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HOST RESPONSE TO MYCOBACTERIUM TUBERCULOSIS

Zahra Toossi and Jerrold J Ellner

Division of Infectious Diseases, Biomedical Research Building, 10th Floor West Administration, Case Western Reserve University School of Medicine, 10900 Euclid Avenue, Cleveland, OHIO 44106-4984

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1. ABSTRACT

Critical to the complete expression of the virulence of M. tuberculosis and thereby its pathogenesis in human infection, is the ability of this pathogen to interact with the host in a specific manner. To date, cytokine circuits during tuberculosis and M. tuberculosis infection have been studied most intensely. With this regard, both the whole M. tuberculosis and its protein and non-protein moieties appear to be influential on the *in situ* cytokine profile, and consequently, to the final outcome of infection. The interplay and final balance of macrophage activating and immuno-enhancing cytokines versus macrophage deactivating and immunosuppressive cytokines most likely determines the final expression of M. tuberculosis infection. Further, cytokine circuits also underlie the immunopathology of tuberculosis. Modulation of the in vivo cytokine milieu may allow the development of more effective vaccines to prevent M. tuberculosis infection, and adjunctive immunotherapy to improve treatment of tuberculosis.

2. INTRODUCTION

Infection with *Mycobacterium tuberculosis* continues to be the most common recognized infectious disease worldwide contributing to significant morbidity and mortality (1). The interaction of the host with M. tuberculosis is likely to be conducive to expression of the virulence of this pathogen. In fact, the very nature of the complex interaction between the host and the organism may underlie the salient features of *M. tuberculosis* infection namely, the persistence of infection within host tissues, and the development of disease. The immune response to *M. tuberculosis* can be characterized to be both salutary to the survival of the host, contributing to control of mycobacterial replication, and damaging to the host, contributing to promotion of tuberculosis. Recent research has allowed a better understanding of the survival of the pathogen after initiation of *M. tuberculosis* infection, and the establishment of immunopathologic circuits during tuberculosis. This knowledge may pave the way for development of both better vaccines to prevent infection with *M. tuberculosis*, and immunomodulatory approaches to aid in treatment of tuberculosis.

3. PATHOGENESIS OF M. TUBERCULOSIS INFECTION

In humans, infection with M. tuberculosis is associated with a wide spectrum of outcomes ranging from containment of infection and development of protective immunity, to development of tuberculosis as a consequence of rapid initial mycobacterial replication or reactivation of latent infection after a period of mycobacterial dormancy. The unique ability of M. tuberculosis to be associated with a pathogenesis allowing such diverse modes of outcome is dependent on M. tuberculosis virulence, which in turn is comprised of and combines the following three different components. The ability of M. tuberculosis to survive and replicate within host mononuclear phagocytes; the specific property of M. tuberculosis dormancy which allows the organism to survive for prolonged periods of time within the tissues of the host; and the intense interaction of M. tuberculosis with the host which contributes both to pathogenesis and protective immunity.

3.1. Infection with M. tuberculosis

In an M. tuberculosis-naive individual, subsequent to an aerosol infection with a few (up to 5) bacilli (2), a primary focus of infection is established which is initially mainly featured by the intracellular multiplication of the organism within the host's most proficient phagocytes, the alveolar macrophages (3). In fact it appears that M. tuberculosis has adapted to employ any of several cellular molecules abundantly present on the surface of macrophages, such as the complement receptors (CR) (CR3 and CR4) (4), fibronectin receptors (5), and mannose

receptors (6), to gain access to the intracellular space of these professional "killer" cells. Through particular modes of intracellular trafficking and a combination of events which may include alkalinization of the phagosome (7), prevention of phagosome-lysosome fusion (8), and escape from the phagosome (9), M. tuberculosis evades powerful intracellular killing mechanisms. Despite induction of several potent macrophage activating molecules such as tumor necrosisa (TNF α) (10) and interleukin-1 (IL-1) (11) by M. tuberculosis, intracellular replication ensues. The biologic basis for the rapid intracellular replication of M. tuberculosis is not fully understood, however, most likely is multifactorial. Potent macrophage deactivating molecules such as transforming growth factor beta (TGFβ) (12) and IL-10 (13) are induced by M. tuberculosis, which counteract all known macrophage activating cytokines (TNF α and IFNγ), and microbicidal molecules (reactive oxygen and nitrogen intermediaries) (14, 15). In addition, M. tuberculosis is well-equipped to scavenge these potent microbicidal molecules (reactive oxygen and nitrogen intermediaries) by moieties such as mycobacterial sulfatides (16) and cell wall lipoarabinomannan (17). Further, the organisms ability to alter intracellular iron concentrations, as by mycobactins (18), may allow modulation of the production of macrophage activating cytokines (19).

Soon after establishment of a focus of M. tuberculosis infection, with the recruitment of blood mononuclear cells, the process of M. tuberculosis "sensitization" of CD4 and CD8 lymphocytes is initiated. However, due to yet to be defined reasons, protective immunity requires up to 3 weeks to become adequately vigorous to contain M. tuberculosis growth. Meanwhile, uncontrolled intracellular replication continues which eventually may culminate in the rupture of infected phagocytes and spread of infection to other cells. Importantly, phagocyte rupture allows the initiation of both extracellular growth, and tissue damage culminating in caseation necrosis. As M. tuberculosis growth expands, lymphohematogenous spread allows the seeding of both pulmonary (upper lobes of the lung) and extrapulmonary sites. Finally, with the development of specific host cell-mediated immune response, mycobacterial replication is controlled and most infected individuals develop a robust life-long immunity to M. tuberculosis. Protective immunity involves the host's capacity to produce T-cell cytokines, that expand M. tuberculosis antigen reactive T-cells (IL-2) and induce macrophage activation (IFNγ), and ultimately to develop microbicidal granulomas. With this regard, the production and activity of cytokines that are crucial to the development of Th1 responses, such as by macrophage IL-12 (20), are critical to the final containment of infection. However, regardless of the development of systemic protective immunity, about 5-10% of M. tuberculosis-infected subjects retain the capacity to reactivate mycobacterial growth and development of tuberculosis after a short (1-2 years) or a prolonged (life-time) period of latency. The ability of M. tuberculosis to persist in a dormant state within host tissues is not well understood, however, most probably relates to the capacity of the pathogen to switch its metabolism from a rapid aerobic growth to a slowa naerobic growth (21). Whether the structure of the initial granulomatous response and/or the nature of cytokines induced by M. tuberculosis are important in the initiation or termination of dormancy is not known. However, conditions that weaken cell-mediated immunity increase the chance of terminating the latent state of infection and development of tuberculosis (22).

The factors that are important in the maintenance of protective immunity against M. tuberculosis infection after primary infection (i.e immunologic surveillance) are not known. In experimental animals live but not dead organisms induce protective immunity (23), and two separate T-cell subsets confer protective immunity and delayed type hypersensitivity (24). It is possible that the sustenance of successful immunological surveillance after M. tuberculosis infection is contingent on a dynamic interaction *in situ* (granuloma) which is permissive to the continuos sensitization of M. tuberculosis-reactive T-cells, which may in turn be dependent on the low grade replication of a few remaining bacilli, periodically. Under this scenario, the co-incidence of the breakdown of the immune system by co-conditions (such as HIV infection) with the low grade periodic mycobacterial replication within "healed" M. tuberculosis-infected foci, is likely to be conducive to initiation of the process of reactivation and exponential growth of M. tuberculosis. Whereas the actual events around reactivation are poorly understood, cytokines induced by the organism or its products may be instrumental in this process. With this regard again the capacity of M. tuberculosis to induce cytokines that are suppressive to T-cell function and deactivate macrophages and damage the tissues is noteworthy.

3.2. Induction of cytokines by M. tuberculosis

M. tuberculosis and its protein and non-protein antigens are strong stimuli for induction of cytokines in human mononuclear phagocytes which most likely affect the outcome of infection at any stage of M. tuberculosis infection. Early studies indicated that purified protein derivative (PPD) of M. tuberculosis induces the pro-inflammatory cytokines, IL-1 (11) and TNFα (10). On the other hand, M. tuberculosis, but not its PPD, was strong in induction of IL-12 in monocytes (25). However, M. tuberculosis (12), its PPD (26), and its cell wall lipoarabinomannan (LAM) (27), which constitutes 0.5% of mycobacterial weight (28), were potent inducers of TGFβ. Further, the effect of LAM on induction of TGFβ appeared to be dominant over induction of the pro-inflammatory cytokines (TNF α, IL-1 beta, and IL-6) and IL-10 (27). Importantly, one of the three moieties of a major secretory component of actively replicating mycobacteria (antigen 85 complex), namely 30 kD antigen (85B), strongly induces TNF α (29). Interestingly, 30 kD antigen is a fibronectin binding protein (30), and its interaction with fibronectin enhances the production of TNF α (29). More recently, 30 KD antigen has been shown to induce TGFβ (31) and IL-10 (32). Of note, TGFβ up-regulates its own production (33), 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 M. tuberculosis, its components, and its state of metabolism.

Whereas it may be argued that the mononuclear phagocyte cytokine-inducing capacity of M. tuberculosis is similar to certain "physiologic" stimuli such as bacterial LPS, it certainly is not a property shared by all bacterial products. For example, early studies showed that tetanus toxoid was an extremely poor stimulus for induction of IL-1 beta as compared to mycobacterial PPD (11). Furthermore, the persistence of M. tuberculosis infection within host tissues allows a dominant role of mononuclear cell phagocyte cytokine profile *in situ* that may not be true in the case of infection with other bacterial pathogens.

However, at any stage of M. tuberculosis infection, within granulomas, the cumulative effect of M. tuberculosis and its moieties on mononuclear cell responses and, in particular, the cytokine profile determines the success of the host in containment of mycobacterial growth. In this regard, the *in situ* balance of macrophage activating and deactivating cytokines is critical. To date, TNF α has been shown to be modest in its anti-M. tuberculosis activity in human mononuclear cell in vitro systems (12, 34). In mice, abrogation of TNF α was associated with loss of microbicidal granulomas (35). Further, M. tuberculosis infection of human alveolar macrophages as compared to autologous blood monocytes, lead to significantly higher induction of TNF α (3), and production of nitric oxide (NO) (36), which correlated with the superiority of the former cell type to contain M. tuberculosis. A role for NO in human mycobacterial infections has been suggested, although it has not been consistently shown (37). On the other hand the ability of alveolar macrophages to produce TGFβ in response to stimulation by lipopolysaccharide (LPS) was limited (38). Importantly, the potency of T-cell IFNγ as a predominant macrophage activating cytokine may be at least partly through upregulation of production of TNF α (39). Both TNF α and IFNγ induce microbicidal pathways (production of reactive oxygen and nitrogen intermediaries) in phagocytes (40). On the other hand, it appears that the main cytokines responsible for macrophage deactivation within maturing granulomas are TGFβ (41) and possibly IL-10. However, the basis for macrophage deactivation by TGFβ and IL-10 appears to be both directly, through inhibition of the generation of microbicidal molecules, and indirectly by counteraction to the effects of the macrophage activating cytokines (IFNγ and TNF α). Abrogation of TGFβ increased (12, 42), and recombinant TGFβ decreased (12) the ability of human monocytes to contain the intracellular growth of M. tuberculosis. Recently, it has also been suggested that the differential gradient of IFNγ activity across granulomas may be important in the fate of M. tuberculosis *in situ* (Orme unpublished). It is possible that other macrophage deactivating cytokines, such as IL-1 receptor antagonist (IL-1Ra) and IL-4, play a role in early M. tuberculosis granulomas. Recent data suggest that IL-1Ra is induced by M. tuberculosis in monocytes (Wilkinson, unpublished), and that both IL-4 (43) and TGFβ (44) enhance the production of IL-1Ra. Whether production and activity of IL-1Ra correlates with particular host inflammatory responses is not known.

3.3. T-cell responses during M. tuberculosis infection

Evidence for the role of T-cells, in particular CD4 cells, in protective immunity against M. tuberculosis was established initially in experimental models of tuberculosis (24). In humans, the strongest evidence for a predominant role of CD4 cells in protective immunity is evidenced by the significant susceptibility of HIV infected populations to development of active tuberculosis (45). Further, in HIV-infected patients with tuberculosis, mycobacterial load increases as CD4 depletion becomes more prominent (46). Also, tuberculous pleuritis, which is both a paucibacillary M. tuberculosis infection and self -resolving, is associated with expansion of CD4 cells and abundance of IFNγ (47) locally. However, the pathogenesis of tuberculous pleuritis likely reflects a state of "hypersensitivity" of the host to M. tuberculosis and its products, rather than true M. tuberculosis infection.

The mechanisms by which CD4 cells contribute to immunosurveillance against M. tuberculosis include the induction of Th1 cytokines, IL-2 and IFNγ, and contact between CD4 cells and mononuclear phagocytes (48). The importance of IFNγ in protective immunity to tuberculosis derives from two separate lines of evidence. Mice with a disrupted gene for IFNγ are extremely susceptible to M. tuberculosis infection (49, 50). Humans who are homozygous for a mutation in IFNγR are extremely sensitive to fatal mycobacterial infection (51). On the other hand, cytolytic activity of M. tuberculosis reactive CD4 cells towards M. tuberculosis infected target cells has been demonstrated in vitro (52). This mechanism may particularly contribute to apoptotic cell death of M. tuberculosis-infected cells at sites of active infection (53), which recently has been shown to be important in containment of intracellular mycobacterial replication (54). However, secondary to the fact that human T-cell responses to M. tuberculosis antigens are heterogeneous, it has been practically impossible to decipher particular M. tuberculosis antigens that confer protective immunity (55).

Despite a well-demonstrated role of CD8 cells in protective immunity against M. tuberculosis in mice (56), the role of this cell type in human infection remains unclear. However, when sensitive assays were employed, MHC class1-restricted CD8 responses to early secreted antigens, which presumably are targets of protective immunity (57) were shown in a subgroup of tuberculosis patients (58). Further, M. tuberculosis-specific CD8 cells appeared to function through cytolytic mechanisms (58). Similarly, whereas γδ Tcells have been shown to demonstrate innate reactivity to M. tuberculosis (59), produce IFNγ and lyse M. tuberculosis-infected targets (60), their role in immunosurveillance against M. tuberculosis remains unclear. On the other hand, both CD8 and γδ T-cells types may contribute to host defense early during primary M. tuberculosis infection.

4. CYTOKINE CIRCUITS DURING ACTIVE TUBERCULOSIS

Up to 60% of patients with active pulmonary tuberculosis, the predominant clinical form of disease,

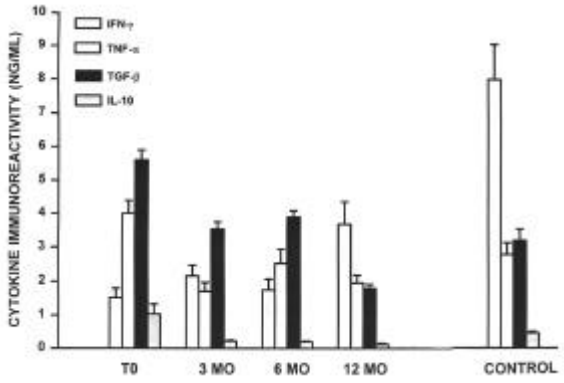

Figure 1. Longitudinal analysis of cytokines induced by PPD of M. tuberculosis in PBMC of patients with pulmonary tuberculosis and healthy PPD skin test reactive control subjects. PBMC were isolated from patients with tuberculosis at the time of diagnosis (T0), and 3, 6, and 12 months (MO) after initiation of anti-tuberculous therapy. PBMC were cultured with and without PPD (10 mg/ml), and culture supernatans harvested. PBMC from control subjects were only assessed for cytokine production concurrent with enrollment of patients at T0. Cytokines (IFNγ, TNF α, TGFβ, and IL-10) were assessed in culture supernatants. All patients were treated successfully (i.e. without treatment failure) with short course chemotherapy (6 months) (Hirsch & Ellner, unpublished data).

demonstrate suppression of their in vitro T-cell responses to mycobacterial antigens (61). Suppression of T-cell responses correlates with the extent of tuberculosis radiographically, and is associated with loss of cutaneous DTH response (i.e. PPD skin test), (62). The basis for low T-cell responses is not due to changes in the relative numbers of the main T-cell populations (63), and despite possible concentration and/or expansion of M. tuberculosis-reactive cells at sites of active infection, low responses are not due to lack of antigen-responsive T-cells in the blood (64). We have described a functional suppression of T-cell responses to mycobacterial antigens that encompasses both low Th1 cytokine production (IL-2 and IFNγ) and T-cell proliferative responses (IL-2 responses) (62, 65). The molecular basis of T-cell suppression during active tuberculosis has been identified to be predominantly TGFβ (62). Levels of TGFβ in plasma in fact correlate with extent of tuberculosis (62), and stimulated release of TGFβ corrects to that seen in healthy subjects by 3-6 months after initiation of therapy (Hirsch unpublished). Importantly, in the latter study stimulated production of IFNγ remained significantly low until time points well after completion of chemotherapy (Figure 1). These data may imply mechanisms other than and in addition to that mediated by immunosuppressive cytokines in modulation of IFNγ response during tuberculosis.

4.1. The role of monocytes in immunosuppression of tuberculosis

Evidence for a predominant role of blood monocytes in suppression of T-cell responses during tuberculosis derives from several studies. Depletion of adherent monocytes from peripheral blood mononuclear cells of patients with pulmonary tuberculosis enhances T-cell responses (65, 66), including the production of IL-2 (65) and IFNγ (31). On the other hand, small numbers of monocytes when added back to T-cell cultures, suppress T-cell functions (67). Further, monocytes from patients with tuberculosis spontaneously express IL-2R on their surface (68), and display an activation of nuclear factor k B (NFkB) (69). When cultured with exogenous IL-2, monocytes from patients remove IL-2 from supernatants (68). Also, the production of the proinflammatory cytokines, are upregulated upon *in vitro* stimulation with PPD or LPS (70, 71).

The mechanisms by which monocytes from patients with tuberculosis suppress T-cell responses are in part known. First, inspite of expression of functional IL-2 R, the blastogenic defect of PBMC is only partly corrected by exogenous IL-2 (68). On the other hand, monocytes from patients with active pulmonary tuberculosis spontaneously express TGFβ (41), and produce augmented amounts of TGFβ upon stimulation by PPD or 30 kD antigen of *M.tuberculosis* (62, 31). By contrast, concentrations of IL-10 in monocyte cultures of patients with tuberculosis were either similar (62), or only slightly increased (figure 1) as compared to that of control subjects. Abrogation of TGFβ by neutralizing antibody or natural inhibitors of TGFβ enhanced T-cell responses to PPD in PBMC of patients (62, 42).

4.2. Cytokine profile of tuberculous granulomas

Recently, Shwander et al have shown an alveolitis featured by the presence of abundant numbers of immature mononuclear phagocytes, which by cytostaing are indistinguishable from monocytes, in bronchoalveolar lavages of the tuberculosis involved lungs (as compared to the uninvolved lung) of patients with tuberculosis (72). Whereas active recruitment of blood monocytes to sites of infection underlies the latter finding, the cytokine profile of monocytes may well be reflected at sites of tuberculous granulomas. We have identified TGFβ, but not TNF α, in tuberculous granulomas of patients with active untreated tuberculosis (41). In the above mentioned study (72), despite the fact that the distribution of lung CD4 and CD8 cells simulated that of the blood, there appeared to be a concentration of naive but not memory T-cells in the tuberculosis involved lungs. However, alveolar T-cells were "hyper-responsive" to M. tuberculosis antigens as compared to PBMNC in vitro (Schwander, unpublished). Whether these dysregulations of mononuclear cells at sites of infection contribute to the inability of the host to contain M. tuberculosis or to immunopathology is not known.

4.3. Role of cytokines in immunopathology of tuberculosis

The clinical hallmark of tuberculosis include features such as fever, weight loss, and inanition. Whereas, the immunopathology of chronic M. tuberculosis infection is characterized by extensive tissue destruction, formation of cavities, and fibrosis. Many of the mentioned features of tuberculosis are likely cytokine-mediated, and correlate with cytokines measured systemically. TNF α is cytotoxic to epithelial cells, reduces the production of surfactant protein

by type II alveolar cells, promotes fibroblast activity, enhances the production of fibroblast collagenases, and promotes the production of reactive oxygen intermediaries that are cytotoxic to tissues (73). Further, TNF α potentiates the cellular toxicity of *M. tuberculosis* (39). On the other hand, excessive production of TGFβ is associated with extensive fibrosis and tissue damage (74). TGFβ is a strong inhibitor of epithelial and endothelial cell growth (75), and it both promotes the production and deposition of collagen matrix(76), and enhances tissue degradation through induction of the production of macrophage collagenases (77). In experimental animals, systemic administration of TGFβ is associated with cachexia and generalized fibrosis (78). Further, in humans undergoing chemotherapy, high plasma TGFβ correlated strongly with the development of liver and lung fibrosis (79). The pathogenesis of many human fibrosing diseases has been in fact associated with TGFβ (80).

4.3. Modulation of the host responses to M. tuberculosis

The elucidation of cytokine pathways during M. tuberculosis infection and during tuberculosis allows the application of immunomodulatory approaches to both vaccination against the organism, and management of tuberculosis. It is of note that certain cytokines, such as IL-12, boost immunologic responses to microbial antigens, and therefore may prove to be ultimately useful as adjuvants in the design of protective vaccines (81). On the other hand, in a study of immunization of mice against *Shistosoma mansoni*, vaccination routes that were conducive to expression of TGFβ were associated with failure of development of protective immunity (82). As noted, M. tuberculosis and its moieties induce TGFβ in mononuclear cells (12, 26, 27, 31). The above considerations need to be incorporated in the design of a new vaccine to successfully induce protective immunity against the M. tuberculosis.

5. M. TUBERCULOSIS INFECTION DURING HIV DISEASE

As noted, conditions that weaken cell-mediated immunity increase the chance of terminating M. tuberculosis latency subsequent to a primary infection, and increase the chance of development of tuberculosis. The fact that infection with human immunodeficiency (HIV) is the strongest risk factor for development of tuberculosis in the M. tuberculosis infected host, underscores the role of T-cells, and in particular CD4 cells, in immunosurveillance against M. tuberculosis infection. On the other hand, the fact that tuberculosis is the commonest and the earliest opportunistic infection in HIV infected subjects worldwide, in turn underscores the virulence of M. tuberculosis in humans.

However, during active M. tuberculosis infection in an HIV-infected subject, an intense interaction is initiated between the host and these pathogens which ultimately culminates in enhanced viral load (83), and augmented HIV-related mortality and morbidity (84). With regard to immunologic responses to M. tuberculosis, patients with dual infection display even more dramatic suppression of IFNγ production (85), and increase in TNF α (86) and TGFβ (85). Enhanced TNF α and TGFβ activity during dual HIV tuberculosis may contribute to augmentation of viral replication and dissemination.

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Send correspondence to: Dr Zahra Toossi, Division of Infectious Diseases, Biomedical Research Building, 10th Floor West Administration, Case Western Reserve University School of Medicine, 10900 Euclid Avenue, Cleveland, Ohio 44106-4984, Tel: (216)-368-4844, Fax:(216)-368-2034, E-mail: zxt2@po.cwru.edu