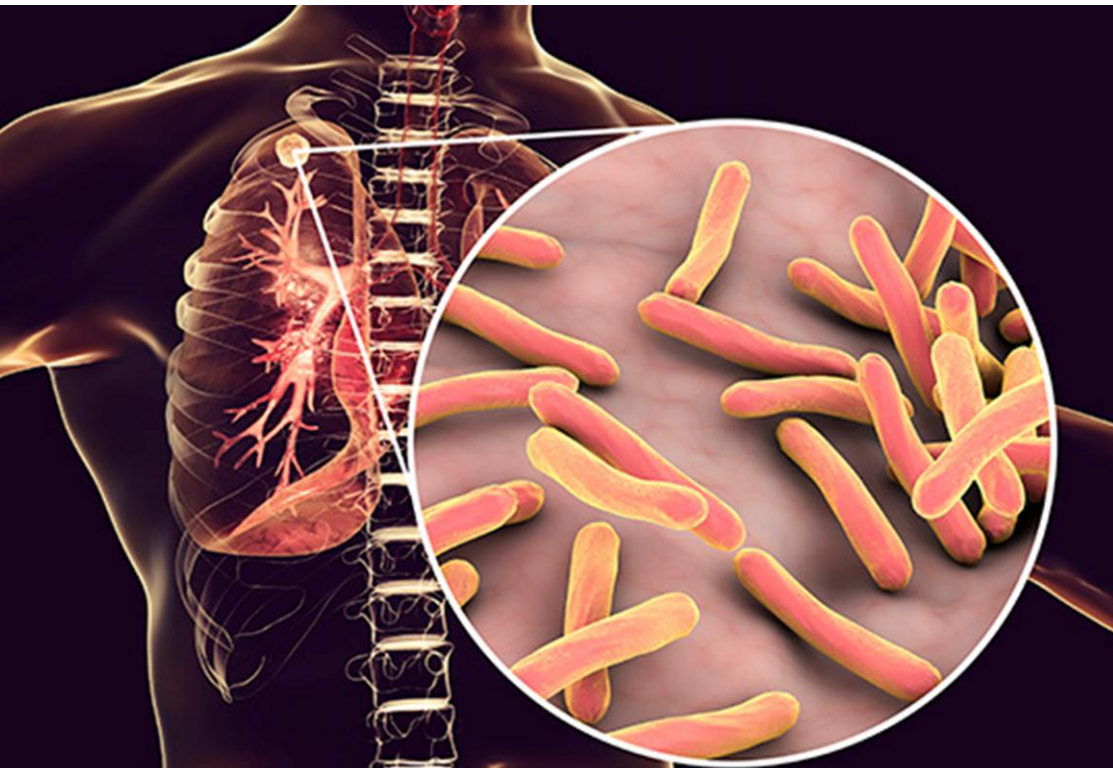


جامعة تكريت

كلية العلوم

قسم علوم الحياة

Immunology of Tuberculosis



الطالب

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INTRODUCTION Protective immunity and varied clinical manifestations of infection with *Mycobacterium tuberculosis* represent a delicate balance between the bacillus and the type as well as magnitude of the immune response elicited by the host. Host immune response is a broad term reflecting complex interactions among various arms of the immunity involving numerous cell types and molecules. This confers a homeostatic balance either in favour of the host, leading to containment of the infection, disease or in parasite's favour resulting in failure of containment of infection. Immunity against tuberculosis [TB] needs to be understood not only in terms of sterilising immunity that eliminates *Mycobacterium tuberculosis* infection at the initial exposure, but also with respect to immunity of granuloma formation that maintains the steady state control over the bacillary spread and prevents the occurrence of clinical disease. Antituberculosis immunity involves innate as well as adaptive immunity at various levels following *Mycobacterium tuberculosis* infection. Both of these will be discussed separately here: first the innate and then the adaptive one. It should be understood that separating these immune mechanisms is only for the sake of better understanding of the complex cross-talk among diverse cell subsets and bio-molecules and obtaining reductionist insights into the antituberculosis immunity in totality. However, *in vivo*, the innate and the various components of adaptive immunity are complementary and work synergistically in concert.

CHRONOLOGY OF IMMUNOPATHOGENESIS OF TUBERCULOSIS

Pulmonary TB can be marked with four distinct phases following *Mycobacterium tuberculosis* infection [Figure 1.1]. Each of these phases is determined by the homeostasis between the bacillary factors and host immune status including both innate and adaptive immunity [cellular as well as humoral]. First, following inhalation of *Mycobacterium tuberculosis*, depending on their intrinsic microbicidal capability alveolar macrophages ingest the pathogen and destroy them. However, bacilli often evade initial destruction by phagocytes and continue to multiply inside them ending in their disruption to cause fresh infection of the bystander macrophages. This heralds the second phase, characterized by recruitment of blood monocytes and other inflammatory cells to the primary disease site, the lungs in most instances. Monocytes ingest the bacilli and differentiate into macrophages, but fail to eliminate them completely. This stage is marked by logarithmic growth of the pathogens with little tissue destruction. Following this, antigen specific T-cells are recruited to the pathologic site[s] that activate the monocytoïd cells leading to their differentiation into either of these two types of giant cells, epithelioid and multi-nucleated Langhans' type giant cells. This is the third stage of granuloma formation, which aims at walling off the infection from the rest of the body and prevents dissemination of bacilli, thus contains the infection. This stage of latency, which disrupts under conditions of failing immune surveillance and gives rise to endogenous reactivation of dormant foci culminating in post-primary TB which is characterized by cessation necrosis [fourth phase]. In summary, after entry into the body, *Mycobacterium tuberculosis* encounters a series of host defense mechanisms with final outcome depending on the balance between bacillary growth and extent of host immunity. Essentially, all these phases of TB infection involve various arms of innate and acquired immunity sequentially in an orchestrated manner.

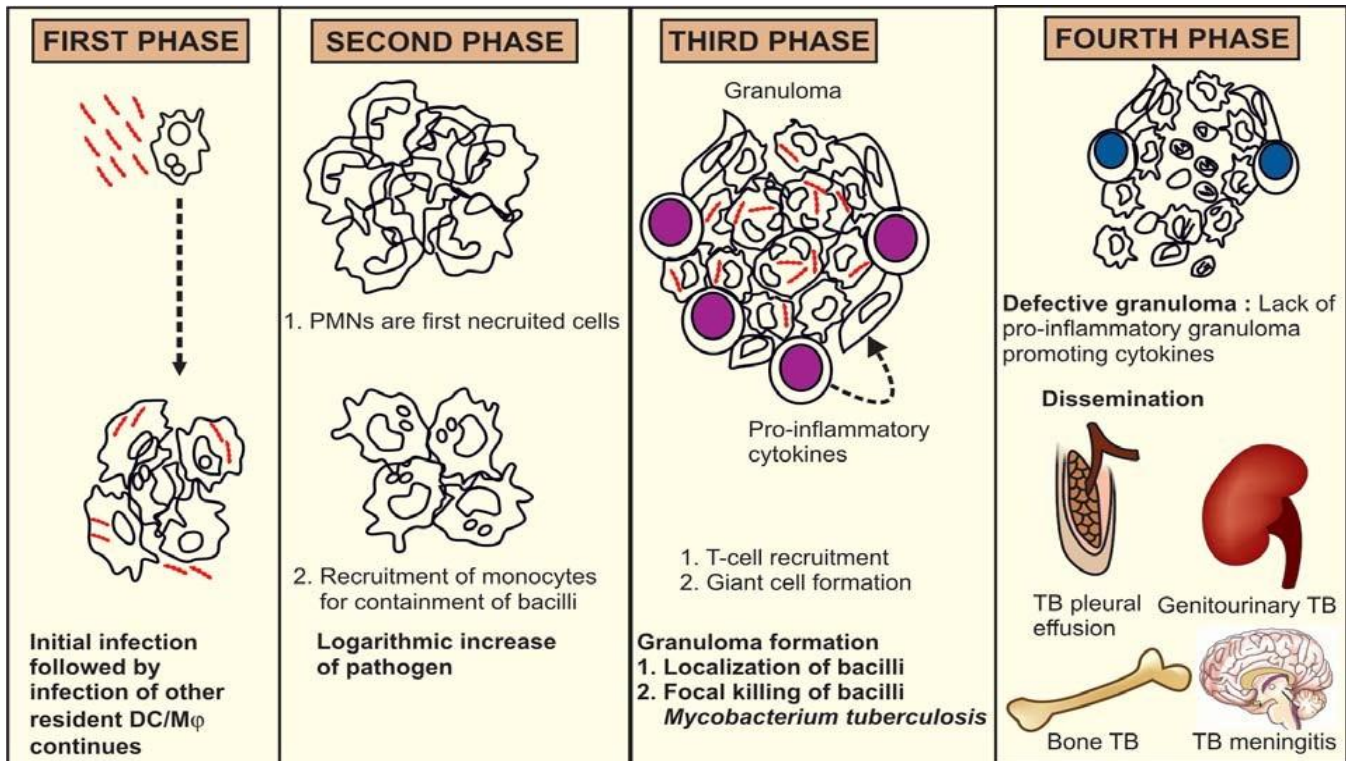


Figure 1.1: Events during TB infection. Broadly, TB infection is divided into four phases: First phase includes an initial establishment of *Mycobacterium tuberculosis* infection in the resident macrophages [alveolar]. This is followed by influx of PMNs, which prevents *Mycobacterium tuberculosis* to escape from the innate immune factors. Subsequently, monocytes are recruited to the site of infection/ pathological site [second phase]. Third phase includes granuloma formation. Core of granuloma is made up of multinucleated giant cells and elongated epithelioid cells. These are surrounded by T-cells. This is aimed at restricting the bacilli from spreading. The fourth and terminal phase includes dissemination of bacilli. Defective granuloma formation promotes release of bacilli from control of immune system. Organs/loci targeted by bacilli after dissemination are listed here
DC = dendritic cells; Mφ = macrophages; PMNs = polymorphonuclear leucocytes; TB = tuberculosis

INITIAL ENCOUNTER AND INNATE IMMUNITY

Mononuclear cells including alveolar macrophages and dendritic cells [DCs] play a crucial role during their initial encounter with *Mycobacterium tuberculosis* by their intrinsic or innate defense mechanism[s]. This has been demonstrated by Lurie in animal model where early infection and bacillary multiplication occur in susceptible rabbits (1). A probable role of DC specific intercellular adhesion molecule 3 [ICAM-3] grabbing nonintegrin [DC-SIGN], a recently discovered type II transmembrane protein has been implicated in DC mediated dissemination of *Mycobacterium tuberculosis*. Subsequently, DCs in the lymph nodes present some of the early secretory antigens such as early secreted antigenic target 6 [ESAT6] and antigen 85 with major histocompatibility complex

[MHC] class II. These antigens presumably serve as the dominant antigens for CD4+ cells, which start accumulating in large numbers in lesions during the early stages of *Mycobacterium tuberculosis* infection. Recent data suggest that probably T-cell dependent acquired immunity is also critical for protection against dissemination and disruption of latency of TB. However, they may not be so critical for eliminating the initial infection with *Mycobacterium tuberculosis* (2,3). This dynamics of effector immune mechanisms fits well with the basic fact that the acquired immunity requires time to develop and until then innate mechanisms attempt to either eliminate or control multiplication of the bacilli so that effective T-cell response eventually may contain the infection through stable granuloma formation (4). A plethora of clinical and experimental evidences suggests

an essential role of innate immune responses during an early phase of infection with *Mycobacterium tuberculosis*. Alveolar macrophages are the first line of cellular elements of initial uptake of aerosolized *Mycobacterium tuberculosis*, although subsequently DCs and monocytes are also involved in the process. Various receptors expressed on the phagocytes mediate endocytosis of the bacilli. Uptake of opsonized bacilli [coated with preformed humoral elements like antibodies or complement split products] is greatly facilitated by complement receptors expressed on macrophages such as complement receptors [CRs] CR1, CR3 and CR4. Bacillary uptake by human macrophages deficient in CRs is found to be reduced up to 80 per cent indicating their role in engulfing *Mycobacterium tuberculosis* (5). Non-opsonized bacilli are engulfed by macrophages through mannose receptors [MRs] that recognize the terminal mannose moieties of mycobacteria (6). Additionally, non-opsonized *Mycobacterium tuberculosis* can be taken up by the macrophages through binding to the scavenger receptor type A, as blocking CRs and MRs could not completely abrogate the bacillary uptake. Several other groups of molecules of the innate immune system may also facilitate binding and uptake of *Mycobacterium tuberculosis*. Collectins, a structurally related group of proteins are important in this regard. Surfactant protein A enhances the uptake while surfactant D blocks it. Another member of collectins, the plasma factor mannose binding lectins is also involved in macrophage uptake of the bacilli. Fibronectins also facilitate uptake of *Mycobacterium tuberculosis* by alveolar epithelial cells through binding to antigenic proteins (7). Thus, multiple mechanisms are operational in the uptake of *Mycobacterium tuberculosis* by mononuclear phagocytes giving them a chance to kill the bacilli. However, all these mechanisms only facilitate in their uptake but fail to elicit any immune recognition leading to macrophage activation. Up-regulation of a battery of surface expressed and soluble molecules determine the shape of the eventual acquired immunity on the surface of macrophages. Toll-like receptors [TLRs] are such family of molecules on the surface of macrophages that play a critical role in immune recognition of *Mycobacterium tuberculosis* and elicitation of an effective innate immune response. The TLRs are phylogenetically conserved molecules mediating the innate immunity and dictating the development of eventual T-cell responses. They are

transmembrane proteins with leucine rich repeat motifs in extracellular domain. Cytoplasmic domains of TLRs are homologous to the signalling domain of IL-1 receptor [IL-1R] and are linked to signalling molecule IL-1R associated protein kinase [IRAK-1], a serine kinase that activates transcription factors of several key immunoregulatory cytokines, such as nuclear factor- kappa beta [NF- κ B]. Of the several TLRs discovered till date TLR2, TLR4, TLR3 and TLR9 appear to elicit cellular response to mycobacterial antigens including the 19-kDa lipoprotein and lipoarabinomannan [LAM]. In context of CD14, TLR2 binds to LAM, a heterodimer of TLR2 and TLR6 binds to CD19 kDa lipoprotein. The TLR4 binds to yet undefined heat labile cell associated factor and TLR9 binds to mycobacterial DNA motifs. Engagement of TLRs by mycobacterial antigens leads to coupling of myeloid differentiation primary response gene [88] [MyD88] and IRAK signalling molecules resulting in multiple signalling events that ultimately translocate transcription factor NF- κ B from cytosol to nucleus and stimulate the production of various cytokine required for innate as well as adaptive immune events. Production of cytokines following TLRs induced activation of macrophages is important for immunity to *Mycobacterium tuberculosis*. Several cytokines are released, some of which take part in non-specific inflammation, and others regulate the functional bias of the relevant T-cells. A brief account of the important cytokines produced by *Mycobacterium tuberculosis* infected macrophages is provided in Figure 7.2. These cytokines eventually induce further activation of immune cells and lead to a complex process of immune regulation. Among the pro-inflammatory cytokines tumour necrosis factor- α [TNF- α], interleukin-1 β [IL-1 β], interleukin-6 [IL-6], interleukin-12 [IL-12], interleukin-15 [IL-15] and interleukin-18 [IL-18] are important. Each of them plays a distinct role in the immune response against TB.

Tumour Necrosis Factor- α

This prototype pro-inflammatory cytokine is produced by macrophages, DCs and Th1 like cells upon infection and stimulation with *Mycobacterium tuberculosis*. It plays key roles in macrophage activation, immune regulation and particularly granuloma formation by induction of appropriate chemokine receptors on the effector T-cells and thus recruiting them to the disease site (8).

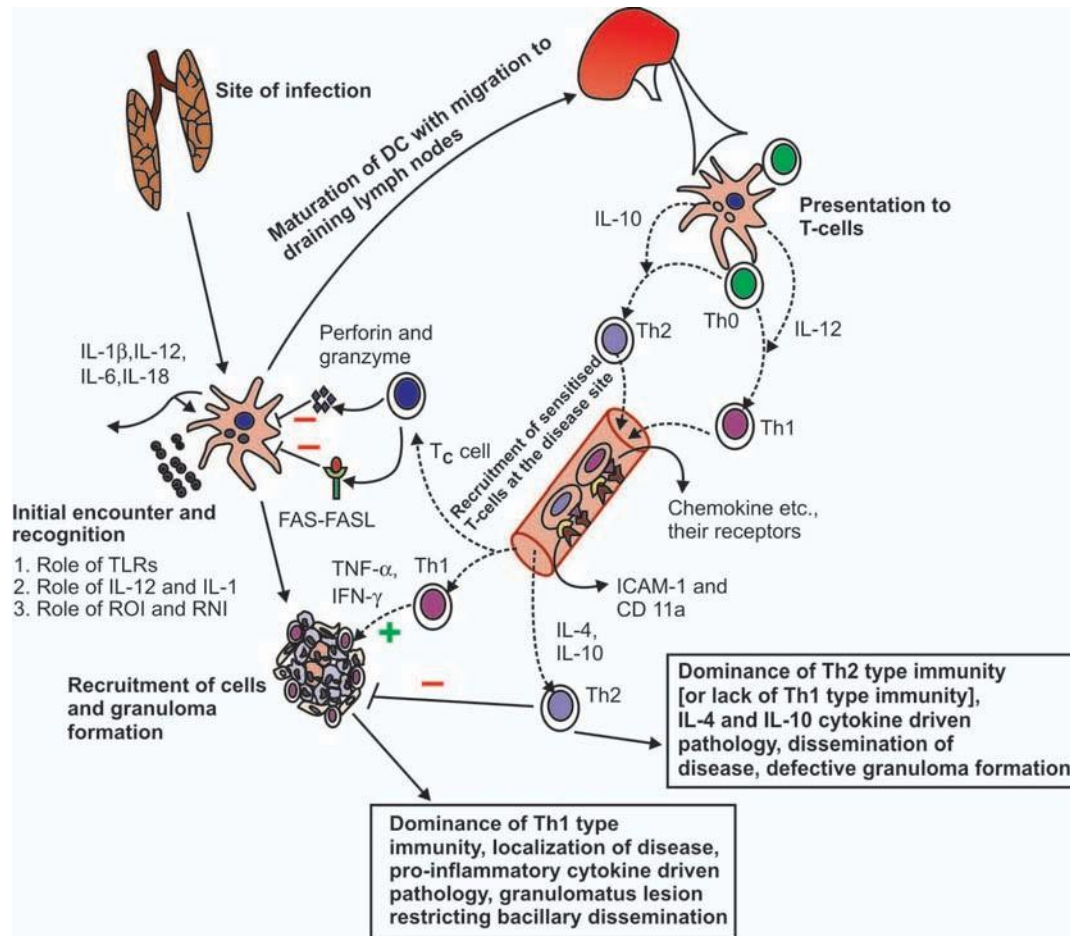


Figure 1.2: Summary of the immune response in tuberculosis pathogenesis. After infection, innate immune responses try to put check on increasing infection. Meanwhile, immature DCs after taking up the antigens, move towards the draining lymph nodes and antigen recognition and presentation to T-cells occur inside the regional/draining lymph nodes. Recruitment of antigen specific T-cells at the pathological sites and production of pro-inflammatory cytokines such as tumour necrosis factor- α [TNF- α], interferon- γ [IFN- γ] etc., lead to granuloma formation to localize the pathogen. Later on, depending on presence/absence of Th1/Th2 skewed response, dissemination or containment of bacilli occurs

DC = dendritic cell; TLRs = toll-like receptors; ROI = reactive oxygen intermediates; RNI = reactive nitrogen intermediates; IL = interleukins; Tc cell = T-cytotoxic cell; FAS-FASL = tumour necrosis factor receptor superfamily, member 6 - tumour necrosis factor receptor superfamily, member 6 ligand; ICAM-1 = intracellular adhesion molecule-1

of TB infection and has been found at the disease site[s]. Its role in humans is best evidenced by occurrence of high incidence of TB among rheumatoid arthritis patients when treated with anti-TNF- α antibodies. However, it is thought to be a “double edged sword” causing bystander damage of the host tissue and cavity formation, particularly when present in relative excess in the milieu.

Interleukin-1 β and Interleukin-6

Interleukin-1 β is another proinflammatory cytokine secreted by the *Mycobacterium tuberculosis* infected

macrophages and DCs and is found in excess at the pathologic site[s] of TB. An increased mycobacterial growth and a defective granuloma formation are observed in IL-1 β knock out mice (9). Interleukin-6 on the other hand, may serve as both pro- and anti-inflammatory cytokines and is found to be present in TB patients (10). Various reports suggest that it may antagonise either TNF- α or interferon- γ [IFN- γ], both of which are widely believed to be critical for protective immune response against TB.

Interleukin-12 and Interleukin-18

Interleukin-12 is the most potent Th1 driving regulatory cytokine produced by infected or stimulated macrophages and DCs and thus, plays a crucial role in the development of protective Th1 type immunity in TB. In TB, IL-12 has been detected in disease sites such as lung infiltrates, pleurisy and granulomas (9,11). Its receptor is also over-expressed in these sites. Children with deleterious mutation of genes encoding IL-12p40 subunit and IL-12 receptor are highly susceptible to recurrent nontuberculous mycobacterial infections (12).

Interleukin-18, another pro-inflammatory cytokine is important for IFN- γ axis of the T-cell response. High susceptibility of IL-18 knock out mice to *Mycobacterium tuberculosis*, bacille Calmette-Guérin [BCG] and *Mycobacterium leprae* strongly suggests a protective role of this cytokine in TB (13). A close parallelism has been noted between the concentration of IL-18 and IFN- γ among patients suffering from TB pleural effusion (14). Protective effect of IL-18 may be mediated by enhanced production of IFN- γ , another potent effector cytokine for macrophage activation leading to killing of the intra-cellular mycobacteria.

Interferon- γ

A protective role of IFN- γ in TB is beyond doubt. But it must be remembered that it is not the only terminal effector cytokine to confer protection in TB. Mycobacterial antigen specific *in vitro* production of IFN- γ by T-cells from patients represents a surrogate marker of immunity against TB. However, there exists a great deal of divergence of opinion in this respect among the researchers and clinicians. In TB, physiologically relevant sources of IFN- γ are natural killer cells [NK-cells], antigen specific T-cells [helper and cytotoxic], macrophages themselves, and other relatively rare fine T-cell subsets such as $\gamma\delta$ T-cells and CD1d restricted NKT-cells (15). The IFN- γ is a potent activator of infected macrophages resulting in potentiation of lytic mechanism[s] responsible for killing of intracellular *Mycobacterium tuberculosis* and enhancement of human leucocyte antigen [HLA] and co-stimulatory molecules which result in efficient presentation of macrophage processed mycobacterial antigens and elicitation of strong T-cells responses (16).

In addition to the production of the above pro-inflammatory cytokines, certain anti-inflammatory

cytokines are produced as well in TB. Some of these, such as interleukin-4 [IL-4], interleukin-10 [IL-10] and transforming growth factor- β [TGF- β], are Th2 like cytokines and their role in the immunopathogenesis of TB has provided the concept of Th1/Th2 paradigm in various forms of TB. These cytokines are believed to antagonise the protective and/or containing immunity, thus suppressing the required immunity. Therefore, they have been implicated in enhanced susceptibility as well as disease severity in terms of clinical manifestation and the extent of the disease.

Interleukin-10

Interleukin-10 is produced by the macrophages after phagocytosis of *Mycobacterium tuberculosis* and also by the Th2 cells following recognition of duly processed mycobacterial antigens. Mononuclear cells from TB patients, particularly those with disseminated disease, produce copious amounts of IL-10 *in vitro* in response to mycobacterial antigens (17). Interleukin-10 transgenic mice supports better bacillary growth. Also in humans, IL-10 production is significantly higher in patients with purified protein derivative [PPD] anergy and severe forms of TB. Interleukin-10 is well known for its ability to suppress IFN- γ , TNF- α and IL-12, all of which are critical for eliciting a desired Th1 type immune response in host's favour.

Interleukin-4

The deleterious influence of IL-4 in TB is well known and is attributed to its suppressive effect on IFN- γ production. *Mycobacterium tuberculosis* infected mice with progressive form of disease shows a significantly higher production of IL-4 (18). Disseminated form of TB, such as miliary TB, is associated with a very high production of IL-4 by T cells derived from peripheral blood and bronchoalveolar lavage [BAL] fluid following *in vitro* stimulation (19,20). However, several studies failed to reproduce similar observation, which may be due to variation in the study subjects and methods used for the detection of IL-4. It is widely believed that IL-4 production is responsible for suppression of Th1 type immune responses against TB. Thus, the host fails to contain the disease, leading to the development of severe and disseminated forms of TB.

In some recent elegant studies, a splice variant of IL-4 gene has been detected. This truncated splice variant called IL-4 δ gives rise to protein isoform, which inhibits

the immunosuppressive Th2 like function of native IL-4. Expression of IL-4 δ messenger ribonucleic acid [mRNA] was very minimal in the peripheral blood mononuclear cells [PBMCs] of the healthy subjects. On the other hand, it was found to be expressed in significantly higher amount in the thymocytes and BAL fluid cells from patients with TB. Tissue specific expression of this splice variant and tight correlation with the disease severity suggests a potential immunoregulatory role in pathogenesis of TB. Plausibly, IL-4 δ functionally inhibits Th2 skewing of the host immune response by antagonising the effect of native IL-4 and facilitates the desired Th1 response. Ratio of IL-4/IL-4 δ may be useful in monitoring the cytokine polarized immune response among TB patients.

Transforming Growth Factor- β

Transforming growth factor- β also appears to inhibit the protective immunity against *Mycobacterium tuberculosis* and aggravates the pathology. The TGF- β is also produced in abundance by TB patients and its expression is observed at the pathologic site[s] (21). The TGF- β is a well known inhibitor of T-cell proliferation, IFN- γ production, macrophage activation and antigen presentation. Moreover, it is also known for its potent host tissue damaging effect and fibrosis (22). The TGF- β along with IL-10 potently suppresses the Th1 function during TB infection and is thought to contribute to the pathogenesis of TB.

IMMUNE EFFECTOR MECHANISMS AGAINST MYCOBACTERIUM TUBERCULOSIS

Activated macrophages are the terminal effector cells responsible for killing of intracellular bacilli in TB. Multiple factors are responsible for this activation finally resulting in triggering the major lytic mechanisms. Among several factors, the most well documented and definitive mediators are IFN- γ and TNF- α . These cytokines are primarily derived from the Th1 cells and activate the macrophages to induce the intracellular messenger molecules, reactive oxygen intermediates [ROIs] and reactive nitrogen intermediates [RNIs] that are thought to be involved in killing of the intracellular mycobacteria. Also important for macrophage activation synergistically with IFN- γ and TNF- α is the active metabolite of vitamin D [1,25-dihydroxy vitamin D] (23).

Several studies have reported reduced levels of vitamin D among TB patients. Recent clinical trials suggest a distinct role for vitamin D in potentiating the immunity against TB. An interesting element involved in macrophage activation as well as killing of intracellular mycobacteria is the solute carrier family 11 [proton- coupled divalent metal ion transporters], member 1 [*Slc11a1*], and its human orthologue, SLC11A1, formerly known as natural resistance associated macrophage protein [*Nramp1*]. It is an integral membrane protein belonging to a family of metal ion transporter, particularly the iron [Fe²⁺]. Following phagocytosis *Slc11a1* becomes integrated to the phagosomes and activates macrophages with generation of toxic anti-microbial radicals, particularly ROIs (24). The *Slc11a1* mutant mice show reduced phagosomal maturation and acidification. In humans, polymorphism of this gene associated with reduced expression of SLC11A1 is associated with susceptibility to TB in West African population (25). Therefore, genetic variation of this gene may in part determine the outcome of *Mycobacterium tuberculosis* infection.

Generation of ROIs appears to be important for killing of intracellular bacilli. However, the conclusive proof is still awaited due to variation among experimental systems, particularly among mice and human studies. *In vitro* mycobacteria are resistant to killing by superoxides and hydrogen peroxides, this may be due to presence of LAM on *Mycobacterium tuberculosis* that can scavenge the ROIs. In mice lacking functional P47 unit of nicotinamide adenine dinucleotide phosphate [NADPH], which is required for the generation of superoxide ions, early and excessive growth of *Mycobacterium tuberculosis* has been demonstrated (26). This points towards an important role of ROIs in mycobacterial killing. On the contrary, patients suffering from chronic granulomatous disease [CGD] with a defect in production of superoxide radicals have not been demonstrated to be susceptible to TB (27)

patients with TB have shown an increased expression of *iNOS* gene (28). But the precise role of over-expression of *iNOS* in patients with TB remains uncertain, as post-translational modification of *iNOS* is required for functional activity.

ACQUIRED T-CELL MEDIATED IMMUNE MECHANISM

T-cell mediated immune response is at the hub of immunity against *Mycobacterium tuberculosis* as antibodies fail to contain the infection due to its intracellular habitat. Upon initial exposure and recognition of immuno-dominant epitopes, naive T-cells are primed and converted into effector and memory T-cells. Effector T-cells contain the initial infectious load. However, dormant foci of *Mycobacterium tuberculosis* within macrophages persist and reactivation of the bacillary foci occurs in the event[s] of perturbation of a delicate balance of T-cell immunity that contained the foci so far (29). Additionally, a fresh exogenous infection may also take place. Whatever is the case, on these subsequent exposures, the memory T-cells generated during the primary infection elicit a strong Th1 response and migrate to the site of the pathogen. These migrated T-cells are further activated by the processed antigens presented by the local infected macrophages and secrete key effector cytokines, such as IFN- γ and TNF- α , which help in activation as well as terminal differentiation of the macrophages into the giant cells. A well-defined architectural aggregation of T-cells and the giant cells [both multinucleated and epithelioid types] results in granuloma formation, the immunopathologic hallmark of TB. An evolution of granuloma occurs through a sequential influx of various types of cells of the immune system (30). Neutrophils migrate quite early in this process followed by the monocytes which differentiate into macrophages within two to three days. Chemokines induced in the granuloma begin to recruit T-cells which are activated to secrete cytokines. Among the T-lymphocyte layer surrounding the granuloma are predominantly of CD4⁺ type, although CD8⁺ T-cells are also present. The T-cell derived cytokines locally trigger the macrophages to terminally differentiate into highly active giant cells. Apoptosis of cells, prominently in epithelioid cells has been demonstrated by terminal deoxynucleotidyl transferase biotin-dUTP nick end labelling [TUNEL] immunostaining (31). Appropriate positioning of various cell types within the granuloma

is mediated by the expression of adhesion molecules and chemokines. Many of these cell homing molecules are known to be induced by *Mycobacterium tuberculosis*. The intracellular adhesion molecule-1 [ICAM-1], a major adhesion molecule involved in granuloma formation, is induced by TNF- α , IL-6 and IFN- γ . Chemokines, such as macrophage inflammatory protein [MIP1- α], regulated on activation, normal T-expressed and secreted [RANTES], monokine induced by interferon γ [Mig], and IFN- γ -inducible protein-10 [IP-10] etc., all associated with inflammation are also induced by the bacilli (32,33). The Th1 cells preferentially recruited by these homing molecules, through the release of cytokines promote terminal differentiation of macrophages into giant cells like multinucleated and epithelioid types. These activated cells of monocyte/macrophage lineage ultimately kill the intracellular *Mycobacterium tuberculosis* through ROIs and RNIs. Mycobacteria are also capable of inducing caseation necrosis in the centre of the granuloma. Conventionally, TNF- α has been thought to be the prime mediator of caseation necrosis. However, recent findings of caseation necrosis among mice lacking functional component of TNF receptor [55 kDa TNF-R] suggest the existence of other mechanism[s] for this phenomenon. Mycobacterial components such as, LAM have been demonstrated to activate interstitial collagenase gene and matrix metalloproteinase-9 [MMP-9]. These extracellular matrix enzymes probably play a major role in caseation necrosis. Interestingly, up-regulation of MMP-9 has been observed in BAL cells recovered from cavitary TB patients (34). Granuloma may be regarded as a localized immune reaction that attempts to wall off the pathogen and prevents its further spread. The dormant foci of infection may remain as such for years or even life-long. However, perturbation of a delicate balance of immunity with suppression of critical mechanism[s] may lead to activation of endogenous foci containing dormant mycobacteria. Alternatively, fresh exogenous infection can also occur. Both of these develop into a clinical disorder such as pulmonary TB. Immune response to *Mycobacterium tuberculosis* continues to play a critical role and dictates the clinical outcome of the disease as well. The extent and severity of the disease appear to be determined by the type and magnitude of the T-cell response elicited eventually.

Th1 and Th2 type of response play an important role to dictate whether the disease will be of limited extent with localized form such as pulmonary TB or will disseminate to give rise to severe forms of disease such as miliary TB, multidrug-resistant TB [MDR-TB] [Figure 1.2] (20,35).

CD4+ AND CD8+ T-CELLS IN TUBERCULOSIS

Importance of CD4+ helper T-cells is best demonstrated by significantly higher incidence and occurrence of severe and disseminated forms of TB among patients co-infected with human immunodeficiency virus [HIV] and *Mycobacterium tuberculosis* (36). It is also suggested by the predominant presence of CD4+ helper T-cells in the granuloma outnumbering the CD8+ T-cells. Functionally, mature or memory helper T-cells are of two distinct types, namely, Th1 and Th2 cells. The Th1 cells preferentially produce IL-2, IFN- γ and TNF- α to stimulate the cell mediated immunity which is crucial for containment of *Mycobacterium tuberculosis*. On the other hand, Th2 cells are biased to produce more of IL-4, interleukin-13 [IL-13], and IL-10, etc., and boost the antibody production, particularly of IgE isotype and suppress the Th1 like immunity. The importance of Th1/Th2 paradigm has been studied in various diseases; the most notable is that among polar leprosy patients. Tuberculoid leprosy, which is characterized by high degree of T-cell reactivity against *Mycobacterium leprae* is associated with Th1 like polarized cytokine response while lepromatous leprosy, hallmarked by T-cell anergy towards *Mycobacterium leprae* strongly correlates with dominant Th2 like cytokine profile (37). Similar association in TB has been proposed and subsequently demonstrated by several groups of investigators. However, conclusive picture is yet awaited. *In vitro* studies of T-cell proliferation and their cytokine production profile upon antigen stimulation of PBMCs obtained from TB patients and control subjects support the role of cytokine polarized immunity in TB. Cytokine profile skewed towards dominant production of the IL-4 along with lower IFN- γ level was found among TB patients compared to that of the controls and healthy contacts. At the same time several other groups failed to observe the same. The reasons for this apparent discrepancy may be the variations of the experimental systems, use of different antigens, different populations with diverse host genetic factors and importantly, a great deal of variation among the study subjects and the extent as

well as severity of their disease. Another major limitation of these studies is that all of them looked either at the soluble and accumulated cytokine level in the peripheral blood or long term *in vitro* stimulation which might have imposed functional bias on the responding T-cell in terms of cytokine production.

To address the issue of Th1/Th2 paradigm in TB, investigators focussed attention to the cells producing the cytokines particularly the T-cells derived from the disease site. Data emanating from these recent studies indicate a possible compartmentalization of T-cell mediated immune response among TB patients and a predominant role of IL-12. Cytokine profile of the pleural fluid revealed excess levels of IFN- γ and IL-12 relative to their levels in the peripheral blood compartment (37). Several investigators have demonstrated a Th1 biased response in the pleural compartment representing the site of a strong T-cell response of local TB pathology.

To understand the Th1/Th2 phenomenon in TB researchers have studied patients suffering from disseminated and severe forms of TB. The T-cells from TB patients in the setting of HIV infection showed predominant IL-4 production with excess of IL-10 that is known to drive the effector T-cell responses towards Th2 and antagonize the Th1 driving cytokine IL-12. Study of T-cells from patients with miliary TB provided strong indication that extent and dissemination of TB tightly correlate with a strong Th2 bias demonstrated by the T-cells derived from BAL fluid representing the pathologic site of disseminated TB (19). Interestingly, IFN- γ production by the T-cells from the BAL fluid could be restored by supplementation with IL-12. Using flow cytometry based assay of intracellular cytokines, Mitra *et al* (20) have demonstrated that the T-cells from TB pleural effusion predominantly produce IFN- γ , the Th1 designate cytokine,

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