Inflamm. Res. (2017) 66:1031–1056
DOI 10.1007/s00011-017-1081-z

Inflammation Research

REVIEW

Striking the right immunological balance prevents progression of tuberculosis

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Received: 18 May 2017 / Revised: 3 July 2017 / Accepted: 7 July 2017 / Published online: 15 July 2017

Abstract
Introduction Tuberculosis (TB) caused by infection with *Mycobacterium tuberculosis* (Mtb) is a major burden for human health worldwide. Current standard treatments for TB require prolonged administration of antimycobacterial drugs leading to exaggerated inflammation and tissue damage. This can result in the reactivation of latent TB culminating in TB progression. Thus, there is an unmet need to develop therapies that would shorten the duration of anti-TB treatment and to induce optimal protective immune responses to control the spread of mycobacterial infection with minimal lung pathology.
Findings Granulomata is the hallmark structure formed by the organized accumulation of immune cells including macrophages, natural killer cells, dendritic cells, neutrophils, T cells, and B cells to the site of Mtb infection. It safeguards the host by containing Mtb in latent form. However, granulomata can undergo caseation and contribute to the reactivation of latent TB, if the immune responses developed to fight mycobacterial infection are not properly controlled. Thus, an optimal balance between innate and adaptive immune cells might play a vital role in containing mycobacteria in latent form for prolonged periods and prevent the spread of Mtb infection from one individual to another.
Conclusion Optimal and well-regulated immune responses against *Mycobacterium tuberculosis* may help to prevent the reactivation of latent TB. Moreover, therapies targeting balanced immune responses could help to improve treatment outcomes among latently infected TB patients and thereby limit the dissemination of mycobacterial infection.

Keywords Tuberculosis · *Mycobacterium tuberculosis* · Macrophages · T cells · Granulomata · Autophagy · Immunopathology · Inflammation · Cytokines · Chemokines · Anti-mycobacterial therapies

Introduction

Tuberculosis (TB) poses a major threat to mankind globally [1]. Despite being curable, TB remains one of the leading causes of death worldwide [2]. There were 1.4 million TB deaths and 10.4 million new TB cases in 2015 [3]. TB is caused by the inhalation of aerosolized droplets containing the rod shaped bacilli *Mycobacterium tuberculosis* (Mtb), released from the sputum of infected TB patients [4, 5]. Mtb predominantly infects lungs, but it may also spread to various other body organs through the lymphatic system or blood to develop extrapulmonary TB [5, 6]. About 15% of TB patients suffer from extrapulmonary TB of lymph nodes, bones, joints, pleura, abdomen, central nervous system, genitourinary system, skin and meninges [5, 6]. Mycobacterial infection can lead to a spectrum of outcomes depending on the competence of the host's immune system [7]. Strong innate immune responses upon encounter with Mtb might lead to effective clearance of mycobacteria (sterilization) preventing mycobacterial infection [8]. However, following infection with mycobacteria, most of the individuals contain Mtb in latent form as a result of an immunological equilibrium between innate and adaptive immune responses [8, 9].

Responsible Editor: John Di Battista.

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Patients suffering from latent mycobacterial infection are tuberculosis skin test and IFN-γ release assay positive (TST^+ and $IGRA^+$) individuals, but are asymptomatic and non-contagious. In this phase people are able to control Mtb infection without complete elimination of mycobacteria [10]. Infection of immunocompromised people with Mtb can lead to the development of active disease within a year of infection known as primary tuberculosis [9, 10]. Moreover, disruption of immunological equilibrium in latently infected individuals might also lead to the reactivation of latent TB. Latently infected TB patients serve as potent reservoirs of Mtb dissemination and have a 5–10% probability of developing active TB disease known as post-primary tuberculosis [9, 10]. Manifestation of active TB is highly variable and is characterized based on their breadth of spread. This could be either limited to the site of infection (tuberculous pleuritis) or dissemination of mycobacteria throughout the body (miliary tuberculosis) [8]. Active TB is contagious and patients suffering from the disease present symptoms including fever, weight loss, fatigue, night sweats and cough resulting in the transmission of mycobacteria via aerosols [10]. This indicates that active TB disease is probably the chief promoter of the spread of mycobacterial infection and measures should be taken to prevent its development. Since the discovery of *Mycobacterium tuberculosis* as the causative agent of TB by Robert Koch, numerous therapeutic advancements have been made to control the spread of TB [11]. However, despite these developments, TB epidemic is increasing worldwide due to the emergence of multi-drug-resistant (MDR) and extensively drug-resistant (XDR) strains of Mtb [11]. In 2015, 480,000 new cases of multi-drug-resistant tuberculosis were reported [3]. This unrestrained spread of MDR TB is due to the limitations of current treatment, which includes prolonged use of combination of toxic anti-TB drugs such as rifampicin, isoniazid and pyrazinamide. These anti-TB drugs are known to cause gastrointestinal intolerance, hepatitis and renal failure [12]. Long-term treatment with these anti-TB drugs can lead to poor treatment outcomes and aberrant inflammatory responses in hosts. This can cause disease exacerbation and reactivation of latent infection to active TB. Hence, novel therapeutic interventions are imperative to decrease TB burden and improve the treatment outcome among TB patients.

Granulomata: wolf in sheep's clothing?

The role of granulomata in TB pathogenesis is complex [3]. Granulomata is known to serve as a shield to protect the host from tuberculous infection by restraining Mtb replication and reducing mycobacterial load [13]. The protective immune responses in the granulomata might enable containment of Mtb in latent form where mycobacteria cannot grow but can survive for prolonged periods without harming the host [13]. However, under conditions of imbalanced immune responses (hypo/hyper immune responses), granulomata might undergo remodeling and form caseum, a cheese-like structure that contains necrotic material [4]. Caseous necrotic granulomata is formed due to calcification and neutrophilic necrosis leading to formation of a caseous centre and peripheral fibrosis [4]. This event can lead to disruption of granulomata and reactivation of latent TB culminating in dissemination of mycobacteria from one individual to the other [3]. This suggests that the immune responses generated in granulomata should be maintained within optimal levels to contain Mtb in latent form and prevent the reactivation of latent TB. Immunocompromised individuals (HIV co-infected TB patients) have diminished immunity (hypo-inflammation) and can be treated with standard anti-TB therapies to boost the immune responses against Mtb [3]. This might help to restore protective immunity to fight mycobacterial infection and contain Mtb in dormant form [3]. However, hyper-inflammatory responses developed due to uncontrolled protective immunity can lead to immunopathology, and hence cannot be treated with the available therapies that involve enhancement of immune responses [3]. Treatment of MDR TB (a major roadblock in the eradication and restriction of TB disease) with standard first- and second-line of anti-TB drugs requires longer duration of treatment leading to poor therapeutic outcomes and exacerbated pro-inflammatory responses among patients [3]. This aggravated inflammation during MDR TB treatment can lead to tissue destruction and granulomata dissociation culminating in the release of Mtb from the latent granulomata [3]. This event might promote progression of active TB disease from the latent Mtb infection leading to the spread of tuberculosis. Thus it is crucial to develop novel therapeutic strategies that would mount tailored immune responses in the granulomata against drug sensitive and MDR Mtb without inducing tissue damage and immunopathology. This paradigm shift in TB therapy will help to treat TB patients with increased efficacy without developing deleterious inflammatory responses. Additionally, the co-morbidities associated with tuberculosis including type I diabetes and lung cancer will also be reduced. This indicates that development and maintenance of granulomata is crucial in TB. We will discuss the host immune responses that play a vital role in granulomata formation with a major focus on adaptive immunity. The balance between various immune components that enable granulomata maintenance and containment of latent Mtb will also be extensively reviewed here.

Host protective immune responses leading to granulomata formation

Granulomata is widely known to be host protective structure formed by the recruitment of Mtb-activated innate and adaptive immune cells particularly monocytes macrophages, neutrophils, CD4+ T cells, CD8+ T cells and B cells to the site of infection (mainly lungs). The innate and adaptive immune responses that lead to granulomata formation have been described below:

Innate immune cells provide early protection against *Mycobacterium tuberculosis*

Mycobacterium tuberculosis is an acid-fast bacterium with a complex cell wall, made up of long chain fatty acids, peptidoglycan, proteins and glycolipids [4]. Pathogenicity of Mtb is due to the presence of certain cell wall components such as mycolic acid, lipoarabinomannan, arabinogalactan, and phenolphthiocerol [14]. It has a slow doubling time of 18–24 h that correlates to chronic form of the disease [14]. Following inhalation of Mtb released from the sputum of infected patients, various Mtb ligands are recognized by pattern recognition receptors such as toll-like receptors, complement receptors, scavenger receptors and others present on the surface of alveolar macrophages and dendritic cells [5, 15, 16]. PRRs that recognize the pathogen-associated molecular patterns present on the surface of Mtb have been extensively reviewed elsewhere [8, 15]. Upon recognition of Mtb ligands, macrophages develop various effector mechanisms including phagocytosis and phagosome maturation, apoptosis and autophagy to kill Mtb and limit the spread of infection [17]. Phagocytosis of Mtb by macrophages results in the formation of phagosomes, which undergo a series of fusion events with various endocytic vesicles and lysosomes to form phagolysosomes [18]. The phagolysosomes attain anti-mycobacterial properties and kill the internalized Mtb due to the action of hydrolytic enzyme (lysozyme) and anti-microbial peptides (cathelicidin, defensins) [18, 19].

Apoptosis is used by activated macrophages to kill the intracellular mycobacteria without disrupting the plasma membrane [20]. Mtb infection elicits TNF-α production by the alveolar macrophages, and thereby stimulates extrinsic apoptotic pathway [20]. The extrinsic apoptotic pathway involves the phosphorylation of FADD-like interleukin-1β–converting enzyme (FLIPs) by TNF-α induced kinase. Phosphorylated FLIPs then undergo proteasomal degradation upon interaction with E3 ubiquitin ligase and ultimately activates caspase 8 [20]. Upon activation, caspase 8 induces caspase 3/7 activation, which then leads to apoptosis of infected macrophages [20]. Thus, apoptosis of alveolar macrophages is involved in restraining Mtb burden during early phase of mycobacterial infection by removing the niche for mycobacterial proliferation. Moreover, it also induces T cell activation by enabling cross-priming by dendritic cells, and thereby also enables activation of adaptive immune response against Mtb [21].

Autophagy is another vital effector mechanism utilized by Mtb-infected macrophages to obliterate the invading mycobacteria. Following recognition of Mtb ligands by various PRRs, numerous pro-inflammatory cytokines including IFN-γ and TNF-α are released and autophagy is induced by the alveolar macrophages [22]. Autophagy primarily augments maturation of Mtb-containing phagosomes [23]. However, it has been widely accepted recently that autophagy also directs Mtb killing by forming autophagosomes. Autophagy induces ubiquitination of the phagocytosed mycobacteria and causes the recruitment of LC3 (Microtubule-associated protein 1A/1B-light chain 3) to the phagosomes and forms autophagosomes [24]. These ubiquitinated Mtb proteins are targeted and degraded by E3 ligase in the autophagosomes, which undergo maturation to form autolysosomes. The autolysosomes thus formed possess anti-microbial peptides such as ubiquicidin, and thereby enable elimination of Mtb [23, 24].

Macrophages, following infection with Mtb also generate free radicals including reactive oxygen species (ROS) and reactive nitrogen intermediates (RNI) to restrict the growth of Mtb [17]. These radicals induce oxidation of DNA, proteins and other membrane lipids, thereby limiting mycobacterial replication [17]. Nitric oxide (NO), a potent RNI produced from L-arginine by the action of inducible nitric oxide synthase enzyme, is effectively used by macrophages to eliminate Mtb [25]. Infected macrophages also induce the activation of various signaling pathways including MAPK, Jak-STAT and NF-κB. These signaling pathways release soluble mediators: cytokines such as IL-1β, TNF-α, IFN-γ and IL-18 and chemokines belonging to CC and CXC family that enable T cell activation and development of adaptive immunity against Mtb to control infection [17]. Moreover, activated macrophages further deprive essential nutrients including glucose, fatty acids and amino acids to prevent mycobacterial growth [17]. It is known that superoxide dismutases of Mtb require zinc/manganese ions to provide protection against oxidative stress generated by the host [17]. Thus, to inhibit the subsistence of mycobacteria against the free radicals produced by the host, Mtb-infected macrophages limit the availability of these essential metals to mycobacteria, thereby preventing mycobacterial replication [17]. These mechanisms collectively contribute to the development of early immune response against Mtb. The mechanisms employed by Mtb-activated macrophages to kill mycobacteria are mentioned in the following Table 1.

Table 1 Defense strategies implemented by alveolar macrophages against *Mycobacterium tuberculosis*

Effector	Mechanism	References
Radical formation: (reactive oxygen species, reactive nitrogen intermediates)	Oxidative and nitrosative destruction of membrane lipids, DNA and tyrosine residues	[25]
Cytokine production: (IFN-γ, TNF-α, IL-1β and IL-18)	Activation of macrophages	[17]
	Adaptive immunity development	
	Nitric oxide synthesis	
Deprivation of essential nutrients: (depletion of glucose, fatty acids, amino acids and essential metals: iron, zinc, copper and manganese)	Deprivation of glucose, tryptophan and fatty acids required for mycobacterial growth	[17]
	Depletion of essential metals required by Mtb as a defense against the radicals produced by the host	

Neutrophils, natural killer cells and dendritic cells also play vital role in developing innate immune response against Mtb. The role of neutrophils in TB pathogenesis is bimodal. Following infection with Mtb, neutrophils are recruited to the site of infection to provide protection against Mtb infection by generating free radicals, releasing granules containing anti-microbial peptides and producing pro-inflammatory cytokines such as IFN-γ and TNF-α [26]. However, neutrophils are also known to be responsible for the progression of pathogenesis and tissue damage during chronic tuberculosis [27, 28]. This suggests that the role of neutrophils in TB pathogenesis is complex, and hence the influx of neutrophils at the site Mtb infection should be regulated to prevent augmentation of mycobacterial infection via inflammation.

Natural killer cells are known to kill intracellular Mtb by different mechanisms including direct release of pro-inflammatory cytokines IFN-γ and TNF-α, secretion of perforin, granulysin and granzyme, and also by the activation of alternative apoptotic pathways in granzyme-independent mechanism [29, 30]. Thus, these innate immune responses provide early control of mycobacterial growth but may not lead to complete elimination of Mtb successfully in most of the infected individuals. Hence, long-term control of Mtb infection can be achieved via dendritic cells, which following Mtb recognition present Mtb ligands to TCR of naïve CD4+ and CD8+ T cells resulting in the initiation and development of adaptive immune response [31].

Dendritic cells and initiation of T cell response

The control of mycobacterial growth depends on the complex interplay between Mtb and host's innate and adaptive immune responses [32]. As previously mentioned, in most of the individuals infected with mycobacteria, the innate immune response is potent enough for the early control of mycobacterial growth but cannot maintain protective immunity against Mtb for prolonged periods [33]. A study involving aerosol Mtb infection of wild-type mice and mice lacking αβ T cells, γδ T cells, MHC I and MHC II unraveled the importance of adaptive immune responses in controlling tuberculosis [34]. Mycobacterial growth was controlled in wild-type mice. However, mice lacking αβ T cells and MHC II succumbed to Mtb infection as a result of uncontrolled Mtb growth, necrosis and neutrophil-driven lung pathology [34]. Similarly, in a study on Mtb-infected cynomolgus macaques (non-human primate), antibody-mediated depletion of CD4+ T cells caused increased bacterial load and pathology [35]. Studies on humans suffering from HIV have also demonstrated that loss of CD4+ T cells leads to increased susceptibility to pulmonary tuberculosis [36, 37]. These studies indicate that T cell-mediated immune responses are crucial for the control of Mtb infection.

Dendritic cells (DCs) are professional antigen presenting cells, which induce T cell activation and development of adaptive immune response against pathogens [38]. The role of DCs in driving T cell activation during tuberculosis was determined in a study, wherein the depletion of CD11c+ (a marker highly expressed by dendritic cells) led to diminished initiation of adaptive immune responses resulting in uncontrolled replication of Mtb [39]. Upon interaction with Mtb, immature dendritic cell subsets in the airway epithelium induce phagocytosis of mycobacterial ligands by various PRRs present on their surface, and thereby activate naïve T cells [15, 40, 41]. Mtb infection of T cell receptor transgenic mice demonstrated that the draining lymph nodes are the site for T cell activation but not the lungs. This suggests that dendritic cells upon interaction and phagocytosis of Mtb ligands have to migrate to the draining lymph nodes to prime activation of naïve T cells [31]. DC subsets involved in priming naïve T cell activation in the mediastinal lymph nodes during tuberculosis are not completely known. DCs are a heterogeneous population of immune cells with distinct functions [42]. Various subsets of dendritic cells, including CD11c+, CD1c+, CD11b+, CD103+ and plasmacytoid DCs, are

known to enable activation of cellular immune responses [42]. However, the precise role of each subset in controlling mycobacterial infection remains unclear. In wild-type mice upon Mtb infection, two different populations of CD11c+ DCs were observed in their lung homogenates [43]. These DC subsets were distinguished based on the distinct surface markers expression, such as CD11c, Ly6C, CD13 and CD282. CD11b+ DCs, and their subpopulations were involved in secreting IL-1α and IL-1β resulting in the control of mycobacterial load and decreased mortality of Mtb-infected mice [43]. This indicates that CD11c+ DCs play a role in secreting pro-inflammatory cytokines, and thereby impart protective immunity against Mtb. Moreover, a study on *plt* mice, (mice lacking CCL19 and CCL21 chemokines) resulted in decreased recruitment of CD11b+ DCs to the lung draining lymph nodes culminating in diminished transport of mycobacteria and impaired naïve T cell activation [40]. Thus, it is suggested that CD11b+ DCs are involved in directing the transport of mycobacteria to the mediastinal lymph nodes to prime naïve T cell activation and development of adaptive immune response against mycobacteria. The role of CD103+ DCs in tuberculosis was determined by the intratracheal administration of Mtb in C57BL/6 mice [44]. It was demonstrated that following Mtb infection, CD103+ DCs transport mycobacteria to mediastinal lymph nodes and prime naïve T cell activation [44]. The importance of CD103+ DCs in restricting mycobacterial growth was also determined in this study using CLEC9A-DTR transgenic mice which lack CD103+ DCs [44]. Depletion of CD103+ DCs led to increased Mtb growth and delayed the activation of naïve CD8+ T cells [44]. Thus, it can be concluded that CD103+ DCs are primarily involved in the migration of mycobacteria to the lung draining lymph nodes, and hence enable mycobacterial growth control by inducing naïve CD8+ T cell activation. Kaufmann et al. identified the function of CD1c+ DCs in priming naïve T cell activation [45]. It was observed that following Mtb infection of PBMC-derived DCs from healthy donors, mycobacterial antigens were phagocytosed by CD1c+ DCs [45]. Mtb-infected CD1c+ DCs induced enhanced expression of CD83, HLA-DR and CD40 and elicited secretion of IL-1β, IL-6 and TNF-α resulting in priming of naïve T cell activation [45]. This study also demonstrated that the proliferation of naïve T cells by CD1c+ DCs was augmented by plasmacytoid dendritic cells during TB [45]. Hence, it is suggested that a cross-talk occurs between CD1c+ and plasmacytoid dendritic cells to induce T cell activation and control mycobacterial load. However, dendritic cells do not have enough potential to migrate to the draining lymph nodes and induce T cell activation till day 8, post-infection [31]. Earlier the reason for this delay was assumed to be low bacterial burden. However, a study involving aerosol Mtb infection of C57BL/6 mice revealed that low bacterial burden was not exclusively responsible for delayed initiation of adaptive immunity as the activation of CD4+ T cells was not accelerated with increased bacterial burden [31]. Moreover, this study also suggested that delayed activation of adaptive immune response might occur due to the presence of mycobacteria in a compartment that prevents their early migration from lungs to lymph nodes [31]. These results demanded further investigations to determine the actual cause for delayed activation of adaptive immune cells. To determine the precise cause for the delayed initiation of adaptive immune response during tuberculosis, DCs isolated from PBMC of healthy donors were infected with Mtb [46]. It was observed that upon Mtb infection there is a reduction in the expression of integrin CD18 [46]. Decreased CD18 expression resulted in impaired adherence of DCs to the walls of lung endothelial cells culminating in diminished migration of dendritic cells to the draining lymph nodes and delayed activation of cellular responses [46]. This implies that downregulation of CD18 integrin expression is an invasive strategy employed by Mtb to promote its growth and delay the onset of adaptive immunity. Ernst and his colleagues also attempted to unravel the cause for the delayed initiation of T cell responses [47]. They observed that upon intratracheal administration of Mtb-infected bone marrow-derived DCs (BMDCs) in the wild-type mice, Mtb antigen (Ag85B) was exported from the infected DCs to the uninfected DCs [47]. They hypothesized that this transport of Mtb antigens to the bystander DCs might be a mechanism utilized to bypass Mtb-induced impaired naïve T cell priming. Interestingly, they observed contradictory results when they further carried out the same study to determine the mechanism of antigen transport. They administered Mtb-infected BMDCs to the wild-type mice [48]. It was observed that the export of antigens by the infected DCs enabled the uninfected DCs to process and present Ag85B to naïve T cells but did not circumvent the delayed T cell priming [48]. Surprisingly, it was also observed that the antigen transport by Mtb-infected DCs instead of enhancing naïve T cell priming, diverted mycobacterial antigens from the classical MHC II presentation pathway [48]. This resulted in the survival and proliferation of mycobacteria as a result of suboptimal T cell activation [48]. Thus, it is suggested that the transport of mycobacterial antigens by Mtb-infected DCs to uninfected DCs might be another invasive strategy employed by mycobacteria to slow down the development of adaptive immune response.

It is also known that DCs can pick Mtb-containing apoptotic macrophages to trigger their migration to draining lymph nodes and undergo maturation [32]. However, Mtb is known to inhibit the apoptosis of macrophages by inducing lipoxin production and thereby prevents cross-

presentation of Mtb ligands to dendritic cells and T cell activation [49]. Hence, therapies inhibiting lipoxin production during early Mtb infection might also enable prevention of delayed T cell activation and ultimately arrest chronic TB development. These findings collectively indicate that impaired antigen presentation and delayed T cell priming is a major bottleneck in the activation of the adaptive immune response against Mtb. Hence, complete elimination of Mtb infection requires vaccine strategies aimed at overcoming the blockade in antigen presentation and T cell priming. Recently, Shabana Khader and her group observed that upon delivery of Mtb-primed DCs into the lungs of vaccinated mice, there was a rapid activation of vaccine-induced CD4+ T cells [50]. Moreover, the delayed activation of vaccine-induced CD4+ T cells was also altered by activating CD103+ DCs and CD40-CD40L pathway [50]. This suggests that such advancements in vaccine strategies can help to prevent early increase in mycobacterial growth by timely activation of cellular immune responses.

These studies altogether conclude that DCs are the innate immune cells that link innate and adaptive immunity by initiating cellular responses and enable efficient control of mycobacterial replication. The innate immune responses developed during Mtb infection have been demonstrated in Fig. 1.

T cell-mediated immune responses are essential for prolonged control of mycobacterial infection

CD4+ T cells and their role in controlling mycobacterial infection

Mtb ligands and apoptotic bodies containing Mtb are recognized and phagocytosed by the immature DCs. Immature DCs migrate to the draining lymph nodes in the presence of CCL19 and CCL21, wherein upregulation of MHC I, MHC II, CD40, CD54, CD58, CD80 and increased production of IL-12, TNF-α and IL-1 occurs [38]. These events indicate maturation of dendritic cells [38]. Matured dendritic cells prime naïve T cell activation, and thereby enable development of adaptive immunity at day 14, post-infection [32]. Activated T cells migrate to the site of infection and develop cellular responses to control mycobacterial infection [51]. CD4+ T cells are the predominant T cells responsible for developing adaptive immune response against Mtb [52]. Upon activation, CD4+ T cells primarily secrete various pro-inflammatory cytokines such as IFN-γ, TNF-α, IL-17, IL-9 and many more to enable tuberculosis control [53]. CD8+ T cells also play an important role in clearing Mtb. Whether these cells require CD4+ T cells for clearing TB is not clear. It was observed that in $CD4^{-/-}$ and $MHC^{-/-}$ mice, CD8+ T cells induced the production of IFN-γ and maintained CD4+ T cell-mediated responses [54, 55]. However, the cytotoxic functions of CD8+ T cells were dampened in the absence of CD4+ T cells, and the secretion of IL-2 and IL-15 required for CD8+ T cell-mediated responses were also attenuated [55]. Similarly, another study on non-human primate macaques demonstrated that CD4+ T cells are essential to maintain CD8+ T cell-mediated cytotoxic effector mechanisms against Mtb [54]. These studies collectively determined that CD4+ T cells play a vital role in controlling mycobacterial growth by secreting various soluble cytokines and by maintaining the immune responses developed by other adaptive immune cells. Moreover, it is also known that a particular subset of CD4+ T cells, Th1 cells are responsible for activating macrophages to develop various defense mechanisms to eliminate Mtb. Thus, owing to the importance of CD4+ T cells in inducing as well as maintaining the innate and adaptive immune responses against Mtb, our primary focus will be on the role of CD4+ T cells and its various subsets in controlling Mtb infection. CD4+ T cells are divided into various subsets based on the type of cytokines they produce. Five major CD4+ T cell subsets have been implicated in immune responses against mycobacteria. Given below are the functions of various T helper cell subsets in limiting Mtb infection.

Th1 cells The differentiation of CD4+ T cells into Th1 cells is induced primarily by IL-12 and IFN-γ. IL-12 enhances T-bet expression, and thereby drives polarization of CD4+ T cells towards Th1 cell phenotype [56]. IL-12 is predominantly produced by macrophages and dendritic cells and is formed by the covalent bond formed between IL-12p40 and IL-12p35 subunits [33, 57]. Studies using murine models and human alveolar macrophages have determined that IL-12 is indispensable for the production of IFN-γ and lack of IL-12p40 subunit leads to increased mycobacterial growth and mortality caused by the bacteria [58–60]. IL-12p40 subunit is shared between IL-12 and IL-23 and is bound to IL-23p19 to form IL-23 (a member of IL-12 cytokine family) [33, 57]. Using murine model of tuberculosis, it was demonstrated that lack of IL-12p35 led to decreased generation of IFN-γ-producing T cells and ultimately impaired bacterial growth control [59]. However, Th1 cells were not entirely exhausted in these mice till IL-12p40 subunit was present [59]. This suggests that IL-23 might enable maintenance of IFN-γ-induced protective responses against Mtb in the absence of IL-12 [61]. These findings collectively conclude that IL-12p40 subunit is essential for Th1-mediated protective immune responses [59]. The importance of IL-12 in inducing Th1 cell activation during TB was also observed in humans, which was evident when lack of IL-12Rβ1 on PBMCs of Mtb-infected patients reduced the accumulation of the effector memory T cells and disrupted Th1 function [62]. IL-27, which

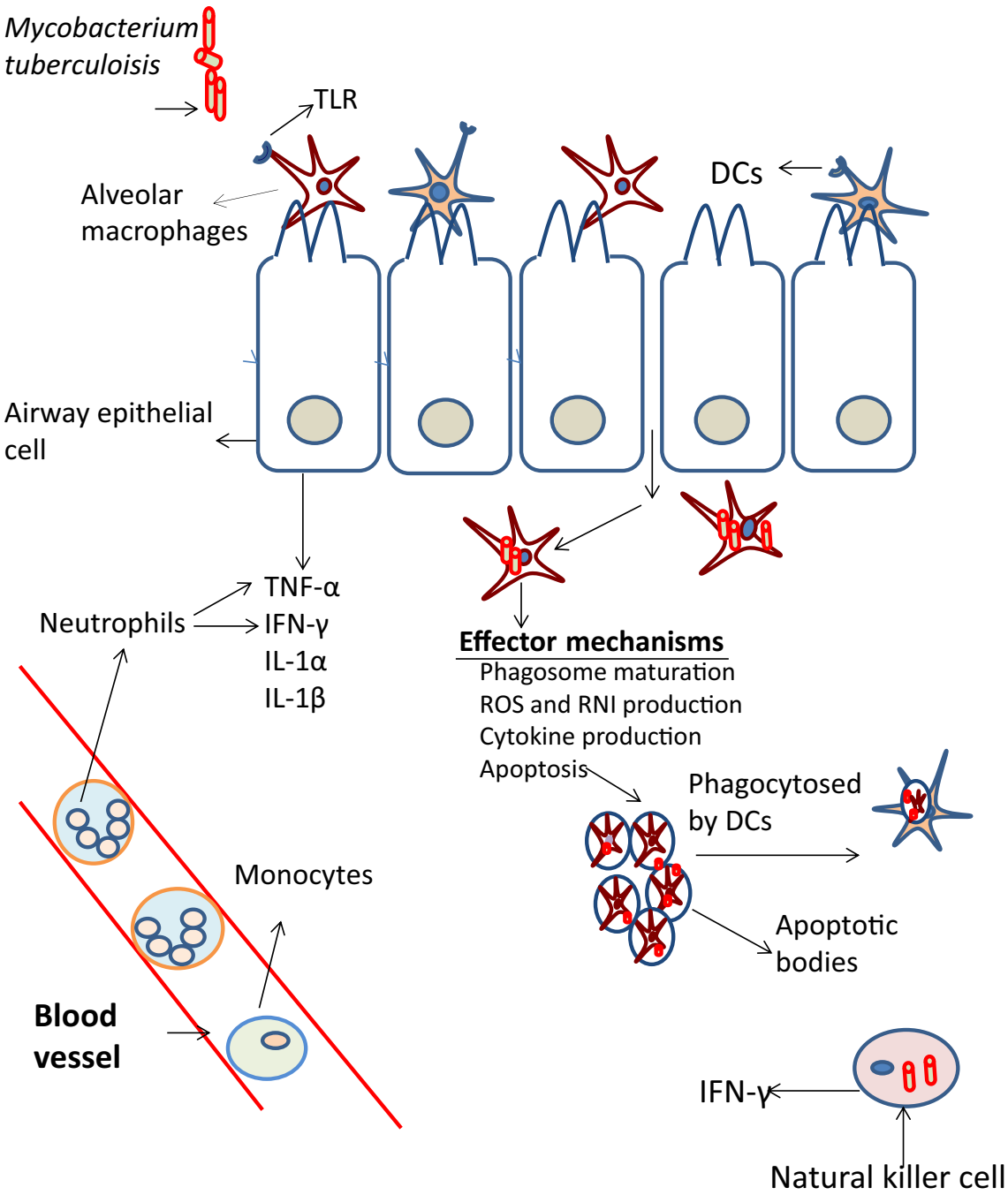


Fig. 1 Development of innate immune response during tuberculosis: recognition of Mtb ligands by TLRs and other pattern recognition receptors present on the surface of innate immune cells leads to the activation of macrophages, dendritic cells, monocytes, neutrophils and natural killer cells. Activated macrophages develop various effector mechanisms such as phagosome maturation, apoptosis, autophagy, radical formation (reactive oxygen species and reactive nitrogen intermediates) and cytokine production (IL-1β, IL-18, IFN-γ and TNF-α) to combat Mtb. Apoptotic bodies formed by the apoptosis of alveolar macrophages are picked up by the Mtb-activated dendritic cells, which then migrate to the draining lymph nodes and thereby evoke adaptive immune response to clear Mtb infection. Neutrophils, monocytes and natural killer cells also release various cytokines including IFN-γ and TNF-α to fight Mtb infection

belongs to the IL-12 family, also induces IFN-γ production from T cells as mice lacking IL-27R have significantly attenuated levels of IFN-γ [63]. However, deficiency of IL-27R did not exhibit any effect on IFN-γ producing T cells, but the expression of IFN-γ and T-bet transcript was significantly reduced, suggesting that IL-27 is responsible for enhancing and not inducing IFN-γ production from CD4+ T cells [63]. Thus, cytokines belonging to the IL-12

cytokine family are primarily responsible for Th1 cell development during tuberculosis.

IFN-γ, a type II IFN produced by immune cells including CD4+ T cells, macrophages and natural killer cells play a crucial role in fighting Mtb infection by employing various effector mechanisms [64, 65]. IFN-γ deficiency in mice led to increased susceptibility to fatal tuberculosis [66]. Moreover, deficiency of IFN-γ and its receptor IFN-γR in humans resulted in enhanced susceptibility to mycobacterial infection indicating crucial role of IFN-γ in controlling mycobacterial infection [67]. IFN-γ released by CD4+ T cells led to an increase in the CD8+ T cell-mediated cytotoxic response in C57BL/6 mice infected with Mtb, resulting in decreased mycobacterial load. This indicates that IFN-γ released by CD4+ T cells is key to enhance CD8+ T cell-mediated protective immune response in TB [68]. IFN-γ also plays a significant role in activating phagocytic macrophages and employs various defense mechanisms such as phagolysosome fusion, and apoptosis to kill Mtb [69]. Autophagy is also a key defense mechanism used by IFN-γ-activated macrophages to kill mycobacteria [23]. In a murine study, IFN-γ-induced nitric oxide production played a critical role in eliminating mycobacteria. This study demonstrated that nitric oxide produced by IFN-γ-activated macrophages led to induction of apoptosis by the activation of caspase 3/7, and thereby decreased the mycobacterial load [70]. However, other mechanisms employed by Th1 cells to control mycobacterial replication were determined in a study using IFN-γR-deficient mice [71]. This study demonstrated that lack of IFN-γ did not increase bacterial load, but loss of Th1 cells prevented mycobacterial growth control [71]. This suggested that Th1 cells possess IFN-γ-dependent as well as IFN-γ-independent mechanisms to control mycobacterial growth [71]. IFN-γ-independent mechanisms employed by Th1 cells were deduced in an experiment on PPD+ (purified protein derivative) patients. This study demonstrated that Th1 cells restrained mycobacterial growth by the release of cytotoxic granules including granulysin and granzyme in perforin and Fas/Fas ligand-independent manner [72].

Mtb-activated Th1 cells also secrete TNF-α, which by various mechanisms enables control of mycobacterial growth [73]. *TNF-α*$^{-/-}$ or *TNFR1*$^{-/-}$ mice are highly susceptible to mycobacterial infection and also succumb within few weeks upon infection with Mtb [74]. Neutralization of TNF-α by monoclonal antibodies resulted in necrosis of alveolar macrophages and prevented NO synthesis and granulomata formation in patients suffering from TB [75]. This implies that TNF-α might enable containment of Mtb in the latent form by inducing granulomata formation [76]. TNF-α enables evasion of mycobacteria by inducing apoptosis of Mtb-infected macrophages [77]. A study involving TB patients determined that TNF-α mediates apoptosis of infected macrophages and their absence leads to necrosis [77]. TNF-α also controls chemokine production, and thereby regulates leukocyte movements. A study on HIV-infected patients showed that the secretion of β chemokines was dependent on TNF-α [78]. However, neutralization of TNF-α by TNF blockers did not completely block the secretion of β chemokines such as MIP-1α and MIP-1β [78]. This result is consistent with a murine study which demonstrated that *TNF-α*$^{-/-}$ mice were not able to produce chemokines, and thereby failed to direct leukocyte migration at least two weeks, post-infection [79]. However, after 28 days chemokine synthesis and secretion was enhanced by Th1-like T cell response and thus compensated for the deficiency of TNF-α [79]. Together these findings indicate that TNF-α plays a crucial role in directing apoptosis of infected macrophages and also induces early granulomata formation. Therefore, Th1 cells play an extremely crucial role in controlling mycobacterial growth as well as in containing Mtb in the granulomata.

Th2 cells IL-4 is the key cytokine that drives Th2 cell polarization [80]. Mycobacterial infection leads to IL-4 cytokine production, and thereby induces Th2 cell generation (characterized by the secretion of IL-4 and IL-13) [80]. Th2 cells activate alternatively activated macrophages (AAMs) that inhibit protective immune response developed by classically activated macrophages (CAMs) [81]. CAMs are activated upon Mtb infection to kill the evading mycobacteria by various effector mechanisms discussed previously. Autophagy is one potential mechanism by which CAMs restrain Mtb infection. AAMs activated by Th2 cells are known to inhibit autophagy indicating a role of Th2 cells in hindering the control of mycobacterial proliferation [82]. A study on pulmonary TB patients reported that Th2 cells act via SOCS3 (suppressor of cytokine signaling 3) to inhibit IFN-γ and enhance the levels of IL-4 (that dampens protective immune response) [83]. A study on IL-13 overexpressing transgenic mice demonstrated that upon infection with Mtb, there was an increase in IL-13 production by Th2 cells in the transgenic mice compared to Th2 cells in wild-type mice [84]. This resulted in the elevation of arginase activity culminating in enhanced tissue remodeling and caseous necrosis of the granulomata [84]. Tissue damage and necrotic granulomata ultimately led to the spread of mycobacteria from the granulomata and reactivation of latent TB. This indicates that Th2 cells could prevent the development of protective immune response as well as can induce tissue injury by inducing the secretion of IL-4 and IL-13. This might lead to reactivation of latent TB. Hence, their production needs to be regulated to fight mycobacterial infection.

Th17 cells Th17 cells are also produced during tuberculosis by CD4+ T cells in the presence of cytokines IL-6 and TGF-β [85]. Th17 cells are also produced by γδ T cells upon mycobacterial infection [86]. Activated Th17 cells secrete IL-17 and other related cytokines including IL-22 [87]. It has been demonstrated that upon Mtb infection, IL-23 maintains Th17 cell differentiation and IL-17 production [33]. However, studies in C57BL/6 mice suggested that IL-23 is not absolutely essential for the development of IL-17-induced protective immune responses during TB [88]. This finding was supported by another study using macrophages generated from PBMCs of healthy donors. It was demonstrated that IL-1 family cytokines IL-2, IL-15 and IL-18 also induce the production of IL-17, indicating that IL-23 is not exclusively required for the production of IL-17. However, despite the production of IL-17 by IL-1 family cytokines, prolonged generation of IL-17 could not be maintained in the absence of IL-23 during mycobacterial infection [89]. Thus, collectively these studies suggest that though IL-23 is not indispensable for the generation of Th17 cells, it is probably essential for the sustenance of the IL-17-induced responses against Mtb for prolonged periods [89]. A study on *IL-17*$^{-/-}$ mice determined that lack of IL-17 prevented neutrophil activation when infected with *M. bovis* BCG [87]. Furthermore, this study also demonstrated that deficiency of IL-17 resulted in impaired granulomata formation and reduced IFN-γ production from Th1 cells, suggesting the importance of Th17 cells in Th1-specific response [87]. Moreover, the bacterial load was also increased in various organs such as lungs, liver and spleen in the absence of IL-17 [87]. In contrast, another study on a murine model of tuberculosis suggested that lack of IL-17 did not have a significant impact on the control of Mtb growth when infected with low bacterial load. However, in case of high bacterial loads, the absence of IL-17 prevented the control of mycobacterial load [90]. This indicates that IL-17 has a protective role in tuberculosis when infected with high bacterial load, but is dispensable in case of low bacterial burden. This paradigm that IL-17 is dispensable for the development of protective immune response was challenged in a study involving *IL-17*$^{-/-}$ and *IL-17R*$^{-/-}$ mice, which showed that infection of mice lacking IL-17 and its receptor with hypervirulent strain of Mtb HN878 leads to increased bacterial burden in lungs [91]. It was also demonstrated that overexpression of IL-17 restored the protective immune response in *IL-17*$^{-/-}$ mice by increasing the expression of CXCL13 and by enhancing the accumulation of iNOS producing macrophages [91]. These reports suggest that the significance of IL-17 in developing protective immune response during TB depends on the strain of the infecting mycobacteria and also on the bacterial load.

Th9 cells Th9 cells are IL-9-secreting subset of T cells, in which IL-9 secretion is regulated by a number of cytokines and transcription factors including TGF-β, IL-4 and STAT6, PU.1 and IRF4 [92]. Th9 cells are primarily associated with the development of allergic inflammation. Additionally, Th9 cells can impart anti-tumor and anti-helminth immune response in murine model [93]. However, the role of Th9 cells in tuberculosis remains obscure. One recent report has suggested a role of Th9 cells in regulating protective immune response against Mtb [94]. In this study, pleural mesothelial cells were shown to function as antigen presenting cells to stimulate Th9 cell differentiation [94]. It was also demonstrated that IL-9 released by Th9 cells promoted pleural mesothelial cell repair [94]. This indicated that Th9 cells might enable tissue repair and thereby prevent immunopathology. This study further demonstrated that IL-9 secreted by Th9 cells also directed inhibition of IFN-γ-induced apoptosis. This finding was corroborated by another study on TB patients, wherein exogenous IL-9 reduced IFN-γ transcript production in PBMCs of TB patients [95]. These contradictory roles of Th9 cells suggest that IL-9 secretion by Th9 cells might either contribute to the progression of TB during early infection by reducing IFN-γ production or may be involved in immunoregulation during chronic TB infection. However, the actual role of Th9 cells in TB pathogenesis is still not completely known due to lack of extensive studies. Hence, further investigations are essential to confirm the role of Th9 cells during tuberculosis.

Treg cells Treg cells are a subset of CD4+ T cells that express Foxp3 transcription factor and secrete anti-inflammatory cytokine IL-10 [96]. They are produced upon infection with Mtb from a population of pre-existing Tregs in the draining lymph nodes of the lungs [97]. These Tregs dampen the immune response by secreting anti-inflammatory cytokines IL-10, IL-35 and TGF-β resulting in delayed accumulation of effector T cells by downregulating CXCR3 expression on CD4+ T cells [98]. IL-10 produced by Treg cells can inhibit innate and adaptive protective immune response during tuberculosis [99]. IL-10 induces reactivation of latent pulmonary tuberculosis in C57BL/6 mice by reducing IFN-γ secretion and decreasing TNF-α and IL-12p40 transcript production [100]. This report was supported by a study on *IL-10*$^{-/-}$ CBA/J mice. It was demonstrated that lack of IL-10 enabled control of TB infection and prolonged the survival time by enhancing the production of Th1 cells [99]. In TB patients, it was shown that IL-10 inhibits IL-12-induced IFN-γ production. It was further demonstrated that treatment of these TB patients with anti-IL-10 reversed the anti-inflammatory effect of IL-10 by increasing IFN-γ production [101]. A study on PMA-treated THP-1 cells, monocyte-derived macrophages and human alveolar macrophages demonstrated that addition of

IL-10 arrested phagosome maturation in a STAT3-dependent manner [102]. This suggests that IL-10 abrogates the effector mechanisms developed by macrophages to prevent mycobacterial growth [102]. IL-10 is also known to reduce TNF-α production in the macrophages of Mtb-infected BALB/c mice and thereby suppresses apoptosis of Mtb-infected macrophages [103]. This result was corroborated by a study on HIV+ patients wherein pre-treatment of Mtb-infected alveolar macrophages with IL-10 led to decreased TNF-α production via upregulation of BCL-3, an inhibitor of NF-κB pathway [104]. This decreased TNF-α prevented apoptosis of Mtb-infected macrophages [104]. IL-35, a member of IL-12 cytokine family, is also known to possess immunoregulatory properties and loss of IL-35 reduces the immunosuppressive capacity of Treg cells; but their role in tuberculosis is yet to be determined [105]. This indicates that Treg cells hamper the development of protective immune response against Mtb. However, IL-10 also counterbalances inflammatory responses developed during TB-associated immune reconstitution inflammatory syndrome (immune response mediated exacerbation of tuberculosis) and thereby prevents immunopathology [106]. This suggests that optimal production of Treg cells is essential to maintain the protective immune responses against Mtb without developing pathological consequences due to hyperinflammation. Thus, it is suggested that various CD4+ T cell subsets can aid in controlling mycobacterial infection. Moreover, anti-inflammatory T cell subset, Tregs also play a vital role in preventing collateral damage that might develop as a result of uncontrolled activation of pro-inflammatory CD4+ T cell subsets.

Role of CD8+ T cells in preventing growth of Mycobacterium tuberculosis

Presentation of Mtb ligands by MHC I can lead to activation of CD8+ T cells in both humans and mice [107]. The importance of CD8+ T cells in controlling Mtb infection was determined using β2-microglobulin knockout mice. It was demonstrated that due to deficiency of β2-microglobulin, mice were unable to express MHC class I molecules, and were highly susceptible to mycobacterial infection [7]. This work is consistent with other studies on mice deficient in β2-microglobulin, TAP1, CD8α, perforin and CD1d. β2-microglobulin mice are highly susceptible to mycobacterial infection, indicating that CD8+ T cells are vital to develop protective immunity against Mtb [108, 109]. Since CD1d-deficient mice were fully resistant to Mtb infection, it was concluded that both TAP1-dependent and TAP1-independent CD8+ T cells are involved in the development of protective immune response against Mtb [109]. An experiment on cynomolgus macaques also demonstrated that depletion of CD8+ T cells resulted in caseous necrosis in the granulomata, indicating impaired containment of Mtb infection [110]. Similarly in humans, upon stimulation of PBMCs from healthy BCG vaccinated individuals with *M. bovis*, CD8+ T cells were activated and imparted protection against mycobacteria by inducing cytolysis [111]. Furthermore, study on various gene knockout mice strains including perforin-deficient, TNFR-deficient and CD95L-deficient mice indicated that a variety of cytolytic mechanisms are induced by Mtb-activated CD8+ T cells [112]. According to this study, perforin-mediated cytolysis plays a vital role for Mtb growth control. Moreover, the cytolytic killing of Mtb depends predominantly on CD95L-mediated mechanism in the absence of perforin [112]. The increased susceptibility of $Fas^{-/-}$, $FasL^{-/-}$ and $perforin^{-/-}$ mice to Mtb corroborate the importance of these pathways for immunity [69]. In humans, CD8+ T cells can kill intracellular mycobacteria via perforin/granulysin/granzyme protein production [113]. Together, these data suggest that killing of mycobacteria by CD8+ T cells is required to control tuberculosis [7].

B cells and their role in tuberculosis

B cells are antigen presenting cells that are involved in the development of humoral immune response [114]. B cells, apart from DCs present Mtb antigens to T cells and thereby enable T cell activation to develop effector mechanisms against Mtb [115]. In mice, B cells prevent excessive neutrophil accumulation during TB infection by manipulating IL-17-mediated immune responses and by negatively regulating CXCL13 secretion by follicular dendritic cells and stromal cells, resulting in the prevention of exacerbated inflammation [116, 117]. Additionally, B cell-deficiency in mice resulted in impaired recruitment of macrophages and CD8+ T cells to the lungs upon Mtb infection. This indicates that B cells play a vital role in the regulation of chemokine secretion and thereby control migration of immune cells to the lungs [4]. This suggests that B cells are probably involved in granulomata formation and also prevent the development of immunopathology following Mtb infection.

Migration of immune cells to the site of infection and granulomata formation

Following infection with Mtb, various pro-inflammatory cytokines secreted by immune cells induce the synthesis and secretion of chemokines to enable control of mycobacterial growth [68]. Chemokines are soluble mediators released by immune cells via NF-κB, p38 and ERK pathways [118, 119]. These mediators bind with their

receptors present on the surface of innate and adaptive immune cells, direct their migration to the site of infection and enable granulomata formation during tuberculosis. Granulomata is a hallmark structure formed during tuberculosis by the accumulation of immune cells to the lungs to restrict the mycobacterial growth [13]. Earlier, granulomata was believed to be formed only after the initiation of adaptive immunity [120]. However, studies on zebrafish embryos suggested that granulomata formed early after infection with *Mycobacterium marinum* is initiated by the migration of macrophages to the lungs [121]. These macrophages in the granulomata might change to multinucleated giant cells by fusion with other macrophages or may be transformed to foam cells due to lipid accumulation [122, 123]. Following development of adaptive immunity, immune cells such as monocytes, macrophages, neutrophils, dendritic cells, natural killer cells, T cells and B cells migrate to the lungs to form organized solid granulomata [68, 124]. Chemokines including CCL2, 3, 4, 5, 8, 12, 13 and CCL16 bind to surface receptors CCR2/CCR5 and direct the migration of monocytes, macrophages, dendritic cells and T cells to lungs [125]. Recruitment of neutrophils (expressing CXCR1 and CXCR2) and natural killer cells to the site of Mtb infection is directed by chemokines CXCL1, 2, 3, 5, 6, 7 and CXCL8, secreted by various innate immune cells [124, 126]. CXCR3, CXCR5 and CCR6 chemokine receptors are expressed on the surface of T cells and B cells, which bind to chemokine ligands CXCL9, 10, 11 and 13 and thereby migrate to lungs to from granulomata limiting the spread of free mycobacteria [126, 127]. Hence, chemokine-directed migration of immune cells to the lungs leads to granulomata formation around the growing mycobacteria and hampers the replication of Mtb. Moreover, it also contains the evading mycobacteria in a quiescent state for prolonged durations. The adaptive immune responses leading to granulomata formation are depicted in Fig. 2.

Balanced immune responses and containment of latent tuberculosis

Tuberculosis is a chronic disease which requires constant activation of immune system to control mycobacterial load [33]. Despite the fact that innate and adaptive immune responses enable containment of Mtb in granulomata and prevent progression of tuberculosis, a tight regulation of these immune responses is critical to prevent excess inflammation and associated pathology [28, 128]. In most of the immunocompetent individuals infected with Mtb or MDR Mtb, both pro- and anti-inflammatory immune responses are thoroughly regulated to prevent tipping off the balance towards either hypo or hyper inflammation [129, 130]. The mechanisms employed by the immune system to maintain optimal protective immune response against Mtb without causing pathological consequences are discussed below.

Balance between cytokines and lipid mediators regulate macrophage cell death patterns

Mycobacteria reside predominantly in alveolar macrophages following entry into the lungs [13]. Thus, they use alveolar macrophages as a niche to grow and replicate [15]. Alveolar macrophages have developed various effector mechanisms to prevent the growth of mycobacteria as mentioned earlier [20]. Apoptosis of alveolar macrophages being one such effector mechanism removes the niche for Mtb growth and also enhances T cell-mediated immunity by cross-presentation of apoptotic vesicles containing Mtb to naïve T cells [20]. This suggests that apoptosis of macrophages plays an important role in the elimination of mycobacteria. However, dysregulation of immune responses during chronic Mtb infection can lead to necrotic death of infected macrophages resulting in the release of free mycobacteria from the granulomata culminating in the spread of Mtb infection [131]. Thus, cell death patterns of alveolar macrophages must be regulated by the host's immune system to contain Mtb in latent form. Cytokines and lipid mediators are known to play a vital role in modulating cell death patterns. Balance between various inflammatory mediators that enable regulation of macrophage death and prevent the dissemination of mycobacteria is discussed below.

Regulation of TNF-α production by lipoxins and leukotrienes and modulation of macrophage cell death pattern

Eicosanoids are known to play a vital role as effectors and regulators of immunological balance during tuberculosis [132, 133]. Eicosanoids are lipid mediators that are produced by oxygenation of arachidonic acid, a polyunsaturated fatty acid [133]. Leukotrienes and lipoxins are lipid mediators that are produced as a result of oxygenation of arachidonic acid by the enzyme 5-lipoxygenase [134]. Leukotriene A4 hydrolase (LTA4H) enzyme drives the production of leukotriene B4 (LTB4) and its deficiency leads to the production of lipoxin A4 (LXA4, member of lipoxin family). In spite of being produced from the same precursor, LTB4 and LXA4 have opposing roles in tuberculosis. LTB4 is involved in augmenting the secretion of TNF-α, whereas LXA4 inhibits TNF-α release during mycobacterial infection [134]. As mentioned previously, TNF-α is widely known to ameliorate apoptosis and its

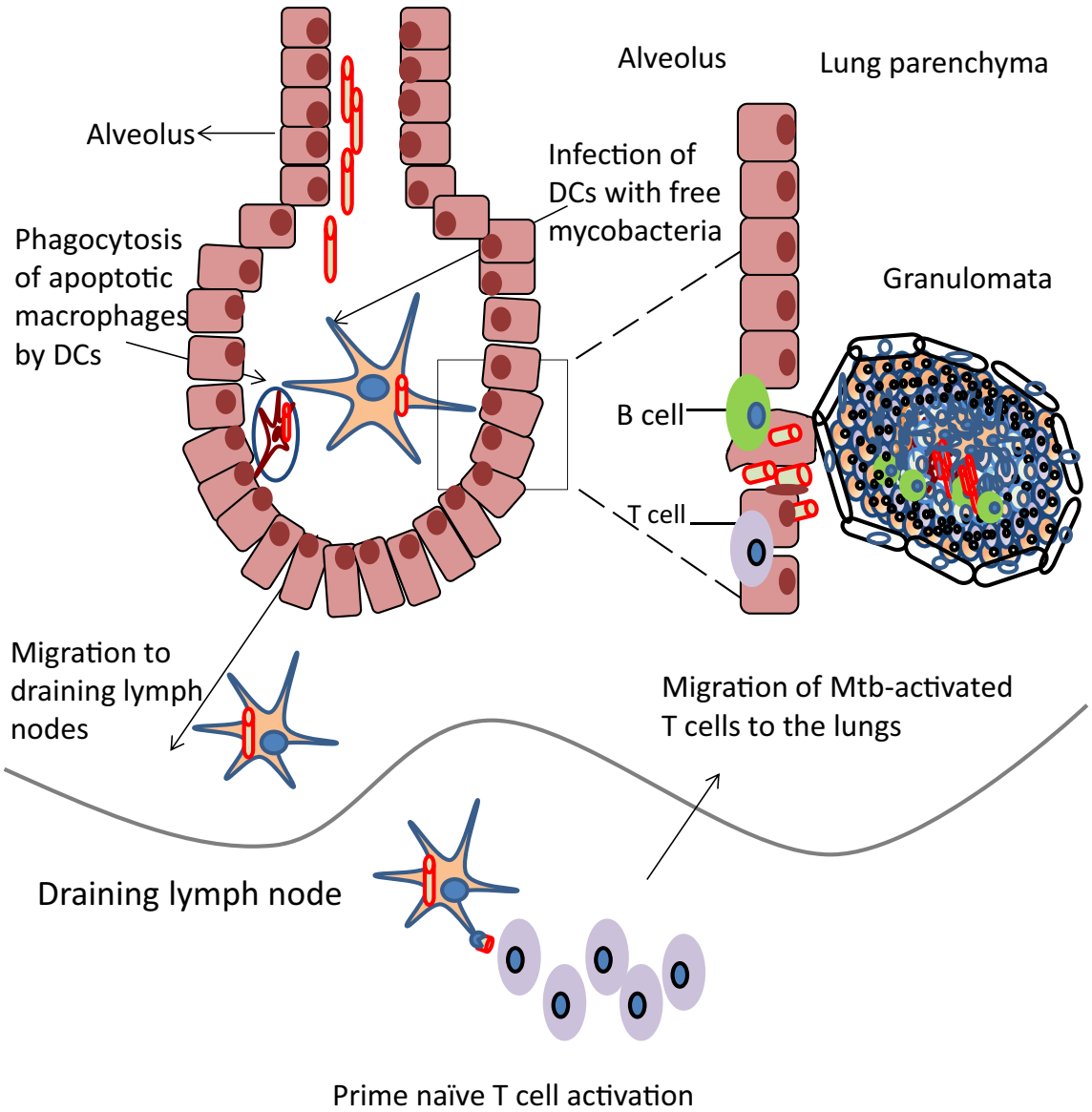


Fig. 2 Development of adaptive immune response and granulomata formation during tuberculosis: innate immune response against Mtb plays a vital role in providing early protection against mycobacterial infection. However, cell-mediated immune response is essential for the long-term control of Mtb infection. Dendritic cells are known to initiate the development of adaptive immune response against Mtb. The interaction of dendritic cells with Mtb directly or indirectly via uptake of apoptotic vesicles leads to the migration of dendritic cells to the draining lymph nodes, where they prime naïve CD4+ and CD8+ T cell activation and induce the development of adaptive immune response. Thus, dendritic cells form a bridge between innate and adaptive immune cells. Following, the development of innate and adaptive immune response against mycobacteria, chemokines generated as a result of the innate immune response against Mtb, direct the migration of monocytes, macrophages, dendritic cells, natural killer cells, neutrophils, CD4+ and CD8+ T cells and B cells to the lungs to form granulomata, which contains Mtb in the dormant form and prevents the spread of infection

absence can lead to necrosis of Mtb-infected macrophages [77]. However, several studies have identified an opposing role of TNF-α in promoting TB pathology. A study demonstrated that infection with high dose of TNF-α expressing BCG led to mycobacterial clearance in TNF-α-deficient mice but also caused severe inflammation in lungs and spleen, culminating in early death of the infected mice [131]. On the contrary, infection of TNF-deficient mice with low dose of BCG-TNF resulted in the control of bacterial growth and survival of mice. This suggests that the role of TNF-α in imparting host protection or immunopathology depends on the abundance of TNF-α at the infection site [131].

Dual role of TNF-α in TB pathogenesis has also been demonstrated in zebrafish and humans recently. A study by David Tobin and colleagues on zebrafish had demonstrated

that LTA4H, responsible for the production of LTB4 (pro-inflammatory eicosanoid), plays a crucial role in maintaining the balance between pro- and anti-inflammatory responses during TB [135]. It was observed that LTA4H locus determines the susceptibility of zebrafish to *Mycobacterium marinum* through two molecular pathways, which upon deregulation can lead to abnormal TNF-α levels [135]. Specific mutation in LTA4H locus resulted in excessive production of LTB4 leading to exuberant TNF-α secretion. High TNF-α production induced excessive inflammation, tissue damage and necroptosis (programmed necrosis) [135]. Excessive secretion of TNF-α induced uncontrolled mitochondrial ROS production via RIP1-RIP3 (receptor-interacting serine–threonine kinase)-dependent pathways [135]. ROS generated by TNF-α induced necroptosis by disrupting the mitochondrial permeability and by inducing ceramide production [136]. In addition, a hyper-susceptible genetic variation in LTA4H locus prevented LTB4 production upon mycobacterial infection, resulting in increased anti-inflammatory LXA4 production. Aberrant production of LXA4 resulted in insufficient TNF-α production and uncontrolled bacterial growth culminating in the arrest of macrophage apoptosis as well as augmented macrophage necrosis due to rampant mycobacterial growth [135]. This study was translated in humans, wherein severe TB meningitis was observed in homozygotic individuals with over or under expression of LTA4H enzyme [134]. Mycobacterial growth was controlled and host immunopathology was not observed in heterozygotes with optimal LTA4H expression [134]. These findings collectively indicate that pro- and anti-inflammatory eicosanoids (LTB4 and LXA4, respectively) regulate the way in which TNF-α modulates host macrophage cell death. Hence, a balance between LTB4 and LXA4 optimizes the level of TNF-α production and directs TNF-α-mediated apoptotic cell death of Mtb-infected macrophages. These studies indicate that finely tuned production of pro- and anti-inflammatory eicosanoids is crucial for the control of mycobacterial growth without aggravating immune responses and maintain the granulomata in the latent state for prolonged periods.

A cross-regulatory network by eicosanoids and cytokines determine the macrophage cell death pattern

As previously discussed, arachidonic acid (AA) is the precursor for various eicosanoids. Oxygenation of AA by cyclooxygenase (COX)-2, instead of lipoxygenase leads to the formation of a lipid mediator known as prostaglandin (PGE2) [134]. Lipoxin A4 is produced by the oxygenation of AA by 5-lipoxygenase (5-LO) enzyme. 5-LO and COX-2 compete for the precursor AA to produce LXA4 and PGE2, respectively. PGE2 is known to promote Mtb-infected macrophage apoptosis as well as prevent necrosis by maintaining mitochondrial membrane stability and stimulating repair of plasma membrane damage [49]. Thus, PGE2 enables early control of mycobacterial infection by promoting macrophage-mediated defense mechanisms. The importance of PGE2 in providing protection against mycobacteria was observed in a study involving a mice lacking prostaglandin E2 synthase ($Ptgs2^{-/-}$ mice) [137]. Deficiency of prostaglandin E2 synthase, which is involved in the final step of PGE2 synthesis led to enhanced susceptibility to Mtb and increased bacterial burden, suggesting the role of PGE2 in controlling Mtb growth [137]. On the contrary, LXA4 is known to inhibit CD4+ T and CD8+ T cell-mediated host immune responses. It was also demonstrated that deficiency of LXA4 in $Alox5^{-/-}$ mice led to early initiation of adaptive immunity [137]. LXA4 induces necrosis of infected macrophages by preventing the development of protective immunity against Mtb and blocking apoptosis by inhibiting pro-apoptotic mediators and caspase activation [138]. This implies that PGE2 and LXA4 have opposing roles and might counter-regulate each other to influence the outcome of mycobacterial infection and direct the macrophage cell death patterns. The production of these eicosanoids is tightly regulated by cytokines IL-1β and IFN-α/β as mentioned below.

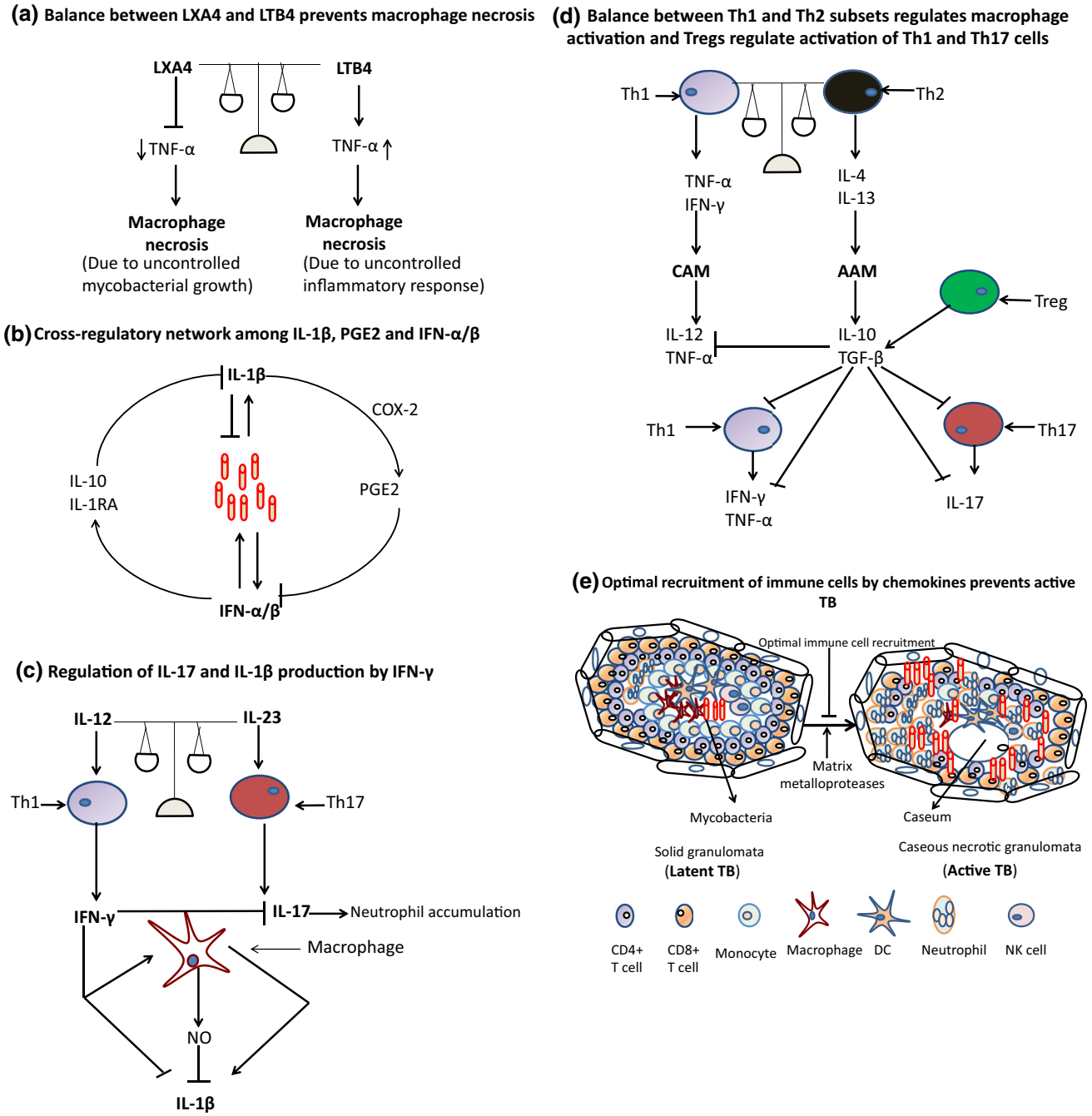
IL-1β produced by the NLRP3/ASC activated inflammasome complex also plays a vital role in developing early protective immunity against Mtb. A study on wild-type, *IL-1r*$^{-/-}$, *IL-1α/β*$^{-/-}$, *IL-1β*$^{-/-}$ *and IL-1α*$^{-/-}$ mice revealed that upon Mtb infection, IL-1β triggers PGE2 production, thereby promoting apoptosis of Mtb-infected macrophages [138]. It was concluded that the presence of both IL-1α and IL-1β prevented acute necrotic pneumonia in mice infected with Mtb and the absence of both of them led to increased inflammatory cell infiltration and acute necrosis in *IL-1α/β*$^{-/-}$ mice [139]. However, deficiency of either IL-1α or IL-1β alone did not cause severe necrosis, indicating that the absence of IL-1β is compensated by IL-1α and vice versa. This study also demonstrated that both IL-1β and PGE2 down-regulate type I interferons that are responsible for impairing mycobacterial growth control. Hence, it is indicated that IL-1β is important in restraining the progression of tuberculosis. However, despite this protective role against Mtb, it is known that hyper-activation of inflammasome pathway can lead to deleterious effects on the host [132]. Knocking out ASC in C57BL/6 mice resulted in decreased inflammation and prevented disease exacerbation by downregulating IL-1β production [140]. Thus, it is suggested that a well-regulated production of IL-1β can control mycobacterial burden and prevent immunopathology during chronic Mtb infection [140].

IFN-α and IFN-β are type I interferons having profound detrimental effect on the host [138]. IFN α/β induce immunopathology by modulating the early innate immune responses at the site of *Mtb* infection [141]. Mtb infection of mice lacking receptor for IFNα/β, display reduced mycobacterial growth [141]. Moreover, a study on patients with active tuberculosis demonstrated a type I IFN signature in the neutrophils, which dissipated upon treatment of the active TB patients [142, 143]. This indicates that type I IFN production corresponds to unregulated mycobacterial growth control [141]. When infected with Mtb, IFN-α/β also induces lipoxin (LXA4) production, resulting in decreased bacterial proliferation control and augmented macrophage necrosis [144]. However, despite their pernicious role, they are also known to induce immunoregulation and prevent the development of pathogenic responses during tuberculosis [145]. A study on mice and human volunteers infected with Mtb demonstrated that type I interferons upregulated the levels of IL-1Ra (IL-1 receptor antagonist) and also induced IL-10 production in bone marrow-derived monocytes and dendritic cells [43]. These IL-1Ra and IL-10 produced by type I interferons inhibited IL-1α and IL-1β production, and thus prevented IL-1-induced lung pathology that occurs due to dysregulated IL-1 cytokine secretion [43]. Therefore, IFNα/β is implicated in maintaining balanced IL-1β-mediated immune response to prevent lung pathology. Moreover, IL-1β counter-regulates type I interferon production by inducing COX-2 resulting in prostaglandin E2 (eicosanoid) synthesis. Prostaglandin inhibits IFN-α/β and thereby enables mycobacterial control. This suggests that a regulatory network exists among IL-1β, PGE2 and IFN-α/β to control macrophage cell death. Excess activity of either of the mediators leads to the activation of this feedback loop, and thus prevents disease exacerbation and latent TB reactivation. Therefore, balanced PGE2 and LXA4 production by IL-1β and IFNα/β will promote macrophage apoptosis without developing IL-1β-induced immunopathology. Furthermore, this balanced immune response will also enable control of mycobacterial replication in the granulomata and prevent reactivation of latent TB. How the balance between cytokines and lipid mediators regulate hyper-inflammation due to TB is described in Fig. 3a, b.

Innate immune effector nitric oxide mediates optimal cytokine production to prevent immunopathology

IL-1β is produced from pro-IL-1β by the NLRP3 activated inflammasome complex [139]. It induces protective immunity against Mtb by enhancing the production of TNF-α resulting in the apoptosis of infected macrophages [146]. As mentioned above, it also augments PGE2-mediated apoptosis and prevents necrosis of Mtb-infected macrophages by inhibiting type I IFNs. IL-1β is also known to be involved in directing neutrophilic migration to

Fig. 3 Balanced immune response in tuberculous granulomata to prevent active TB due to hyper immune response: balance between cytokines, lipid mediators, innate and adaptive immune cells enables development of optimal immune response required to control Mtb growth without developing immunopathology **a** increased LXA4 production can cause diminished TNF-α secretion resulting in macrophage necrosis due to increased mycobacterial burden. On the contrary, enhanced LTB4 production can cause hyper-secretion of TNF-α leading to macrophage necrosis due to increased inflammatory responses. A balance between LTB4 and LXA4 can help to regulate optimal production of TNF-α to impart protective immunity against Mtb without developing immunopathology. **b** A cross-regulatory network occurs among IL-1β, PGE2 and IFNα/β to control mycobacterial growth. IL-1β augments PGE2 production which in turn inhibits secretion of IFNα/β. IFNα/β activated upon Mtb infection secrete IL-10 and IL-1 receptor antagonist, and thus inhibit IL-1β production. This indicates that a feedback loop occurs among IL-1β, PGE2 and IFNα/β to maintain the protective immune responses developed against Mtb within optimal levels and prevents the immunopathology. **c** IFN-γ secreted by Th1 cells can activate macrophages to produce nitric oxide. This nitric oxide in turn prevents the secretion of active IL-1β by macrophages to prevent the aggravated inflammation that might develop due to unrestrained IL-1β secretion. IL-12 induces Th1 cell activation and IL-23 is responsible for activating Th17 cells. Th1 cells upon activation secrete pro-inflammatory cytokines IFN-γ and TNF-α. Th17 cells induce the secretion of IL-17 which is responsible for the recruitment of neutrophils to the lungs. Excessive accumulation of neutrophils can lead to immunopathology and tissue damage. IFN-γ secreted by Th1 cells inhibits IL-17 production from Th17 cells and thereby prevents granulomata disruption due to lung tissue damage. Thus, balance between IL-12 and IL-23 can lead to optimal Th1 and Th17 cell activation and prevent neutrophil-mediated lung damage during tuberculosis. **d** Th1 cells secrete IFN-γ and TNF-α upon Mtb infection, which in turn activates classically activated macrophages. Th2 cells secrete IL-4 and IL-13 leading to activation of alternatively activated macrophages. CAMs are pro-inflammatory, while AAMs are anti-inflammatory. Hyper-activation of either CAMs or AAMs can lead to dysregulated or impaired protective immunity against Mtb resulting in increased mycobacterial burden and progression to active TB. Thus, balance between Th1 and Th2 cell subsets can induce optimal activation of CAMs and AAMs to contain Mtb in latent form with minimal pathological consequences. Tregs are known to secrete anti-inflammatory cytokines IL-10 and TGF-β and thereby dampen the immune response against Mtb. Th1 and Th17 cells are required to develop protective immune response against Mtb. Even so, over-activation of both of them can lead to caseous necrosis and pulmonary damage. Tregs via secretion of IL-10 and TGF-β regulate the levels of Th1 and Th17 cells within optimal levels to prevent disintegration of granulomata and latent TB reactivation due to exacerbated inflammation. **e** Chemokines play a vital role in granulomata formation by directing migration of immune cells to the site of infection. Excessive chemokine production can lead to uncontrolled recruitment of immune cells to the lungs culminating in the secretion of matrix metalloproteinases which can degrade the lung tissue leading to granulomata disruption and latent TB reactivation. Thus, optimal secretion of chemokines by immune cells can maintain tissue integrity and latent TB for prolonged periods



the site of infection, and thus plays a vital role in granulomata formation. Even so, excessive recruitment of neutrophils in the granulomata can cause exaggerated release of hydrolytic enzymes such as collagenase and enhanced ROS production leading to tissue damage and pathology in the host [147]. Thus, unchecked activation of inflammasome pathway can be detrimental for the host and the production of IL-1β should be regulated to prevent immunopathology during chronic TB infection [140]. INF-γ secreted by Th1 cells and macrophages is known to play dual roles in TB: (1) protective response to prevent bacterial replication by inducing numerous anti-microbial mechanisms, and (2) immunoregulatory response to prevent tissue damage by suppressing pro-inflammatory pathways [147]. In the study by Ernst et al., bone marrow chimeras were developed using C57BL/6 wild-type and *IFNγR1*$^{-/-}$ mice. They observed that following infection with mycobacteria, IFN-γ induced production of NO by the Mtb-infected macrophages [147]. NO inhibited the NLRP3-ASC-caspase-1 complex and thereby prevented the

activation of pro-IL-1β to IL-1β [147]. This suggests that INF-γ-mediated nitric oxide balances the level of IL-1β within optimal range to prevent the development of pathogenic immune response and associated risk of active disease progression. The role of nitric oxide in regulating IL-1β production is depicted in Fig. 3c.

Balance between cytokines and T helper cell subsets regulates migration of neutrophils to the site of infection

IL-12 and IL-23 induce activation of Th1 and Th17 cells, respectively, upon mycobacterial infection. Both Th1 and Th17 cells release potent pro-inflammatory cytokines IFN-γ and IL-17, respectively, to control mycobacterial load [91, 148]. IFN-γ activates macrophages and enables mycobacterial growth control by various effector mechanisms [65]. IL-17 induces neutrophil accumulation at the site of infection and also plays a role in maintaining the expression of chemokines and effectors such as CXCL13 and iNOS to prevent mycobacterial growth [149]. However, during chronic infection increased Th17 cell polarization induced by IL-23 causes increased secretion of IL-17 leading to unrestrained recruitment of neutrophils to the lungs [150]. This uncontrolled neutrophil accumulation is regulated by IFN-γ produced by macrophages and Th1 cells. It acts as a regulator of protective immune responses against Mtb by inhibiting IL-17-producing T cells [149]. In a study using mice bone marrow-derived dendritic cells (BMDDCs), exogenous IFN-γ increased the production of IL-12 and reduced the levels of IL-23 production by BMDDCs [149]. Decreased IL-23 production led to reduction in the levels of IL-17 producing T cells [149]. This data was supported by a study in *IFN-γR*-deficient mice. It was observed that addition of IFN-γ to Mtb-infected *IFN-γR*$^{-/-}$ mice prevented IL-17 production from CD4+ T cells and thereby inhibited neutrophil migration to the lungs during chronic TB infection [28]. Hence, it is indicated that IFN-γ counter-regulates IL-17 production and prevents tissue damage during chronic infection. Moreover, IFN-γ produced by Th1 cells upon IL-12 stimulation also induces indoleamine 2,3-dioxygenase enzyme (IDO). IDO causes tryptophan catabolism and its by-products inhibit IL-23-dependent IL-17 production [147]. This prevents excessive recruitment of neutrophils to the lungs and restrains tissue damage in tuberculous granulomata. Hence, IL-12 and IL-23-mediated regulation of T helper cell production (Th1 and Th17) establishes a balance between secretory cytokines IFN-γ and IL-17 and thereby maintains optimal recruitment of neutrophils to the site of infection. These balanced immune responses will ultimately enable maintenance of latent TB granulomata and prevent disintegration of the granulomatous structure that occurs due to unregulated migration of neutrophils to the granulomata and associated immunopathology. The parity between T helper cells and cytokine production in preventing active TB due to hyperinflammation is described in Fig. 3c.

Balance between Th1 and Th2 cells regulates macrophage activation and controls Mtb infection

It is known that CAMs upon mycobacterial infection induce secretion of IL-12 and TNF-α resulting in Th1 cell activation. Activated Th1 cells secrete pro-inflammatory cytokines such as IFN-γ and TNF-α, which play a crucial role in controlling mycobacterial load [151]. In mice, IFN-γ released by Th1 cells in turn induces CAM activation leading to nitric oxide production as an effector molecule to impede the growth of mycobacteria. This indicates that IFN-γ acts as a bridge between innate and adaptive immunity, and is vital for the maintenance of innate and adaptive immune responses. However, it has been reported that excessive recruitment of IFN-γ-producing CD4+ T cells during chronic TB might lead to the development of Mtb-induced pulmonary caseous necrosis, suggesting that their activation should be regulated to inhibit exaggerated immune responses [152]. Moreover, uncontrolled activation of CAMs can also lead to caseous necrosis resulting in granulomata disruption and release of free Mtb, suggesting that the activation of CAMs too needs to be controlled during chronic phase of Mtb infection. AAMs are also known to have an impact on tuberculosis outcome. They possess anti-inflammatory properties and are produced as a result of activation by Th2 cytokines IL-4 and IL-13 [153]. They induce secretion of anti-inflammatory cytokines such as IL-10 and TGF-β and thereby inhibit Th1-mediated immune responses by inhibiting IFN-γ and TNF-α secretion [81]. AAMs induce arginase to block NO synthesis, thereby preventing mycobacterial load control by CAMs [81]. Thus, it is indicated that CAMs and AAMs have opposite roles during tuberculosis. These studies also suggest that even though AAMs inhibit Mtb killing and antagonize the effector mechanisms employed by CAMs, they might also prevent immunopathology that arises as a result of exacerbated immune response. This indicates that a balance between Th1 and Th2 subsets and their secretory cytokines in turn maintains the activation of CAMs (pro-inflammatory) and AAMs (anti-inflammatory). Optimal activation of CAMs and AAMs will control TB without developing immunopathology and latent TB reactivation that might arise due to aggravated IFN-γ-mediated immune responses and uncontrolled activation of CAMs. The balance between Th1 and Th2 cells responsible for regulating macrophage activation is depicted in Fig. 3d.

Regulation of Th1 and Th17 cell activation by regulatory T cells

Th1 cells play a vital in developing adaptive immune responses against Mtb by the secretion of IFN-γ and TNF-α. However, it has also been reported that unrestrained IFN-γ producing CD4+ T cells activation might lead to the development of Mtb-induced pulmonary caseous necrosis, suggesting that their activation should be regulated to inhibit exaggerated immune responses [152, 154]. Moreover, excessive secretion of TNF-α can also lead to macrophage necrosis leading to granulomata disruption and latent TB reactivation. Thus, it is important to curb the exaggerated inflammation that can occur due to over-activation of Th1 cells and their secretory cytokines. Similarly, Th17 cells are also known to direct neutrophil migration to the lungs to form granulomata, enabling containment of Mtb in quiescent state. However, excessive neutrophilic infiltration upon Mtb infection in C3HeB/FeJ mice led to increased mycobacterial load, pulmonary necrosis and progression to active TB [155]. Thus, balanced recruitment of neutrophils by Th17 cells is obligatory to prevent inflammation-mediated lung pathology. Tregs are produced upon Mtb infection from a population of pre-existing Tregs in the draining lymph nodes of the lungs [96]. These Tregs dampen the immune response by secreting IL-10 [97]. This indicates that the preliminary function of Treg cells is to prevent bacterial growth control which might play a role in the development of active TB. However, this belief is challenged owing to recent studies in mice and non-human primates, which indicate that Tregs are essential for regulating inflammation during chronic Mtb infection [156]. It was observed that deficiency of Tregs resulted in high mycobacterial burden and increased mortality [157]. A study supporting the beneficial role of Tregs in tuberculosis demonstrated that Tregs inhibited the exacerbation of immune response by decreasing IFN-γ secretion from Th1 cells and by inducing the expression of CCR4 culminating in decreased bacterial load [101]. Tregs are also known to down-regulate TNF-α secretion by Th1 cells and in turn helps in preventing macrophage necrosis induced by exaggerated inflammatory response [158]. Recently, it was observed that a balance between TNF-α and IL-10 helps to contain Mtb in latent form with minimal collateral damage [158]. This indicates that Tregs help to regulate the production of Th1 cells within optimal levels and the balance between Th1 and Treg cells will probably enable control of Mtb infection, without causing tissue damage due to over-exuberant immune responses. Tregs are also known to regulate the activation of Th17 cells [159]. It was observed that increased Treg production led to reduced mycobacterial burden by restricting neutrophilic infiltration [159]. PD-1 pathway activation is another mechanism by which the T cell-mediated immune response is regulated. PD-1 is an exhaustion marker on T cells that controls hyper-inflammatory responses generated during chronic mycobacterial infection. Thus, it is supposed that regulated expression of PD-1 might establish a balance between protective and pathogenic immune response during chronic TB infection [152]. A study on PD-1 knockout and TCR knockout mice revealed that following Mtb infection, PD-1 knockout mice produce abnormally high levels of CD4+ T cells, and are incapable of controlling mycobacterial infection [152]. Furthermore, PD-1-knockout mice also develop necrotic pulmonary lesions and exhibit increased susceptibility to TB infection [160]. Additionally, CD4+ T cell depletion prevented early mortality of PD-1 knockout mice, suggesting a role of PD-1 in protecting Mtb-infected mice from pathologic effects of aberrant CD4+ T cells production [160]. This indicates that PD-1 probably regulates the production of CD4+ T cells during TB infection to prevent disease exacerbation. The role of Tregs in regulating Th1 and Th17 cell activation is highlighted in Fig. 3d.

Autophagy: an anti-mycobacterial effector mechanism that regulates inflammation and prevents active TB

Autophagy is a mechanism by which intracellular homeostasis is maintained during starvation or infectious stress [161]. Moreover, it is also known to play an essential role in controlling mycobacterial growth by enabling phagolysosome fusion and autolysosomal degradation of mycobacterial proteins [22, 23]. Recently, autophagy has been known to play a dual role in eradicating mycobacteria as well as in preventing lung damage. Autophagy is induced in response to pro-inflammatory cytokines including IFN-γ and TNF-α [22, 23]. Upon activation, autophagy is known to down-regulate the inflammasome formation and thereby inhibiting the development of active IL-1β and IL-18 [162]. As mentioned earlier, IL-1β despite being host protective can lead to immunopathology upon excessive production [147]. Hence, autophagy helps to regulate the level of IL-1β to develop an optimal immune response with minimal collateral damage [162]. Deletion of Atg5 (autophagy related protein) gene in mice led to increased IL-1α/β levels and increased polarization of naïve T cells towards IL-17 producing Th17 cells [163]. Moreover, deficiency of Atg5 also resulted in increased mycobacterial load and lung inflammation, indicating that autophagy is highly essential to regulate the production of IL-1α/β and prevent hyperinflammation and associated tissue damage [163].

Optimal recruitment of immune cells by chemokines prevents tissue damage

Following Mtb infection, pro-inflammatory mediators including chemokines are secreted by various immune cells. These chemokines bind to the receptors present on the surface of immune cells and direct their migration to the lungs to from a well-organized aggregate of immune cells (granulomata). Thus, chemokines play a critical role in the formation of granulomata and thereby enable containment of Mtb in latent form [13]. This indicates that chemokines are essential in controlling mycobacterial growth and preventing Mtb dissemination. However, excessive accumulation of immune cells to the lungs during chronic mycobacterial infection can lead to the production and secretion of matrix metalloproteinases (MMPs) [124]. MMPs are zinc-containing proteases which are responsible for destroying the extracellular matrix surrounding the granulomata culminating in tissue damage and latent TB reactivation [124]. Amongst MMPs, MMP 7, 8 and 9 are widely known to induce tissue destruction during tuberculosis [124]. MMPs can also modulate chemokines to enhance their chemotactic activity leading to the exacerbation of inflammatory responses (cavitary TB). Cavitary TB is characterized by increased lung tissue destruction resulting in the progression to active TB among latently infected individuals [124]. This suggests that a tightly regulated secretion of chemokines by optimally activated immune cells can help to prevent cavitary TB and aid to contain Mtb in latent form for prolonged periods. The optimal recruitment of immune cells by chemokines to prevent active TB is depicted in Fig. 3e.

Improvement in anti-tuberculosis therapies to control the progression of latent to active TB

Tuberculosis is a chronic disease caused by infection with Mtb and is prevalent since decades. In spite of tremendous efforts, no gold standard therapeutic intervention has been developed to cure TB. Presently, treatment of drug-susceptible tuberculosis involves the use of four standard anti-TB drugs, rifampicin, isoniazid, pyrazinamide and ethambutol for 6–8 months [3]. Long-term administration of these first-line drugs have toxic side-effects and can lead to non-adherence amongst patients. This increases the probability of failure of anti-TB treatment and might lead to the development of multi-drug-resistant tuberculosis. Moreover, the lack of sterilization following the administration of standard first-line drugs is due to the presence of mycobacteria in a quiescent (non-replicating) state during chronic TB [164]. Rifampicin, isoniazid and ethambutol target replicating mycobacteria and the presence of non-replicating Mtb hampers their action and imparts drug resistance to the mycobacteria residing in the latent granulomata [165]. Moreover, the available first-line drugs are ineffective in treating patients latently infected with drug-susceptible and drug-resistant strains of Mtb and thereby increase the risk of reactivation of latent TB [166]. Thus, there is an urgent need to develop new therapies to treat tuberculosis with increased efficacy without aggravating the emergence of multi-drug-resistant strains of tuberculosis. Novel anti-TB drugs are also essential to improve the therapeutic outcome among latent TB patients. The future therapeutic interventions should shorten the duration of treatment to reduce the toxic side-effects of the anti-TB drugs and thereby increase the patient compliance. Furthermore, the new drugs should regulate the inflammatory responses developed due to chronic infection during latent TB. This will aid in retaining mycobacteria in latent form for prolonged periods and prevent Mtb dissemination and spread of mycobacterial infection from one individual to another. The emerging pipeline for anti-TB drugs has been reviewed elsewhere [167, 168]. To shorten the duration of the standard anti-TB drugs and curb the excessive inflammation associated with the chronic TB infection, host-directed therapies targeting various inflammatory signaling pathways are widely used recently [169]. These host-directed therapies are being used in combination with the first-line anti-TB drugs to increase their therapeutic efficacy.

As mentioned earlier, autophagy plays an antibacterial as well as an anti-inflammatory role during tuberculosis [163]. This indicates that induction of autophagy will help to clear mycobacterial infection and limit the tissue damage that arises as a result of over secretion of IL-1β during chronic TB. Recently, it has been proposed that autophagy inducers might play an essential role in controlling Mtb growth with minimal collateral damage [170]. It was observed that carbamazepine and valproic acid (autophagy inducers) led to reduced mycobacterial growth and prevented lung pathology in MDR- Mtb-infected mice, suggesting autophagy as a crucial anti-mycobacterial mechanism for regulating immune response within optimal levels [170]. Vitamin D is also known to induce autophagy to control the secretion of pro-inflammatory cytokines during tuberculosis and thereby prevents exacerbated inflammation [171]. This suggests that autophagy inducers might be used as adjunct host-directed therapies to improve the therapeutic efficacy with limited immunopathology among latent TB patients.

Lipid mediators also play a vital role in maintaining the balanced immune response during tuberculosis by regulating the death modalities of Mtb-infected macrophages. Disruption of the balance between these eicosanoids can lead to granulomata disintegration and latent TB reactivation. This suggests that adjunct therapies targeting

eicosanoids might enable development of a well-regulated optimal immune response during tuberculosis [132, 133]. PGE2 is involved in promoting macrophage apoptosis and maintaining tissue repair and thereby prevents latent TB reactivation. However, surprisingly treatment with NSAIDs such as ibuprofen that inhibits COX-2 led to diminished bacterial burden and decreased the mortality in mice model of active TB [172]. It was observed that deficiency of COX-2 resulted in increased production of LXA4, which in turn impaired TNF-α secretion. Diminished TNF-α levels prevented macrophage necrosis that occurred due to exaggerated TNF-α secretion culminating in improved survival rate and decreased mycobacterial load. On the contrary, administration of PGE2 and zileuton (5-LO inhibitor) led to decreased mycobacterial proliferation and improved survival in IL-1-deficient mice [173]. This suggests that ibuprofen and zileuton (opposing effects) can be used as adjunct therapies depending on the inflammatory status of the host to prevent macrophage necrosis and contain Mtb in latent form. As mentioned earlier, hyper-secretion of TNF-α can lead to macrophage necrosis culminating in granulomata disruption and latent TB reactivation. Thus, it is suggested that regulation of TNF-α secretion might help to prevent active TB progression during chronic Mtb infection. Administration of TNF inhibitor (etanercept) as an adjunct therapy to the standard anti-TB drugs in C3HeB/FeJ mice led to reduced mycobacterial burden [174]. Therefore, TNF-α inhibitors might be used to prevent Mtb dissemination and active TB progression.

Tissue damage induced by matrix metalloproteinases can lead to release of free Mtb from the granulomata culminating in the spread of mycobacterial infection from one individual to another. Thus, to prevent disruption of granulomata due to lung tissue destruction, MMP inhibitors could be used to maintain tissue integrity. Recently, it was observed that administration of doxycycline (MMP inhibitor) as an adjunct therapy to the anti-TB drugs led to reduced Mtb burden in guinea pigs [1].

Thus, it can be concluded that adjunct host-directed therapies directed at various inflammatory signaling pathways would help to curb overt inflammation during chronic TB. This will ultimately prevent the progression of active TB among the latently infected individuals and contain Mtb in quiescent state for prolonged periods. Therefore, development and testing of such adjunct therapies targeting various host signaling pathways should be carried out to shorten the duration of anti-TB drugs. This will help to increase the therapeutic efficacy of the available drugs and thereby eradicate TB by preventing its progression within and between the individuals. Host-directed adjunct therapies targeting various signaling pathways have been highlighted in Table 2.

Advancements in vaccines to eradicate Mtb

Since 1920, BCG is the only WHO approved vaccine used to provide long-term control of Mtb infection [174]. It imparts protective immunity against childhood TB and helps to prevent mycobacterial growth. However, it exhibits decreased efficacy to treat adult pulmonary TB (a widely prevalent form of TB). Thus, there is an urgent need to develop improved vaccines to control the spread of pulmonary TB. Progress in TB vaccines is hampered due to the lack of proper knowledge of the immune responses developed during mycobacterial infection. The vaccine-inducible immune responses that impart protection against tuberculosis remain unclear. BCG induces Th1 responses to control Mtb proliferation [175]. However, enhancements of Th1-mediated protective immune responses do not correlate with protective efficacy. The necrotic lesions observed during pulmonary TB arise as a result of hyper inflammation [176]. Furthermore, it is known that progression of active TB occurs due to excessive increase in Mtb-specific Th1 responses [175–177]. Therefore, neither diminished nor excessive inflammatory responses are imperative for the successful containment of Mtb in latent form. Vaccines targeting this fine-tuned balance between various immune components are essential to decrease TB burden.

There are mainly two types of vaccine candidates based on their target population: therapeutic and preventive

Table 2 Host-directed adjunct therapies to control mycobacterial growth with minimal immunopathology

Drug	Target	Biological effect	References
Carbamazepine Valproic acid Vitamin D supplements	Autophagy	These anti-convulsants and vitamin D induce autophagy to regulate IL-1β mediated inflammation to develop balanced protective immune responses	[170, 171]
Ibuprofen Zileuton Etanercept	Eicosanoid pathway	These drugs help to maintain a balance between eicosanoids and TNF-α secretion to prevent overt inflammation and granulomata disruption	[172–174]
Doxycycline	MMP inhibitor	It inhibits MMP-mediated lung tissue damage to maintain intact granulomata for prolonged periods	[1]

vaccines. Therapeutic vaccines have been developed to treat patients already infected with Mtb [169]. Therapeutic vaccines can be Mtb-directed and host-directed. Mtb-directed vaccines are immunogenic and are involved in limiting mycobacterial proliferation [169]. On the contrary, host-directed vaccines target regulation of inflammation and prevent tissue damage [169]. Recently, RUTI and *Mycobacterium indicus pranii* and are in phase II and phase III of clinical trials to determine their efficacy in killing mycobacteria and decrease Mtb burden [169, 178]. Presently, two host-directed therapeutic vaccines namely *Mycobacterium vaccae* and *Mycobacterium manresensis* are used to curb hyper-inflammation during chronic TB and prevent reactivation of latent TB. Administration of heat-killed *M. vaccae* led to conversion of necrotizing granulomata to protective form by striking the right balance between Th1 and Th2 cells [179]. Moreover, oral administration *M. manresensis* resulted in increased Treg cell production and simultaneous reduction in Th17 cell production [159]. This enabled regulation of inflammation and prevented the development of active TB [159]. Thus, development of such host-directed therapeutic vaccines will enable control of mycobacterial growth by containing Mtb in quiescent state with minimal tissue damage.

Conclusions

Following infection with Mtb, the disease can progress in either of two ways: (1) Mtb might manipulate the activation of pro-inflammatory signaling pathways and thereby prevent the development of protective immunity. This can lead to uncontrolled TB infection and tissue damage or (2) the mounted immune response may not be well regulated, and hence the hyper immune response might damage organs. Thus, a balance between mycobacterial killing and regulation of immune response is critical to maintain the health of the patient. In this review, we have extensively focused on the protective immune response developed during mycobacterial infection by various adaptive immune cells and particularly CD4+ T cells. It is evident that various cytokines, chemokines, lipid mediators and innate and adaptive immune cells are required to provide protection against Mtb. In spite of the knowledge about mechanisms by which various immune components control TB infection, no gold standard vaccines or therapies have been developed [180]. This is due to the lack of information regarding how these immune components interact with each other to maintain optimal levels of the inflammatory responses developed to fight Mtb infection. To fill this gap, we have mentioned the mechanisms by which the balance between various immune components is maintained. This balanced immune response will enable containment of TB infection in the latent form for prolonged periods without causing tissue damage and Mtb dissemination. This information will boost the development of vaccines and therapies targeting balanced immune responses. The novel therapeutic strategies discussed in this review will further boost the development of similar treatments that provide protection against Mtb with minimal pathology.

Future directions

Mycobacterial infection in humans can result into various possible outcomes depending on the complex interplay between Mtb and the immune system of the host as discussed previously. Most of the individuals infected with Mtb contain the evading bacteria in latent form which upon reactivation can lead to the spread of mycobacteria from one individual to another. Hence, there is an urgent need to develop therapies targeting the patients infected with latent Mtb infection to prevent further progression and dissemination of mycobacterial infection. Murine models are used to determine the efficacy of the new anti-TB drugs and therapies. However, mice models poorly mimic the tuberculous lesions that are present in humans during various stages of Mtb infection [178, 181]. As a result, translation of the anti-TB drug efficacy results from mice models to humans is difficult. Thus, more studies should be carried out on model organisms including rabbits, guinea pigs and non-human primates which are more likely to mimic the granulomatous lesions prevalent during latent Mtb infection in humans.

There are several factors that predispose a patient suffering from latent TB infection to develop active disease including various environmental factors, variations in genetic makeup of the individual, aberrant immune responses as well as the strain of infecting mycobacteria. Amongst these, the genetic polymorphism is widely known to be responsible for inter-patient variability to the anti-TB treatments. Hence, identification of these genetic variants amongst the TB patients will aid in determining the gene polymorphisms that are responsible for increased susceptibility to Mtb infection and latent TB reactivation [182]. Furthermore, this information will also enable development of personalized anti-TB therapy based on the genetic-makeup of the individuals, and hence may play a vital role in curbing the aggravated inflammation and immunopathology associated with chronic Mtb infection.

In addition to the genetic factors, hormones have also been involved in influencing the outcome of Mtb infection. Recently, the levels of sex steroid hormones such as estrogen, progesterone and testosterone are known to determine the susceptibility of an individual to mycobacterial infection. Further investigations on the role of hormones in developing

and maintaining immune responses during TB will help to develop new therapeutic interventions by targeting hormones to control mycobacterial infection.

The protective immune response against Mtb involves the activation of innate and adaptive immune cells. Adaptive immune cells include conventional T cells and unconventional T cells including invariant natural killer T cells, γδ T cells and mucosal-associated invariant T cells. These unconventional T cells also play a role in developing protective immunity against TB. However, there are limited reports regarding their role in TB. Moreover, non-immune cells such as alveolar epithelial cells are also involved in imparting protection against Mtb. Even so, their exact role in TB remains obscure due to limited studies. This suggests that further investigations are essential to determine the roles of these unconventional and non-immune cells in tuberculosis to identify new targets for the development of TB vaccines and thereby enable control of mycobacterial infection.

Recently, biomarkers are widely used to determine the state of Mtb infection (i.e., latent vs active TB) as well as to determine the efficacy of anti-TB treatment among the TB patients [183]. Several biomarkers with the use of Mtb lipoarabinomannan have been developed [183]. Also, host biomarkers including matrix metalloproteinases, neutrophil abundance and type I IFN levels are widely used to differentiate latent Mtb infection from active form [142]. Despite the advancements in biomarker development, the TB epidemic is progressing worldwide at an alarming rate. Hence, to prevent the further spread of the destructive infectious disease, more biomarkers are essential. Progression of latent infection to active TB is associated with changes in the gene expression of specific genes. microRNAs (miRNA) are widely known to regulate gene expression of various genes and their dysregulation is known to be associated with various diseased conditions including cancer [184]. This suggests that miRNAs could also be used as biomarkers to differentiate latent TB infection from active form of TB. This will help diagnose the state of Mtb infection, and hence can guide the therapeutic interventions required to treat the TB patients. Few miRNA-based biomarkers are already discovered [185]. Identification of more miRNA-based biomarkers and investigations to determine the efficacy of these miRNAs in predicting the TB infection state will help to overcome a major roadblock in the diagnosis of Mtb infection.

Several diseases such as diabetes mellitus, AIDS and lung cancer are known to enhance the risk of TB development [186]. Such co-morbidities can have severe consequences on patients and can even lead to death [186]. Thus, further investigations are essential to determine the mechanisms by which the risk of developing TB increases. This information will enable the development of therapies that prevent TB progression in patients suffering from diseases such as diabetes, AIDS and lung cancer and thereby will prevent the fatal consequences that arise as a result of such co-morbidities.

T helper cells are divided into various subsets based on the secretion of specific cytokines. However, T cells are also known to secrete lineage non-specific cytokines during tuberculosis, i.e., they possess plasticity. Harnessing this T helper cell plasticity might play a beneficial role in directing the immune response based on the inflammatory status of the host. Secretion of lineage non-specific IFN-γ by Th17 cells might control the tissue damage associated with IL-17 driven excessive recruitment of neutrophils and thereby might prevent latent TB reactivation.

Recently, nanoparticles are being used to deliver anti-TB drugs to the site of Mtb infection. Biodegradable polymeric nanoparticles have been used to increase the therapeutic efficacy of available anti-TB drugs [187]. Use of nanoparticles also reduces the dose of anti-TB drugs required to control mycobacterial growth and thereby prevents the toxic side-effects associated with high doses of first-line anti-TB therapies.

Thus, research on human subjects and biomarkers will enable development of new diagnostic and prophylactic strategies to cure TB with increased efficiency. Furthermore, knowledge regarding the genetic polymorphisms in TB patients will enable host-directed treatment of TB disease without undesirable consequences. Identification of the roles of hormones, unconventional T cells and alveolar epithelial cells will provide new targets for therapeutic interventions to cure TB. Advancement in drug delivery and harnessing the immune responses to shift the balance towards protection from pathology will help to control Mtb infection with minimal immunopathology.

These advancements in TB research will allow generation of vaccines and therapies that have better treatment outcomes and thereby will improve treatment regime against *Mycobacterium tuberculosis* by overcoming the limitations of conventional therapies.

Compliance with ethical standards

Conflict of interest The authors declare no conflict of interest.

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