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REVIEW

Th1 cytokines, true functional signatures for protective immunity against TB?

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The lack of an effective preventative vaccine against tuberculosis (TB) presents a great challenge to TB control. Since it takes an extremely long time to accurately determine the protective efficacy of TB vaccines, there is a great need to identify the surrogate signatures of protection to facilitate vaccine development. Unfortunately, antigen-specific Th1 cytokines that are currently used to evaluate the protective efficacy of the TB vaccine, do not align with the protection and failure of TB vaccine candidates in clinical trials. In this review, we discuss the limitation of current Th1 cytokines as surrogates of protection and address the potential elements that should be considered to finalize the true functional signatures of protective immunity against TB.
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INTRODUCTION

In the past 50 years, accumulating evidence has demonstrated that T cells play critical roles in host defense against *Mycobacterium tuberculosis* (Mtb) infection. An insufficient T-cell response renders the host unable to clear Mtb and therefore results in the establishment of persistent Mtb infection. In both systematic and aerosol-challenged murine TB models, T cells were shown to be required for host protective immunity against TB.[1–3] While CD8+T cells play a critical role in mediating immune protection against TB, the protective role of T cells was initially shown to be mainly mediated by CD4 T cells (Figure 1).[4,5] Interestingly, CD4+ cells can act as innate-like cells to contain the very early extrapulmonary dissemination of Mtb and slow down the rapid progression of TB. Protective roles against TB can possibly be attributed to CD4+ cells' master helper function to sustain the systemic and pulmonary anti-TB responses of CD8+ T cells and CD3-non-T lymphocytes.[6] In agreement with these findings, clinical observations suggested that HIV-1-induced loss of CD4 T cells renders TB susceptibility and increases reactivation of latent Mtb infection, further highlighting the importance of T cells in defense against TB.[7,8]

After encountering the Mtb antigen presented by antigen-presenting cells (APCs), naive CD4 T cells differentiate into effector and/or memory cells. Depending on the specificity and affinity of TCR, availability of cognate Mtb antigens, co-stimulation signaling, and so on, naive CD4 T cells can be differentiated into various subsets, including at least Th1, Th2, Th17, Treg and T_{FH} cells. Among these subsets, IFN-γ-producing Th1 cells are accepted as the major population that mediates protective immunity against TB. Indeed, mice deficient in Th1 cytokines (for example, IFN-γ, IL-12p40) succumbed early to Mtb infection with high bacillus loads.[9–11] Furthermore, mice with defects in IFN-γ-dependent enzymes show a similar susceptible phenotype.[12–15] Rapid clonal expansion, pulmonary trafficking and the accumulation of many PPD Ag-specific IFN-γ+CD4+ and few CD8+ T effector cells in BCG-vaccinated macaques upon pulmonary Mtb challenge further highlighted the critical importance of Th1 cytokines in mediating protective immunity against TB infection.[16] In humans, individuals carrying genotypes (that is, IFNGR1, IL-12B, IL12RB1) with impaired Th1 immune response are associated with increased susceptibility to mycobacterial diseases.[17–19]

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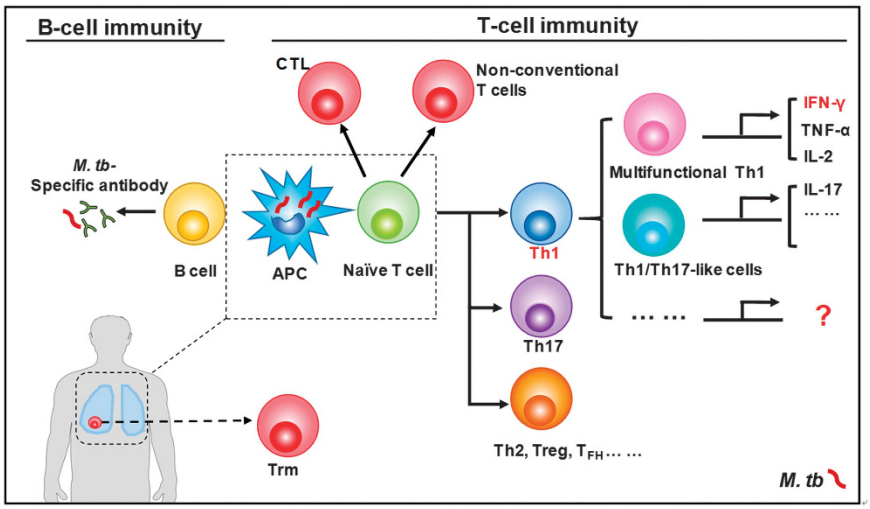

Figure 1 Protective immunity against Mtb infection. Upon exposure to Mtb, antigen-presenting cells (APC) in the lungs process bacterial antigens and present them to naive T cells, which become activated shortly thereafter. Both B-cell immunity and T-cell immunity are essential for the successful clearance of bacteria. B-cell-mediated immune responses are represented by the activation of B cells and subsequently the elevated production of Mtb-specific antibodies. T-cell immunity can be mediated by a variety of T cells, including CTLs, non-conventional T cells, Th1, Th17, Th2, Treg, T_{FH} and other cells. Among them, cells involved in Th1 responses against Mtb are the best characterized, and they are composed of multifunctional Th1 cells that secrete IFN-γ, TNF-α and IL-2, Th1/Th17-like cells that secrete IL-17 and other cytokines, and other uncharacterized T cells. Recent studies also emphasize the protective role of Trm in TB. Trm cells are a subset of T cells that permanently reside in lung tissue to respond rapidly to re-exposure to cognate antigens.

Nevertheless, it is noteworthy that IFN-γ is essential, but not sufficient, for bacterial control after Mtb infection, as mice with intact IFN-γ but which were deficient in TNF-α, GM-CSF, IL-1 or IL-6 all succumbed to Mtb infection. In other words, these results suggest that additional cellular responses are involved in protective immunity against TB. In contrast to its protective role, recent evidence showed that Th1-mediated IFN-γ response inhibited inflammation during TB and was involved in TB immunopathology.[15,20,21]

Due to the discrepant efficacy of *Mycobacterium bovis* Bacille Calmette-Guerin (BCG) in preventing reactivation or re-infection of Mtb in adults, at least 15 vaccine candidates have entered clinical trials within the last decades.[22] Although they are excellent in induction of Mtb antigen-specific IFN-γ-producing Th1 immune responses after systematic administration, none were proven to be more effective at preventing TB than BCG. One of the most promising vaccine candidates, a modified Vaccinia Ankara vector expressing Mtb antigen 85A (MVA85A), was able to elicit powerful Th1 responses, but no significant protection beyond BCG alone was observed.[23]

While these TB vaccine candidates varied in antigen selection or strategies (such as vaccination routes), the fact that they all failed to elicit efficient protection against TB argues against the consensus that Th1 cytokines are useful surrogate markers of protective immunity against TB in humans. Clearly, fine delineation of the surrogate markers of protective immunity against TB, alternative to the current Th1 cytokine IFN-γ, is fundamental for the development of a TB vaccine. In this review, we discuss potential questions that need to be addressed to envision useful signatures for protective immunity against TB.

DO PERIPHERAL TH1 RESPONSES REPRESENT TRUE PROTECTIVE IMMUNITY IN THE LUNGS?

The acquisition of T-cell immunity in the lung upon Mtb infection is exceedingly slow after aerosol challenge. Generally, Mtb enters deeper alveoli in the form of minute alveoli, where they are engulfed by alveolar macrophages. After ~9 days, the bacteria can be transported into draining lymph nodes, where dendritic cells (DCs) present Mtb antigens to T lymphocytes.[24,25] Once T cells are activated in the lymph nodes, they differentiate into effector cells, which can migrate into the lungs.[26] Effective T-cell-mediated protection is initiated by day 14 post infection in mice.[27] In humans, T-cell responses were detected after 42 days post exposure.[28,29] Therefore, it has been postulated that although systematic immunization elicits peripheral Th1 cell responses, delayed immune responses in the lung provide an excellent timeframe for Mtb growth and persistent infection establishment, rendering adaptive immunity unable to successfully eradicate the bacterial infection. In support of this concept, previous studies have indicated that it is not the magnitude of the Th1 response, but the rate of Th1 cell migration into the lung, that determines protective immunity against TB.[30–32] In other words, earlier arrival of effector Th1 cells (measured as antigen-specific IFN-γ-producing CD4 T cells) to the site of infection correlated with earlier restriction of mycobacterial growth in the lung of BCG-vaccinated mice.[33] Consequently, the Th1 responses in peripheral blood, as assayed by the frequency of Th1 cells or the ability to produce INF-γ after Mtb antigen stimulation *ex vivo*, cannot faithfully reflect the immune responses that occur in the lung or at least cannot accurately reflect the rate of Th1 cell migration.

Given that the lung is the place where infected bacillus is cleared, it has been increasingly recognized that tissue resident memory T cells (Trm) in the lung are critical for protection against TB [34–36] in addition to the T cells that have migrated into the lung. Trm cells, expressing mainly the surface markers of CD69 and CD103, have been recognized as the third subset of memory T cells with distinct properties of phenotype, migration, retention, and functional maintenance that are different from peripheral T cells. Unlike central memory T cells, which migrate to lymphoid organs in response to L-selectin ligands, and effector memory T cells, which recirculate between blood and peripheral tissues, Trm cells permanently reside in non-lymphoid tissues and therefore cannot be detected in peripheral blood.[37] They are clonally expanded memory T cells that have the ability to respond rapidly to re-exposure to cognate antigens.[38]

Although early findings did not characterize the exact role of Trm in protective immunity against TB using the surface markers of CD69 and CD103, it has been observed that the inhibition of mycobacterial growth coincides with the presence of activated CD4 T cells detected in the lung.[39] In addition, it has been found that memory CD8 T cells in the airway can confer protection in the absence of peripheral T-cell recruitment.[34] By using fingolimod, a ligand for sphingosine-1 phosphate receptors, to prevent CD4 T-cell egress from the existing lymph node, it was further demonstrated that memory T cells residing in the lung were sufficient for earlier BCG-driven control of an intranasal BCG challenge and that they did not require help from T cells recruited from the lymph node.[35] Notably, the protection did not correlate with the magnitude of either IFN-γ- or IFN-γ/TNF-α/IL-2-producing Th1 cell responses, arguing against the concept that these typical Th1 cytokines, even those produced in the lung, act as sufficient surrogates for protective immunity against TB. The protective role of lung Trm was recently established by adoptive transfer of Trm collected from mice that received mucosal BCG vaccination.[36] Phenotypic analysis showed that these Mtb antigen-specific CD8 Trm (CD8+CD103+CD69+) cells displayed prototypical Trm features with significantly higher levels of IFNG, TNFA and CXCR6 compared to CD8 effector memory counterparts. In contrast, CD4 Trm cells, defined as CD4 +CD103+CD69+ cells, comprise a mixture of regulatory and effector T cells, specifically T-bet- and FoxP3-expressing T-cell subsets, with enhanced IL-10 transcripts. These findings suggest that lung CD4 T cells confer diverse functions well beyond the classical Th1 responses that were previously considered to be correlated with protection; therefore, CD4 T-cell activities cannot be reflected by typical peripheral Th1 cytokines.[36]

However, most of the current studies showing the importance of Trm in Mtb infection are correlational studies. There is a notable lack of direct evidence demonstrating a causal relationship between the immune characteristics of Trm and the protective roles of Trm against TB. Additionally, we cannot rule out the possibility that Trm may play a pathological role during TB infection. Furthermore, CD8+Trm and CD4+Trm may display distinct immune features during microbial infections. Further studies are required to identify accurate molecular markers (not limited to CD surface makers) for TB-specific CD8+Trm and CD4+Trm. Considering that CD103 was expressed in CD8+ T cells for maintenance in different types of locations or epithelium, the existence of transcriptional factors or other biomarkers dictating the differentiation and maintenance of Trm in the lung is highly possible. In addition, details on Trm-mediated immunological events and underlying mechanisms during Mtb infection remain to be fully characterized. Specifically, these details are related to antigen-presentation, interactions between immune cells (for example, macrophages (MΦ)-Trm) or with local cells, such as epithelial cells, cross-talk between different signaling pathways, interweaving between different molecular events for the maintenance of Trm in the lungs, and Trm reactivation and deletion during reencounters with TB antigens.

DO CLASSICAL TH1 CYTOKINES REPRESENT THE TRUE FUNCTIONAL SIGNATURE OF PROTECTIVE IMMUNITY?

As discussed above, classical Th1 cytokines are thought to be inadequate as surrogate protective markers, and new functional signatures for protective immunity against TB need to be identified. With the emergence of omics science, TB protective biomarkers can be identified through large-scale omics studies, which involve gene expression (transcriptomics), proteins (proteomics) and metabolites (metabolomics) in the blood and lungs. Omics approaches provide a great opportunity to potentially discover new protective biomarkers. Interestingly, a previously unrecognized signature of type I interferon signaling has been identified as a key immune mediator for human TB.[40] In addition, a 144-transcript signature associated with pulmonary TB has also been identified.[41]

Up until now, a series of cytokines, chemokines and other factors have been shown to be implicated in protective immunity against TB (Table 1) in addition to IFN-γ. Among them, IL-17 represents the best characterized cytokine in the expanding signature panel that illustrates TB protective immunity. IL-17, which is mainly produced by Th17 cells, has been shown to drive Th1 cell responses by overcoming IL-10-mediated inhibition after BCG vaccination.[84] In addition, BCG vaccination expands lung resident IL-17-producing CD4 T cells that produce chemokines, recruiting IFN-γ-producing CD4 T cells after Mtb challenge.[39] Although an early study with Mtb-infected mice shows that Th17 cells had little effect on infection control,[85] IL-17$^{-/-}$ mice infected with hypervirulent Mtb strain HN878 exhibited increased bacterial burden in lungs compared to WT B6 mice.[56] The fact that adoptive transfer of ESAT-6-specific Th17 cells partially inhibits Mtb growth supports the involvement of Th17 in TB protection.[86] Human studies also support a protective role for Th17/IL-17 responses by comparing the responses in TB patients and healthy individuals. It has been reported that the frequency of Th17 cells in active TB patients is significantly lower than that in healthy controls and LTB individuals, implicating that Th17 cells may contribute to the protection.[87] Taken together, these results suggest that IL-17 also represents an important mediator

Table 1 List of representative mediators with potential protective roles in TB

Gene	*Identification method*	*Animal study evidence*	*Clinical evidence*	*Reference*
IL-1	Real-time PCR, ELISA, WB	IL-1beta/alpha-deficient mice displayed acute mortality with highly increased pulmonary bacterial burden compared to WT mice.	IL-1β and IL-18 were significantly reduced in TB compared to LTB, whereas they were increased following anti-TB treatment.	Elnaggar *et al.*[42] Kathamuthu *et al.*[43]
		Plasma IL-1β was higher in Mbv-infected bovines than non-TB mycobacteria (NTM) or uninfected animals.	IL-1β was higher in TB (MRD-TB >DS-TB) than in HC.	Wang *et al.*[44]
			IL-1β +3953C/T and −511T/C polymorphisms were associated with tuberculosis susceptibility.	Mayer-Barber *et al.*[45]
				Mayer-Barber *et al.*[46]
IL-17/22	Real-time PCR, flow cytometry, WB, ELISA, SNP genotyping	IL-17−/− mice infected with hypervirulent Mtb (HN878) exhibited increased bacterial burden in lungs compared to WT B6 mice.	IL-17 and IL-23 expression was lower in patients than in LTB, and IL-17 producing CD4+ T-cell frequency was lower in active TB than in LTB.	Hu *et al.*[47] Wozniak *et al.*[48] Ahmed *et al.*[49]
		Neutrophil autocrine IL-17 was vital to inhibiting H37Rv growth in mice via reactive oxygen species.	IL-17 TB Ag response had the highest ability to distinguish active TB.	Pereira *et al.*[50] Heidarnezhad *et al.*[51]
		Vaccines inducing robust Th17/IL-17 responses provided superior protection against Mtb infection.	SNPs of rs3819024 in IL-17A and rs763780 in IL-17F were weakly related to a prognosis of tuberculosis.	You *et al.*[52] Wang *et al.*[53]
		In IFN-γ−/− mice, BCG-specific Th17 cells still conferred partial protection against Mtb infection.	Plasma IL-17 was substantially decreased after TB treatment and smear conversion.	Xu *et al.*[54] Zhao *et al.*[55] Gopal *et al.*[56]
		pValac: ESAT-6 boosted BCG vaccine immune response via increased IL-17 cytokine secretion.	IL-17F rs763780 allele C and IL-17A rs3748067 allele C were related to the susceptibility to TB.	Treerat *et al.*[57]
		IL22−/− mice exhibited increased bacterial CFU during chronic Mtb HN878 infection and increased Mtb dissemination to the spleen.		
IL-32	Real-time PCR, ELISA, WB	IL-32-Tg mice showed fewer Mtb in the lungs and exhibited greater survival compared to WT.	IL-32 is associated with vitamin D antimicrobial pathway and Mtb defense.	Bai *et al.*[58] Montoya *et al.*[59]
		ShRNA-IL-32 decreased TNF-alpha and increased Mtb number, whereas rIL-32 reduced Mtb via caspase-3.		
PD-1	Real-time PCR, Flow cytometry	PD-1−/− mice are extraordinarily sensitive to tuberculosis.	Anti-PD-1 therapy increases TB risk.	Lazar-Molnar *et al.*[60]
		PD-1−/− mice had less differentiated Mtb-specific CD4 T cells and succumbed rapidly to Mtb infection with high levels of IFN-γ in their lungs.		Barber *et al.*[61] Fujita K *et al.*[62] Sakai *et al.*[63]

Table 1 (Continued)

Gene	*Identification method*	*Animal study evidence*	*Clinical evidence*	*Reference*
IL-26	Microarray	IL-26 was differentially expressed (up-regulated) in BCG-vaccinated cynomolgus macaques and accounted for control of TB infection.	IL-26 was lower in monocytes of Mtb-infected patients than in HC controls.	Guerra-Laso *et al.*[64]
				Che *et al.*[65]
			Levels of BAL IL-26 correlated with innate effector cells in the lungs of TB patients.	Wareham *et al.*[66]
IL-7/15	ELISA, cytometric bead assay	Enhanced Mtb survival was shown in IL-7- or IL-15-treated mice.	TB patients had lower soluble IL-7R and higher IL-7 than HC.	Maeurer *et al.*[67]
				Singh *et al.*[68]
		Compared to BCG alone, mice injected with BCG supplemented with IL-7 and IL-15 showed enhanced T-cell proliferation, Th1 cytokine production, and more Mtb-specific memory T cells, which were combined with a more efficient reduction in the mycobacterial burden.	High IL-6 and low IL-15 marked the presence of TB infection.	Lundtoft *et al.*[69]
				Rane *et al.*[70]
			IL-15 acts as a co-stimulator in IFN-γ production by NK cells and is important for Mtb clearance. Ag85A-containing vaccine was more efficient than ESAT-6 vaccine for induction of IL-15 and conferred more protection in TB patients.	Lazarevic *et al.*[71]
				Saito *et al.*[72]
		IL-15 −/− mice had slightly higher bacterial numbers during chronic infection.		Umemura *et al.*[73]
				Chandrashekara *et al.*[74]
		IL-15 −/− mice supported more bacterial growth and exhibited impaired protection in the lung on day 120 after BCG infection.		Pydi *et al.*[75]
		IL-15-Tg mice showed enhanced resistance against BCG infection.		
IL-10	PCR, EIA, ELISA	Mice deficient in T-cell-derived (but not monocyte-derived) IL-10 showed significantly reduced lung bacterial loads during chronic Mtb infection compared to IL-10-competent mice.	IL-10 was elevated in the lungs and serum of active PTB patients.	Moreira-Teixeira *et al.*[76]
			IL-10 and TGF-beta were elevated in BAL of TB patients.	Turner *et al.*[77]
				Cyktor *et al.*[78]
		Transgenic mice overexpressing IL-10 exhibited increasing bacterial numbers in the lungs and showed evidence of TB reactivation during chronic infection compared to WT controls.		Beamer *et al.*[79]
				Barnes *et al.*[80]
				Huard *et al.*[81]
				Almeida *et al.*[82]
		IL-10 deficiency in CBA/J mice resulted in the development of mature granulomas and long-term control of Mtb infection.		Bonecini-Almeida *et al.*[83]
		Blocking IL-10 action via anti-IL-10R1 during chronic infection stabilized the pulmonary bacterial load and improved survival in Mtb-infected CBA/J mice.		

of resistance to Mtb, which could be considered to be a surrogate of protection.

During chronic Mtb infection, immune-mediated tissue damage is frequently more harmful to the host than the pathogen itself. The balance between pro- and anti-inflammatory signals determines TB development.[88] PD-1, as a mediator of T-cell exhaustion, has been proven to play a central role in anti-TB immune responses. Inhibition of PD-1 signaling rescues Mtb-specific IFN-γ-producing T cells from apoptosis and enhances the specific degranulation of CD8 T cells for more efficient protection.[89,90] However, PD-1$^{-/-}$ mice are extraordinarily sensitive to TB.[60] Dysregulation of CD4 T cells promotes rather than controls TB in the absence of PD-1 signaling.[61] Therefore, PD-1 deficient mouse studies suggest that PD-1-mediated T-cell exhaustion is essential to controlling excessive immunopathology after Mtb infection. Interestingly, one case report shows that a patient with advanced NSCLC rapidly developed pulmonary TB during anti-PD-1 therapy.[62] The above studies suggest that immune modulatory molecules, such as PD-1, should also be considered in the design of a successful TB vaccine.

Recent studies have demonstrated that it is feasible to identify novel functional signatures for protective immunity through a comparison of different immune phenotypes of LTBI, active TB, etc. Although approximately 1/4 of the world population is estimated to be infected with Mtb, less than 5–10% of affected individuals eventually develop active TB diseases. In addition, a perspective cohort study shows that over half of newly identified IGRAs-positive subjects reversed to IGRAs-negative in the second year of follow-up, suggesting that self-clearance of Mtb infection commonly occurs.[91] Thus, representative immunity in the LTBI population, including transiently IGRAs-positive individuals, potentially represents an immune status that is more effective at containing Mtb infection compared to that in the active TB population. LTBI patients represent a valuable pool for potential biomarker discovery for protective immunity. Following this selective strategy, one study found that IL-32 was a mediator of IFN-γ-vitamin D-dependent antimicrobial immunity.[59] In addition, IL-2, MCP-2, IP-10, IFN-γ, TNFSF14, MIG, and granzyme B have also been identified as associated with LTBI.[92] A plasma proteomic fingerprint that distinguished active TB from LTBI has also been identified.[93] However, it is also possible that the immune status in LTBI only represents protective outcomes, not true protective immunity that can successfully control the development of active TB in humans.

The distinct immune profiles between LTBI and active TB infection do not appear to be limited to circulating cytokine/chemokines. Metabolic products may also be involved in TB development. It was shown that decreased serum 5-oxoproline in TB patients is associated with pathological damage in the lung.[94] Metabolic profiles of decreased activity of indoleamine 2,3 dioxygenase 1 (IDO1) and increased phospholipase activity were observed specifically in LTBI but not in active TB patients.[95] These results suggest that LTBI and active TB display different metabolic profiles. However, we still cannot conclude that these differentiated profiles of cytokine/chemokines and metabolic products are driving immune protection or simply reflecting the outcomes of successful control of Mtb infection. Further animal and human studies should be implemented to investigate the exact roles of the above signatures during Mtb infection.

DO HETEROGENEOUS TH1 CELLS CONFER COMPREHENSIVE T-CELL-MEDIATED PROTECTIVE IMMUNITY?

While the Mtb-specific Th1 population is highly heterogeneous, it still remains unknown which subpopulation producing IFN-γ plays a more critical role against Mtb infection. First, Mtb contains more than 4000 protein antigens, and there is extensive diversity of immunodominant responses in infected individuals. In addition, gene expression profiles of Mtb are highly dynamic, depending on different immunological/physiological stresses or microenvironments. Mtb could change its gene expression profile from active replication to slow or non-replication status during infection to fit certain immunological/physiological stresses or microenvironments, and the resulting protein expression variations might therefore impact the protective capacity of antigen-induced T cells.[96] Thus, the immune responses of T cells derived from PBMC or BAL may not be the ones that are specifically desired for currently existing pathological Mtb antigens. In other words, the T-cell immune responses that are driven by current TB vaccine candidates may not truly cover protective Mtb antigens.

One study provided evidence that protective CD4 T cells targeting Mtb cryptic epitopes conferred superior protection to those recognizing immunodominant Mtb epitopes by eliciting a higher proportion of T-bet^{int}KLRG1^{-} CD4 T cells.[97] By comparing the protective efficiency of ESAT-6 and a truncated ESAT-6 molecule (Δ15 ESAT-6) that lacks the immunodominant ESAT-6 epitope, it was shown that the most efficient protection against Mtb aerosol challenge is mediated by the subdominant T-cell repertoire primed by Δ15 ESAT-6.[98] In this regard, definition of true protective or pathological Mtb antigens and/or epitopes is certainly critical for an improved understanding of TB immunobiology and for the development of vaccines and immunodiagnostics.

Second, Th1 cells can gain the capacity to secrete several cytokines, and these 'multifunctional' Th1 cells mainly produce IFN-γ, TNF-α, and IL-2 (Figure 1).[99] However, the exact roles of multifunctional Th1 cells in TB diseases remain controversial. Several studies show higher frequencies of IFN-γ^{+}TNF-α^{+}IL-2^{+} Mtb-specific CD4 T cells in LTBI compared to TB patients.[100–103] However, other studies have found that frequencies of IFN-γ^{+}TNF-α^{+}IL-2^{+} CD4 T cells increased in active TB patients and normalized after anti-TB treatment.[104–106] In addition, there is evidence indicating that most multifunctional T cells produce cytokines in a sequential fashion. For example, it was proven that multifunctional Th1-skewed cytokine responses (IFN-γ, IL-2 andTNF-α) are initiated asynchronously and that TNF-α production generally precedes IFN-γ and IL-2 synthesis through time-dependent, single-cell analysis of primary human T cells.[107]

Third, the heterogeneity of Th1 cells also comes from a new subset of Th1/Th17-like cells coexpressing T-bet and RORγt,

which exhibit Th1-like and Th17-like characteristics.[108] Although Th1/Th17-like cells were proven to be pathogenic in the gut of patients with Crohn's disease,[15] the exact roles of Th1/Th17-like cells in TB patients are unclear. One study demonstrated that higher frequencies of Th1/Th17-like cells, the main source of IL-17, are highly correlated with disease severity in TB patients.[109] Studies of Mtb-specific memory T cells have revealed that $CCR6^+CXCR3^+CD4^+$ T cells, displaying hallmarks of both Th1 and Th17 transcriptional programs, are significantly increased in LTBI donors compared to healthy controls.[110] Furthermore, isolated $CXCR3^+CCR6^+$ $CD4^+$ T cells from LTBI individuals exhibit higher proliferative ability upon stimulation with Mtb antigens.[111]

Finally, deep sequencing T-cell receptor (TCR) of T cells stimulated by Mtb antigens demonstrates that human memory CD4+ T-cell clones are highly heterogenic in function, and different patterns of clonotype sharing among three Mtb-specific CCR6+ T-cell subsets were observed.[112] Thus, Mtb-specific Th cells are comprised of not only clones polarized toward a single fate but also clones whose progenies have acquired multiple fates. However, the exact molecular mechanisms by which the heterogeneities of Mtb-specific Th cells are shaped and how these heterogeneities dictate the pathological or protective outcomes of Mtb infection remain to be elucidated.

Thus, the current Th1 cell responses defined by limited known antigen specificity and classical cytokines profiles cannot represent comprehensive protective immunity due to the heterogeneity of Th1 cells. It is reasonable to hypothesize that only some TB-specific Th1 cells or T cells are truly and fully protective and that only these T cells should serve as targets for new TB vaccines. To overcome this limit, a better solution should analyze not only Th1 cells but also other T cells, at single-cell level, to clearly delineate TCR specificity and characterize each TB-specific T-cell in Mtb infection.

DO HUMORAL IMMUNE RESPONSES PROVIDE PROTECTION AGAINST MTB INFECTION?

Unlike well-established T-cell-mediated immune responses, the role of humoral immune responses remains largely controversial. Humoral immunity has long been believed to play little or no function against Mtb, an intracellular pathogen that is traditionally considered to be out of the reach of antibodies. However, this view has been progressively changing. Recently, an antibody profiling study on Mtb-specific humoral responses revealed that LTBI individuals have unique antibody Fc functional profiles, selective binding to FcγRIII, and distinct antibody glycosylation patterns that clearly distinguish them from active TB patients.[113] Most notably, the PPD-specific antibody purified from the sera of LTBI, but not active TB, could inhibit Mtb growth in macrophages.[113] The potential involvement of humoral immunity in Mtb defense is further supported by evidence that passive transfer of Mtb-specific monoclonal antibodies,[114–117] intravenous immunoglobulins,[118] and homologous immune sera[119,120] is efficient at providing protection against Mtb infection in murine models. On the other hand, another study has shown that the blocking activity of antibodies against Mtb is dependent on antibody isotype and independent of Fc alpha receptor expression on host cells.[121] Furthermore, antibodies from uninfected healthcare workers who had no prior evidence of latent TB infection, a subset of 'restrictors', show moderate protection against Mtb in aerosol-challenged mice, but this protection is absent with antibodies derived from Mtb active patients.[122]

Taken together, the exact protective role and underlying mechanisms of antibodies against TB need to further defined, as antibody-mediated potential protection might be an important contributor to the true functional signature of protective immunity. More importantly, emerging evidence suggesting the protective effects of antibodies implicates that our current view of TB immunobiology is still in its infancy stage. More protective paradigms are still hidden and waiting to be identified.

CONCLUDING REMARKS

The lack of useful surrogate markers for protective immunity hinders TB vaccine development. Although antigen-specific Th1 cytokines have been currently used to determine the protective efficacy of TB vaccines, vaccine candidates with a strong ability to induce Th1 cytokine production did not confer full protection against TB in humans. Future studies using integrated omics and single-cell sequencing to elucidate the comprehensive, delicate, precise T-cell responses in the lung will eventually discover true protective functional signatures. The recent findings on the protective role of antibodies suggest that antibodies might be an important contributor to the true functional signature of protective immunity.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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