T cells and adaptive immunity to 
*Mycobacterium tuberculosis* in humans

**Summary:** The adaptive immune response mediated by T cells is critical for control of *Mycobacterium tuberculosis* (*M. tuberculosis*) infection in humans. However, the *M. tuberculosis* antigens and host T-cell responses that are required for an effective adaptive immune response to *M. tuberculosis* infection are yet to be defined. Here, we review recent findings on CD4<sup>+</sup> and CD8<sup>+</sup> T-cell responses to *M. tuberculosis* infection and examine the roles of distinct *M. tuberculosis*-specific T-cell subsets in control of *de novo* and latent *M. tuberculosis* infection, and in the evolution of T-cell immunity to *M. tuberculosis* in response to tuberculosis treatment. In addition, we discuss recent studies that elucidate aspects of *M. tuberculosis*-specific adaptive immunity during human immunodeficiency virus co-infection and summarize recent findings from vaccine trials that provide insight into effective adaptive immune responses to *M. tuberculosis* infection.

**Keywords:** TB, CD4<sup>+</sup>, CD8<sup>+</sup>, IL-10-producing immunosuppressive and Treg cells, Th1 and Th17 cells, HIV co-infection

**T-cell responses and detection of *M. tuberculosis* infection**

In humans, the adaptive immune responses to *Mycobacterium tuberculosis* (*M. tuberculosis*), which become detectable 3–8 weeks after infection and play a critical role in the ultimate outcome of *M. tuberculosis* infection, are primarily comprised of antigen-specific CD4<sup>+</sup> T-cell responses. In the majority of *M. tuberculosis*-infected persons, a CD4<sup>+</sup> T-cell-driven type IV delayed hypersensitivity reaction that is characterized by visible skin induration can be detected 24–72 h after intradermal administration of *M. tuberculosis* purified protein derivative (PPD), or tuberculin [tuberculin skin test (TST)] (reviewed in 1). Careful clinical observation of individuals exposed to persons with infectious TB suggests that this immunological sensitivity to PPD frequently coincides with fever and erythema nodosum (2). Through measurement of IFN-γ release assay (IGRA) positivity, which is currently used as a proxy for latent *M. tuberculosis* infection, and based on the prevalence of persons with immunological sensitization to PPD, it has been estimated that more than 2 billion persons globally may be infected with
M. tuberculosis (3). Although one can argue that sensitization is dependent on some level of exposure to M. tuberculosis or, as is more likely, infection with M. tuberculosis, the nature of immunological memory suggests that persons with self-cured disease, aborted infection, or sterilization may also retain antigen-specific immune responses (4) (see below). It thus has been argued that the real prevalence of persons with persistent M. tuberculosis infection is likely lower than typically reported (5).

On the other hand, antigen-specific anergy as measured by lack of TST responses in otherwise immunocompetent TB patients has been well-documented in several populations of distinct ethnicity and can confound estimates of TB prevalence and TB diagnosis itself. For example, the Yanomami indigenous population of Brazil was shown to have a high rate of anergy (approximately 46%) to PPD by TST skin response (<10 mm induration) despite evidence of active TB disease (6). In another example, among otherwise immunocompetent Cambodian patients with acid-fast bacilli (AFB)-positive TB, approximately 20% displayed a lack of TST responses, which was associated with an expansion of immunosuppressive CD4+ IL-10-producing T regulatory cells (7, 8). There is also evidence for ethnic specific influences in the magnitude of TST responses (9, 10). TST anergy in patients with active TB disease appears to be associated with elevated circulating levels of Th2 cytokines such as IL-10 and IL-4 (7, 8, 11–13), a lack of IFN-γ responses to PPD in vitro (7, 8), selective loss of Vβ2 γδ T cells (13, 14), expansion of IL-10-producing immunosuppressive T cells (7, 8), and polymorphisms in Th1 and Th2 cytokine genes and specific HLA genes (15–18). Taken together, these studies indicate that impaired Th1 responses and elevated IL-10 production are major mechanisms underlying anergic responses.

Another issue in evaluating T-cell responses to M. tuberculosis infection is that viable M. tuberculosis can persist for years in healthy individuals, as was reported in 1927 when M. tuberculosis was cultured from apparently healthy tissues of individuals who exhibited no pathological evidence of TB and who died from other causes (19). An additional example demonstrating that viable M. tuberculosis asymptptomatically persists for decades after initial infection is provided by the reactivation of latent M. tuberculosis infection in rheumatoid arthritis patients who receive anti-TNF blocking antibodies and other biologic therapies (20, 21). The risk of developing TB disease among this massive reservoir of latent M. tuberculosis infection is typically reported as a single value, such as approximately 10%, or as approximately 5% in the first 12–24 months with a 5% lifetime risk among the general infected population (22–24). In reality, risk of disease appears to depend on many variables including size of the infectious dose, the phenotype and genotype of the pathogen, time since exposure, gender, age, and a multitude of environmental and host risk factors, as well as host adaptive immune mechanisms and their impairment by, for example, steroids, chemotherapy, biologics, and HIV co-infection (reviewed in 25, 26).

Another limitation to estimating risk of TB disease following infection is that subsequent re-exposure to infectious TB and reinfection may increase risk of disease. For example, in post-World War II Norway, in the setting of a very rapidly declining TB epidemic, the incidence of TB disease peaked 12–24 months after M. tuberculosis infection and thereafter steadily waned (27). This study provided good evidence for consistent waning of latent M. tuberculosis infection and risk of disease over time, suggesting that host mechanisms are capable of sterilizing M. tuberculosis infection. By contrast, in endemic settings where ongoing exposure to infectious TB cases drives a high annual risk of infection, continuous reinfection may maintain a consistently high risk for progression to disease, with reinfection risk dramatically exacerbated by HIV co-infection (28–32).

There is evidence that positive TST or IGRA tests in latently infected individuals and in patients who are treated for TB spontaneously revert to negative (reviewed in 33), and this phenomenon has been reported at rates of 10–50% throughout the last century (34–37). Although reversion could be caused by immune suppression, the egress of M. tuberculosis-specific T cells from the blood and sequestering at sites of disease, or by lowering of M. tuberculosis bacillary load during treatment, it may also indicate that in certain cases humans are able to clear M. tuberculosis infection. A lower incidence of TB disease in reverters with latent TB infection compared with persistently TST-positive or IGRA-positive persons would support this hypothesis. The most comprehensive study of the clinical significance of TST reversion was performed by Arthur Dahlstrom in household contacts of TB index cases in the 1920s (38). Among individuals in which at least two TSTs were performed, 11.1% reverted to negative. These reverters had a minimal risk of developing active TB over 5 years (0.72%), whereas 23.3% of the entire cohort developed disease. By contrast, a high risk of TB disease was observed in Quantiferon Gold In-Tube (QFT) assay reverters (annual reversion rates of 5.1%) in a recent analysis of South African adolescents (39). Although the number of TB cases was too low for robust stratification of disease risk in this study, incident TB was eightfold higher among QFT reverters compared
with persons with consistently negative QFT results (1.47 versus 0.18 cases/100 person-years, \( P = 0.011 \)) (39). Additional studies are necessary to establish the clinical significance of TST and/or IGRA reversion and implications for adaptive immunity to TB.

**T-cell activation after *M. tuberculosis* infection**

Our understanding of the mechanisms involved in the initial CD4+ T-cell response to *M. tuberculosis* infection comes primarily from murine studies and so findings from these reports are briefly reviewed here. In mice, dendritic cells, which are the classical antigen-presenting cells (APC) that activate and induce proliferation of antigen-specific naive CD4+ T cells, are among the first host cells in the lung to encounter and phagocytose *M. tuberculosis* after infection. It is only after these dendritic cells traffic to the mediastinal lymph node that *M. tuberculosis*-specific CD4+ T-cell expansion is detected (40–42). Surprisingly, *M. tuberculosis*-infected dendritic cells in the murine lymph node show profound impairment in their ability to activate naive *M. tuberculosis*-specific CD4+ T cells to proliferate, and this appears to be due in part to reduced MHC class II-peptide presentation by these infected antigen presenting cells (APCs) (43). A recent study found that *M. tuberculosis*-infected dendritic cells in the lymph node release soluble, intact *M. tuberculosis*-infected dendritic cells in the lymph node release soluble, intact *M. tuberculosis* antigens that are taken up by uninfected dendritic cells, which can then efficiently present these antigens to naive CD4+ T cells and initiate the adaptive immune response (44).

In humans, several host-specific differences in the ability to present specific antigens involved in containment of *M. tuberculosis* infection have been uncovered. For example, several alleles of the HLA class II DR2 serotype (45–53), an HLA-DQB1*0503 allele (54), and alleles that encode an aspartic acid (HLA-DQ-β7-Asp) at the P9 binding pocket of HLA-DQβ (18), have been associated with susceptibility to progression to clinical tuberculosis from latency in diverse populations. Furthermore, APCs encoding HLA-DQ-β7-Asp alleles elicit significantly reduced IFN-γ responses to *M. tuberculosis* antigens in *in vitro* studies (18).

The critical role for T-cell immunity in the control of *M. tuberculosis* infection has been demonstrated in a number of human and non-human primate studies (55–58), and defects in Th1 cytokine production, particularly IFN-γ, are well-established risk factors for *M. tuberculosis* infection and TB disease progression in humans (59). Here, we focus on recent findings that shed light on the character of an effective versus insufficient adaptive T-cell response to *M. tuberculosis* infection, and how TB treatment affects the *M. tuberculosis*-specific memory CD4+ and CD8+ T-cell repertoires.

* *M. tuberculosis*-specific Th1 responses and T-cell polyfunctionality during natural infection

A critical factor in containment versus spread of *M. tuberculosis* is the character of the T-cell response that develops in response to infection. During the course of antigen-driven differentiation, a CD4+ or CD8+ T cell can gain the capacity to simultaneously produce two or more cytokines. These ‘polyfunctional’ T cells are generally considered to be superior effectors as compared to cells that produce only one cytokine. The prototypical ‘good or desirable’ polyfunctional T cell is thought to be trifunctional and to produce IFN-γ, TNF, and IL-2, indicating capacity for effector and proliferative functions (60). Although several lines of evidence suggest that induction of polyfunctional CD4+ and/or CD8+ T cells is important for sustained vaccine-induced memory responses (see more below), the role of these multitaskers is less clear during natural TB infection.

Among studies that have examined trifunctional versus mono- and bifunctional CD4+ T-cell activity in *M. tuberculosis* infection and TB disease, several have reported that active TB disease is associated with elevated frequencies of *M. tuberculosis*-specific CD4+ T cells with single or dual function, particularly TNF+ or TNF+ IFN-γ+. By contrast, persons with latent infection exhibit higher frequencies of trifunctional IFN-γ+ TNF+ IL-2+ *M. tuberculosis*-specific CD4+ T cells (61–66). Furthermore, TB treatment appears to drive the CD4+ T-cell compartment to a more trifunctional IFN-γ+ TNF+ IL-2+ state (65, 67). Other studies, however, have found that active TB disease is accompanied by an expansion in the frequency of TNF+ IFN-γ+ IL-2+ CD4+ T cells, as compared to latent infection (68–71) or to after TB treatment (72).

A single study in BCG-vaccinated infants sought to determine if frequency or polyfunctionality of mycobacteria-specific CD4+ T cells was associated with subsequent risk of TB disease. BCG-specific CD4+ and CD8+ T-cell expression of IFN-γ, TNF, IL-2, and IL-17 was measured at 10 weeks of age. A comparison of trifunctional, bifunctional, and monofunctional T cells was performed in infants who ultimately developed TB disease during 2 years of follow-up and control infants who remained healthy. This study however, revealed no association between T-cell expression of IFN-γ, TNF, IL-2, and/or IL-17 and risk of TB disease (73).
The role of mycobacterial antigen load in driving trifunctional CD4⁺ T-cell expansion also remains unclear. While pulmonary, smear-positive TB patients presented with lower M. tuberculosis-specific trifunctional CD4⁺ T cells as compared to smear-negative TB patients in one study (65), in another study patients with multi-focal tuberculous lymphadenitis exhibited higher IFN-γ⁺ TNF⁺ IL-2⁺ CD4⁺ T-cell frequencies compared to patients with pulmonary TB disease (74). Intriguingly, it has recently been reported that Indian adolescents who displayed strong and persistent responses in the QFT whole blood assay had correspondingly higher frequencies of trifunctional CD4⁺ T cells in response to M. tuberculosis antigens in in vitro assays as compared to individuals who exhibited a strong initial QFT response but later reverted to a negative QFT (75). Thus, although additional work is required to establish the relationship between M. tuberculosis antigen burden and the quality of the CD4⁺ T-cell response, these studies suggest a link between these phenomena.

Examinations of T-cell polyfunctionality in TB disease indicate that M. tuberculosis-specific IL-2⁺ CD4⁺ T-cell frequencies decline during active TB disease, both as single expressers and as a component of polyfunctional subsets. By contrast, TNF-expressing M. tuberculosis-specific CD4⁺ T-cell percentages appear to increase. These data suggest that the effector CD4⁺ T-cell response during active TB gradually shifts to a highly differentiated character, with limited proliferative potential. This conclusion is supported by findings that active TB disease is associated with an expansion in effector memory (EM) as well as short-lived effector CD4⁺ T cells (62, 63, 70, 76–80), which becomes more pronounced once TB treatment is initiated (81), and can be evident even decades after spontaneous control of active TB disease (4). By contrast, M. tuberculosis-specific CD4⁺ T-cell recall responses in persons with latent M. tuberculosis infection have been shown to vary from this pattern and to consist primarily of EM, CM, or EM⁺CM cells, depending on the antigen(s) used for activation (63, 66, 79, 82, 83).

Although polyfunctional T cells are capable of producing two or more cytokines in response to stimulation, it appears that in most polyfunctional T cells each cytokine is produced in a sequential fashion. TNF production generally precedes IFN-γ and IL-2 synthesis (84), and longer incubations with antigen prior to intracellular cytokine staining (ICS), may enhance detection of polyfunctionality (85). Thus, in addition to differences in antigens used for stimulation, even minor differences in the length of stimulation during ICS assays may lead to discrepancies in detection and quantification of distinct polyfunctional T-cell populations. Furthermore, variation can also be caused by difference in antibody sources, and variations in human and mycobacterial populations (85). It will thus be important for future studies aimed at understanding the importance of polyfunctional T cells and their role in the human immune response to M. tuberculosis infection to standardize assay protocols in order to directly compare results.

Studies of T-cell subsets and M. tuberculosis antigens that dominate recall immunity to natural M. tuberculosis infection have recently revealed that M. tuberculosis-specific memory CD4⁺ T cells are enriched in the subset of CXCR3⁺ CCR6⁺ CD4⁺ T cells, which exhibit Th1-like and partial Th17-like characteristics (86). Furthermore, of the 80 M. tuberculosis epitopes that accounted for >75% of the total CD4⁺ T-cell recall response in patients with latent M. tuberculosis infection, >80% of the circulating CD4⁺ T cells that recognized these epitopes were CXCR3⁺ CCR6⁺ CD4⁺ T cells (87). Furthermore, isolated CXCR3⁺ CCR6⁺ CD4⁺ T cells from patients with latent M. tuberculosis infection proliferated strongly upon stimulation with M. tuberculosis antigens when compared to CXCR3⁺ CCR6⁺ and CCR6⁻ CD4⁺ T cells that were stimulated with the same antigens. Notably, nearly half of these epitopes were derived from M. tuberculosis proteins that had not previously been identified as antigens (87). These findings indicate that a distinct Th1/Th17-like subset that can respond potently to a relatively restricted assortment of M. tuberculosis antigens dominates the CD4⁺ T-cell memory compartment in latently infected individuals. Future studies are necessary to validate these findings in different geographic areas, evaluate their association with M. tuberculosis disease risk patterns, and determine their role in limiting breakthrough of M. tuberculosis from latency.

**M. tuberculosis-specific CD8⁺ T cells during natural infection**

Although less studied, changes in CD8⁺ T-cell function during active TB have also been described. Both circulating M. tuberculosis-specific CD8⁺ T cells (88) and CD8⁺ T cells located at sites of active TB disease (89), show reduced cytotoxic activity compared to those found in healthy, latently infected patients. Although a recent report found enrichment of IFN-γ⁺ TNF⁺ IL-2⁺ trifunctional M. tuberculosis-specific CD69⁺ CD8⁺ T cells in pleural fluid spaces of TB patients (90), an earlier study reported that these cells were reduced in patients with active TB versus persons with latent infection and that high M. tuberculosis antigen burden appeared to exacerbate CD8⁺ T-cell dysfunction (65).

Terminally differentiated CD45RA⁺ CCR7⁻ CD8⁺ T cells with potent antimycobacterial capacity have also been...
described and appear to be reduced in active TB patients compared to persons with latent M. tuberculosis infection, and this phenotype corresponded with diminished IFN-γ+ IL-2+ CD8+ T-cell percentages (91). In addition, M. tuberculosis-specific CD8+ T cells from South African patients with active TB were primarily CD45RA+ CCR7+ (92). In this latter study, senescent and pro-apoptotic markers were upregulated on M. tuberculosis-specific CD8+ T cells to a greater extent in patients with active TB as compared to those who had latent infection. Furthermore, in another report proliferative deficiencies persisted in M. tuberculosis-specific CD8+ T cells after TB therapy (93). These data thus suggest that, during active TB disease, the M. tuberculosis-specific CD8+ T-cell population is arrested at an intermediate differentiation point characterized by both reduced proliferative potential and reduced cytotoxic activity. Notably, infliximab anti-TNF treatment in patients with autoimmune disorders who also had latent TB infection led to a reduction in, the numbers of terminally differentiated, TNF-dependent, CD45RA+ CD8+ T cells with potent antitubercular capacity (94). It is thus intriguing to speculate that prophylactic and/or early TB treatment may limit CD8+ T-cell effector dysfunction, and promote both improved control of latent infection and reduce the risk of TB re-infection.

The role of IL-17-producing cells in natural M. tuberculosis infection

Over the past decade, the role of IL-17-producing (Th17) cells in M. tuberculosis infection has been described in several studies in the murine TB model. IL-23 drives differentiation and functionality of Th17 cells (95), and an early report in mice found that knockout of the IL-23 subunit p19, which promotes the production of IL-17, resulted in almost complete loss of M. tuberculosis-specific Th17 cells, but intriguingly had little effect on infection control, which was driven by Th1-derived IFN-γ (96). Later murine studies found that, early after infection, IL-17 is primarily produced by γδ T cells (97, 98), and implicated as a positive player in granuloma formation in response to high dose intratracheal infection (97, 99). Dysregulated IL-17 production in the murine lung led to immunopathology driven by excess neutrophil recruitment (100, 101); however, IL-17 signaling appears to be critical for proper neutrophil targeting to the infection site early after infection for control of M. tuberculosis in murine models (102). Recently, it has been demonstrated that IL-17−/− mice are unable to control infection by the hypervirulent M. tuberculosis strain HN878 but survive infection by less pathogenic strains. This outcome was associated with reduced T-cell recruitment to lymphoid follicles in the lung in the absence of IL-17 (103).

In humans, most studies have found that ex vivo stimulation of whole blood or PBMC from individuals with latent TB infection as compared to active TB patients leads to greater IL-17 production and/or increased frequencies of IL-17+ CD4+ T cells (104, 105, 106, 107, 108, 109, 110). One report, however, found higher IL-17 release in response to PPD stimulation in whole blood of active TB patients versus those with latent infection (111). Longer durations of ex vivo stimulation appear to enhance the ability to detect IL-17 synthesis (104, 111, 112), and it is has been reported recently that M. tuberculosis-induced expansion of PBMC from latent TB patients leads to the gradual accumulation of IFN-γ+/IL-17+ CD4+ T cells, and the surface expression of CXCR3 and CCR6 (104). This observation suggested that the capacity to produce IL-17 is delayed in comparison to IFN-γ in M. tuberculosis-specific memory CD4+ T cells and that that M. tuberculosis lysate in patients with more severe TB disease (113). The latter finding suggested that greater bacterial burden and/or extrapulmonary M. tuberculosis growth may lead to increased recirculation of IL-17+ CD4+ T cells, which are normally sequestered at the infection site, leading to reduced presence in the peripheral blood (107). As has been reported in mice, IL-17+ γδ T cells have also been found to be increased in human TB patients (114). Notably, the M. tuberculosis antigen HBHA can also activate TH17 cells; HBHA stimulation ex vivo induced activation of a greater frequency of CD4+ T cells co-expressing IFN-γ, IL-2, and IL-17 in household contacts as compared to active TB patients (115). In summary, Th17 and other IL-17-producing cells appear to contribute to M. tuberculosis control, most likely at an early stage of infection, and differences in pathogenic M. tuberculosis strains may play a role in differential Th17 responses observed in humans.

The role of immunosuppressive IL-10-producing CD4+ T cells and FoxP3+ CD4+ Tregs during natural infection

As described above, a link between immunosuppressive CD4+ T-regulatory cells (Tregs) and reduced immunity against M. tuberculosis was first described through the identification of IL-10-producing Tregs in otherwise immunocompetent Cambodian patients with active TB who showed anergy to PPD by skin test (7). T cells from these patients produced high levels of IL-10 in response to stimulation by
autologous PPD-loaded APCs, which inhibited T-cell proliferation in a contact-dependent and -independent manner. By contrast, T cells from PPD-responsive TB patients produced both IFN-γ and IL-10 and proliferated. Blocking IL-10 resulted in restoration of proliferation of T cells from anergic patients (7). Anergy to PPD in a portion of the anergic patients was transient. PPD responsiveness was regained in this group of patients and was reflected in regaining a T cell proliferative response to PPD in in vitro studies. Strikingly, a subset of patients remained persistently anergic to PPD by TST but did respond with a positive skin test to Candida antigen. Furthermore, PBMC from these persistently PPD-ergic patients in vitro retained their inability to respond to Candida antigen stimulation with IFN-γ production and proliferation (8).

The subsequent identification of natural CD4+ regulatory Tregs, which express FoxP3 and can suppress effector responses in a contact-dependent and IL-10 contact-independent manners (116–118), led to further work aimed at finding additional immunosuppressive T-cell populations in TB patients. CD4+ Treg frequencies were shown to be increased in the peripheral blood and at disease sites of patients with active TB and these cells could suppress M. tuberculosis-specific IFN-γ production by Th1 cells ex vivo (112, 119–123). A study of granulomatous lesions in children with TB found that FoxP3+ CD4+ Treg cells were present in high numbers and that their quantity correlated inversely with CD8+ T-cell numbers at infection sites (124).

At the same time, studies in mice found that CD4+ Tregs prevented M. tuberculosis bacterial control (125–127), and M. tuberculosis-specific murine CD4+ Tregs were shown to expand alongside the expansion of M. tuberculosis-specific Th1 cells in pulmonary lymph nodes, leading to reduced effector CD4+ and CD8+ T-cell recruitment to the lung during early M. tuberculosis infection (128). In macaques, redistribution of CD4+ Tregs from the peripheral blood to the lung early after M. tuberculosis infection was shown to be associated with subsequent elevation of CD4+ Tregs in the circulation (129). In a second macaque study, simultaneous IL-2-mediated expansion of M. tuberculosis-specific Th1 cells and CD4+ Tregs was detected during early TB infection (130).

A population of FoxP3+ Tregs in humans co-expresses the surface ectonucleosidase CD39, which metabolizes pro-inflammatory extracellular ATP (131). These cells are CCR6+ CD45RO+, indicating that they are antigen-experienced (131). CD39+ Tregs were found to expand disproportionately in response to M. tuberculosis-specific stimulation of PBMCs from active TB patients and to potently suppress M. tuberculosis-specific IFN-γ production (132). Furthermore, it was recently demonstrated that CD39+ Tregs are enriched in the M. tuberculosis-specific memory CD4+ T-cell compartment of active TB patients compared to persons with latent M. tuberculosis, and these cells promoted IL-10 production in PBMC cultures stimulated with M. tuberculosis antigens (133). Intriguingly, a direct correlation between CD39+ CD4+ Tregs and M. tuberculosis-specific Th1 and Th17 responses was demonstrated in humans immunized with the experimental TB vaccine MVA-85A, as a proportional decrease in this cellular subpopulation within PBMCs coincided with a peak in antigen-specific IL-17 and IFN-γ production by CD4+ T cells (134, 135).

A major takeaway from these findings is that CD4+ Tregs contribute to impaired M. tuberculosis control/clearance as well as reduced inflammation at infection sites. Moreover, IL-10 derived from Tregs or other cell types (see section about type I interferon responses below) is linked to poor immunity to M. tuberculosis. As a potent suppressor of macrophage activation, especially activation by IFN-γ, excess IL-10 production by T-cell subsets may impair pulmonary macrophage killing of engulfed M. tuberculosis and lead to reduced antigen-presenting capacity and diminished production of IL-12/β, IL-12, and TNF by myeloid cells, with concomitant impairment of M. tuberculosis-specific CD4+ and CD8+ T-cell activation and proliferation (136–141). Thus, limited expansion of CD4+ Tregs and other immunosuppressive T-cell subsets may contribute to an optimal response to M. tuberculosis characterized by modulated inflammation in the first weeks after infection is established (142, 143), but too much Treg expansion leads to impaired TB immunity.

**Inflammation and TB**

Active TB is characterized by inflammation and the upregulation of acute phase proteins, cytokines, chemokines, and matrix metalloproteinases (MMPs) (144–146), which in turn can affect T-cell function and development in the blood, sputum, and at sites of disease. Cavitation, which is a hallmark of adult pulmonary TB disease, is driven by elevated expression of inflammatory matrix metalloproteinase-1 (MMP-1) and MMP-3, which are found at greater levels in sputum and bronchoalveolar lavage fluid from patients with active TB (147). Whole blood transcriptomic analyses have also been applied to investigate human inflammatory responses in M. tuberculosis infection and TB disease. Several reports have described strong upregulation of genes
associated with type I/II interferon responses, inflammation, and myeloid cell activation, and the downregulation of lymphoid cell genes, in blood from TB patients compared with latently M. tuberculosis-infected controls (148–153). An inflammatory signature appears to also occur in children with TB, and a transcriptomic signature consisting largely of type I/II interferon genes has recently been applied as a diagnostic test for TB in children (154).

Strong type I IFN production during M. tuberculosis infection is detrimental in mice. For example, mice lacking the common type I IFN receptor exhibit better control of M. tuberculosis infection as compared to wild type controls (155–158). Furthermore, agonistic activation of type I IFN synthesis in the lung impaired M. tuberculosis immunity and control of mycobacterial burden (159). It has been reported that type I IFNs drive enhanced neutrophil recruitment to the lung in hypersusceptible 129S2 mice, leading to local tissue damage and increased M. tuberculosis dissemination (160). Type I IFN responses that were experimentally induced by influenza infection also resulted in greater susceptibility to subsequent M. tuberculosis infection in mice (161). Another recent murine study established proof of concept that augmentation of prostaglandin E2 levels, which were shown to promote IL-1 production and restrict excessive type I interferon production, led to containment of M. tuberculosis and prevented mortality (162). IL-10 has also been implicated in the negative repercussions of type I IFN signaling in murine models, as type I IFN induction of IL-10 in myeloid subsets in the lung during M. tuberculosis infection was found to repress IL-1 production (142). Furthermore, M. tuberculosis-infected murine macrophages were shown to synthesize IL-10 after type I IFN treatment, leading to reduced mycobacterial killing in these cells in response to lymphoid-derived IFN-γ (143). It should be noted, however, that type I IFNs may also serve a protective role once infection is established in mice by reducing the number of M. tuberculosis-susceptible target cells in the lung, thereby limiting disease progression at the site of infection (163).

In humans, a recent study of the effects of type I interferons on human macrophages in vitro showed that IFN-β upregulated IL-10 expression, which in turn inhibited IFN-γ-mediated macrophage activation and killing of intracellular mycobacteria (164). Combined with several reports that have found augmented type I IFN production to correspond with active TB disease development and/or severity (149, 165, 166), these findings provide further support for a negative impact of type I IFNs on host control of M. tuberculosis.

Indeed, although more research is necessary, it is likely that the adaptive T-cell response is inhibited by type I IFNs, as reduced production of critical T cell-activating factors like IL-12, TNF, and IL-1β due to type I IFN-mediated IL-10 upregulation would be expected to drive a markedly diminished M. tuberculosis-specific Th1 response.

### T-cell responses and M. tuberculosis/HIV co-infection

Recent studies on TB/HIV co-infection have elucidated new insights into adaptive T-cell responses to TB. M. tuberculosis-specific CD4+ T cells are depleted from the periphery early after HIV infection in humans, and evidence suggests that this is partially due to preferential targeting of M. tuberculosis-specific memory CD4+ T cells by HIV (167, 168). A number of reports have found that TB disease enhances CCR5+ expression and/or the frequency of CCR5+ CD4+ T cells in the absence (169–172) and presence of HIV co-infection (169, 173, 174, Haridas et al., manuscript submitted), indicating that the chronic immune activation that accompanies active TB leads to a larger CCR5+ CD4+ T-cell population that is presumably susceptible to highly efficient infection by HIV. Furthermore, a strong CD4+ T-cell response to M. tuberculosis might simultaneously lead to enhanced HIV replication, as demonstrated in in vitro studies where a primary subtype AE HIV-1 isolate replicated to a greater extent in CD4+ T cells from HIV-negative PPD-responsive donors compared to CD4+ T cells from HIV-negative PPD-energic donors after PPD stimulation (175). Notably, this process was partly mediated by PPD-induced IL-10 suppression of viral replication in the anergic donor cells (175). Furthermore, distinct M. tuberculosis strains can drive differential HIV replication in ex vivo PBMCs, as less virulent strains that drive strong proinflammatory cytokine production were found to promote enhanced replication of primary subtype B, C, and AE viruses, while more virulent M. tuberculosis strains that are less immunogenic drove lower levels of viral replication (176). These data indicate that antigen-specific responses to M. tuberculosis would be expected to enhance HIV replication, consistent with the higher viral loads that are detected in TB/HIV co-infected patients versus patients singly infected with HIV who exhibit similar levels of immunosuppression (177, 178, Haridas et al., manuscript submitted).

Examination of polyfunctional T-cell responses in M. tuberculosis/HIV co-infection found that viral load in co-infected TB patients with latent TB inversely correlated with M. tuberculosis-specific IL-2-expressing CD4+ T cells that were positive for TNF or IFN-γ, while the M. tuberculosis-specific
CD8+ T-cell population was highly enriched in IL-2− cells (179). A later report confirmed that increased immunosuppression in HIV+ patients with latent TB infection was associated with a loss of polyfunctional M. tuberculosis-specific CD4+ T-cell responses, which were restored following ART (180). Bronchoalveolar lavage (BAL) fluid from HIV+ patients with latent M. tuberculosis infection also exhibited reduced M. tuberculosis-specific polyfunctional as well as bi- and mono-functional CD4+ T-cell frequencies compared to those from patients free of HIV (181, 182). Another report found that ART did not fully restore M. tuberculosis-specific polyfunctional CD4+ T-cell responses in bronchoalveolar cells (183). Notably, however, BCG-vaccinated HIV+ patients who maintained M. tuberculosis-specific IFN-γ-secretting T cells had significantly reduced risk of future active TB disease (184).

Recapitulating what has been established in human ex vivo studies, the cynomolgus macaque model of latent M. tuberculosis and SIV infection has linked depletion of peripheral CD4+ T cells to TB reactivation (185). Furthermore, it was reported that animals who experience early TB reactivation relative to those who reactivate at later time points exhibit an elevation in M. tuberculosis-specific trifunctional IFN-γ+ TNF+ IL-2+ CD4+ T-cell frequencies (186). Again, this latter finding is consistent with the reports described above that demonstrated that elevated human M. tuberculosis-specific trifunctional IFN-γ+ TNF+ IL-2+ CD4+ T cells are a consequence of high M. tuberculosis burden and not necessarily a sign of effective M. tuberculosis-restrictive immunity.

Lessons from TB-associated immune reconstitution syndrome

M. tuberculosis-specific T-cell responses also play a critical role in TB-associated immune reconstitution inflammatory syndrome (TB-IRIS), a condition that commonly occurs in HIV+ patients who are undergoing simultaneous TB therapy and ART or during the unmasking of occult TB in HIV patients who are initiating ART (187). Several studies have demonstrated that ex vivo M. tuberculosis antigen stimulation of blood or PBMCs from TB-IRIS patients collected prior to, at the time of, or soon after the TB-IRIS event results in strong M. tuberculosis-specific Th1 responses (188–191). In one investigation, ex vivo stimulation of PBMCs from TB-IRIS patients with M. tuberculosis antigens led to a strong enrichment in IFN-γ+ TNF+ IL-2− CD4+ T cells (192). In a recent report, higher soluble IL-2 receptor plasma levels were associated with risk of TB-IRIS, further supporting a role for CD4+ T-cell activation in the development of TB-IRIS (193).

In a prospective and large immunological study of T-cell evolution in TB-IRIS patients, which was nested within the Cambodian Early versus Late Introduction of Antiretrovirals (CAMELIA) randomized clinical trial in Cambodia (194), whole blood immunophenotypes were analyzed prior to ART initiation and at several time points following ART initiation in severely immunocompromised (median CD4+ T-cell count of 25/μl) patients with documented TB who had initiated TB therapy (195). Patients who developed TB-IRIS began ART with significantly higher frequencies of activated (HLA-DR+ CD45RO+) CD4+ T-cells and CCR5+ CD4+ T-cells than patients who avoided TB-IRIS (195). In addition, elevated pre-ART OX40+ CD4+ T-cell proportions were a specific risk factor for TB-IRIS. Furthermore, after ART initiation, patients who experienced TB-IRIS exhibited a significantly greater expansion in effector memory (CD62L− CD45RA−) CD4+ T-cell frequencies and a reciprocal contraction in central memory (CD62L+ CD45RA−) T-cell frequencies (195). These latter findings indicate that TB-IRIS drives the formation of a CD4+ T-cell memory compartment that is distinct from that seen in similarly immune compromised patients who avoid TB-IRIS. In summary, TB-IRIS appears to represent the far end of a spectrum of potent MTb-specific CD4+ T-cell responses that may provide superior long-term protection from TB reactivation and/or reinfection (194).

T-cell responses and vaccination against TB

The only current vaccine against TB is Bacillus Calmette Guerin (BCG), a live attenuated strain of Mycobacterium bovis. This vaccine is given to the majority of the world’s newborns soon after birth, who are then protected against disseminated forms of tuberculosis, such as meningitis or miliary disease. BCG induces a Th1-like CD4+ T-cell response (196) and following vaccination multiple patterns of CD4+ T-cell cytokine production are common. Combined expression of IFN-γ, TNF, and IL-2 and single expression of IFN-γ have been described most frequently (73, 196, 197). In addition, IL-17 expression, mostly distinct from Th1 cytokine production, is detectable (73). BCG also activates CD8+ T cells, but at quantitatively much lower levels than CD4+ T cells, with IFN-γ production dominating in these cells (73, 196, 197). Vaccine-induced CD4+ and CD8+ T cells may also have cytotoxic potential, a function that appears distinct from cytokine producing capacity, and
induced cells have proliferative potential (197, 198). The peak response to newborn BCG vaccination appears to be around 6 weeks of age, after which the response declines but remains detectable until at least 12 months of age (197). Delaying BCG vaccination from the first day of life to later in infancy, and administration of the vaccine to adults may affect quantitative, qualitative, and kinetic characteristics of the immune response to the vaccine (199–201).

The relative contribution of BCG-induced T-cell immunity to protection against childhood TB disease is unclear. A large, prospective study of BCG-vaccinated infants showed that, 10 weeks after newborn vaccination, there was no difference in any measure of BCG-specific T-cell immunity, including markers of Th1 and Th17 activation, between infants who subsequently developed TB disease in their first 2 years of life and those who did not (73). The IFN-γ/IL-12 axis is clearly essential for protection against mycobacteria, as shown by congenital deficiencies in humans and knockouts of components of this pathway in animals; however, as this study implies, it is unclear whether this immunity is sufficient for protection (202). Alternatively, the correlates of risk of TB disease might have been measured at a suboptimal time point in this particular infant study.

The same dilemma complicates elucidation of the factors that contribute to an effective T-cell response after immunization with novel vaccines formulations, and we do not know whether, and which, T-cell responses correlate with protection against TB disease. Most novel vaccines in the current clinical portfolio, including whole mycobacterial candidates, adjuvanted proteins, and viral vectored vaccines, induce type 1 CD4+ T-cell responses comprised mainly of IFN-γ+ TNF+ IL-2+ or at least Th1 responses (203–206). In some cases, CD8+ T-cell immunity is also elicited. One viral vectored candidate, MVA85A, induced easily detectable CD4+ T-cell responses in a randomized, placebo-controlled phase IIb trial of infants who received the vaccine or placebo about 4 months after newborn BCG; regardless, there was no difference in protection against TB disease between the two groups (207). This result again questions the role of these cells in protection against TB disease, and challenges the common practice of measuring conventional CD4+ and CD8+ T-cell responses as immunogenicity outcomes in studies of novel TB vaccines. Similarly, although the hypothesis is attractive, there is no evidence that qualitative and quantitative differences in CD4+ or CD8+ T-cell immunity induced by current novel vaccines are important.

Until we have correlates of protection against TB disease, which is possible only following a successful placebo-controlled efficacy trial of a novel TB vaccine, we will have to devise alternate strategies to gain insights into immune responses that are necessary for effective immunity. First, in every clinical study, much more detailed assessment of induced immunity is important for iterative learning and effort should be made to measure global immunological signatures characteristic of an effective adaptive immune response, thereby reducing the risk that preconceived experimental biases will obscure or preclude unanticipated observations. Further, results from prospective studies of human populations at elevated risk of TB disease might guide the search of correlates of protection. At the very least, results could be used to learn whether individual candidates induce immunity that might be detrimental rather than protective, such as induction of lung inflammation leading to increased recruitment of target cells for M. tuberculosis infection. Finally, as we do not know whether conventional CD4+ and CD8+ T-cell immunity is sufficient for protection, nor to which antigens these responses should be directed, an attractive alternate strategy in vaccination against TB could involve eliciting ‘uncommon’ or non-conventional T-cell responses, particularly if antigen presentation involved is less polymorphic. Examples include induction of CD1 and HLA-E-mediated T-cell responses, γδ T-cell responses, or populations of T cells that primarily reside in the lung, such as mucosal-associated T cells (208–210). The development of improved vaccination approaches will require greater characterization of the antigens involved in the early phases of M. tuberculosis infection and that play a part in the early stages of latent mycobacterial reactivation combined with. They will also require a deeper understanding of the memory characteristics of induced T-cell populations necessary for the induction of an effective and long-lasting adaptive immune response in human TB disease.

References


57. Lin PL, et al. CD4 T cell depletion exacerbates acute Mycobacterium tuberculosis while reactivation of latent infection is dependent on severity of tissue depletion in cynomolgus macaques. AIDS Res Hum Retroviruses 2012;28:1693–1702.

58. Chen CY, et al. A critical role for CD8 T cells in


98. Lockhart E, Green AM, Flynn JL. IL-17 production is dominated by gammadelta T cells rather than CD4 T cells during Mycobacterium tuberculosis infection. J Immunol 2006;177:4662–4669.


111. Sutherland JS, de Jong BC, Jeffries DJ, Adetifa IM, Ota MO. Production of TNF-alpha, IL-12(p40) and IL-17 can discriminate between active TB disease and latent infection in a West African cohort. PLoS ONE 2010;5:e12365.


