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Immunology of tuberculosis

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Tuberculosis is a major health problem throughout the world causing large number of deaths, more than that from any other single infectious disease. The review attempts to summarize the information available on host immune response to *Mycobacterium tuberculosis*. Since the main route of entry of the causative agent is the respiratory route, alveolar macrophages are the important cell types, which combat the pathogen. Various aspects of macrophage-mycobacterium interactions and the role of macrophage in host response such as binding of *M. tuberculosis* to macrophages via surface receptors, phagosome-lysosome fusion, mycobacterial growth inhibition/killing through free radical based mechanisms such as reactive oxygen and nitrogen intermediates; cytokine-mediated mechanisms; recruitment of accessory immune cells for local inflammatory response and presentation of antigens to T cells for development of acquired immunity have been described. The role of macrophage apoptosis in containing the growth of the bacilli is also discussed. The role of other components of innate immune response such as natural resistance associated macrophage protein (Nramp), neutrophils, and natural killer cells has been discussed. The specific acquired immune response through CD4 T cells, mainly responsible for protective Th1 cytokines and through CD8 cells bringing about cytotoxicity, also has been described. The role of CD-1 restricted $CD8^+$ T cells and non-MHC restricted γ/δ T cells has been described although it is incompletely understood at the present time. Humoral immune response is seen though not implicated in protection. The value of cytokine therapy has also been reviewed. Influence of the host human leucocyte antigens (HLA) on the susceptibility to disease is discussed.

Mycobacteria are endowed with mechanisms through which they can evade the onslaught of host defense response. These mechanisms are discussed including diminishing the ability of antigen presenting cells to present antigens to $CD4^+$ T cells; production of suppressive cytokines; escape from fused phagosomes and inducing T cell apoptosis.

The review brings out the complexity of the host-pathogen interaction and underlines the importance of identifying the mechanisms involved in protection, in order to design vaccine strategies and find out surrogate markers to be measured as *in vitro* correlate of protective immunity.

Key words Immunology - *Mycobacterium tuberculosis* - tuberculosis

Tuberculosis (TB) remains the single largest infectious disease causing high mortality in humans, leading to 3 million deaths annually, about five deaths every minute. Approximately 8-10 million people are infected with this pathogen every year[1]. Out of the total number of cases, 40 per cent of cases are accommodated in South East Asia alone. In India, there are about 500,000 deaths occurring annually due to TB[2], with the incidence and prevalence being 1.5 and 3.5 millions per year.

This review summarizes the information available on host immune response to the causative bacteria, complexity of host-pathogen interaction and highlights the importance of identifying mechanisms involved in protection.

Pathogenesis of TB

Route and site of infection: *Mycobacterium tuberculosis* is an obligatory aerobic, intracellular pathogen, which has a predilection for the lung tissue rich in oxygen supply. The tubercle bacilli enter the body via the respiratory route. The bacilli spread from the site of initial infection in the lung through the lymphatics or blood to other parts of the body, the apex of the lung and the regional lymph node being favoured sites. Extrapulmonary TB of the pleura, lymphatics, bone, genito-urinary system, meninges, peritoneum, or skin occurs in about 15 per cent of TB patients.

Events following entry of bacilli: Phagocytosis of *M. tuberculosis* by alveolar macrophages is the first event in the host-pathogen relationship that decides outcome of infection. Within 2 to 6 wk of infection, cell-mediated immunity (CMI) develops, and there is an influx of lymphocytes and activated macrophages into the lesion resulting in granuloma formation. The exponential growth of the bacilli is checked and dead macrophages form a caseum. The bacilli are contained in the caseous centers of the granuloma. The bacilli may remain forever within the granuloma, get re-activated later or may get discharged into the airways after enormous increase in number, necrosis of bronchi and cavitation. Fibrosis represents the last-ditch defense mechanism of the host, where it occurs surrounding a central area of necrosis to wall off the infection when all other mechanisms failed. In our laboratory, in guineapigs infected with *M. tuberculosis*, collagen, elastin and hexosamines showed an initial decrease followed by an increase in level. Collagen stainable by Van Gieson's method was found to be increased in the lung from the 4th wk onwards[3].

Macrophage-*Mycobacterium* interactions and the role of macrophage in host response can be summarized under the following headings: surface binding of *M. tuberculosis* to macrophages; phagosome-lysosome fusion; mycobacterial growth inhibition/killing; recruitment of accessory immune cells for local inflammatory response and presentation of antigens to T cells for development of acquired immunity.

Binding of M. tuberculosis to monocytes / macrophages: Complement receptors (CR1, CR2, CR3 and CR4), mannose receptors (MR) and other cell surface receptor molecules play an important role in binding of the organisms to the phagocytes[4]. The interaction between MR on phagocytic cells and mycobacteria seems to be mediated through the mycobacterial surface glycoprotein lipoarabinomannan (LAM)[5]. Prostaglandin E2 (PGE2) and interleukin (IL)-4, a Th2-type cytokine, upregulate CR and MR receptor expression and function, and interferon-γ (IFN-γ) decreases the receptor expression, resulting in diminished ability of the mycobacteria to adhere to macrophages[6]. There is also a role for surfactant protein receptors, CD14 receptor[7] and the scavenger receptors in mediating bacterial binding[8].

Phagolysosome fusion: Phagocytosed microorganisms are subject to degradation by intralysosomal acidic hydrolases upon phagolysosome fusion[9]. This highly regulated event[10] constitutes a significant antimicrobial mechanism of phagocytes. Hart *et al*[11] hypothesized that prevention of phagolysosomal fusion is a mechanism by which *M. tuberculosis* survives inside macrophages[11]. It has been reported that mycobacterial sulphatides[12], derivatives of multiacylated trehalose 2-sulphate[13], have the ability to inhibit phagolysosomal fusion. *In vitro* studies demonstrated that *M. tuberculosis* generates copious amounts of ammonia in cultures, which is thought to be responsible for the inhibitory effect[14].

How do the macrophages handle the engulfed M. tuberculosis?: Many antimycobacterial effector functions of macrophages such as generation of reactive oxygen intermediates (ROI), reactive nitrogen intermediates (RNI), mechanisms mediated by cytokines, have been described.

Reactive oxygen intermediates (ROI): Hydrogen peroxide (H_2O_2), one of the ROI generated by macrophages via the oxidative burst, was the first identified effector molecule that mediated mycobactericidal effects of mononuclear phagocytes[15]. However, the ability of ROI to kill *M.*

tuberculosis has been demonstrated only in mice[16] and remains to be confirmed in humans. Studies carried out in our laboratory have shown that *M. tuberculosis* infection induces the accumulation of macrophages in the lung and also H_2O_2 production[17]. Similar local immune response in tuberculous ascitic fluid has also been demonstrated[18]. However, the increased production of hydrogen peroxide by alveolar macrophages is not specific for TB[19]. Moreover, the alveolar macrophages produced less H_2O_2 than the corresponding blood monocytes.

Reactive nitrogen intermediates (RNI): Phagocytes, upon activation by IFN-γ and tumor necrosis factor-α (TNF-α), generate nitric oxide (NO) and related RNI via inducible nitric oxide synthase (iNOS2) using L-arginine as the substrate. The significance of these toxic nitrogen oxides in host defense against *M. tuberculosis* has been well documented, both *in vitro* and *in vivo*, particularly in the murine system[20]. In genetically altered iNOS gene knock-out (GKO) mice *M. tuberculosis* replicates much faster than in wild type animals, implying a significant role for NO in mycobacterial host defense[21].

In our study, rat peritoneal macrophages were infected *in vitro* with *M. tuberculosis* and their fate inside macrophages was monitored. Alteration in the levels of NO, H_2O_2 and lysosomal enzymes such as acid phosphatase, cathepsin-D and β-glucuronidase was also studied. Elevation in the levels of nitrite was observed along with the increase in the level of acid phosphatase and β-glucuronidase. However, these microbicidal agents did not alter the intracellular viability of *M. tuberculosis*[22].

The role of RNI in human infection is controversial and differs from that of mice. 1, 25 dihydroxy vitamin D3 [1, 25-$(OH)_2D_3$] was reported to induce the expression of the NOS2 and *M. tuberculosis* inhibitory activity in the human HL-60 macrophage-like cell line[23]. This observation thus identifies NO and related RNI as the putative antimycobacterial effectors produced by human macrophages. This notion is further supported by another study in which IFN-γ stimulated human macrophages co-cultured with lymphocytes (*M. tuberculosis* lysate/IFN-γ primed) exhibited mycobactericidal activity concomitant with the expression of NOS2[24]. High level expression of NOS2 has been detected immunohistochemically in macrophages obtained by broncho alveolar lavage (BAL) from individuals with active pulmonary TB[25].

Other mechanisms of growth inhibition/killing: IFN-γ and TNF-α mediated antimycobacterial effects have been reported. In our laboratory studies, we were unable to demonstrate mycobacterial killing in presence of IFN-γ, TNF-α and a cocktail of other stimulants[26].There is lack of an experimental system in which the killing of *M. tuberculosis* by macrophages can be reproducibly demonstrated *in vitro*. The reports of the effect of IFN-γ treated human macrophages on the replication of *M. tuberculosis* range from its being inhibitory[27] to enhancing[28]. Later it was demonstrated that 1,25-$(OH)_2D_3$, alone or in combination with IFN-γ and TNF-α, was able to activate macrophages to inhibit and/or kill *M. tuberculosis* in the human system[29]. In our comparative study of immune response after vaccination with BCG, in subjects from Chengalput, India and London, *M. bovis* BCG vaccination did not enhance bacteriostasis with the Indians, but did so with the subjects from London.

Macrophage apoptosis

Another potential mechanism involved in macrophage defense against *M. tuberculosis* is apoptosis or programmed cell death. Placido *et al*[30] found that using the virulent strain H37Rv, apoptosis was induced in a dose-dependent fashion in BAL cells recovered from patients with TB, particularly in macrophages from HIV-infected patients. Klingler *et al*[31] have demonstrated that apoptosis associated with TB is mediated through a downregulation of bcl-2, an inhibitor of apoptosis. Within the granuloma, apoptosis is prominent in the epithelioid cells as demonstrated by condensed chromatin viewed by light microscopy or with the *in situ* terminal transferase mediated nick end labeling (TUNEL) technique[32].

Molloy *et al*[33] have shown that macrophage apoptosis results in reduced viability of mycobacteria. The effects of Fas L- mediated or

TNF-α-induced apoptosis on *M. tuberculosis* viability in human and mouse macrophages is controversial; some studies report reduced bacterial numbers within macrophages after apoptosis[34] and others indicate this mechanism has little antimycobacterial effect[35].

Evasion of host immune response by *M. tuberculosis*

M. tuberculosis is equipped with numerous immune evasion strategies, including modulation of antigen presentation to avoid elimination by T cells. Protein secreted by *M. tuberculosis* such as superoxide dismutase and catalase are antagonistic to ROI[36]. Mycobacterial components such as sulphatides, LAM and phenolic- glycolipid I (PGL-I) are potent oxygen radical scavengers[37,38]. *M. tuberculosis*-infected macrophages appear to be diminished in their ability to present antigens to $CD4^+$ T cells, which leads to persistent infection[39]. Another mechanism by which antigen presenting cells (APCs) contribute to defective T cell proliferation and function is by the production of cytokines, including TGF-β, IL-10[40] or IL-6[41]. In addition, it has also been reported that virulent mycobacteria were able to escape from fused phagosomes and multiply[42].

Host immune mechanisms in TB

Innate immune response: The phagocytosis and the subsequent secretion of IL-12 are processes initiated in the absence of prior exposure to the antigen and hence form a component of innate immunity. The other components of innate immunity are natural resistance associated macrophage protein (Nramp), neutrophils, natural killer cells (NK) *etc*. Our previous work showed that plasma lysozyme and other enzymes may play an important role in the first line defense, of innate immunity to *M. tuberculosis*[43]. The role of CD-1 restricted $CD8^+$ T cells and non-MHC restricted T cells have been implicated but incompletely understood.

Nramp: Nramp is crucial in transporting nitrite from intracellular compartments such as the cytosol to more acidic environments like phagolysosome, where it can be converted to NO. Defects in Nramp production increase susceptibility to mycobacteria. Newport *et al*[44] studied a group of children with susceptibility to mycobacterial infection and found Nramp1 mutations as the cause for it. Our laboratory study on pulmonary and spinal TB patients and control subjects suggested that NRAMP1 gene might not be associated with the susceptibility to pulmonary and spinal TB in the Indian population[45].

Neutrophils: Increased accumulation of neutrophil in the granuloma and increased chemotaxis has suggested a role for neutrophils[46]. At the site of multiplication of bacilli, neutrophils are the first cells to arrive followed by NK cells, γ/δ cells and α/β cells. There is evidence to show that granulocyte-macrophage-colony stimulating factor (GM-CSF) enhances phagocytosis of bacteria by neutrophils[47]. Human studies have demonstrated that neutrophils provide agents such as defensins, which is lacking for macrophage-mediated killing[48]. Majeed *et al*[49] have shown that neutrophils can bring about killing of *M. tuberculosis* in the presence of calcium under *in vivo* conditions.

Natural killer (NK) cells: NK cells are also the effector cells of innate immunity. These cells may directly lyse the pathogens or can lyse infected monocytes. *In vitro* culture with live *M. tuberculosis* brought about the expansion of NK cells implicating that they may be important responders to *M. tuberculosis* infection *in vivo*[50]. During early infection, NK cells are capable of activating phagocytic cells at the site of infection. A significant reduction in NK activity is associated with multidrug-resistant TB (MDR-TB). NK activity in BAL has revealed that different types of pulmonary TB are accompanied by varying degrees of depression[51]. IL-2 activated NK cells can bring about mycobactericidal activity in macrophages infected with *M. avium* complex (MAC) as a non specific response[52]. Apoptosis is a likely mechanism of NK cytotoxicity. NK cells produce IFN-γ and can lyse mycobacterium pulsed target cells[53]. Our studies[54] demonstrate that lowered NK activity during TB infection is probably the 'effect' and not the 'cause' for the disease as demonstrated by the follow up study. Augmentation of NK activity with cytokines implicates them as potential adjuncts to TB chemotherapy[54].

The Toll-like receptors (TLR): The recent discovery of the importance of the TLR protein family in immune responses in insects, plants and vertebrates has provided new insight into the link between innate and adaptive immunity. Medzhitov *et al*[55] showed that a human homologue of the Drosophila Toll protein signals activation of adaptive immunity. The interactions between *M. tuberculosis* and TLRs are complex and it appears that distinct mycobacterial components may interact with different members of the TLR family. *M. tuberculosis* can immunologically activate cells via either TLR2 or TLR4 in a CD 14-independent, ligand-specific manner[56].

Acquired immune response

Humoral immune response: Since *M. tuberculosis* is an intracellular pathogen, the serum components may not get access and may not play any protective role. Although many researchers have dismissed a role for B cells or antibody in protection against TB[57], recent studies suggest that these may contribute to the response to TB[58].

Mycobacterial antigens inducing humoral response in humans have been studied, mainly with a view to identify diagnostically relevant antigens. Several protein antigens of *M. tuberculosis* have been identified using murine monoclonal antibodies[59]. The immunodominant antigens for mice include 71, 65, 38, 23, 19, 14 and 12 kDa proteins. The major protein antigens of *M. leprae* and *M. tuberculosis* have been cloned in vectors such as *Escherichia coli*. Not all the antigens identified based on mouse immune response were useful to study human immune response.

In our laboratory a number of *M. tuberculosis* antigens have been purified and used for diagnosis of adult and childhood TB[60-66]. Combination of antigens were also found to be useful in the diagnosis of HIV-TB[67,68]. Detection of circulating immune complex bound antibody was found to be more sensitive as compared to serum antibodies. The purified antigens were evaluated for their utility in diagnosing infection[69,70].

Cellular immune response

T cells: *M. tuberculosis* is a classic example of a pathogen for which the protective response relies on CMI. In the mouse model, within 1 wk of infection with virulent *M. tuberculosis*, the number of activated $CD4^+$ and $CD8^+$ T cells in the lung draining lymph nodes increases[71]. Between 2 and 4 wk post-infection, both $CD4^+$ and $CD8^+$ T cells migrate to the lungs and demonstrate an effector/memory phenotype ($CD44^{hi}CD45^{lo}CD62L^-$); approximately 50 per cent of these cells are $CD69^+$. This indicates that activated T cells migrate to the site of infection and are interacting with APCs. The tuberculous granulomas contain both $CD4^+$ and $CD8^+$ T cells[72] that contains the infection within the granuloma and prevent reactivation.

CD4 T cells: *M. tuberculosis* resides primarily in a vacuole within the macrophage, and thus, major histocompatibility complex (MHC) class II presentation of mycobacterial antigens to $CD4^+$ T cells is an obvious outcome of infection. These cells are most important in the protective response against *M. tuberculosis*. Murine studies with antibody depletion of $CD4^+$T cells[73], adoptive transfer[74], or the use of gene-disrupted mice[75] have shown that the $CD4^+$ T cell subset is required for control of infection. In humans, the pathogenesis of HIV infection has demonstrated that the loss of $CD4^+$ T cells greatly increases susceptibility to both acute and re-activation TB[76]. The primary effector function of $CD4^+$ T cells is the production of IFN-γ and possibly other cytokines, sufficient to activate macrophages. In MHC class II-/- or CD4-/- mice, levels of IFN-γ were severely diminished very early in infection[75]. NOS2 expression by macrophages was also delayed in the $CD4^+$ T cell deficient mice, but returned to wild type levels in conjunction with IFNγ expression[75].

In a murine model of chronic persistent *M. tuberculosis* infection[77], CD4 T cell depletion caused rapid re-activation of the infection. IFN-γ levels overall were similar in the lungs of $CD4^+$ T cell-depleted and control mice, due to IFNγ production by CD8+ T cells. Moreover, there was no apparent change in macrophage NOS2 production or activity in the $CD4^+$ T cell-depleted mice. This indicated that there are IFN-γ and NOS2-independent, $CD4^+$ T cell-dependent mechanisms for control of TB. Apoptosis

or lysis of infected cells by $CD4^+$ T cells may also play a role in controlling infection[32]. Therefore, other functions of $CD4^+$ T cells are likely to be important in the protective response and must be understood as correlates of immunity and as targets for vaccine design.

CD8 T cells: $CD8^+$ cells are also capable of secreting cytokines such as IFN-γ and IL-4 and thus may play a role in regulating the balance of Th1 and Th2 cells in the lungs of patients with pulmonary TB. The mechanism by which mycobacterial proteins gain access to the MHC class I molecules is not fully understood. Bacilli in macrophages have been found outside the phagosome 4-5 days after infection[78], but presentation of mycobacterial antigen by infected macrophages to CD8 T cells can occur as early as 12 h after infection. 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[79].

Yu *et al*[80] analyzed CD4 and CD8 populations from patients with rapid, slow, or intermediate regression of disease while receiving therapy and found that slow regression was associated with an increase in $CD8^+$ cells in the BAL. Taha *et al*[81] found increased $CD8^+$ T cells in the BAL of patients with active TB, along with striking increases in the number of BAL cells expressing IFNγ and IL-12 mRNA. These studies point to a potential role for $CD8^+$ T cells in the immune response to TB. Lysis of infected human dendritic cells and macrophages by CD1- and MHC class I-restricted $CD8^+$ T cells specific for *M. tuberculosis* antigens reduced intracellular bacterial numbers[82]. The killing of intracellular bacteria was dependent on perforin /granulysin[83]. Lysis through the Fas/Fas L pathway did not reproduce this effect[82]. At high effector-to-target ratio (50:1), this lysis reduced bacterial numbers[84]. It is shown that IFN-γ production in the lungs by the CD8 T cell subset was increased at least four-fold in the perforin deficient (P-/-) mice, suggesting that a compensatory effect protects P-/- mice from acute infection[85].

Studies defining antigens recognized by $CD8^+$ T cells from infected hosts without active TB provide attractive vaccine candidates and support the notion that $CD8^+$ T cell responses, as well as $CD4^+$ T cell responses must be stimulated to provide protective immunity.

T cell apoptosis: A wide variety of pathogens can attenuate CMI by inducing T cell apoptosis. Emerging evidence indicates that apoptosis of T cells does occur in murine[86] and human TB[87]. In *in vitro* studies using peripheral blood mononuclear cells (PBMC) from tuberculous patients[88], the phenomenon of T cell hypo-responsiveness has been linked to spontaneous or *M. tuberculosis*-induced apoptosis of T cells. The observed apoptosis is associated with diminished *M. tuberculosis*-stimulated IFN-γ and IL-2 production. In tuberculous infection, CD95-mediated Th1 depletion occurs, resulting in attenuation of protective immunity against *M. tuberculosis*, thereby enhancing disease susceptibility[89]. Detailed analysis of para formaldehyde-fixed human tuberculous tissues revealed that apoptotic $CD3^+$, $CD45RO^+$ cells are present in productive tuberculous granulomas, particularly those harbouring a necrotic centre[90]. Studies carried out in our laboratory have demonstrated the ability of mycobacterial antigens to bring about apoptosis in animal models[91]. In addition, increased spontaneous apoptosis, which is further enhanced by mycobacterial antigens, has also been shown to occur in pleural fluid cells[92].

Nonclassically restricted CD8 T cells: CD1 molecules are nonpolymorphic antigen presenting molecules that present lipids or glycolipids to T cells. There is evidence of a recall T cell response to a CD1-restricted antigen in *M. tuberculosis*-exposed purified protein derivative (PPD) positive subjects[93]. CD1 molecules are usually found on dendritic cells *in vivo*[94], and dendritic cells present in the lungs may be stimulating CD1-restricted cells in the granuloma that can then have a bystander effect on infected macrophages. Further investigation of the processing and presentation of mycobacterial antigens to CD1-restricted CD8 T cells is necessary to understand the potential contribution of this subset to protection.

γ/δ *T-cells in TB*: The role of γ/δ T cells in the host response in TB has been incompletely worked

out. These cells are large granular lymphocytes that can develop a dendritic morphology in lymphoid tissues; some γ/δ T cells may be CD8+. In general, γ/δ T cells are felt to be non-MHC restricted and they function largely as cytotoxic T cells.

Animal data suggest that γ/δ cells play a significant role in the host response to TB in mice and in other species[95], including humans. *M. tuberculosis* reactive γ/δ T cells can be found in the peripheral blood of tuberculin positive healthy subjects and these cells are cytotoxic for monocytes pulsed with mycobacterial antigens and secrete cytokines that may be involved in granuloma formation[96]. Studies[97,98] demonstrated that γ/δ cells were relatively more common (25 to 30% of the total) in patients with protective immunity as compared to patients with ineffective immunity. Our study in childhood TB patients showed that the proportion of T cells expressing the γ/δ T cell receptor was similar in TB patients and controls[99]. Thus γ/δ cells may indeed play a role in early immune response against TB and is an important part of the protective immunity in patients with latent infection[100].

Th1 and Th2 dichotomy in TB: Two broad (possibly overlapping) categories of T cells have been described: Th1 type and Th2 type, based on the pattern of cytokines they secrete, upon antigen stimulation. Th1 cells secrete IL-2, IFN-γ and play a protective role in intracellular infections. Th2 type cells secrete IL-4, IL-5 and IL-10 and are either irrelevant or exert a negative influence on the immune response. The balance between the two types of response is reflected in the resultant host resistance against infection. The type of Th0 cells shows an intermediate cytokine secretion pattern. The differentiation of Th1 and Th2 from these precursor cells may be under the control of cytokines such as IL-12.

In mice infected with virulent strain of *M. tuberculosis*, initially Th1 like and later Th2 like response has been demonstrated[101]. There are inconsistent reports in literature on preponderance of Th1 type of cytokines, of Th2 type, increase of both, decrease of Th1, but not increase of Th2 *etc*. Moreover, the response seems to vary between peripheral blood and site of lesion; among the different stages of the disease depending on the severity.

It has been reported that PBMC from TB patients, when stimulated *in vitro* with PPD, release lower levels of IFN-γ and IL-2, as compared to tuberculin positive healthy subjects[102]. Other studies have also reported reduced IFN-γ[103] increased IL-4 secretion[104] or increased number of IL-4 secreting cells[105]. These studies concluded that patients with TB had a Th2-type response in their peripheral blood, whereas tuberculin positive patients had a Th1-type response.

More recently, cellular response at the actual sites of disease has been examined. Zhang *et al*[106] studied cytokine production in pleural fluid and found high levels of IL-12 after stimulation of pleural fluid cells with *M. tuberculosis*. IL-12 is known to induce a Th1-type response in undifferentiated CD4+ cells and hence there is a Th1 response at the actual site of disease. The same group[107] observed that TB patients showed evidence of high IFNγ production and no IL-4 secretion by the lymphocytes in the lymph nodes. There was no enhancement of Th2 responses at the site of disease in human TB. Robinson *et al*[108] found increased levels of IFN-γ mRNA *in situ* in BAL cells from patients with active pulmonary TB.

In addition, reports suggest that in humans with TB, the strength of the Th1-type immune response relate directly to the clinical manifestations of the disease. Sodhi *et al*[109] have demonstrated that low levels of circulating IFN-γ in peripheral blood were associated with severe clinical TB. Patients with limited TB have an alveolar lymphocytosis in infected regions of the lung and these lymphocytes produce high levels of IFN-γ[34]. In patients with far advanced or cavitary disease, no Th1-type lymphocytosis is present.

Cytokines

Interleukin-12: IL-12 is induced following phagocytosis of *M. tuberculosis* bacilli by macrophages and dendritic cells[110], which leads to development of a Th1 response with production of IFN-γ. *IL-12p40*-gene deficient mice were susceptible to infection and had increased bacterial burden, and

decreased survival time, probably due to reduced IFN-γ production[111]. Humans with mutations in *IL-12p40* or the *IL-12R* genes present with reduced IFN-γ production from T cells and are more susceptible to disseminated BCG and *M. avium* infections[112]. An intriguing study indicated that administration of IL-12 DNA could substantially reduce bacterial numbers in mice with a chronic *M. tuberculosis* infection[113], suggesting that induction of this cytokine is an important factor in the design of a TB vaccine.

McDyer *et al*[114] found that stimulated PBMC from MDR-TB patients had less secretion of IL-2 and IFN-γ than did cells from healthy control subjects. IFN-γ production could be restored if PBMC were supplemented with IL-12 prior to stimulation and antibodies to IL-12 caused a further decrease in IFN-γ upon stimulation. Taha *et al*[81] demonstrated that in patients with drug susceptible active TB both IFN-γ and IL-12 producing BAL cells were abundant as compared with BAL cells from patients with inactive TB.

Interferon-γ: IFN-γ, a key cytokine in control of *M. tuberculosis* infection is produced by both $CD4^+$ and $CD8^+$ T cells, as well as by NK cells. IFN-γ might augment antigen presentation, leading to recruitment of $CD4^+$ T-lymphocytes and/or cytotoxic T-lymphocytes, which might participate in mycobacterial killing. Although IFN-γ production alone is insufficient to control *M. tuberculosis* infection, it is required for the protective response to this pathogen. IFN-γ is the major activator of macrophages and it causes mouse but not human macrophages to inhibit the growth of *M. tuberculosis in vitro*[16]. IL-4, IL-6 and GM-CSF could bring about *in vitro* killing of mycobacteria by macrophages either alone or in synergy with IFN-γ in the murine system[115]. IFN-γ GKO mice are most susceptible to virulent *M. tuberculosis*[116].

Humans defective in genes for IFN-γ or the IFN-γ receptor are prone to serious mycobacterial infections, including *M. tuberculosis*[117]. Although IFN-γ production may vary among subjects, some studies suggest that IFN-γ levels are depressed in patients with active TB[107,118]. Another study demonstrated that *M. tuberculosis* could prevent macrophages from responding adequately to IFN-γ[119]. This suggests that the amount of IFN-γ produced by T cells may be less predictive of outcome than the ability of the cells to respond to this cytokine.

Our study comparing the immune response to pre- and post- BCG vaccination, has shown that BCG had little effect in driving the immune response towards IFN-γ and a protective Th1 response[120]. In another study on tuberculous pleuritis, a condition which may resolve without therapy, a protective Th1 type of response with increased IFN-γ is seen at the site of lesion (pleural fluid), while a Th0 type of response with both IFN-γ and IL-4 is seen under *in vitro* conditions[121].

To determine if the manifestations of initial infection with *M. tuberculosis* reflect changes in the balance of T cell cytokines, we evaluated *in vitro* cytokine production of children with TB and healthy tuberculin reactors[122]. IFN-γ production was most severely depressed in patients with moderately advanced and far advanced pulmonary disease and in malnourished patients. Production of IL-12, IL-4 and IL-10 was similar in TB patients and healthy tuberculin reactors. These results indicate that the initial immune response to *M. tuberculosis* is associated with diminished IFN-γ production, which is not due to reduced production of IL-12 or enhanced production of IL-4 or IL-10.

Tumor necrosis factor (TNF-α): TNF-α is believed to play multiple roles in immune and pathologic responses in TB. *M. tuberculosis* induces TNF-α secretion by macrophages, dendritic cells and T cells. In mice deficient in TNF-α or the TNF receptor, *M. tuberculosis* infection resulted in rapid death of the mice, with substantially higher bacterial burdens compared to control mice[123]. TNF-α in synergy with IFN-γ induces NOS2 expression[124].

TNF-α is important for walling off infection and preventing dissemination. Convincing data on the importance of this cytokine in granuloma formation in TB and other mycobacterial diseases has been reported[123,125]. TNF-α affects cell migration and localization within tissues in *M. tuberculosis* infection. TNF-α influence expression of adhesion molecules as well as chemokines and chemokine

receptors, and this is certain to affect the formation of functional granuloma in infected tissues.

TNF-α has also been implicated in immunopathologic response and is often a major factor in host-mediated destruction of lung tissue[126]. In our studies, increased level of TNF-α was found at the site of lesion (pleural fluid), as compared to systemic response (blood) showing that the compartmentalized immune response must be containing the infection[127].

Interleukin-1: IL-1, along with TNF-α, plays an important role in the acute phase response such as fever and cachexia, prominent in TB. In addition, IL-1 facilitates T lymphocyte expression of IL-2 receptors and IL-2 release. The major antigens of mycobacteria triggering IL-1 release and TNF-α have been identified[128]. IL-1 has been implicated in immunosuppressive mechanisms which is an important feature in tuberculoimmunity[129].

Interleukin-2: IL-2 has a pivotal role in generating an immune response by inducing an expansion of the pool of lymphocytes specific for an antigen. Therefore, IL-2 secretion by the protective CD4 Th1 cells is an important parameter to be measured and several studies have demonstrated that IL-2 can influence the course of mycobacterial infections, either alone or in combination with other cytokines[130].

Interleukin-4: Th2 responses and IL-4 in TB are subjects of some controversy. In human studies, a depressed Th1 response, but not an enhanced Th2 response was observed in PBMC from TB patients[107,118]. Elevated IFN-γ expression was detected in granuloma within lymph nodes of patients with tuberculous lymphadenitis, but little IL-4 mRNA was detected[107]. These results indicated that in humans a strong Th2 response is not associated with TB. Data from mice studies[116] suggest that the absence of a Th1 response to *M. tuberculosis* does not necessarily promote a Th2 response and an IFN-γ deficiency, rather than the presence of IL-4 or other Th2 cytokines, prevents control of infection. In a study of cytokine gene expression in the granuloma of patients with advanced TB by *in situ* hybridization, IL-4 was detected in 3 of 5 patients, but never in the absence of IFN-γ expression[131]. The presence or absence of IL-4 did not correlate with improved clinical outcome or differences in granuloma stages or pathology.

Interleukin-6: IL-6 has also been implicated in the host response to *M. tuberculosis*. This cytokine has multiple roles in the immune response, including inflammation, hematopoiesis and differentiation of T cells. A potential role for IL-6 in suppression of T cell responses was reported[41]. Early increase in lung burden in IL-6 $^{-/-}$ mice suggests that IL-6 is important in the initial innate response to the pathogen[132].

Interleukin-10: IL-10 is considered to be an anti-inflammatory cytokine. 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 IFN-γ production by T cells. IL-10 directly inhibits $CD4^+$ T cell responses, as well as by inhibiting APC function of cells infected with mycobacteria[133]. Transgenic mice constitutively expressing IL-10 were less capable of clearing a BCG infection, although T cell responses including IFN-γ production were unimpaired[134]. These results suggested that IL-10 might counter the macrophage activating properties of IFN-γ.

Transforming growth factor-beta (TGF-β): TGF-β is present in the granulomatous lesions of TB patients and is produced by human monocytes after stimulation with *M. tuberculosis*[135] or lipoarabinomannan[136]. TGF-β has important anti-inflammatory effects, including deactivation of macrophage production of ROI and RNI[137], inhibition of T cell proliferation[40], interference with NK and CTL function and downregulation of IFN-γ, TNF-α and IL-1 release[138]. Toossi *et al*[135] have shown that when TGF-β is added to co-cultures of mononuclear phagocytes and *M. tuberculosis*, both phagocytosis and growth inhibition were inhibited in a dose-dependent manner. Part of the ability of macrophages to inhibit mycobacterial growth may depend on the relative influence of IFN-γ and TGF-β in any given focus of infection.

Cell migration and granuloma formation

A successful host inflammatory response to invading microbes requires precise coordination of

myriad immunologic elements. An important first step is to recruit intravascular immune cells to the proximity of the infective focus and prepare them for extravasation. This is controlled by adhesion molecules and chemokines. Chemokines contribute to cell migration and localization, as well as affect priming and differentiation of T cell responses[139].

Granuloma: $CD4^+$ T cells are prominent in the lymphocytic layer surrounding the granuloma and $CD8^+$ T cells are also noted[140]. In mature granulomas in humans, dendritic cells displaying long filopodia are seen interspersed among epithelioid cells. Apoptosis is prominent in the epithelioid cells[32]. Proliferation of mycobacteria *in situ* occurs in both the lymphocyte and macrophage derived cells in the granuloma[141]. Heterotypic and homotypic cell adhesion in the developing granuloma is mediated at least in part by the intracellular adhesion molecule (ICAM-1), a surface molecule that is up regulated by *M. tuberculosis* or LAM[142]. The differentiated epithelioid cells produce extracellular matrix proteins (*i.e.*, osteopontin, fibronectin), that provide a cellular anchor through integrin molecules[143].

In our experience[144], the lymph node biopsy specimens showing histological evidence of TB could be classified into four groups based on the organization of the granuloma, the type and numbers of participating cells and the nature of necrosis. These were (*i*) hyperplastic (22.4%) - a well-formed epithelioid cell granuloma with very little necrosis; (*ii*) reactive (54.3%) - a well-formed granuloma consisting of epithelioid cells, macrophages, lymphocytes and plasma cells with fine, eosinophilic caseation necrosis; (*iii*) hyporeactive (17.7%) - a poorly organized granuloma with macrophages, immature epithelioid cells, lymphocytes and plasma cells and coarse, predominantly basophilic caseation necrosis; and (*iv*) nonreactive (3.6%) - unorganized granuloma with macrophages, lymphocytes, plasma cells and polymorphs with non caseating necrosis. It is likely that the spectrum of histological responses seen in tuberculous lymphadenitis is the end result of different pathogenic mechanisms underlying the disease[144].

Chemokines: The interaction of macrophages with other effector cells occurs in the milieu of both cytokines and chemokines. These molecules serve both to attract other inflammatory effector cells such as lymphocytes and to activate them.

Interleukin-8: An important chemokine in the mycobacterial host-pathogen interaction appears to be IL-8. It recruits neutrophils, T lymphocytes, and basophils in response to a variety of stimuli. It is released primarily by monocytes/macrophages, but it can also be expressed by fibroblasts, keratinocytes, and lymphocytes[145]. IL-8 is the neutrophil activating factor.

Elevated levels of IL-8 in BAL fluid and supernatants from alveolar macrophages were seen in patients[140]. IL-8 gene expression was also increased in the macrophages as compared with those in normal control subjects. In a series of *in vitro* experiments it was also demonstrated that intact *M. tuberculosis* or LAM, but not deacylated LAM, could stimulate IL-8 release from macrophages[146].

Friedland *et al*[147] studied a group of mainly HIV-positive patients, and reported that both plasma IL-8 and secretion of IL-8 after *ex vivo* stimulation of peripheral blood leukocytes with lipopolysaccharide remained elevated throughout therapy for TB. Other investigators had previously shown that IL-8 was also present at other sites of disease, such as the pleural space in patients with TB pleurisy[148].

Other chemokines: Other chemokines that have been implicated in the host response to TB include monocyte chemoattractant protein-1 (MCP-1) and regulated on activation normal T cell expressed and secreted (RANTES), which both decrease in the convalescent phase of treatment, as opposed to IL-8. Chemokine and chemokine receptor expression must contribute to the formation and maintenance of granuloma in chronic infections such as TB. In *in vitro* and *in vivo* murine models, *M. tuberculosis* induced production of a variety of chemokines, including RANTES, macrophage inflammatory protein1-α (MIP-α), MIP2, MCP-1, MCP-3, MCP-5 and IP10[149]. Mice over expressing MCP-1[150], but not MCP-/- mice[151], were more susceptible to *M. tuberculosis* infection than were wild type mice. C-C chemokine receptor 2 (CCR2) is a receptor for

MCP-1, 3 and 5 and is present on macrophages and activated T cells. CCR2-/- mice are extraordinarily susceptible to *M. tuberculosis* infection and they exhibit a defect in macrophage recruitment to the lungs. The current literature indicates that TNF-α can upregulate expression of MIP1-α, MIP1-β, MIP2, MCP-1, cytokine-induced neutrophil chemoattractant (CINC) and RANTES[152], and it can affect recruitment of neutrophils, lymphocytes and monocytes/ macrophages to certain sites.

RANTES, MCP-1, MIP1-α and IL-8 were released by human alveolar macrophages upon infection with *M. tuberculosis in vitro*[153] and monocytes, lymph node cells and BAL fluid from pulmonary TB patients had increased levels of a subset of these chemokines compared to healthy controls[153,154]. In human studies, CCR5, the receptor for RANTES, MIP-α and MIP-β, was increased on macrophages following *in vitro M. tuberculosis* infection and on alveolar macrophages in BAL from TB patients[155].

HIV-TB coinfection

Studies from many parts of the world have shown higher incidence of TB among HIV infected individuals, ranging from 5 to 10 per year of observation[156], which is in sharp contrast to the lifetime risk of 10 per cent among people without HIV. Persons with HIV infection are at increased risk of rapid progression of a recently acquired infection, as well as of re-activation of latent infection. TB is the commonest opportunistic infection occurring among HIV-positive persons in India and studies from different parts of the country have estimated that 60 to 70 per cent of HIV positive patients will develop TB in their lifetime[157]. Differences in HIV-positive TB, as opposed to HIV-negative TB, include a higher proportion of cases with extra-pulmonary or disseminated disease, a higher frequency of false-negative tuberculin skin tests, atypical features on chest radiographs, fewer cavitating lung lesions, a higher rate of adverse drug reactions, the presence of other AIDS-associated manifestations and a higher death rate.

TB and HIV infections are both intracellular and known to have profound influence on the progression of each other. HIV infection brings about the reduction in $CD4^+$ T cells, which play a main role in immunity to TB. This is reflected in the integrity of the cellular immune response, namely the granuloma. Apart from the reduction in number, HIV also causes functional abnormality of $CD4^+$ and $CD8^+$ cells. Likewise, TB infection also accelerates the progression of HIV disease from asymptomatic infection to AIDS to death. A potent activator of HIV replication within T cells is TNF-α, which is produced by activated macrophages within granuloma as a response to tubercle infection[158]. Because the clinical features of HIV infected patients with TB are often non specific, diagnosis can be difficult. The method most widely used, detection of acid-fast bacilli by microscopic examination of sputum smears, is of little use, since 50 per cent of the HIV-TB cases are negative by acid fast staining[159]. Chest radiograph is normal in up to 10-20 per cent of patients with AIDS[160]. Alternative diagnostic tests, based on serology, using crude mycobacterial antigens[161], purified lipid[162] and protein antigens[163], have been tried with varying results. Our results with purified 38, 30, 16 and 27kDa antigens to study the antibody response to different isotypes have yielded an improved sensitivity and specificity[67,68].

Since the $CD4^+$ receptors of the T cells are bound by the HIV through the gp120 antigen, interaction of these cells with APC presenting antigen in the context of Class II MHC molecules is impaired, which results in hypo-responsiveness to soluble tubercle antigens. HIV infection also downregulates the Th1 response, not affecting or increasing the Th2 response. In patients co-infected with TB and HIV, expression of IFN-γ, IL-2 and IL-4 in PBMCs is suppressed, but IL-10 levels do not differ from patients with HIV infection[164]. The suppressed Th1 response paves the way for susceptibility to many intracellular infections. A role for NK cells also has been implicated in the immune response to HIV. It has been reported that NK cells from normal and HIV positive donors produce C-C chemokines and other factors that can inhibit both macrophage and T cell tropic HIV replication *in vitro*[165]. Another group reported a decline in NK activity, which strongly correlated with the disease progression in HIV patients[166]. Our studies demonstrate that even though

there is no difference in the per cent of NK cells, there is lowered NK activity during TB and HIV-TB infection[54].

Though most patients respond very well to antituberculous treatment initially, they develop other opportunistic infections and deteriorate rapidly within a few months. Further, recurrence of TB is more frequent than in immunocompetent population, due to both endogenous reactivation or exogenous reinfection.

Immunogenetics of TB

Yet another important area in understanding the immunology of TB is host genetics, which is briefly discussed here. Susceptibility to TB is multifactorial. Finding out the host genetic factors such as human leucocyte antigens (HLA) and non-HLA genes/ gene products that are associated with the susceptibility to TB will serve as genetic markers to understand predisposition to the development of the disease.

A number of studies on host genetics have been carried out in our laboratory. Our studies on HLA in pulmonary TB patients and their spouses revealed the association of HLA-DR2 (subtype DRB1*1501) and -DQ1 antigens with the susceptibility to pulmonary TB[167,168]. Further studies on various non-HLA gene polymorphisms such as mannose binding lectin (MBL)[169], vitamin D receptor (VDR)[170,171], TNF-α and β[172], IL-1 receptor antagonist[170] and Nramp[45] genes revealed that functional mutant homozygotes (FMHs) of MBL are associated with the susceptibility to pulmonary TB. The polymorphic *BsmI*, *ApaI*, *TaqI* and *FokI* gene variants of VDR showed differential susceptibility or resistance with male or female subjects. These studies suggest that multicandidate genes are associated with the susceptibility to pulmonary TB.

The role of HLA-DR2 and the variant genotypes of MBL on the immunity to TB revealed that in a susceptible host (HLA-DR2, FMHs of MBL-positive subjects) the innate immunity (lysozyme, mannose binding lectin, *etc*.) play an important role[173-176]. If the innate immunity fails, HLA-DR2 plays an important role on the specific immune response against the pathogen.

Conclusion

The protective and pathologic response of host to *M. tuberculosis* is complex and multifaceted, involving many components of the immune system. Because of this complexity, it becomes extremely difficult to identify the mechanism(s) involved in protection and design surrogate markers to be measured as *in vitro* correlate of protective immunity. A clear picture of the network of immune responses to this pathogen, as well as an understanding of the effector functions of these components, is essential to the design and implementation of effective vaccines and treatments for TB. The combination of studies in animal models and human subjects, as well as technical advances in genetic manipulation of the organism, will be instrumental in enhancing our understanding of this immensely successful pathogen in the future.

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