+, but a recent study indicated that CD1 could also present antigen to CD4+ T cells (Sieling et al, 2000). The first described non-protein antigen presented by CD1b was mycolic acid (Beckman et al, 1994), a mycobacterial cell wall component. Since then, additional mycobacterial lipid-containing antigens presented by CD1 have been reported, including LAM (Sieling et al, 1995). Group I CD1 molecules appear to sample different compartments of the cell for antigen presentation (Fig VII). Differences in CD1 localization may be important in determining which lipid antigens are presented by each molecule, and this, together with MHC class I and class II molecule presentation, allows a full survey of mycobacterial antigens by T cells. CD1 molecules are usually found on dendritic cells in vivo (Sieling et al, 1999).

**Effector Functions of CD8+ T Cells:**

There appear to be two primary effector functions for CD8+ T cells in tuberculosis: lysis of infected cells and production of cytokines, namely IFNγ. CD8+ T cells from the lungs of infected mice are primed to produce IFNγ, which secrete this cytokine upon TCR ligation or by interaction with *M. tuberculosis* infected dendritic cells (Serbina and Flynn, 1999). However, unlike CD4+ T cells, spontaneous ex vivo production of IFNγ by CD8+ T cells is very low, suggesting that production of this cytokine by CD8+ T cells in the lungs is limited. Lysis of target cells by CD8+ T cells can occur via perforin and granzymes or the Fas/FasL pathway. Perforin is required to form a pore in the host cell or organelle membrane, but the molecule responsible for killing of intracellular organisms was granulysin, another cytotoxic granule (Stenger et al, 1998). But Fas/FasL pathway is unable to kill the bacteria inside (Stenger et al, 1997).
Granuloma Formation- the *in vivo* situation:

Granulomas are cellular frameworks which provide a site for T cells (which produce cytokines) and macrophages (which limit the growth of bacteria) to be in close proximity for effective macrophage activation. In addition, granulomas restrict the toxic environment, which is required to control Mycobacteria, such that the delicate alveolar tissue is protected. A third and crucial function is to limit the dissemination of the infection from the lungs.

After being inhaled, the pathogen is engulfed by alveolar macrophages and dendritic cells. The dendritic cells transport the pathogen to the draining lymph nodes, where the priming of T cells occurs (Saunders and Cooper 2000). Infected macrophages produce chemokines that cause the extravasation of additional phagocytes (Oppenheim et al 1991). These activated macrophages also secrete TNFα, which initiates granuloma formation (Kindler et al 1989). Eventually, T cells activated in draining lymph nodes as well as NK cells are attracted to the site of inflammation (Boom 1996, Feng et al 1999 and 2000). Although NK cells and γδ T lymphocytes seem to precede αβ T cells, the former two are soon outnumbered by the last. A productive granuloma with a high cellular turnover develops: bacteria are confined in it, and their growth is restrained. Although these granulomas effectively inhibit bacterial multiplication, they are generally unable to eradicate the pathogens. Later, a fibrotic wall may encapsulate the productive granuloma, and the centre of the granuloma may undergo necrosis. Encapsulation further contributes to microbial containment and the low partial oxygen pressure in the necrotic centre provides unfavourable growth conditions for *M. tuberculosis*. 