Cellular and Humoral Immune Responses During Tuberculosis Infection: Useful Knowledge in the Era of Biological Agents

Andrea Matucci, Enrico Maggi, and Alessandra Vultaggio

ABSTRACT. In this review, recent insights into innate and adaptive cellular and humoral immune response to *Mycobacterium tuberculosis* (Mtb) are discussed and the role of specific cytokines such as tumor necrosis factor-α (TNF-α) is highlighted. According to recent findings, the immune system plays a key role in avoiding mycobacteria dissemination. The importance of different cell types (macrophages, dendritic cells, interferon-γ-producing T cells) as well as the production of pro-inflammatory cytokines such as interleukin 6 (IL-6), IL-12, and IL-23/IL-17 have been demonstrated. Alveolar macrophages are considered the first cells infected by Mtb during respiratory infection. Mtb proliferates within alveolar macrophages and dendritic cells and induces the release of cytokines such as TNF-α, IL-1, IL-6, and IL-12. Toll-like receptors-stimulated dendritic cells link innate and adaptive immunity by promoting polarization of effector T cells. The efficient induction of Th1 immunity is decisive in defense against Mtb. In fact, host effector immune response against Mtb is related to the presence of a Th1 response. The definition of the cellular and molecular mechanisms involved in the immune response to Mtb can be helpful in developing new preventive strategies to avoid infection relapse, particularly in patients treated with biological agents. (JRheumatol Suppl. 2014 May; 91:17–23; doi:10.3899/jrheum.140098)

Key Indexing Terms:
TUMOR NECROSIS FACTOR-α BLOCKERS
MYCOBACTERIUM
LATENT TUBERCULOSIS
T CELLS

*Mycobacterium tuberculosis* (Mtb) infection is a major public health problem. A large body of evidence indicates that the immune system plays a key role in avoiding mycobacteria dissemination as well as in the pathogenesis of the full-blown disease. Inhaled bacteria are intercepted by macrophages in the lung, wherein they can replicate resisting innate defense mechanisms. As confirmed in experimental models, selective T cell depletion and reconstitution suggest the involvement of different immune pathways. The importance of different cell types such as macrophages, dendritic cells (DC), interferon-γ (IFN-γ)-producing T cells in the early phase of Mtb infection, as well as the production of regulatory and proinflammatory cytokines such as interleukin 6 (IL-6), IL-10, IL-12, and IL-23/IL-17, has been demonstrated. Infection with mycobacteria results in the formation of granuloma, a complex cellular structure that requires the presence of tumor necrosis factor-α (TNF-α).

We discuss the mechanisms linking the innate and adaptive (cellular and humoral) immune response involved in the pathogenesis of latent and active tuberculosis (TB), including practical clinical applications related to the use of biological agents such as anti-TNF-α blockers.

Innate Immune Response

Role of Toll-like receptors (TLR) as key receptors in the innate response to *Mycobacterium*. Among the pattern recognition receptors (PRR), TLR are expressed by many types of cells and represent crucial triggers for adaptive immune response. The activation of innate immunity is dependent on recognition of Mtb structural components of the wall such as mycolic acid, peptidoglycans, mannan, through TLR.

Among them TLR2, TLR4, and TLR9 seem to play key roles, even if studies performed in mouse models displayed conflicting results. In fact, the secondary immune response to Mtb has been shown to be efficient in TLR2–/– mice, and similarly, macrophages obtained from triple knockout mice (TLR2, TLR4, TLR9) display a normal capacity to control Mtb infection as wild type mice. On the other hand, TLR4–/– mice (C3H/HeJ) are more susceptible to lethal
infection in comparison to wild type mice, thus demonstrating a critical role of TLR4 in the development of efficient innate host response to Mtb.\(^{21}\)

The complexity of the innate response to Mtb is evident from the results on MyD88 (myeloid differentiation factor 88), an adaptor molecule bound to the cytoplasmic portion of all TLRs.\(^{22,23}\) In experimental models the lack of MyD88 is associated with a high susceptibility to infections including TB,\(^{24,25,26,27}\) probably because of defective signaling in response to IL-1\(\alpha\) and IL-1\(\beta\). MyD88 is also involved in the differentiation of effector Th17 cells, a key protective T cell subset against Mtb.\(^{29,30,31}\) Structural components of Mtb are also recognized by specific family members of C-type lectin receptor (CLR) family. Single deletion of CLR members such as DC-SIGN, dectin1, or mannose receptor has been associated with no significant effects during Mtb infection. On the other hand, in experimental models the deletion of the gene encoding the CLR adaptor molecule CAR9, shared among the different members of the CLR family, is associated with lethal Mtb infection.\(^{32}\) Recently, gene polymorphisms of these innate receptors have been demonstrated and associated with susceptibility to TB.\(^{35}\)

**Macrophages and Dendritic Cells in the Early Phase of Tuberculosis Infection**

Alveolar macrophages are considered the first cells infected by inhaled Mtb. They actively produce antimicrobial factors and they initiate the immune response against Mtb itself.\(^{36,37}\)

The early phase of infection is characterized by a progressive recruitment and accumulation of cells, all of which are progressively infected by the expanding population of Mtb.\(^{38}\) DC are likely to become infected during Mtb aerosol exposure and seem to play a key role in triggering T cell responses.\(^{39,40}\) Mtb impairs DC migration to lymph nodes and their antigen presentation capacity through the downregulation of MHC class II molecules, thus limiting an efficient adaptive immune response.\(^{41}\)

It is generally agreed that Mtb proliferates within alveolar macrophages and DC and induces release of cytokines such as TNF-\(\alpha\), IL-1, IL-6, and IL-12, which in turn activate macrophages to induce Mtb killing.\(^{42}\) IL-12 and IL-18 produced by macrophages and DC induce the production of IFN-\(\gamma\) by many T cell subsets.\(^{37,43,44,45}\) The activation of macrophages induced by IFN-\(\gamma\) produced by T cells appears to be the central event in the elimination of Mtb.\(^{46}\)

**Recruitment and Activation of Natural Killer Cells During TB Infection**

Natural killer (NK) cells, through the production of IFN-\(\gamma\), activate macrophages, which produce IL-12, IL-15, and IL-18 and expand CD8+ T and NK T cells (NKT)\(^{37,48,49}\). NK cells recognize Mtb-infected macrophages through NKP44, NKP46, and NKG2D molecules, which are the principal receptors involved in the lysis Mtb-infected cells.\(^{50,51,52}\)

NKT cells recognizing lipid antigens presented in the context of CD1a molecules, have been distinguished into invariant (iNKT) and noninvariant NKT cells.\(^{53}\) iNKT cells play a major role in the recognition of glycolipids of the Mtb wall and may be activated by microenvironmental cytokines. There is growing evidence that NKT cell deficiency might be crucial for the development of active TB in patients infected with Mtb.\(^{54}\) In fact, NKT cell levels were significantly lower in the peripheral blood of patients with pulmonary TB and extrapulmonary TB.\(^{55}\) TNF-\(\alpha\)-blockers are able to downregulate the NK- and NKT-driven mechanisms leading to an efficient Mtb killing.

**New Insight into the Role of Cytokines and Chemokines in Tuberculosis Infection**

Cytokines have regulatory effects and participate in the host defense against infectious agents. Actual participation of a number of cytokines has already been identified in TB. In the last few years, the existence of functionally polarized CD4+ T cell subsets based on their profile of cytokine secretion has accumulated. Type 1 T helper (Th1) cells produce IFN-\(\gamma\), IL-2, and TNF-\(\alpha\), which activate macrophages and are responsible for cell-mediated immunity to intracellular pathogens. By contrast, Th2 cells produce IL-4, IL-5, IL-10, and IL-13, which are responsible for promoting antibody responses and inhibiting macrophage functions.\(^{56}\) Th17 cells represent the third arm of CD4+ T cell effectors and complement the function of the Th1 and Th2 cell lineages. They selectively produce IL-17A and F and play a critical role for host defense to extracellular pathogens and fungi and are associated to autoimmunity.\(^{57}\) The classic pattern is represented by IL-1\(\alpha\) and IL-1\(\beta\); IL-2; IL-6; TNF-\(\alpha\); and IL-12.\(^{43,44,45,48,58,59,60,61,62}\) TNF-\(\alpha\) induces the production of other cytokines, such as IL-1, IL-6, and indirectly, of IFN-\(\alpha\) and IL-2, which in turn can amplify TNF-\(\alpha\) production.\(^{63}\) Although Mtb infection in the lungs is largely characterized by the presence of inflammatory cytokines, there is some evidence of regulatory activity as shown by the production of IL-10. These data are crucial, considering that IL-10 is able to downregulate the transcription of TNF-\(\alpha\) mRNA.\(^{63}\) There has been accumulating evidence that anti-TNF-\(\alpha\) therapy increases the risk of reactivating infections that are normally maintained in a latent state such as TB, and in which TNF-\(\alpha\) plays a central role.

The synthesis of IFN-\(\gamma\) is another crucial event during Mtb infection. Such a cytokine, produced by activated T, NK, and NKT cells, promotes cellular proliferation, activation of the respiratory burst, and expression of adhesion molecules and PRR by macrophages and DC.\(^{64,65}\)
The role of IFN-γ in Mtb infection has been confirmed in models of IFN-γ transgenic mice. These animals showed an increased ability to hold out against high-dose challenge with Mtb. Accordingly, IFN-γ-/- of IFN-γR KO mice are susceptible to Mtb dissemination and death. In agreement with such data, subjects with a deficiency of IFN-γR gene displayed an increased clinical severity of the infection, poor formation of granuloma, and microorganism dissemination. The role of IFN-γ was also confirmed by the results obtained in subjects with active or latent form of TB. In the latent form the production of IFN-γ and IL-2 is higher than in the active form. Recent data have also demonstrated that the IL-23/IL-17 pathway may play a role in the immune response against Mtb, particularly in maintaining the response to the microorganism and in improving the development of Th1 cells. IL-12 is the key molecule for the response to the microorganism and in improving the development of Th1 cells. IL-12 is the key molecule for the regulation of inflammatory response during Mtb infection. The effector functions of CD8+ T cells during Mtb infection are represented by the ability (1) to lyse infected cells as macrophages and DC; (2) to produce IFN-γ, although to a lesser extent than CD4+ T cells; and (3) to directly kill intracellular bacteria through the production of granzymes and perforins. It is important to note that the longterm development and function of CD8+ T cell response, in Mtb as well as in other agents infecting individuals, is closely dependent on the amounts and profile (Th1) of memory CD4+ T cells93,94,95,96. The proliferation of naive Mtb-specific T cells first occurs in the draining lymph nodes after the activation induced by the DC. Mtb-specific T cells migrate to the blood and then to the primary areas of infection in the lung (driven by tissue chemokines) and actively participate in controlling the infection. A persistent Th cell function during an acute and chronic Mtb infection is crucial for an efficient protective immune response to the microorganism.

**CD8+ T cells and Mycobacterium infection.** CD8+ T lymphocytes are another subset involved in the immune response to intracellular pathogens. The role of Mtb-specific CD8+ T cells was confirmed by their appearance in the airway lumen at the beginning of the infection. The effector functions of CD8+ T cells during Mtb infection are represented by the ability (1) to lyse infected cells as macrophages and DC; (2) to produce IFN-γ, although to a lesser extent than CD4+ T cells; and (3) to directly kill intracellular bacteria through the production of granzymes and perforins. It is important to note that the longterm development and function of CD8+ T cell response, in Mtb as well as in other agents infecting individuals, is closely dependent on the amounts and profile (Th1) of memory CD4+ T cells.

**Other lymphocytes in Mtb infection.** While the role of CD4+ T cells is well known, the importance of other lymphocytes in Mtb infection has to be better defined. Among them, γδT cells are primarily involved and are capable of lysing...
infected macrophages. When stimulated, γδT cells develop cytolytic activity and produce cytokines such as IFN-γ, TNF-α, and IL-10. IFN-γ-producing and TNF-α-producing cells have been observed in high frequency in CD4–CD8–γδT cells, particularly in patients presenting the non-severe form of the disease, while the modulatory part of the cells producing IL-10 is evident in severe TB. Through Fas ligand-dependent and independent mechanisms, γδT cells also release granzymes and perforins that exert a direct effect on Mtb and infected cells. Experimental models of Mtb infection have also demonstrated that γδT cells are the main source of IL-17, particularly during the early immune response at the mucosal level. It is very likely that TNF-α blockers also impair these cells, which actively produce TNF-α.

New insights into the role of humoral immune response to Mtb. A reappraisal of humoral immune response to Mtb in humans in addition to experimental models has been done. In fact, Mtb-specific antibodies neutralize pathogen toxins and promote opsonization and complement activation. The antibody-mediated protective mechanisms also include the ability to interfere with the adhesion of Mtb to cells. A correlation between humoral immune response and protection against Mtb infection is confirmed by results reported in children: the disseminated form of TB was associated with low levels of Mtb-specific IgG antibodies compared to children with localized infection who showed high titer of antibodies. In vitro studies have also demonstrated that the levels of anti-PPD IgG antibodies correlate with the reduction of proliferative response to tuberculin.

An efficient cross-talk between innate and adaptive immune responses is crucial in the control of Mtb infection. The specific contributions of IFN-γ CD4+ Th1 cells is well documented during Mtb infection. However, the role of other cell populations has been extensively described including Th17, NKT cells, NK cells, and B cells. Among cytokines, TNF-α is fundamental in the control of Mtb infection. The relevance of this cytokine during Mtb infection has generated new interest because of the risk of Mtb reactivation during therapy with TNF-α blockers. The definition of the cellular and molecular mechanisms involved in the immune response to Mtb in patients treated with these biologic agents can further aid in the development of new preventive strategies to avoid infection relapse.

REFERENCES


69. Katti MK. Assessment of serum IL-1, IL-2 and IFN-γ levels in patients with tuberculosis. Lung 2001;179:30-5.


