Cross-Talk Between the Immune System and Tuberculosis Pathogenesis; A Review With Emphasis on the Immune Based Treatment

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Abstract

As a globally major health problem, tuberculosis (TB) causes almost two million cases of death annually. Epidemiological studies demonstrate that a third of the world's individuals is infected with *Mycobacterium tuberculosis*. Approximately 10% of infected patients with *M. tuberculosis* develop chronic manifestation as TB. Due to HIV coinfection and emerging the drug-resistant TB, the disease has been increasing and its control has been frustrated in several parts of the world. Current diagnostic techniques and therapeutic tools for TB are not satisfactory. Consequently, it is urgently essential to establish new therapies concerning vaccines, immunotherapeutic agents to provide prosperous attempts for TB controlling. To achieve this goal, it is required to be armed with comprehensive understanding of immunobiology and immunopathogenesis of TB. This would be beneficial in designing new immune-based protections, drug discoveries, personalized medicine by choosing highly-effective immunotherapeutic interventions, identification and development of novel drug candidates. Hopefully, immunotherapies could be advantageous in modulating the immune system in patients with TB, providing efficient control of *M. tuberculosis* infection perpetuation and, therefore, its pathogenesis. This review herein attempts to describe the function of immune system in response to TB that is of the therapeutical and clinical importance. Moreover, new insights based on therapeutics to resolve TB with immunological orientation will be discussed.

Keywords: Mycobacterium tuberculosis, Tuberculosis, Therapeutic agents, Immune system.

Introduction

Tuberculosis (TB) is a major global health issue and one of the leading causes of death mediated by infectious agents. Despite enormous efforts to control the disease, estimations show that there are about 9 million new cases of TB infection with 1.5 million deaths per year.[1] Although TB affects several organs of the body, it majorly and specifically affects the lungs. In most circumstances, infections with *Mycobacterium tuberculosis* do not demonstrate symptoms and clinical manifestations, which is known as latent tuberculosis (LTBI). However, almost 10% of cases with latent infections further develop to active disease. The most common clinical symptoms of active TB include a prolonged cough combined with blood-containing sputum, sweating, fever, and weight loss.[2]

Initiation of TB infection occurs once *M. tuberculosis* reaches the pulmonary alveoli, where the bacterium invades and then replicates within the endosomes of alveolar-resident macrophages. The behavior of immune response determines the fate of infection by modifying the rate of actively replicating *M. tuberculosis* in patients with concomitant alterations in TB disease risks. During latent and early activated phases of TB, the infection is mainly intracellular and, consequently, T-cell responses play important roles for

the protective immunity. In this immune response, CD8+ T cells as well as CD4+ helper 1 T cells (Th1) through producing cytokines including tumor necrosis factor (TNF)-α, interferon (IFN)-γ, and interleukin (IL)-2 participate in controlling the *M. tuberculosis* replication.[3-5] Development of TB is likely due to dysregulated immune response as well as impaired immune regulation. In addition, a bulk of the lung damage related to TB is due to host-mediated immunopathology instead of direct virulence factors derived from *M. tuberculosis*. The modulation of immune regulation through divergence of a protective response by Th1 and CD8+ T cells, with Th2-related cytokines, TGF-β, regulatory T cells (Treg), and other immunosuppressive mediators is a key player in this regard.[6-8] Thus, host-directed therapy by applying immunomodulators might be a promising therapeutic approach to control TB. This paper aimed to focus on the immunology of TB and the immune-based therapies to control this disease.

Methods

Mechanisms of the Innate Immunity to *M. tuberculosis*

Following *M. tuberculosis* entrance into the host lungs, the surface antigens interact with several receptors like pattern recognition receptors (PRRs) including toll-like receptors (TLRs),[9] mannose receptor,[10] complement receptor 3 (CR3),[11] scavenger receptor,[10] and dendritic cell (DC)-specific intercellular-adhesion-molecule-3-grabbing non-integrin (DC-SIGN), which are commonly located on the surface of macrophages and DCs. The antigens of *M. tuberculosis* which are recognized with these receptors include CpG-containing DNA, lipoprotein, phosphatidylinositol mannoside, and mannose-capped lipoarabinomannan. Furthermore, *M. tuberculosis* surface lipoarabinomannan is recognized by the pulmonary surfactant protein D (SP-D), resulting in limitation of the intracellular growth of *M. tuberculosis* by means of promoted infusion of phagosome and lysosome.[12] Moreover, there is a cooperation among TLR-2, C-type lectin dectin-1 that interacts with *M. tuberculosis*, and cytosolic nucleotide binding and oligomerization domain-like receptors like NOD2 that binds to muramyl dipeptide, which eventuates in activation of the nuclear factor-kappa B (NF-κB) signaling pathway, which in turn facilitates the production of pro-inflammatory cytokine.[13] The cytokines which are produced and secreted upon the activation of NF-κB include IL-1, IL-12, IL-18, TNF-α, and chemokines. These chemokines mediate the recruitment of immune cells such as natural killer (NK) cells, neutrophils, T cells, DC, and macrophages to the infected tissue.[14,15] *M. tuberculosis* secrets protein ESAT-6, which suppresses the activation of NF-kB signaling pathway by inhibiting the interaction of MyD88 with the downstream molecule, namely, interleukin-1 receptor-associated kinase 4(IRAK4).[16] Activation of TLR signaling causes up-modulation of expression of both the vitamin D receptor (VDR) and the vitamin D-1-hydroxylase genes, which mediate the conversion of pro-vitamin D into the active form of vitamin D, 1,25(OH)2D3. Overexpression of these genes ultimately eventuates in production of the antimicrobial peptides such as β-defensin and cathelicidin to kill the intracellular pathogen.[17-20] On the other side, activation of DC-SIGN signaling causes the production of immunosuppressive cytokines like IL-10 and transforming growth factor (TGF)-β.[14]

Macrophages are heterogeneous and play different roles during immune response toward *M. tuberculosis* infection. The type 1 IL-23-producing macrophages promote a protective Th1 mediated immunity against infection, and type 2 IL-10-producing macrophages play a role in the suppression of immune response to *M. tuberculosis*.[21] In addition, type 2 macrophages participate in the induction of CD4+ T cells to be converted to CD25+FoxP3+mTGFβ-1+Tregs, which further suppress the immune system.[22]

Activated T cells and NK cells both secret IFN-γ which plays a role in the activation of macrophages to kill bacteria by promoting the phagosomal maturation and production of reactive oxygen species (ROS) and reactive nitrogen intermediates.[23,24] While IFN-γ contributes to the fusion of phagosome and lysosome by cell signaling pathway IRGm1 (LRG-47)[25,26] and PI3K,[27] both IL-4 and IL-13 secreted by Th2 inhibit autophagy-associated killing of bacteria by Akt signaling pathway.[28] TNF-αhas also a role in killing the intracellular *M. tuberculosis* by activating the reactive nitrogen species (RNS), thereby participating in the development of granuloma.[29]

Neutrophils are the first immune cells attracted to the infected sites that express many surface receptors as well as antimicrobial agents.[14] In vitro experiments on neutrophils demonstrate that these cells are activated during incubation with *M. tuberculosis* and are capable of limiting the bacterial growth.[29] Neutrophils produce the cathelicidin LL-37, human neutrophil peptides 1-3, and lipocalin 2, which can either kill or limit the growth of *M. tuberculosis*.[30] On the other hand, apoptotic neutrophils have a role in the activation of macrophages by producing the heat shock protein 72,[31] and granule proteins.[32] Interestingly, some studies have suggested that neutrophils have no beneficial roles in infection with *M. tuberculosis* and show a pathological function rather than a protective role in the control of active TB[30,33] (Figure 1).

Mechanisms of the Adaptive Immunity to *M. tuberculosis*

DCs and macrophages infected with *M. tuberculosis* are common antigen presenting cells (APCs), which present bacterial antigens to T and B cells of the adaptive immunity. It has been shown that IL-12p40 plays an important role in the activation of pulmonary DC during pathogen induced stimulation.[34] Furthermore, more efficient antigen presentation could occur by released apoptotic vesicles from the macrophage apoptosis procedures that provide bacterial antigens to primary mycobacteria uninfected DCs. Activation of CD8+ T cells can be diminished by decreased antigen delivery through blocking the macrophage apoptosis.[35]

The protective immune response by adaptive immunity
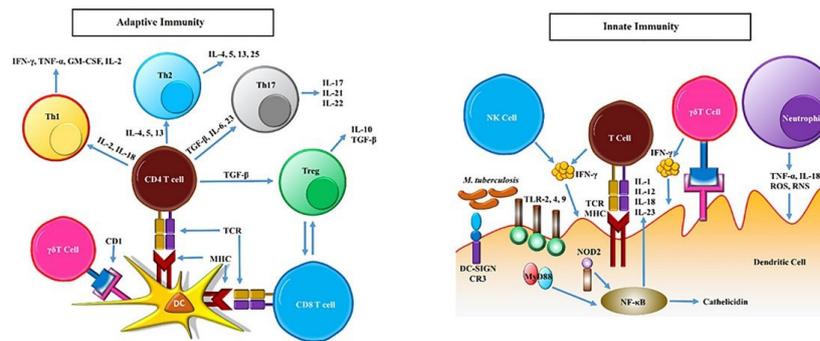
Figure 1. Cells and Molecules Involved in the Adaptive and Innate Immunity Against *Mycobacterium tuberculosis*.

during *M. tuberculosis* infection is carried out by the MHC restricted CD4^{+} and CD8^{+} T cells as well as γ-δ T lymphocytes, which participate in IFN-γ production. Thus, CD4^{+} Th1 cells are major players in TB protection and demonstrate powerful IFN-γ response, compared with CD8^{+} T cells in patients with mycobacterial infection.[36,37] Moreover, depletion of CD4^{+} T cells in *M. tuberculosis* infection culminates in the procrastinated chemotaxis of activated CD8^{+} T cells from the lymph nodes, resulting in the impaired immune protection.[38] During *M. tuberculosis* infection, CD4^{+} T cells can differentiate into several subtypes of Th1, Th2, Th17, and even regulatory T (Treg) cells. Cytokines produced by Th1 cells, namelyIL-2, IFN-γ, TNF-α, TNF-β, and granulocyte-monocyte colony-stimulating factor (GM-CSF)cause further differentiation and activation of Th1 cells, cytotoxic T lymphocytes (CTL), macrophages, and granulocytes. On the other hand, Th2 cytokines including IL-4, IL-5, IL-6, IL-9, andIL-13 cause activation and stimulation of B cells, providing antibody response. Additionally, Th2 cytokines suppress the Th1 associated immune response, that mediates a non-protective immune response toward TB. The Th17 cells play a role in the early phase of inflammatory response by producing specific cytokines such as IL-17F, IL-21, and IL-22. These cytokines are involved in the production of defensin as well as recruitment of inflammatory cells like neutrophils and monocytes to the site of infection.[39-41]

Tregs mainly belong to CD4^{+}CD25^{+}FoxP3^{+} T cells, which suppress numerous mechanisms of immune response by producing IL-10 and TGF-β.[42,43] Moreover, CD8^{+} Treg cells are involved in the inhibition of T cell proliferation and function.[44] Tregs play a role in the modulation of CD4^{+} T cell differentiation to the Th1, Th2, or Th17 subsets. The natural FoxP3 expressing Tregs have been observed to show greater expansion during *M. tuberculosis* infection.[45] These cells participate in the inhibition of IFN-γ production from γ/δ memory T cells responding to antigens from *M. tuberculosis*.[46] Treg cells also mediate their action by producing TGF-β, which prevents the activation and proliferation of CD4^{+} T cell,[47] facilitating mycobacterial infection dissemination and therefore, exacerbation of infection manifestations.[48,49] Increased numbers of Treg cells have been observed in patients with

active TB,[49] and depletion of Treg cells has been observed to improve the protective efficacy of vaccines toward infection with *M. tuberculosis*.[50,51]

For many years, B cells have been thought to have little impact in protection against TB. Animal studies have revealed a beneficial role of B cells through interactions with players of the cellular immunity as well as activation of complements, which provide an optimal protection in mice with *M. tuberculosis* infection.[52,53] Activation of the three complement alternative, classical, and lectin pathways by Bacillus Calmette–Guérin (BCG) causes the fixation of the main complement component of C3b on the surface antigens of bacteria, contributing to phagocytosis and killing of mycobacteria.[54]

Various profiles of secreted cytokines during *M. tuberculosis* infection determine the fate of CD[+] T cell differentiation and therefore play roles in the beneficial or deleterious quality of immune response to TB. A protective Th1 related response is mediated by IFN-γ, IL-12, and IL-18, whereas Th2 development is carried out by another cytokine set including IL-4, IL-5, and IL-13. TGF-β in lower doses alongside with IL-6, IL-21, and IL-23 are involved in Th17 development; however, TGF-β at higher concentrations in combination with IL-2 promote Treg differentiation.[42,43] IL-6 is involved in inflammatory responses by suppressing the TGF-β induced Treg cell development as well as by inducing the Th17 differentiation alongside with TGF-β[55] (Figure 1).

Results
Immune System-Based Therapeutics of Tuberculosis
Other Uncommon Mycobacteria
Application of *Mycobacterium vaccae* as an immunotherapeutic tool has been shown. Investigations demonstrated that single injection of *M. vaccae* could increase the conversion of sputum culture after 1 month and cause a significant improvement, regarding the radiographic manifestations, after 6 months.[56] However, a study could not show the beneficial effects of *M. vaccae* injection.[57] On the other hand, a meta-analysis concluded that intradermal injection of *M. vaccae* based immunotherapy was beneficial, considering the sputum conversion enhancement as well as amelioration of radiographic outcomes.[58] In addition, enhanced sputum

conversion in newly treated cases with TB was shown after oral administration of *M. vaccae*.[59] Promising observations have been reported using other mycobacteria like *M. indicuspranii* in the animal models of TB.[60]

Vaccines

DNA vaccines have been explored to treat the TB. Several DNA vaccines, based on genes expressing *M. tuberculosis* proteins such as ESAT-6, Hsp65, and Ag85A have been indicated to have fruitful results in mice with *M. tuberculosis* infection.[61-64] A developed DNA vaccine with cDNA3.1 plasmid as a vector that expresses *IL-2* as well as *Hsp65* genes that integrate into the virus-free envelopes, originated from the hemagglutinating virus of Japan. The intramuscular administration of the above-mentioned DNA vaccine that contained both *Hsp65* and *IL-12* genes resulted in the promoted survival rate of mice, which were infected with multidrug resistance (MDR) or extensively drug-resistant (XDR) TB. Moreover, almost the same beneficial effects were reported in another survey, which demonstrated that the DNA vaccine containing *Hsp65* and *IL-12* genes improved the survival rate of primates with *M. tuberculosis* infection.[65]

On the other side, vaccines containing other molecules have been explored to see if there are potential benefits. RUTI is a vaccine, which is built using the detoxified cellular fragments of *M. tuberculosis* and liposomes as a delivery approach.[66] It has been reported that RUTI has the potential to be used as a tool for the immunotherapy and prophylaxis against *M. tuberculosis* infection in animal models.[67] The immunogenicity and safety of this vaccine have been established in Phase I and II clinical trials.[68,69]

Mesenchymal Stem Cells

Mesenchymal stem cells (MSCs) are multipotent stromal cells that differentiate into several cell types.[70,71] There are MSCs in several tissues and organs of the body like lungs,[72,73] providing a potential in the repair of damaged tissues.[74,75] MSCs modify a tissue with chronic inflammation to a condition with capability to stimulate a strong pathogen-specific immune response. The cell-to-cell contact as well as release of mediators like prostaglandin E2 and TGF-β have been considered as the functional pathways of MSCs.[76] Administration of MSCs during 4 weeks of TB treatment as a phase I clinical trial demonstrated beneficial effects by measuring the radiological changes.[71]

V5 Immunitor

V5 immunitor is derived from the blood of patients with hepatitis B and C virus-positive and is inactivated by chemical agents and heat. It was first developed to treat patients with chronic hepatitis B and C infections.[77] It has been proposed that blood donors may have circulating *M. tuberculosis* cell fragments and antigens, which drive immune responsestoTB.[78] Moreover, the circulating cytokines and other immune mediators from the blood of donors could increase the T-cell responses to *M. tuberculosis*. However, there may be some other unknown agents, providing adjuvant function. V5 immunitor oral therapy, during a Phase I clinical trial, culminated in a remarkable conversion of sputum culture after 1 month of treatment.[79,80]

Cytokines and Inhibitors

Application of IFN-γ and IL-12 as adjuncts resulted in beneficial outcomes in some cases with MDR TB.[81-83] Moreover, IFN-γ administration in combination with intranasal IgA in murine models of TB caused a decreased load of *M. tuberculosis* in the lungs.[84] There are controversial observations in the advantageous effects of IL-2to treat TB patients.[85,86] However, intradermal injection of IL-2 to patients with MDR TB resulted in improved sputum conversion.[87] Moreover, IL-2 caused an increased activity of a pyrophosphate to promote γδ T cell responses as well as a diminished load of *M. tuberculosis* in the lungs of monkeys infected with the bacterium.[88] Anti-TNF-α antibodies play a role in increasing the risk of TB reactivation.[89] However, anti-TNF-α therapy is beneficial in the patients with active TB alongside with TB multidrug therapy. The benefits of anti-TNF-α therapy may be mediated by increased susceptibility of *M. tuberculosis* to the bactericidal activity of other drugs.[90] IL-4 and TGF-β blockade can improve Th1 type immunity and contribute to decline in the bacterial load of *M. tuberculosis* in the lungs of mice infected with the bacterium.[91,92]

Antibodies

B-cell-deficiency causes a higher bacterial load and, therefore, severe manifestations upon *M. tuberculosis* infection.[93,94] Use of monoclonal antibodies against *M. tuberculosis* antigens has conflicting outcomes. This issue may be due to differences in the strategies of administration as well as the types of antibodies.[95-97] Improved phagocytosis rate of mycobacteria has been shown following the administration of sera from the vaccinated patients with BCG. This intervention could also enhance the ability of macrophages in killing the intracellular *M. tuberculosis*.[98]

Conclusion

Many studies have been performed on the promising immunotherapy of TB. The immunotherapy may contribute to improve the management and control of MDR/XDR TB cases. A third of population are infected with *M. tuberculosis*. Hence, immunotherapeutic approaches which can help to eliminate the latent infection with *M. tuberculosis* could have a marked impression on TB control. The impacts of novel immunotherapeutics/vaccines on the reactivation or the progression of latent cases of TB in humans need to be further studied.

Ethical Approval

Not applicable.

Competing Interests
Authors declare that they have no competing interests.

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