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The Role of T cells In Mucosal Immunity against *Mycobacterium tuberculosis (Mtb)* Infection: A Review of Current Understanding

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Abstract

Tuberculosis (TB) caused by *Mycobacterium tuberculosis (Mtb)* remains one of the most important infectious diseases globally. Because Bacille Calmette-Guerin (BCG) vaccine is short of preventing transmission, the search for an efficacious vaccine to either augment that of BCG or to replace has been intensified globally. Currently, there are many candidate vaccines in different stages of clinical trials but none of these has achieved the desired efficacy to control TB. One central issue in TB immunology is lack of clear understanding as to what leads to immune protection or development of clinical disease. Tuberculosis is primarily a disease of the lungs and the main portal of entry of *Mtb* to the lungs and systemic organs is the respiratory tract and its mucosal surfaces. Therefore, the role of mucosal immunity against *Mtb* infection has been the subject of several investigations quite for some time. There is emerging evidence that mucosal immunity against TB may be critical for the control of *Mtb* infection and development of an efficacious vaccine. Although it is generally believed that type helper T (Th1) cells are critical for protection against *Mtb* infection, emerging data show that non-conventional T cells could play a vital role in protection against TB, particularly in the respiratory mucosa. In this review, current understanding on the role of various T cell subsets, with focus on lymphocytes collectively called "innate-like lymphocytes", including gamma-delta (γδ) T cells, natural killer T (NKT) cells, and mucosal-associated invariant T (MAIT) cells will be presented.

Keywords: Tuberculosis; Mucosa; Immunity; T cells; Respiratory; BCG

Introduction

Tuberculosis caused by *Mtb* remains one of the most important infectious diseases globally. According to World Health Organization (WHO), there were 8.6 million new cases and 1.3 million deaths due to TB in 2012 [1]. Over 100 million children are given BCG vaccination every year in over 120 countries, but the vaccine has no apparent effect on TB transmission. Efforts to augment the efficacy of BCG or to replace it with new efficacious vaccine have achieved little, partly because of lack of basic understanding about immune parameters that lead to protection or clinical TB [2]. Currently, tuberculosis control depends on case detection and treatment following WHO directly observed treatment short course (DOTS) strategy [3].

On the other hand, efforts to develop an efficacious vaccine has led to new understanding about different T cell subsets, molecules expressed, antigens recognized by the different T cells population, mechanisms of antigen recognition, and resulting immune responses. *Mtb*, the causative agent of TB, enters the lung and other organs mainly through the mucosal tissue of the respiratory tract, which makes mucosal immune responses critical in defense against the pathogen. However, compared to the peripheral blood and injection based antigens, research on mucosal immunity and development of mucosal vaccines is relatively slow. This is partly because administration of mucosal vaccines and measurement of immune responses are demanding, and functional testing of mucosal T cells is labor intensive and technically challenging [4].

However, mucosal immunity against TB has been the subject of several investigations, with special focus on mucosal route (mainly intranasal) of vaccine delivery [5]. Several lines of evidence have suggested that mucosal immunity can provide unique advantage for protection against *Mtb* infections [6-8]. Since it is beyond the scope of this paper, readers are referred to review articles on the role of mucosal immunity against infectious diseases in general [9,10] and that of TB in particular [5-7].

Generally it is believed that a strong Th1 response is critical to effectively control *Mtb* replication within phagocytes (macrophages and dendritic cells). The widely held view is that Th1 cells produce interferon-gamma (IFN-γ) required for activation of antigen presenting cells (APCs) to control *Mtb* replication [11,12]. However, it has been observed that these Th1 cells do not enter the lung in sufficient quantities until 8 to 20 days following *Mtb* exposure, resulting in delayed lung protection and this has been attributed to delay in T cell priming in the draining lymph nodes [13-16]. This lag in the generation of adaptive immune responses is a critical time for the pathogen and the host. Several nonconventional T cells act during this critical lag phase. These cells include mucosal associated invariant T (MAIT) cells, natural killer T (NKT) cells, and gamma-delta (γδ) T cells, collectively termed innate-like lymphocytes, which recognize molecular patterns, and have the capacity to immediately express effector functions [6,9].

These different subsets of T cells are involved in early infection protection, either by directly killing infected cells, or by secretion of cytokines and influencing the effector role of other immune cells. In this review, current understanding about the different T cell populations involved in mucosal immunity against TB will be presented.

T cells

T cells or T lymphocytes develop in the thymus and are distinguished from other lymphocytes, such as B lymphocytes and natural killer (NK) cells by the presence of T cell receptor (TCR). There are several subsets of T cells, including $CD4^+$, $CD8^+$, MAIT cells, NKT, invariant NKT (iNKT), γδ T cells based on surface molecules expressed. However, T lymphocytes, NK cells, and NKT also share common expression of specific molecules, including the C-type lectin, CD161. CD161 expression is not restricted to any pre-defined T cell subset. It is expressed by the majority of NK cells and approximately 24% of peripheral T cells, including both γδ and αβ TCR expressing subsets and NKT cells, which comprise less than 1% of human peripheral blood cells, and the remaining $CD161^+$ T cells represent a distinct lineage of T lymphocytes. However, it is not well understood whether or not CD161 is a phenotypic marker, identifying T cells of common activation state and/or lineage. For instance, it has been demonstrated that CD161 expression is significantly up-regulated in Th17 clones and there is a correlation between IL-17 secretion and CD161 expression, reviewed in [17]. In the following sections, the significance of these T cells in response to *Mtb* infection in the respiratory mucosa will be presented.

$CD4^+$ T cells (helper T cells)

Traditionally there were two lineages of $CD4^+$ T cells, namely helper type 1 (Th1) and helper type 2 (Th2) [18]. However, this number has grown to include other Th cells (Th3, Th17, regulatory T (Treg) cells, Th9, T follicular helper (Tfh), and Th22 [19]. $CD4^+$ T cells are central for generation of acquired immune responses against pathogens. $CD4^+$ T cells help B cells to produce antibody and undergo class switching and affinity maturation, they recruit $CD8^+$ T cells, macrophages, neutrophils, eosinophils, basophils and other effector cells. $CD4^+$ T cells activate dendritic cells (DCs) [19]. Based on the Th1/Th2 paradigm Th1 and Th2 T cells have been given distinctive division of labor, Th1 cells protect the host from intracellular pathogens, whereas Th2 cells protect extracellular pathogens, or parasites [18]. In addition, two early studies [11,12] in experimental model suggested an essential role of Th1 cells that produce interferon-gamma (IFN-γ) and activate macrophage to *Mtb*. Therefore, IFN-γ secreting Th1 cells remain the most studied T cell subset. In recent years however, other T cell subsets such as Th17 T cells are gaining increased attention [2,19] and work done on the role of these two Th subsets in protection against TB in the mucosa will be covered.

Th1 cells

As indicated earlier, there are few studies that focused on the role of Th1 T cells on mucoal immunity against *Mtb* infection because recovery and functional testing of mucosal T cells is labour intensive and technically challenging [4]. However, limited data, mainly focusing on intransal immunization using BCG and/or different antigens, with or without adjuvants are beginning to emerge [20-30].

Some of these studies focused on intranasal adjuvants for boosting prior BCG immunity to *Mtb* infection [27-33]. Anderesen and co-workers [28] demonstrated that an intranasal vaccine, comprising the antigen fusion protein Ag85B-ESAT-6 and the mucosal combined adjuvant vector CTA1-DD/ISCOMs has not only promoted a Th1-specific immune response, dominated by IFN-γ-producing CD4 T cells, but also strongly boosted prior BCG immunity, resulting in reduced bacterial burden in the lungs of challenged mice. In another study, the same group [29] showed that administration of mucosal adjuvant, mutant *E. coli* enterotoxin (LTK63) and the Ag85B-ESAT-6 induced a strong Th1 response mediated by IFN-γ producing cells, and that the same mucosal vaccine boosted BCG induced immunity in vaccinated mice. Similarly, Takahashi et al. [30] have shown that a single nasal immunization of mice with killed BCG and LTK63 induced expansion of CD4 cells producing cytokines IL-2, IFN-γ and TNF-α.

Among these studies some were devoted to comparing parenteral and intranasal routes of immunizations in inducing CD4 based protective immunity as well as boosting prime BCG vaccination [31-33].

Using BCG as a prime vaccine and adenoviral vector expressing Ag85A (Adag85A) as a boost vaccine, it was reported that a single intranasal immunization with Ag85A was able to induce potent protection from pulmonary *Mtb* challenge in a mouse model. Such enhanced protection was correlated with the numbers of IFN-γ-producing CD4 and CD8 T cells. While protection by BCG priming was boosted by intranasal AdAg85A, the same was not effectively achieved by subcutaneous BCG or intramuscular Ag85A immunization [31].

Similarly, Goonetilleke et al. [33] by comparing intranasal and parentral routes of BCG immunization and booster recombinant vacinia virus Ankara expressing Ag85A, induced stronger CD4 T cell responses compared to parenteral immunization. Protection conferred was correlated with Ag85A-specific, IFN-Y producing CD4 T cells in the lung lymph nodes of *Mtb* challenged mice [33].

In another example, Forbes et al. [23] have shown that boosting BCG-primed mice with *Mtb* Ag85B through intradermal and intransal routes gave different results. Accordingly, mice boosted through intradermal route of vaccination made a very strong splenic CD4 and CD8 Th1 cytokine responses to Ag85A but showed no change in lung mycobacterial burden over BCG primed animals. In contrast, intranasally boosted mice showed reduced mycobacterial burden and made much weaker splenic response but a very strong lung CD4 and CD8 responses to Ag85A [23].

Contrary to the above observations, one interesting report is that persistent mucosal population of CD4 effector memory cells (T_{EM}) can be induced by parenteral immunization, a feature only previously attributed to mucosal immunization routes [22]. A single systemic BCG vaccination of mice has resulted in distinct systemic and mucosal populations of T_{EM} ($CD4CD44^{hi}CD62L^{lo}CD27^-$) cells capable of producing IFN-γ and TNF-α, or IFN-γ, IL-12 and TNF-α. It has also been shown that these multifunctional T_{EM} cells are strongly associated with protection and can be maintained for a period of over 16 months [22].

In addition to the studies aimed at boosting BCG, there are studies that used *Mtb* specific antigens, or antigen conjugates, with or without adjuvants, the most widely studied being ESAT-6, Ag85B and Ag85A, or their conjugates [20,21,24,26,34]. The study by Zhang et al. [24] showed that intranasal immunization of mice with chimeric flagellin component FliC of *Salmonella typhimurium*, containing ESAT-6 (SL5928 (fliC/esat) can induce strong Th1 and cytotoxic immune responses in mucosal lymphoid tissues such as nasopharynx-associated lymph nodes, lung and Peyer`s patches, but a mixed Th1/Th2 responses in spleen and mesenteric lymph nodes [24].

Ballester et al. [34] also compared intradermal and pulmonary administration of nanoparticles (NP)-ag85B conjugation in eliciting protective immunity. It has been shown that pulmonary administration of NP-Ag85B with CpG led to enhanced induction of antigen-specific poly-functional Th1 responses in the spleen, lungs-draining lymph nodes as compared to soluble Ag85B with CpG and to the intradermally-delivered formulations [34].

In addition to route of immunization, the importance of antigenic epitopes (dominant and cryptic) in CD4 T cell response has also been documented. A recent study [20] that compared functionality of CD4 T cell responses against dominant and cryptic eiptopes of *Mtb* ESAT-6 pre-and-post-infection showed that protective T cells lacking the dominant eitopes not targeted during natural infection were induced by vaccinating mice. Vaccination of mice with truncated ESAT-6 protein and subsequent aerosol *Mtb* challenge has resulted in recruitment and expansion of CD4 T cells that produced increased Th1 cytokines. In a similar study [21], mice vaccinated with conjugated *Mtb* cutinate-like proteins (Culp)1 and 6 and MTP83 had increased recruitment of effector CD4 and CD8 T cells to mesenteric lymph nodes and systemic antigen-specific IFN-γ producing T lymphocyte and IgG response [21]. Pulmonary immunization with either culp1-6-lipokel or MPT83-lipokel powder vaccines generated protective responses in the lung against aerosol *Mtb* challenge [21]. Intranasal immunization of mice with yeast expressed recombinant heparin binding haemagglutnin adhesion (rHBHA), with mucosal adjuvant cholera toxin (CT) has been shown to result in enhanced production of specific antibody and Th1 immunity against challenge infection in the lungs of immunized mice [26].

Put together, the above studies show that mucosal immunization using different antigens, with or without adjuvant can lead to the recruitment and expansion of protective Th1 cells and assert the importance of these cells in mucosal immunity. However, it should be borne in mind that although the critical role of CD4 T cells in protective immunity against *Mtb* is well documented, immune mechanisms that lead to immune protection or development of clinical disease are yet to be determined.

Th17 cells

The discovery and inclusion of Th17 into CD4 lineages has brought some new insights into our understanding of TB immunology and immuno-pathology. Currently, two variants of Th17 cells are recognized, namely, natural (nTh17) and induced Th17 (iTh17) cells, which share similar functional properties such as production of IL-17 and IL-22 following TCR cross-linking but are distinctly regulated by phosphatitidyliositol 3-kinase (PI3K)/Akt and mammalian target protein of rapamycin (mTOR) Akt pathways [35,36]. The newly identified natural (nTh17) cell is IL-17$^+$CD4$^+$ T cell acquires the ability to secrete IL-17 during thymic development [36,37] and that retinoic acid related orphan receptor γt (ROCγt) and the transcription factor promyelocytic leukemia zinf finger (PLZF) are valuable new markers to identify nTh17 cell population [38]. One characteristic feature observed by this study was that, in addition to TCR cross-linking, nTh17 cells secrete IL-17 and IL-22 when stimulated with IL-23 and IL-1β, either in recombinant form or in supernatants from TLR-4-activated DCs [38]. However, this innate-like ability of ROCγt$^+$ nTh17 cells to respond to TLR-4 induced cytokine was not shared by induced Th17 (iTh17) cells. The second distinct feature of nTh17 cells is their high expression of PLZF, and their absence from lamina propria, where iTh17 cells are abundant [38].

Although the distinctive role of these two Th17 cell variants in protection and/or development of clinical TB is yet to be known, experimental and clinical evidence suggest that certain infections require a Th17 immune response to achieve a proper clearance and that immunization by the intranasal route could be beneficial in case of certain pathogens, and that they are capable of regulating the production of antimicrobial proteins in mucosal epithelium and clearing various pathogens [39-42]. One characteristic feature that makes Th17 cells important is their distribution in mucosal associated tissues and production of cytokines such as IL-17A, IL-17F, IL-21, IL-22, and IL-26 [43-45], and IL-17 is produced early against *Mtb* infection and is critical in controlling TB [31]. However, it must be noted that although Th17 cells are the main source of IL-17, cells from the innate-like lymphocytes, including include MAIT cells, γδ T cells, and NK T cells, are also important sources of IL-17 production in the early phases of *Mtb* infection in the respiratory tract [46].

As first noted by cooper and co-workers, the protective role of Th17 largely depends on IL-23. Simultaneous loss of IL-12 and IL-23 during *Mtb* infection leads to increased bacterial burden and reduced IFN-γ production and higher mortality as compared with the loss of IL-12 alone [47]. In a related development it has been shown that pulmonary IL-23 gene delivery reduces both mycobacterial burden and inflammation in the lungs and augments the expansion of mycobacterial-specific IFN-γ and IL-17 producing CD4 T cells [48]. In addition, when IL-23 is co-administered with coding sequence of Ag85B, a strong IFN-γ response is induced in mice deficient in IL-12p40 subunit [49].

It has been shown that IL-23-dependent Th17 plays a key role in Th1 recruitment and vaccine-induced protection in a parenteral [41] and mucosal [39,40,50,51] routes of vaccine-induced immunity to *Mtb* infection.

One study has shown that mice deficient in IL-17 are unable to control TB after high intra-tracheal infection and that IL-17 also plays a crucial role in the establishment of protective pulmonary CD4$^+$ T cell responses after vaccination or infection with *Mtb* [41].

The study by Copal and co-workers not only shows the critical role of Th17 cells in vaccine induced mucosal immunity against *Mtb* infection but also that protection imparted is independent of IFN-γ [50]. Using wild-type C57BL/6 mice, the study demonstrates that mucosal adjuvants such as type II heat labile enterotoxin (LT-Iib), delivered through the mucosal route induce pulmonary *Mtb*-specific Th17 responses and provide vaccine-induced protection against *Mtb* infection. Importantly, this study shows that it is IL-17 but not IFN-γ that is critical for protection for mucosal-induced immunity against *Mtb* challenge [50]. IL-17 is believed to mediate C-X-C motif chemokine ligand 13 (CXCL13) induction in the lung of pro-inflammatory cytokine producing C-X-C motif chemokine receptor-5-positive (CXCR5$^+$) T cells, thereby promoting early efficient macrophage activation and control of *Mtb* infection. CXCL13 induces the expression of inducible bronchus-associated lymphoid tissue (iBALT) required during granuloma formation in the lungs [52-54].

In addition, the activated CD4$^+$ T cells also showed an up-regulated expression of the chemokine CCR6 (which is a marker for murine Th17 cells) on the surface of CD4$^+$ T cells and that this phenotype is independent of the kind of adjuvant used [39].

Despite reports on the critical role Th17 cells in protection against *Mtb* infection, especially in the mucosa associated tissues as indicated above, there is also a wealth of data on the role of Th17 cells in the

induction of immuno-pathology [44,53]. One of the mechanisms by which *Mtb* infection is controlled by Th17 cells is through the recruitment of neutrophils to the site of infection and production of neutrophil chemo-attractants [55]. While the recruitment of neutrophils to site of infection by IL-17 is crucial for pathogen clearance, accumulation of large numbers of neutrophils in the lung is also associated with increased lung pathology, reviewed in [56,57]. It has been shown that neutrophils are the prominent infected cell types in active human TB patients [58] and in genetically susceptible mice [59]. Therefore, more work is required to understand whether or not new TB candidate vaccines should aim at inducing the recruitment and expansion of Th17 T cells.

CD8 T cells

There were contending views regarding the role of CD8 T cells in immune protection against TB for some time. Some early studies have suggested that *Mtb* antigens do not induce CD8 T cell response [60,61]. But support for the role of CD8 T cells in protection against *Mtb* infection comes from animal studies which showed the critical role of CD8 T cell responses for the control of *Mtb* infection [62,63]. For instance, Flynn et al. [63] have shown that mice with disruption of genes of β-2 microglobulin gene, which lacked MHC-I molecule and consequently failed to develop functional CD8 T cells failed to control *Mtb* multiplication. When these mutant mice were infected with virulent form of *Mtb*, 70% were dead or were moribund 6 weeks post infection, while control mice expressing β-2 gene remained alive for more than 20 weeks [63].

Another support for the role of CD8 T cells in protection against *Mtb* infection comes from adoptive transfer experiments. Adoptive transfer or *in vivo* depletion of CD8 cells showed that these T cells could confer protection against subsequent challenge [64,65]. One study that compared the relative role of CD4 and CD8 T cells during acute and latent *Mtb* infection in mice has shown that anti-CD8 treatment resulted in a 10-fold increase in bacterial numbers in lungs of *Mtb* infected mice, suggesting the critical role played by CD8 cells in controlling *Mtb* infection [66]. According to a recent study [67], in BCG-immunized transgenic RAG-knock-out mice adults (in contrast to infants), CD8 T cells are the main IFN-γ producing cells, suggesting the crucial role of these cells in protection against TB in adults.

The importance of CD8 T cells in mucosal immunity was shown by the appearance of *Mtb* antigen-specific CD8 T cells in the airway lumen at the time of *Mtb* infection [68-70]. When a recombinant adenovirus-based TB vaccine expressing *Mtb* Ag85A was administered either intranasally or intra-mascularly to mice, it elicited higher number of antigen-specific CD4 and CD8 T cell responses in the airway lumen that were capable of IFN-γ-production and cytolytic activities, as assessed by an intra-tracheal *in vivo* Cytolytic assay. This murine study suggested that intranasal delivery of low doses of soluble antigen was able to recruit and retain antigen-specific CD8 T cells in the airway lumen over time, while the antigens delivered via intramuscular route failed to induce the antigen-speicificCD8 T cells [69].

In another study, Jeynathan et al. [47] have shown that airway delivery of soluble *Mtb* antigens restores protective mucosal immunity in previously vaccinated mice, effectively mobilizes systematically activated T cells into the airway lumen. A recent study by Wu and co-workers also found that CD4 T cell depleted mice intranasally vaccinated with adenovirus vector expressing *Mtb* antigen Ag85A led to suboptimal generation of antigen-specific CD8 T cells in the lung and spleen at the early time following the immunization [71].

There seems to be three primary effector functions of CD8 T cells in TB as compared to CD4 T cells: these include lysis of infected cells in the mucosal surfaces (e.g., macrophages and DCs), direct killing of the intracellular bacteria, and production of IFN-γ cytokines.

One interesting observation in recent years is that in humans, *Mtb*-specific $CD8^+$ T cells are present at high frequencies in both *Mtb*-infected and uninfected individuals and that these cells recognize *Mtb*-infected cells [72,73]. These cells are mucosal associated invariant (MAIT) cells, and their role in tuberculosis mucosal immunity will be presented in the following section.

MAIT cells

Two sub-populations of innate-like T lymphocytes display conservation between species of both TCR and their respective MHC-1b restricting element. These are the newly defined MHC-related 1 (MR1)-restricted MAIT cell and CD1d-restrcited natural killer T (NKT) cell [74-76]. MAIT cells are identifiable by the high expression of CD161, which is a C-type lectin-like membrane receptor [77,78].

The development of human MAIT cells is not well understood but a recent study [78] shows that MAIT cells are rare and immature in the fetal thymus, spleen and mesenteric lymph nodes, whereas mature IL-18Ra+ and CD8aa MAIT cells are enriched in the fetal small intestine, liver and lung [77]. Human and mouse experiments using anti-human Va7.2 antibody and MAIT cell–specific iTCRα and TCR-β transgenic mice of different genetic backgrounds show that MAIT cell development is a step-wise process, with an intra-thymic selection followed by peripheral expansion [78]. MAIT cells are selected in an MR1-dependent manner both in fetal thymic organ culture and in double iTCRα and TCRβ. In humans MAIT cells display a naïve phenotype in the thymus as well as in cord blood [78].

Despite their location, MAIT cells express CD127 and ki67 *in vivo* and readily proliferate in response to *E. coli in vitro*. Maturation is accompanied by the gradual post-thymic acquisition of the PLZT transcription factor and the ability to produce IFN-γ and IL-22 in response to bacteria in mucosal, thus, acquiring their innate-like responsiveness in mucosa before exposure to environmental microbes and the commensal microbiota [77].

It has been shown that MAIT cells are activated by cells infected with various types of bacteria and yeast in both humans and mice, and this activation requires cognate interaction between the invariant TCR and MR1, which can present bacteria-derived ligand [79]. MAIT cells have wide specificity for antigens of phylogenetically distant microbial entities and that observed cross-reactivity between humans and mice, suggesting that MAIT cells recognize a conserved antigen [79]. A recent study shows that MAIT cells react to metabolites of the riboflavin (vitamin B2), which represents a class of antigens found in broad spectrum of microorganisms [80]. This discovery that MR1 presents vitamin metabolites, presumably from pathogenic and/or commensal bacteria, distinguishes MAIT cells from peptide or lipid-recognizing αβ T cells in the immune system. However, the recognition by MAIT cells is not specific to *Mtb* since they can detect cells infected with a variety of other bacteria [80-83].

MAIT cells may be abundant in mucosal associated tissues but there are contending reports on their protective role against *Mtb* infection.

A study by Le Bouhris and colleagues has shown that MAIT cells can be up to 10% of blood T cells in healthy individuals but this number is substantially reduced in peripheral blood of TB patients [79]. This study also demonstrates that MAIT cells can lyse MR1 expressing epithelial cells in the presence of bacteria or bacterial ligand [79].

On the other hand, Gold et al. [60] have shown that MAIT cells, which have no previously known *in vivo* function, make up a proportion of *Mtb*-reactive $CD8^+$ T cells and detect *Mtb*-infected cells via MR1. *Mtb*-reactive MAIT cells are enriched in the lungs and detect primary *Mtb*-infected lung epithelial cells from the airway where initial exposure to *Mtb* occurs [82].

In humans, MAIT cells have been found in the lungs of patients infected with *Mtb*, whereas MR1-deficient mice exhibited transiently elevated lung bacterial burdens following aerosol *M. bovis* BCG infection [79,82,84]. One significant observation was that primary human large airway epithelial cells infected with *Mtb* can induce a robust response by MR1-resricted MAIT cells. Following inhalation, *Mtb* is far more likely to encounter airway epithelium than alveolar macrophages. As a result the capacity of large resident MAIT cells to respond directly *ex vivo* to *Mtb*-infected lung epithelial cells suggests these cells could play a physiological role in directly controlling *Mtb* in the lung early in infection. *Mtb*-reactive MAIT cells not only produced IFN-γ but also TNF-α and granzyme in response to infected targets. MAIT cells could influence *Mtb*-reactive DCs by producing IFN-γ which could facilitate optimal priming of *Mtb*-specific $CD8^+$ and $CD4^+$ responses [82]. Similarly, a study by Chua et al. [84] showed that purified polyclonal MAIT cells potently inhibit intracellular bacterial growth of *M. bovis* BCG in macrophages in co-culture assays, and this inhibitory activity was dependent upon MAIT cell selection by MR1, secretion of IFN-γ, and innate interleukin-12 (IL-12) signal from infected macrophages. The same group has also shown that MAIT cell-deficient mice had higher bacterial load at early times after infection compared to wild-type mice, demonstrating that MAIT cells play a unique role among innate lymphocytes in protective against bacterial infection [62].

A recent study in South Africa found low levels of peripheral $CD161^{++}CD8^+$ MAIT in HIV and HIV-TB co-infection. These cells expressed high levels of the HIV co-receptor CCR5, the tissue homing marker CCR6, and MAIT TCR VaVa7.2. Acute and chronic HIV were associated with lower frequencies of $CD161^{++}CD8^+$ T cells, which did not correlate with CD4 count or HIV viral load [85].

Another study [86] shows that MAIT cells are activated by B cells infected with various bacteria strains (commensals and pathogens from the Enterobacteriaceae family), but not by uninfected cells. These results provide important insights into the role of B cells as a source of antigen-presenting cells to MAIT cells and the gut immune surveillance of commensal micro-biota.

Natural killer T (NKT) cells

The term "NK T cells" was first used in mice to define a subset of T cells that expressed the NK cell-associated marker NK1.1 (CD161). It is now generally accepted that the term "NKT cells" refers to CD1d-restricted T cells, present in mice and humans, some of which co-express a heavily biased, semi-invariant TCR and NK cell markers [87].

NKT cells recognize a wide range of endogenous and exogenous lipid antigens presented by monomorphic major MHCI-like CD1d molecule. There are no reports on the role of NKT cells in mucosa-related TB immunity but their role in pulmonary immunology has been reviewed by Paget and Trottein [88].

However, one recent study by Rothchild et al. [89] shows that when invariant natural killer T (iNKT) cells are co-cultured with infected macrophages, iNKT cell activation, as measured by CD25 up-regulation and IFN-γ production, was primarily driven by IL-12 and IL-18. In contrast, iNKT cell control of *Mtb* growth was CD1d-dependent, and did not require IL-12, IL-18, or IFN-γ. This demonstrated that conventional activation markers did not correlate with iNKT cell effector function during *Mtb* infection. Invariant NKT cell control of *Mtb* replication was also independent of TNF and cell-mediated cytotoxicity. By dissociating cytokine-driven activation and CD1d-restricted effector function, it was shown that iNKT cell antimicrobial activity depended on granulocyte-monocyte colony stimulating factor (GM-CSF). These cells produced GM-CSF *in vitro* and *in vivo* in a CD1d-dependent manner during *Mtb* infection, and GM-CSF was both necessary and sufficient to control *Mtb* growth [89].

Gamma-delta (γδ) T cells

γδ T cells are the most widely studied among innate-like lymphocytes. Although γδ T cells account for approximately 3-5% of all lymphoid cells found in the secondary lymphoid tissues and the blood, they are more prevalent at mucosal epithelial sites [90].

It is now well established that γδ T cells which are abundant in mucosal surfaces play important roles in the mucosal immunity against *Mtb*. They are involved in early defense against *Mtb* infection, linking innate and adaptive immune responses [91]. Their effector role includes induction of CD4 T cell expansion; act as APCs and cross-presenting to CD8 T cells; production of cytokines such as IFN-γ and IL-17, and cytotoxic activity and lysis of *Mtb*-infected phagocytic cells [91].

In mucosal surfaces, γδ T cells are capable of lysing infected macrophages and containing mycobacterial growth. By using pulmonary murine model of *Mtb*, Lockhart and co-workers found that the major production of IL-17 in T cells isolated from the lungs of infected mice were γδ T cells, suggesting that γδ T cells are more potent producers of IL-17 during the early immune response at mucosal sites following the infection [92]. In addition, these cells are the first immune cells found in the fetus and provide immunity to newborns prior to activation of the adaptive immune system [93]. γδ T cells are a more potent source of IL-17 as compared with activated $CD4^+$ T cells, which had previously been identified as the main producers of IL-17. In addition at least, one report indicates that IL-17-producing γδ T cells are increased in patients with active pulmonary TB [94].

One of the challenges during the past few decades was to determine antigens recognized by γδ T cells and mechanisms of antigen recognition. Originally it was thought that antigen recognition by Vγδ T cells was restricted to *Mtb*-derived non-peptidic, phosphorylated metabolites (Phospho-antigens) [95].

In recent years, however, significant progress has been made in determining antigens recognized by these cells and mechanisms of antigen recognition. Emerging data show that γδ T cells recognize a diverse array of antigens, including peptidic and non-peptidic molecules, and that antigen recognition by γδ T cells resembles that of B cell antigen receptor [78]. A recent study [96] identified two distinct *Mtb* protein antigens (p1 and P2). P1 is an enzyme (1-deoxy-D-

xyluose-5-phosphate synthase 2 (DXs2), and TP2 is an extracellular peptide (EP) of Rv2272, which is trans-membrane protein. These two antigens have been found to bind to γδ TCR and effectively activate γδ T cells isolated from PTB patients. In addition to binding and activation, both DXS2 and EP induced not only expansion of Yδ T cells from PTB patients but also induced the production of IFN-γ and monocyte-chemo-attraction protein 1 (MCP-1), which stimulates monocyte chemotaxis towards the site of infection and mediating cytotoxicity against *Mtb* [81]. In addition to *Mtb* antigens, γδ T cells recognize tetanus toxoid, staphylococcal enterotoxin, and toxin listeriolysin [97].

However, mechanisms of γδ T cell antigen recognition and activation is far from complete. Recent studies suggest that γδ T cells are incapable of direct response to mycobacteria and that the involvement of APCs is essential [95-100]. Some studies suggest that presentation is required by a putative antigen presenting molecule only found in human and non-human primates [95,99,100]. A study by spencer et al. [95] has demonstrated a restricted TCR diversity in mycobacteria-reactive γδ T cells compared with those that are phospho-antigen-reactive, and that only a subset of phospho-antigen-reactive γδ T cells respond to mycobacteria. In another study that used BCG for activation [100], it has been shown that both DCs and memory CD4 T cells are required for γδ T cell effector response to BCG and that γδ T cells are activated by supernatants from BCG-treated DCs and CD4 T cells in an IL-12p70-depndent manner. In addition, DC production of IL-12p70 is dependent on processing of BCG antigen and presentation to memory CD4 cells.

Put together, the fact that T γδ CRs recognize qualitatively distinct antigens from αβ T cells, their abundance in mucosal tissues, a wide range of antigen recognition, early responses to *Mtb* infection, especially in young animals, make γδ T cells attractive targets and deserve attention in vaccine development against TB. However, there are many questions that need answers regarding antigens recognized by γδ T cells, mechanisms of antigen presentation and recognition. More work is also required to determine the exact role of γδ T cells during *Mtb* infection.

Conclusion

At present, the only licensed TB vaccine is BCG but it has no impact in controlling TB transmission. Therefore, TB control depends on case detection and treatment based on DOTS strategy. However, control programs based on the DOTS strategy are facing challenges due to emerging drug resistant *Mtb* strains, and HIV/AIDS epidemics. On the other hand, efforts to develop efficacious vaccines are underway, and some TB candidate vaccines are in the different phases of clinical trials. Although development of mucosal vaccine against TB has attracted attention for several decades, work done so far is very limited. In addition to the ease of administration and acceptance of needle-free vaccines by the public, the respiratory mucosa is the portal of entry for *Mtb* to the lungs and other organs. Therefore, more effort should be made to develop vaccines that use the mucosal route. In addition to the well documented IFN-γ secreting Th1 cells, the critical role of innate-like lymphocytes is being recognized, and their antigen receptors, mode of antigen recognition and their immune protective role in the fight against TB are being unraveled. These cells are MAIT cells, NKT cells and γδ T cells. The fact that these cells are distributed abundantly in mucosal tissues, respond early to *Mtb* infection (especially in infants), recognition of different antigens (but not MHC-restricted peptides), development of immunological memory, production of essential cytokines, and efficient immune protection against *Mtb* infection, make them vital in the fight against TB, and they deserve the right attention in the development efficacious vaccines against TB.

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