Role of Cytokines in Immune Response to Pulmonary Tuberculosis

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There has been a consistent high incidence of tuberculosis in many developing countries. This along with emergence of multidrug resistant strains of Mycobacterium tuberculosis and the opportunistic behavior of M. tuberculosis during HIV infection have sharpened scientific interest in this ancient scourge. Protective immunity against M. tuberculosis in animal models is based on cell-mediated immunity involving interaction between T cells and macrophages. Several lines of evidence indicate that similar mechanisms operate in humans too.

Infection with M. tuberculosis yields a wide spectrum of outcomes from asymptomatic infection to systemic and fatal disease. This is due to interaction of M. tuberculosis and human immune defenses. During active pulmonary tuberculosis, signs of both immune depression and immune activation have been reported to be present simultaneously. Decreased tuberculin skin test reactivity in vivo and deficient IFN-γ production by M. tuberculosis stimulated mononuclear cells ex vivo have been observed to exist concomitantly. On the other hand, the serum levels of cytokines, including TNF-α and other inflammatory mediators, may be increased and circulatory monocytes and T-cells may show phenotypic and functional evidence of in vivo activation.

Cytokines are central to acquired resistance against intracell-
malar bacteria. These are secreted and regulated by both monocytes/macrophages (Mn/Mφ) and CD4+ lymphocytes. Cytokines involved in the immunoinflammatory mechanism are divided into two groups. Th1 type includes interleukin-2 (IL-2) and IFN-γ and is central to cell-mediated immunity against intracellular pathogens. These are responsible for delayed type hypersensitivity (DTH) and activation of cytotoxic T cells. Th2 type includes IL-4 and IL-5. These cytokines serve as helper for B cells and are elevated in allergic diseases and helminthic infections. The cytokine environment present at the time determines the differentiation of Th cells. If IL-12 is present along with antigen stimuli Th1 cells develop and if IL-4 is present along with antigen stimuli Th2 cells develop. Most of the M. tuberculosis reactive CD4+ T cells propagated in vitro are found to be Th1-like and the concentration of mRNA for Th1 cytokines dominates over that for Th2 cytokines in lungs. Development of newer antituberculosis vaccines and immunotherapeutic modalities, to enhance immune response and mitigate immunopathology, requires an understanding of the cytokines involved. In this article, we will review the current understanding of the role of cytokines in the immune response to human infection of M. tuberculosis.

**Immune response to M. tuberculosis**

Pulmonary tuberculosis is characterized by granulomatous inflammation, which may result in extensive fibrosis and tissue damage. Monocytes(Mn)/macrophages (Mφ) particularly the alveolar macrophages are the natural hosts for M. tuberculosis. They have a limited intrinsic capacity to reduce the growth of mycobacteria and additional acquired immune activation by CD4+ and CD8+ T-cells is necessary to control the infection. To achieve this, M. tuberculosis antigens have to be presented to specific CD4+ and CD8+ T-cells by professional antigen presenting cells including dendritic cells and other cells of Mn/Mφ lineage. CD4+ T-cells secrete cytokines, most importantly IL-2 and IFN-γ which can activate macrophages to ingest and digest mycobacteria and to secrete other cytokines such as IL-8, IL-6 and TNF-α which activate the acute phase inflammatory response. TNF-α assists in granuloma formation and thus performs a protective role by physically containing the tubercle bacilli. The role of CD8+ T cells in the immunity to TB is less well understood. CD8+ MHC class I restricted cytotoxic T cells, γδT cells, or the αβ receptor bearing CD1 restricted (CD4 CD8+) T cells, can all recognize mycobacterial lipids and glycolipids and other mycobacterial antigens. These various cell types can secrete IFN-γ and probably have some role in the release of bacteria from effete macrophages. They probably are important in the chronic phase of the disease; γδT cells also help in efficient formation of granuloma through their secretion of chemokine MCP-1. Experiments with neutralizing antibodies and with gene knockout mice have also shown that the immunity requires MHC class II, a Th1 cytokine pattern and TNF-α. It is probable that in humans, as in mice, immunity is associated with a Th1 type of response leading to macrophage activation and to cytotoxic removal of effete infected cells. Studies of cells in tuberculosis pleural effusions support this concept, since this is a high resistance form of the disease, and the Th1 type pattern dominates.

**Cytokine production in tuberculosis**

Cytokines are key mediator molecules in the expression of acquired immunity in the lungs. Acquired immunity to tuberculosis is generated when CD4+ T-cells recognize antigens presented in context of MHC class II molecules, respectively. They are bathed in macrophage derived IL-1, which promotes IL-2 production, IL-2 receptor expression and subsequent clonal expansion of the CD4+ and CD8+ cells, as well as macrophage derived IL-12 which promotes IFN-γ secretion by the CD4+ cells. The IFN-γ then stimulates macrophages to produce TNF-α and other reactive oxygen and nitrogen radicals for elimination of mycobacteria.

**Macrophage derived cytokines**

**Interleukin-1:** IL-1 is produced on stimulation of human monocytes with M. tuberculosis and specific mycobacterial components, including lipoarabinomannan (LAM) and mycobacterial proteins of 20 and 46 kDa. The main action of this cytokine is attraction of phagocytes. IL-1 is an endogenous pyrogen and may contribute to the fever that is characteristic of tuberculosis. IL-1 exhibits other properties that enhance inflammatory responses, e.g. production of the proinflammatory cytokines IL-6 and TNF-α. An increased production of IL-1β, IL-6, and TNF-α has been observed in broncho-alveolar lavage (BAL) cells of tuberculosis patients. It may be possible that these cytokines are involved in granuloma formation. Among the mycobacterial antigens...
ManLAM is significantly less potent than AraLAM in induction of TNF-α, IL-1, IL-6 and IL-10.24 IL-1 also stimulates T-cell proliferation by up-regulation of T-cell expression of IL-2 receptor and IL-2 production.25,26

**Interleukin-12:** IL-12 is a disulfide linked heterodimeric cytokine composed of two subunits of 40 kDa both of which are concentrated at the site of disease in patients with tuberculous pleuritis.27 IL-12 is produced in these patients by pleural fluid cells in response to M. tuberculosis. Increased number of IL-12 producing cells is found in human tuberculosis patients.24,29 IL-12 is induced rapidly and readily after infection with M. tuberculosis, phagocytosis is a potent signal for IL-12 production by monocytes. IL-12 favors development of precursor T cells into Th1 cells which are thought to mediate resistance against mycobacteria.30 IL-12 is essential to the generation of a protective immune response to M. tuberculosis, with its main functions being the induction of IFN-γ and the activation of antigen-specific lymphocytes capable of creating a protective granuloma.31,32 IL-12 may also enhance cytotoxicity by augmenting proliferation of antigen specific cytolytic T-cells and NK cells.33-35 IL-12 induces proliferation of cytolytic T-cells only on co-stimulation of T cell receptors with antigen or anti CD3 in contrast to IL-2 and IL-7.36 IL-12 can modulate IFN-γ production and cytotoxicity by CD4+ T-cells. Addition of IL-12 strongly increased the IFN-γ production in PPD-stimulated PBMC cultures from both patients and controls, indicating normal IL-12 function.37

**Interleukin-18:** IL-18 has recently been identified as an IFN-γ inducing factor for T cells and NK cells and plays an important role in the Th1 response. Significantly increased levels of IL-18 and IFN-γ were found in both blood and pleural fluid of pulmonary TB patients whereas M. tuberculosis stimulated PBMC from TB patients secreted less IL-18 and IFN-γ.38-40

**TNF-α:** Human mononuclear cells and alveolar macrophages produce large quantities of TNF in response to tuberculosis and specific mycobacterial components such as LAM and proteins of molecular size 20, 44, 58 and 65 kDa.20,41 Induction of TNF-α is also dependent on Mφ CD14 molecule.19,21,42,43 Addition of TNF-α in vitro to human macrophages enhances antimycobacterial activity.44 Peripheral blood monocytes from patients with chronic refractory pulmonary TB produce a significantly lower amount of TNF than do monocytes from patients with newly diagnosed TB.45 Clinical and experimental data in humans and animals suggest that TNF-α contributes both to protection against tuberculosis and to immunopathology. Tuberculosis is characterized by fever, weight loss, a prolonged acute-phase protein response and granuloma formation. These characteristics may partly be due to action of proinflammatory cytokines, e.g. TNF-α, IL-6 and IL-8.46,47 Excessive local production of TNF may cause marked tissue necrosis that is characteristic of progressive TB and may result in TNF-α release into the circulation contributing to systemic manifestations of TB such as fever and cachexia. In contrast, a physiological concentration of TNF-α contributes to antimycobacterial defense, and local production leads to granuloma formation, control of infection and mycobacterial elimination.

**Interleukin-6:** IL-6 is a B-cell growth and differentiation factor that induces immunoglobulin production by activated B-cells and is thought to mediate polyclonal B cell expansion and immunoglobulin production in infection and neoplastic diseases.48 IL-6 may mediate the hyperglobulinemia that is characteristic of TB. Addition of IL-6 to human monocytes also enhances intracellular and extracellular mycobacterial growth.49

**Interleukin-8:** IL-8, a chemokine, functions as a chemotactic factor for neutrophils, T-lymphocytes, macrophages and basophils; it belongs to a family of 8 kDa polypeptides.50 Exaggerated release of IL-8 could lead to the increased number of neutrophils observed in tuberculous infiltrates and recruitment of T-lymphocytes involved in granuloma formation.51 In addition, IL-8 activates inflammatory cells and may contribute to the necrotic destruction of lung tissue.

**Interleukin-10:** IL-10 is an anti-inflammatory cytokine that is produced by human macrophages exposed to M. tuberculosis in vitro.19,52,53 IL-10 suppresses antigen specific T-cell proliferation by down regulation of Mφ class II MHC expression.54 IL-10 reverses the mycobacterialidal effects of TNF. These findings suggest that IL-10 may play a role in inhibiting the immune response to M. tuberculosis in human and may contribute to the anergy and failure of lymphocytes to proliferate in response to M. tuberculosis. Antigen induced IFN-γ induction is found to be decreased in TB patients which is attributed to IL-10.55
Transforming Growth Factor-β: TGF-β exhibits both pro-inflammatory and anti-inflammatory activities. TGF-β promotes the migration of undifferentiated leucocytes to the sites of inflammation, induces their activation and maturation. TGF-β inhibits synthesis and activity of Th1 cytokines, e.g. IL-2 and IFN-γ. By down regulating class II MHC expression it inhibits the proliferation of T-cells as well as decreases IL-2 production and IL-2 receptor expression by T-cells. It suppresses both the generation and effector functions of cytotoxic T cells, natural killer (NK) cells and LAK cells. TGF-β is constitutively overproduced by monocytes from tuberculosis patients. Langerhan's giant cells and epithelioid cells in tuberculous granulomas also express mRNA for TGF-β, suggesting that local production of TGF-β may result in deactivation of macrophages and immunopathology. In human macrophages infected with M. avium, production of TGF-β is highest in those infected with the most virulent strains and addition of recombinant TGF-α inhibits the antimycobacterial effects of TNF.

T-cell derived cytokines

Interferon-γ: IFN-γ contributes to protective immunity against M. tuberculosis by activating macrophages to more effectively eliminate these organisms. It is also observed that individuals with IFN-γ and IFN-γ receptor deficiency have disseminated mycobacterial diseases. Recently, we have shown that IFN-γ is the most potent cytokine to induce release of nitric oxide from human cultured monocytes/macrophages and this NO is toxic to mycobacteria. IFN-γ and IL-2 concentrations are found to be higher at the site of disease in patients with tuberculosis. Further studies have shown that M. tuberculosis inhibits the effects of IFN-γ by affecting the distal parts of the signaling by IFN-γ. The inhibitory effect of M. tuberculosis is directed at the transcription of IFN-γ responsive genes, but does not affect proximal steps in the Janus Kinase-STAT pathway. STAT-1 tyrosine phosphorylation, dimerization, nuclear translocation and DNA binding are found to be intact in M. tuberculosis infected cells whereas there is a marked decrease in association of STAT-1 with transcriptional co-activators CREB binding protein (CRB) and p300. This study indicates that M. tuberculosis directly or indirectly disrupts this protein-protein interaction that is essential for the transcriptional response to IFN-γ.

Interleukin-2: IL-2 is a critical T cell growth factor that expands population of antigen reactive T cells and is likely to increase the local concentration of Mφ activating factors secreted by T cells, mainly TNF-α. The number of CD4+ T cells as well as M. tuberculosis induced production of IFN-γ and IL-2 by PBMC is depressed in TB patients. Pleural fluid lymphocytes stimulated with M. tuberculosis secrete more IFN-γ and IL-2 than PBMC from these patients. Tuberculosis patients receiving recombinant human IL-2 showed clinical improvement and reduced bacterial load. Also there was an increased mRNA production for IL-2 and IFN-γ without an increase in TNF-α.

Interleukin-4: IL-4 deactivates macrophages and blocks T-cell proliferation by down regulation of IL-2 receptor expression and inhibition of transcription of the IL-2 gene. IL-4, therefore, has the capacity to inhibit the immune response to M. tuberculosis. Peripheral blood lymphocytes from TB patients express IL-4 gene, while there is a deficit in IL-2 expression. Tuberculosis patients have specific immunoglobulin E (IgE) and IgG4 antibodies, which are IL-4 dependent.

TNF-α: Mycobacterium reactive human CD4+ T cells secrete a factor that synergizes with GM-CSF in inducing macrophage aggregation and its effects are abrogated by neutralizing antibodies to TNF-α. It is a homologue of TNF-α, also known as lymphotoxin. Its effects are normally restricted to paracrine and autocrine functions because of production of lower concentration of TNF-α by T-cells.

CONCLUSION

It is essential to understand the host-parasite interaction and the mechanisms of immunomodulation in TB. Such knowledge is the key to developing effective vaccine and immunotherapeutic strategies upon which the eradication of tuberculosis may rely. Whereas T cells are mediators of immunity, macrophages are the effector cells. They harbour mycobacteria within their phagosomes and upon activation by cytokines reduce the intraphagosomal pH and expose bacilli to oxygen and nitrogen radicals. Immunity to tuberculosis involves protective immunity as well as...
delayed type hypersensitivity (DTH). The protective immunity is considered to be mediated by cytokines while DTH is mainly controlled by chemokines under the influence of TNF-α. Cell-mediated protective immunity against active tuberculosis is associated with a Th1 type T-cell response characterized by IFN-γ, IL-2 and IL-12. The mechanism by which these cytokines mediate anti-mycobacterial activity appears to be the production of nitric oxide (NO) by the macrophages. This metabolite is particularly induced by IFN-γ and TNF-α and is crucial for protection against tuberculosis. It is seen that acquired deficiency of type I response, e.g. HIV infection, leads to clinical reactivation of tuberculosis. Also human genetic deficiencies in IL-12, IFN-γ, IFN-γ receptor axis result in increased susceptibility to mycobacterial diseases. TNF-α has a dual role in tuberculosis, being needed for protection, and playing a role in immunopathology. In tuberculosis also, the immune inhibitory functions of the TGF-β and IL-10 prevail. These cytokines appear to be involved in the termination of inflammatory responses and thus limit tissue injury. Primary tuberculosis patients produce sufficient amount of macrophage cytokines to enhance mycobacterial clearance and these show mild clinical manifestation of TB. Early production of IL-12 favors expansion of Th1 cells that limit the extent of disease and mediate a delayed type hypersensitivity response. Patients with progressive primary TB with extensive pulmonary infiltrates and cavitation show an active immune response that fails to eliminate bacteria perhaps because of both Th1 and Th2 cell activation. Early secretion of IL-4 (Th2) or IL-10 and TGF-β by Mφ prevents Th1 cells from clearing mycobacteria despite a prominent inflammatory response. Alternatively, there may be minimal activation of Th1 or Th2 cells and immune response may be dominated by high concentrations of inflammatory cytokines produced by mononuclear phagocytes which are unable to clear the infection in the absence of a Th1 response. Absence of a Th1 response and predominance of immunosuppressive cytokines may be associated with the development of military tuberculosis.

Therefore, it is the balance between the inflammatory and protective immune responses that determines whether patients will develop active disease. Furthermore, the extent and duration of the inflammatory versus the protective response will determine how severe the disease manifestations become in the absence of therapy. The outcome is predicted as a balance between the protective and inflammatory responses. Thus, increased IFN-γ in the context of reduced TNF-α probably suggests a better outcome.

Although BCG is the only globally used vaccine against tuberculosis at the moment, the search is on to develop a better and improved vaccine. An effective vaccine has to stimulate a precise combination of T-cells and cytokines required for the different tasks. Th1 cytokines, such as IFN-γ, IL-2 and IL-12 through enhancement of T-cell function and macrophage activation may prove to be potent immuno-therapeutic agents. On the other hand, agents that inhibit deactivated cytokines (such as TGF-β) or reduce the production and effect of pro-inflammatory molecules (such as TNF-α) may also prove to be useful. Therefore, an ideal vaccine should promote increased protective immunity (Th1 response) while not increasing inflammatory responses.

REFERENCES


50. Matsushima K, Baldwin ET, Mukaide N. Interleukin-8 and MCAF: novel