



Review

The Inflammatory Response in *Mycobacterium tuberculosis* Infection

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Abstract. Infection with *Mycobacterium tuberculosis* (MTB) is accompanied by an intense local inflammatory response which may be critical to the pathogenesis of tuberculosis. Activation of components of the innate immune response, such as recruitment of polymorphonuclear (PMN) and mononuclear phagocytes and induction of pro-inflammatory cytokines, such as tumor necrosis factor α (TNF- α), by MTB occurs early after MTB infection, however, may persist as the organism establishes itself within granulomas. MTB and its protein and non-protein components are potent in induction of cytokines and chemokines from PMN and monocytes. This review focuses on the interaction of MTB and the host with regard to activation of the innate immune response. It also attempts to identify the potential impact of this early response on the subsequent pathogenesis of MTB, and its role in development and extent of tuberculosis. Insights into the initiation and persistence of the inflammatory response may allow the application of anti-inflammatory agents as adjuncts in the treatment of tuberculosis.

Key words: polymorphonuclear; monocyte; cytokine; chemokine; tumor necrosis factor α ; *Mycobacterium tuberculosis*; tuberculosis.

Introduction

In humans, *Mycobacterium tuberculosis* (MTB) is the commonest infectious cause of morbidity and mortality⁵⁴. However, the underlying basis for the pathogenic proficiency of MTB is still poorly understood. Despite an absolute requirement for acquired cell-mediated immunity for both the initial containment and prolonged immunosurveillance against re-activation of MTB¹⁴, the contribution of the host immune responses that are conducive to the success of MTB as an intracellular pathogen is not clear. However, MTB infection is accompanied by both an initial and a persistent intense local inflammatory response. Recent research has

begun to elucidate the nature of the innate immune response to MTB and indicates a predominance of pro-inflammatory cytokines, such as tumor necrosis factor α (TNF- α), and an intense and prolonged infiltration by polymorphonuclear (PMN) *in situ*. This review focuses on recent findings on the interaction of MTB and the host with regard to the activation of the innate immune response. Further, it attempts to identify the potential impact of this early response on the subsequent pathogenesis of MTB and its role in the development and extent of tuberculosis.

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Inflammation during MTB Infection and Tuberculosis

In an MTB-uninfected individual, an aerosol infection (with as few as 5 bacilli)⁵³ leads to a primary focus of infection which is initially characterized by intracellular multiplication of the organism within the host's most proficient phagocytes, the alveolar macrophages²². However, a vigorous inflammatory response is initiated from the onset, featured by newly recruited PMN cells and monocytes (MN), and their products⁴¹. Over time, the characteristics of this local response change and mononuclear cells, especially lymphocytes and macrophages, become predominant, although, PMN continue to be present up to the time of resolution of MTB infection². Soon after establishment of an MTB focus of infection, the process of "sensitization" of CD4⁺ and CD8⁺ lymphocytes is initiated, most likely within lymph nodes draining the sites of infection. However, specific protective immunity requires up to 3 weeks to become sufficient to contain MTB growth at sites of infection. Meanwhile, bacillary replication continues and, thus, the recruitment and activation of the components of the innate immune response persist.

The biologic basis for the rapid intracellular replication of MTB is not clear; however, it is most likely multifactorial. MTB evades powerful intracellular killing mechanisms subsequent to infection¹⁰ and, despite induction of several macrophage-activating molecules, such TNF- α ⁶⁵ and interleukin 1 (IL-1)⁶⁹ and, reactive oxygen and nitrogen intermediaries (ROI and RNI)^{36, 44}, intracellular replication ensues. In addition, anti-inflammatory molecules, such as transforming growth factor β (TGF- β)²³ and IL-10³⁵, are also induced by MTB which counteract macrophage activation and microbicidal molecules^{6, 12}. Meanwhile, uncontrolled intracellular replication continues and, eventually, lymphohematogenous spread allows the seeding of both pulmonary and extrapulmonary sites. With the development of the specific host cell-mediated immune response, mycobacterial replication is controlled and most infected individuals develop a robust life-long immunity to MTB¹⁴. Protective immunity depends in part on the host's capacity to produce T cell cytokines that expand MTB antigen reactive T cells (IL-2) and induce macrophage activation (IFN- γ) to ultimately develop microbicidal granulomas.

Data from animal models of MTB infection provide an understanding of the dynamics of cell recruitment and interaction at sites of primary infection^{19, 25, 38, 39}. Several studies have now shown that, subsequent to infection of mycobacteria-susceptible strains of mice,

there is a dramatic recruitment of PMN and lymphocytes to the lung^{19, 39}. Interestingly, the peak numbers of PMN appeared about a week before the peak levels of lymphocytes¹⁹. Also, cytokine expression *in situ* is characterized by excess pro-inflammatory molecules^{25, 37}. On the other hand, data from limited studies of human primary infection indicate that, among the household contacts of smear-positive TB patients, the majority experience granulocytosis, and produce excess TNF- α , and have a higher frequency of IFN- γ -producing cells in response to MTB antigens in their broncho-alveolar lavage (BAL) fluid⁵⁰. Cumulatively, these data suggest that primary MTB infection, similar to acute infection by other pathogens, is associated with an intense inflammatory response.

In addition, in murine models of progressive MTB infection, PMN and pro-inflammatory cytokines continue to be persistent features of the granulomas⁴³, indicating that uncontrolled mycobacterial replication is associated with a continuous recruitment and activation of the innate components of the immune response. In support of this contention, BAL fluids from the TB-involved lungs from patients are enriched with PMN and immature macrophages⁴⁹ and are rich in pro-inflammatory cytokines¹⁵, which correlate with the extent of lung disease⁶⁴.

Activation of Components of the Innate Immune Response during MTB Infection

As noted, PMN and macrophages are predominant cell types not only in early MTB infection, but also later within well-formed granulomas. Other cell types of the innate branch of the immune response that feature early MTB lesions are natural killer (NK) and $\gamma\delta$ T cells⁷.

The recruitment of the components of inflammation to sites of MTB infection is orchestrated by chemokines. Specifically, IL-8⁷⁴, monocyte chemoattractant protein-1 (MCP-1)⁴⁸ and growth-related gene product (GRO)- α ^{45, 74} are promptly induced by MTB in alveolar macrophages and allow the recruitment of PMN, MN, NK cells, and $\gamma\delta$ T cells. The recruited PMN and mononuclear cells then become secondary power houses for the generation of both chemokines and cytokines. For example, upon stimulation with MTB, PMN release abundant IL-8 and GRO- α ⁴⁵. Also, production of MCP-1 by PMN has been shown to be in response to live but not heat-killed MTB²⁹. Further, the abundant MTB glycolipid²⁶, lipoarabinomannan (LAM), induces IL-8 and GRO- α through activation of lipoxygenase pathways⁴⁵. In addition, amplifying loops may lead to the

excess production of certain chemokines. For example, the release of IL-8, but not MIP-1a, was potentiated by TNF- α ²⁸. Chemokines such as IL-8 and GRO- α are powerful in the activation of PMN, including degranulation and increased expression of adhesion molecules^{31, 32}.

MTB Induction of Pro- and Anti-Inflammatory Cytokines

MTB and its protein and non-protein antigens are strong stimuli for the induction of cytokines in human phagocytes. Early studies indicated that purified protein derivative (PPD) of MTB induces the pro-inflammatory cytokines IL-1 β and TNF- α ^{65, 69} in MN. In addition, MTB also strongly induces IL-12 in MN³³. Importantly, a major secretory component of actively replicating mycobacteria, the 30 kDa antigen (85B), induces TNF- α ³. Interestingly, the 30 kDa antigen is a fibronectin-binding protein¹, and its interaction with fibronectin enhances the production of TNF- α by MN³. Further, LAM⁴ and other antigens of MTB⁷¹ also potently induce TNF- α and IL-1. In addition, MTB induces pro-inflammatory cytokines in PMN⁹.

However, MTB²⁴ and its cell wall LAM¹¹ are also potent inducers of the anti-inflammatory cytokine TGF- β . Further, the effect of LAM on the induction of TGF- β appears to be dominant over the induction of the pro-inflammatory cytokines (TNF- α , IL-1 β and IL-6) and IL-10¹¹. More recently, the 30 kDa antigen has also been shown to induce TGF- β (HIRSCH, unpublished) and IL-10⁶². Further, cytokines may regulate the production of one another or amplify the immunologic effects of one another. For example, TGF- β induces IL-10 in human MN, and these molecules synergize in inhibition of PPD-induced production of IFN- γ ⁴². In addition, cytokines such as TNF- α and TGF- β up-regulate their own production^{32, 75}, thereby allowing a mechanism for predominance over other cytokines *in situ*. Thus, mechanisms for induction and amplification of cytokine circuits appear to be inherent to MTB and its components and to its state of metabolism.

Both the *in situ* balance of pro- and anti-inflammatory cytokines and the predominance of each cytokine in relationship to the stage of MTB infection are critical to the progression and/or resolution of MTB infection. Despite the fact that, on a per cell basis, PMN are weaker than mononuclear phagocytes in the production of pro-inflammatory molecules⁹, their early and persistent presence at sites of MTB infection assures that their secretory and functional profile is well-reflected *in situ*.

TNF- α ; a double-edged sword in MTB infection

TNF- α has been shown to be modest at best in its anti-MTB activity in human mononuclear phagocytes in *in vitro* systems²⁴. However, MTB infection of human alveolar macrophages as compared with autologous MN, leads to a significantly higher induction of TNF- α , and inhibition of TNF- α leads to a lesser ability to contain MTB growth²⁴. Others have shown an MTB growth-promoting effect of TNF- α in human MN^{8, 52}. Our recent data indicate that TNF- α enhances MTB gene transcription in newly infected blood MN *in vitro* (WILKINSON, unpublished).

In mice, TNF- α tends to favour bactericidal granuloma formation and retards the growth of intracellular mycobacteria^{17, 33}. In MTB-infected mice, abrogation of TNF- α by neutralizing antibody³³ was associated with a loss of microbicidal granulomas. Further, TNF- α /lymphotoxin double knock-out mice²⁸ and TNF receptor deficient mice¹³ developed fatal TB. Both the TNF- α -dependent production of RNI and the induction of apoptosis of cells infected by MTB appears to be the mechanism for limiting the growth of this pathogen^{18, 44}.

In addition, IFN- γ , which is produced by all classes of lymphocytes³¹ and, potentially, even by alveolar macrophages¹⁶, up-regulates the production of TNF- α ⁵⁸. It is therefore possible that the continuous presence of TNF- α *in situ* is linked to the production of IFN- γ by mononuclear cells and PMN responding to MTB and its various components. On the other hand, TGF- β and IL-10 down-modulate and counter-act TNF- α ⁵⁸. However, in contrast to TNF- α , and as compared with blood MN, the ability of alveolar macrophages from healthy subjects to produce TGF- β was limited⁵⁹. Further, the kinetics of production of IL-10 by alveolar macrophages⁵⁹ indicates that, its activity may only become significant later at sites of MTB infection. Therefore, it is possible that at least in the initial days of MTB infection the production and activity of MTB-induced TNF- α is unopposed, contributing to immunopathogenic networks. On the other hand, as blood MN which produce TGF- β are recruited to sites of MTB infection, at least some of the effects of TNF- α may be ameliorated *in situ* later within well-established MTB foci.

Recently we have observed that TNF- α induced the expression of MTB mRNA, especially that of 85B mRNA, in MN (WILKINSON, unpublished). In complex with fibronectin, which binds antigen 85B, this MTB protein itself induces TNF- α secretion directly from MN³. Thus, a positive feedback loop may be in operation at sites of MTB infection. This interaction may

be further augmented by the subsequent arrival of T cells *in situ*, as 85B is a powerful stimulus for IFN- γ production²⁷ and, as noted, this cytokine induces TNF- α . Thus, within MTB lesions TNF- α may be both associated with macrophage activation and the formation of granulomas and with enhancement of MTB gene expression.

The role of TNF- α in the pathology of TB

Extensive tissue destruction and necrosis, formation of cavities, and fibrosis are characteristic of the pathology of human tuberculosis. Whereas some components of MTB may be directly involved in activating cellular proteases, most of the pathology induced by the organism is probably due to the induction of tissue-damaging cytokines, such as TNF- α and TGF- β .

TNF- α is cytotoxic to epithelial cells, reduces the production of surfactant protein by type II alveolar cells, promotes fibroblast activity and enhances the production of fibroblast collagenases. Further, by promoting the production of reactive oxygen intermediaries that are cytotoxic to tissues⁵⁵, it further enhances damage. Also, TNF- α potentiates the cellular toxicity of MTB⁴⁷. Excess TNF- α (and other pro-inflammatory cytokines) may be involved in the tissue damage (edema and necrosis) of MTB lesions, thus giving rise to organ dysfunction. In addition, some of the constitutional signs and symptoms of tuberculosis, such as fever, night sweats, weight loss and anorexia, indicate excessive circulating inflammatory cytokines. On the other hand, excessive production of TGF- β is associated with extensive fibrosis and tissue damage. TGF- β is a strong inhibitor of epithelial and endothelial cell growth⁵⁶ and, while it promotes the production and deposition of collagen matrix³⁰, it also has been shown to increase the production of macrophage collagenases⁶⁸. Mice injected intraperitoneally with TGF- β for 10 days developed cachexia and generalized fibrosis⁷⁶.

During active TB the production of both TNF- α and TGF- β are enhanced and, therefore, may synergize in cachexia and tissue destruction in patients. Numerous studies have reported high and sustained circulating TNF- α in patients with TB and its correlation with disease activity³⁹. High TNF- α and other pro-inflammatory cytokine levels have been reported in BAL fluid of TB patients, and the levels correlate with the extent of cavity formation^{15, 69}. On the other hand, expression of TGF- β is a feature of TB granulomas⁵⁹, and increased circulating TGF- β has also been reported in TB patients²³.

Immunomodulation of TNF- α

The use of anti-TNF- α agents as adjuncts in the management of patients with tuberculosis has been investigated only in small fractions of TB patients with specific forms of TB⁵¹. However, a more generalized role for anti-TNF- α adjunctive therapy in the management of TB is not known. Theoretical concerns about the use of TNF- α inhibitors are that the similarities in the genetic regulation of pro-inflammatory cytokines and other physiological molecules in general may lead to untoward effects. Further, as TNF- α has some mycobactericidal activity, use of these agents may potentially be associated with slower responses to anti-mycobacterial chemotherapy.

Agents that inhibit pro-inflammatory cytokines effectively usually act by transcriptional inhibition of cytokine genes. With this regard, corticosteroids are presently the strongest inhibitors of the pro-inflammatory cytokines. Corticosteroids have been used successfully as part of the medical management of many forms of tuberculosis, including tuberculous pericarditis, tuberculous meningitis, and advanced pulmonary tuberculosis⁵¹. The consensus of studies on the use of corticosteroids as adjunctive therapy in TB is that it is beneficiary to most forms of TB. Corticosteroids, when added to anti-tuberculous chemotherapy, has been associated with a faster clinical response and with the prompt cessation of fever³⁸. Other agents that inhibit TNF- α include thalidomide⁵⁷ and pentoxifylline⁷³. A recent controlled study indicates that thalidomide, when added to the anti-tuberculous regimen for 2–3 weeks improved the systemic symptoms and weight gain of patients with tuberculosis without affecting their cellular responses to MTB⁶³. Also, pentoxifylline was not associated with a worsening of mycobacteriologic parameters⁷⁰.

The role of TNF- α in up-regulation of HIV-1 in patients with HIV/TB

Active tuberculosis in HIV-1-infected subjects is associated with increased HIV-1-related immunodeficiency and mortality⁷². Whereas generalized immune activation has been found to correlate with HIV-1 activity in HIV/TB patients⁶⁶, as TNF- α is a major HIV-1-inducing cytokine⁶⁷, a major role for TNF- α in the interaction of HIV-1 and MTB is now clear. TNF- α is up-regulated at sites of MTB infection⁵ and the cytokine profile of the tuberculous microenvironment is conducive to HIV-1 replication²¹. Importantly, inhibition of TNF- α *in vitro*⁶¹ reduces MTB-induced HIV-1

activity. Results from studies using thalidomide⁶³ or pentoxifylline⁷⁰ indicate that induction of HIV-1 in HIV/TB patients can also be controlled to a certain degree. Results from studies using stronger TNF- α inhibitors may show an even more impressive effect on reduction of HIV-1.

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