Review

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Exploring the potential of adjunct therapy in tuberculosis

Geetha Vani Rayasam[1] **and Tanjore S Balganesh**[2]

[1] CSIR-Open Source Drug Discovery (OSDD) Unit, Council for Scientific and Industrial Research, Anusandhan Bhavan, 2 Rafi Marg, New Delhi, India
[2] CSIR-OSDD Unit, SID, Indian Institute of Science, Bangalore, India

A critical unmet need for treatment of drug-resistant tuberculosis (TB) is to find novel therapies that are efficacious, safe, and shorten the duration of treatment. Drug discovery approaches for TB primarily target essential genes of the pathogen *Mycobacterium tuberculosis* (Mtb) but novel strategies such as host-directed therapies and nonmicrobicidal targets are necessary to bring about a paradigm shift in treatment. Drugs targeting the host pathways and nonmicrobicidal proteins can be used only in conjunction with existing drugs as adjunct therapies. Significantly, host-directed adjunct therapies have the potential to decrease duration of treatment, as they are less prone to drug resistance, target the immune responses, and act via novel mechanism of action. Recent advances in targeting host–pathogen interactions have implicated pathways such as eicosanoid regulation and angiogenesis. Furthermore, several approved drugs such as metformin and verapamil have been identified that appear suitable for repurposing for the treatment of TB. These findings and the challenges in the area of host- and/or pathogen-directed adjunct therapies and their implications for TB therapy are discussed.

Burden of TB and need for adjunct therapy

TB remains one of the deadliest communicable diseases, with an estimated 1.5 million lives lost to the disease in 2013 [1]. Drug-resistant TB is a major bottleneck in the control and eradication of TB, with an estimated 480 000 new cases of multidrug resistant (MDR) TB and 210 000 deaths in 2013 [2]. Extensively drug-resistant TB (XDR TB) has been reported from several countries and an estimated 9.0% of MDR TB patients have XDR TB that is characterized by resistance to MDR TB drugs and poor treatment outcomes [2].

TB therapy is characterized by long duration of treatment to achieve sterilization and administration of multiple drugs to overcome drug resistance [1,2]. The first-line therapy typically lasts for 6 months, with an initial intensive phase for 2 months with four drugs: isoniazid (INH), rifampicin (Rif), ethambutol (ETH), and pyrazinamide, followed by 4 months continuation with Rif and INH [3]. Side effects of drugs, such as hepatitis, gastrointestinal intolerance, rash, and renal failure [4], coupled with long duration of treatment may lead to non-compliance to treatment, increasing the probability of developing drug-resistant TB. The second-line treatment of MDR patients who are resistant to INH and Rif, comprises pyrazinamide, a fluoroquinolone, an injectable antibiotic (amikacin or kanamycin or capreomycin), ethionamide (or prothionamide), and either cycloserine or para-aminosalicylic acid with treatment lasting for 18–20 months [5]. Drugs that target the causative pathogen Mtb, which were discovered more than four decades ago, still constitute the mainstay of therapy, with the exception of bedaquiline and delamanid that have received approval for MDR TB recently [6–8]. Novel drugs for TB with enhanced efficacy and an improved safety profile as compared to the current standard of care with the ability to shorten the duration of therapy are needed [6]. Only a significant decrease in duration of treatment can lead to a paradigm shift in TB therapy for which innovative approaches are needed. In addition to discovery of novel anti-TB drugs, therapy must harness advances in systems-level analysis of host–pathogen interactions towards development of novel therapies. TB therapy should expand to include drugs that are not microbicidal on their own but modulate host pathways that combat Mtb and synergistically enhance the activity of the anti-Mtb drugs. These new approaches are referred to as adjunct therapy [9]. Adjunct therapies could be either host-directed or pathogen-directed. Host-directed therapies are attractive options as they are not prone to the resistance associated with antibiotics, pathogen-directed targets such as virulence factors that are essential for pathogenicity *in vivo* but are not microbicidal by themselves are alternative therapeutic options. We explore adjunct therapy as a potential therapeutic strategy and discuss the associated advantages and challenges with this approach.

Host immune response and its modulation by pathogen

A brief overview of Mtb life cycle is discussed (Box 1) and depicted in Figure 1 to illustrate the context of action of adjunct therapies in TB [10–12]. While one-third of the global population is infected by latent Mtb, only 10% of those go on to develop active disease, highlighting the complexity in host–pathogen interactions. The incidence and progression of TB is closely related to the host immune response, and Mtb modulates both the innate and adaptive

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Corresponding author: Rayasam, G.V. (gvrayasam@csir.res.in).

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Box 1. Illustration of Mtb life cycle and host interactions as potential targets for adjunct therapies

Initiation of infection

Uptake of Mtb via different types of receptors on the alveolar macrophages initiates infection, with the survival chances of the pathogen being dependent upon the receptor involved in entry [14,60]. Upon phagocytosis, the microbicidal activity of the macrophages may contain the infection, or the pathogen virulence factors may allow escape from lysosome-mediated destruction, by preventing fusion with phagosomes [14,60]. The outcome of infection is influenced by Mtb strain and host genetic background. Interestingly, fate of Mtb in the macrophages is influenced by the death modality, with apoptosis and autophagy being beneficial to the host, while necroptosis favors the pathogen [11,61–63]. Mtb triggers anti-inflammatory responses and blocks the reactive oxygen and nitrogen intermediates generated upon macrophage activation [12]. Bacteria that escape the phagocyte endocytic trafficking replicate within macrophages, and recent studies have demonstrated that Mtb can be present in various intracellular niches [12,14].

Granuloma and TB pathogenesis

Adaptive immune response develops and bacterial growth reaches a plateau in 14–21 days [12,14,60]. Early granulomas are formed that contain infected macrophages, surrounded by mononuclear phagocytes and T lymphocytes. While host immunity contains the infection and restricts Mtb to granulomas, it is unable to eradicate the bacteria. Mtb within the granulomas are believed to exist in a dormant/latent state and can remain so for many decades in immunocompetent individuals [12,14,60]. However, when the immune system is compromised, reactivation of Mtb can lead to active disease. The precise signals and mechanisms triggering these are poorly understood.

Mature granulomas have macrophages, foamy macrophages, neutrophils, dendritic cells, B and T cells, and natural killer cells, and are characterized by regions of necrosis due to death of participating cells [64]. Extracellular Mtb within the caseous central liquefied necrotic core in the granuloma can multiply in the hypoxic, lipid-rich conditions. The growing granuloma leads to fibrosis and destroys tissues around the granuloma, leading to cavitation and release of infectious Mtb into airways where they are aerosolized in cough droplets and can spread the infection [10,12,14,60,63].

immune response [11–13]. The host mounts an initial intense proinflammatory response, followed by production of anti-inflammatory mediators to regulate tissue damage. However, Mtb has adapted to survive within this environment and subvert the immune response to its advantage. Although the immune response plays a critical role in control of infection, it is unable to eliminate Mtb and restricts the pathogen to the granulomas, thereby paradoxically aiding the persistence of the bacteria in latent form within the granulomas [11].

Macrophages play a central role in TB pathogenesis. Upon infection with Mtb, macrophages mount an innate immune response with secretion of proinflammatory cytokines and chemokines. In response to Mtb infection, there is a release of cytokines such as tumor necrosis factor (TNF)-α, interleukin (IL)-6, IL-12, IL-1β, interferon (IFN)-α, and IFN-β by macrophages and dendritic cells [10–12]. Chemokines such as chemokine CC ligand (CCL)2, CCL5 and chemokine CXC (CXC)8 that function by recruitment of inflammatory cells such as phagocytes and T and B lymphocytes, and play a role in granuloma formation and adaptive immune responses [11–13]. Upon migration of Mtb-infected cells to pulmonary lymph nodes, adaptive immune response sets in and an Mtb antigen-specific T cell response is initiated. Interestingly, onset of adaptive immune response is delayed by Mtb by deferring T cell priming, and their arrival and activity in the granuloma, which allows Mtb to expand, giving it an advantage over the host [14]. T helper (Th)1 cells of adaptive immunity play a critical role in the control of Mtb infection by producing IFN-γ while Th2 cells counter-regulate Th1 cells and produce IL-4, IL-5, and IL-13 [14–16]. In addition, regulatory T cells (Tregs) also contribute to the downmodulation of the immune response to the pathogen and to TB reactivation by blocking IFN-γ production through production of transforming growth factor (TGF)-β and IL-10 [15–17]. The goal of immunotherapy is to restore the Th1/Th2 balance by enhancing Th1 response and/or suppressing Th2 response [18]. This suggests that immunotherapy for TB can either enhance proinflammatory or suppress/neutralize anti-inflammatory responses or involve use of immunomodulators [18].

Host-directed adjunct therapies

The majority of the host-directed therapies are immunotherapies that have been reviewed extensively [13,19,20]. Here, we focus on recent developments with an emphasis on novel and repurposed drugs and pathways for which a proof-of-principle has been achieved in animal models or in the clinical setting. Therapeutic vaccines as adjunct therapy and supplementation with vitamins are not covered in this review and readers are referred to other reviews [20–22].

The lipid mediators implicated in inflammation, such as eicosanoids and prostaglandins, have emerged as potential therapeutic targets. Lipoxins (LXAs) and leukotrienes are generated from arachidoinc acid (AA) by 5- and 15-lipoxygenases (LOs), while prostagladins (PGEs) are generated from AA by cyclooxygenase (COX) I and II and prostaglandin synthases [23,24]. The balance of PGE2 and LXA4 regulates the relative amounts of apoptosis and necrosis after Mtb infection, with PGE2 protecting against necrosis and promoting apoptosis, while LXA4 inhibits COX2 and reduces PGE2 and promotes necrosis [23,24].

In studies using *Mycobacterium marinum*, a natural fish pathogen, and zebrafish as model systems, it was shown that levels of leukotriene A4 hydrolase (LTA4H), which catalyzes the production of the proinflammatory leukotriene LTB4, determines the susceptibility to *M. marinum* infection [25]. Absence or reduced activity of LTA4H leads to increased anti-inflammatory lipoxin LXA4 synthesis and TNF-α deficiency that contributes to hypersusceptibility to *M. marinum* infection [25]. Interestingly, both inadequate inflammation caused by excess lipoxins and TNF-α deficiency and hyperinflammation caused by excess LTB4 and TNF-α excess promotes bacterial growth and macrophage necrosis. Polymorphisms in LTA4H in humans influence responses to adjunct glucocorticoid therapy in TB meningitis; only patients with the proinflammatory LTA4H-high genotype respond to treatment [25]. It was hypothesized that distinct therapies would be needed for the LXA4 and LTB4 excess conditions [24]. In the high LTA4H genotype characterized by excess LTB4 levels, aspirin, which induces formation of LXA4, is beneficial, while inhibitor of 15-LO benefits the low LTA4H