INTRODUCTION

The goal of the End TB Strategy is to reduce TB deaths by 90% and TB incidence of new cases per year by 80% by year 2030, compared with 2015.1 The ambitious goal of a fast deceleration of disease incidence could be achieved by a multipronged approach including increases in access to TB medical care, addressing socioeconomic factors, as well as research and technological breakthroughs especially in diagnostics, therapeutics, and vaccines.

Despite significant worldwide control efforts over the last 20 years, the progress toward elimination of tuberculosis has slowed. The World Health Organization (WHO) estimates that approximately one-quarter of the world’s population (1.7 billion total) is infected with Mtb. Mtb is responsible for 1.3 million deaths among HIV-negative individuals.
annually and ~ 10 million new infections are reported each year. In 2019, tuberculosis was the ninth leading cause of death worldwide and the leading cause from a single infectious agent, ranking above HIV/AIDS. The severity of this situation is compounded by the fact that many cases in low-income and middle-income countries go undiagnosed and thus untreated. Additionally, with the COVID-19 pandemic in 2020/21, tuberculosis management has been neglected; patients have discontinued their treatment due to lockdowns, new cases are not visiting clinics despite symptoms, and co-infection with SARS-CoV-2 may lead to increased mortality. Thus, it is expected that the number of tuberculosis cases will rise further in the coming years.

2 | THE SPECTRUM OF MTB INFECTIONS

*Mtb* infections are traditionally classified into active TB (ATB) infection or a quiescent/latent state (LTBI). ATB is typically defined as the presence of symptoms and/or *Mtbi* smear/culture positivity. However, it is now well accepted that *Mtbi* infection should be seen as a continuous spectrum with high heterogeneity and no clear segregation between the LTBI and ATB group.4–6

The distinction between ATB and LTBI is often made based on presence of symptoms and culture positivity for simplicity in clinical and research settings. Typically, LTBI will have a positive tuberculin skin test (TST) and/or Interferon Gamma Release assay (IGRA), but this may also be true for individuals who have eliminated their infection. Therefore, the IGRA+ group contains a spectrum of individuals from those who have cleared their infection to individuals with subclinical TB disease, and not all within this group have the same likelihood of developing active disease. Moreover, the TST and IGRA tests have limited usefulness in areas with high TB burden and TB endemic areas. LTBI individuals with a more recent infection, or with presence of co-morbidity factors such as HIV, or diabetes are at higher risk of developing active disease. In addition to co-morbidities and time since infection, severity of ATB is also dependent on additional factors, such as the infecting *Mtbi* strain and how far the infection has progressed. In this review, we compare *Mtbi*-specific classical CD4 T cell immune responses in LTBI (usually defined as IGRA+) vs ATB (individuals with symptoms) as this is commonplace in the scientific literature. However, we realize that improved diagnostics and molecular tools to allow more granularity on the disease spectrum will allow more in-depth studies in the future and the characterization of immune correlates of protection that can more closely cover this spectrum.

3 | THE NEED FOR IMPROVED VACCINATION AND IMMUNODIAGNOSTICS

The majority of infected individuals control the pathogen by mounting a successful, long-lived, and protective immune response, leading to either elimination of the bacteria or a persistent latent infection which is not associated with significant clinical symptoms. However, approximately 10% of latently infected individuals eventually develop active disease. The risk of developing ATB is higher in individuals that are immunocompromised (due to age, corticosteroid use, malnutrition, and HIV infection). The lengthy treatment is expensive and requires a combination of multiple antibiotics.

In many parts of the world, access to these drugs is limited and compliance with the drug regime is often poor, thus favoring the development of drug resistant strains. The development of drug resistant strains of *Mtbi* pose a threat to global health and the success of the End TB Strategy. Worldwide, 4.1% of new cases and 19% of previously treated cases are infected with rifampicin-resistant (RR-TB) or multidrug-resistant TB (MDR-TB). About 6.2% of these MDR-TB cases are classified as extensively drug-resistant TB (XDR-TB), which has been identified in 123 WHO member states. The prevalence of MDR cases both complicates the schedule and increases cost of treatment. Most importantly, the existence of antibiotic resistant strains emphasizes the need for development of a vaccine solution to curb their spread.

The vaccination of children with *M. bovis* BCG results in a 60%–80% decrease in the incidence of active TB. However, in most developed countries BCG vaccination is not recommended due to the relatively low incidence of disease and variable effectiveness in preventing first time pulmonary TB in adults, a large fraction of active disease cases. A new vaccine against TB is required, preferably targeting adolescents and adults who represent the vast majority of new cases and are responsible for spreading *Mtbi* infection. Ideally, a vaccine should be protective irrespective of *Mtbi* infection status, that is, both in individuals with and without evidence of latent infection, and prevent progression to active disease, reinfection, and reactivation. A recent prevention of infection trial using BCG and a subunit TB vaccine candidate provided encouraging results showing reduced rates of sustained QuantiFERON conversions.

As a complimentary approach to developing a new vaccine, advanced diagnostic tools could theoretically identify individuals at high risk of developing active TB disease through systematic screening. The high-risk individuals could then be treated before they become infectious, which would also contribute to a reduction of TB cases. Immunodiagnostic tests for TB rely on the detection of an immune response against mycobacterial antigens, either by delayed hypersensitivity reaction (Tuberculin skin test; TST), or by detection of IFNy following in vitro stimulation (IGRA). A TST can produce a false-positive result due to prior BCG vaccination, and may produce a false-negative result due to other factors such as immunosuppression or malnutrition.

In summary, new vaccines and immunodiagnostics could provide a quantum leap in the fight against TB. However, to accomplish these goals a precise understanding of the characteristics of immune responses and their impact on disease progression and susceptibility is required. The rest of this review will focus on these issues.

4 | CD4 T CELLS AND THEIR IMPORTANCE IN MTB INFECTION

Human T cell responses to *Mtbi* involve classically restricted CD4 and CD8 αβ T cells, and non-classically restricted T cells such as NKT (CD1), MAIT (MR1) and γδ T cells. Depletion of CD4 T cells
demonstrated that while CD8+ T cells and other immune cells also play a protective role against Mtb, they alone cannot compensate for the lack of a dominant CD4 T cell response.17 The importance of CD4 T cells in the defense against Mtb is also supported by the fact that patients with HIV infection (which leads to reduced CD4 T cell counts) are more susceptible to primary Mtb infection, reinfection, and reactivation.18

CD4 T cells primarily act by secreting a variety of cytokines that attract other immune cells to the site of infection and initiate the differentiation of different CD4 T cell subsets capable of performing effector functions. The ability of T cells to recognize Mtb-infected antigen presenting cells is a key step in containing the infection. Srivastava et al was able to demonstrate using mouse models that direct recognition of Mtb-infected cells by CD4 T cells is required for control of the infection.19

IFNγ production by CD4 T cells is commonly associated with control of Mtb infection.8,20-23 The essential role of IFNγ in the protective immunity to mycobacteria is made apparent in individuals with genetic defects in the IFNγ receptor, who have an increased susceptibility to infection with mycobacteria.24 However, several reports demonstrated other anti-tuberculosis CD4 T cell effector functions not accounted for by IFNγ production. The likelihood of developing ATB does not correlate with either the amount of produced IFNγ or the pattern of co-production with other cytokines.25 Accordingly, the focus of our review is on classical CD4 T cells, since they represent a major component of the T cell response against Mtb. We consider both IFNγ production, as well as other effector and phenotypic functions associated with CD4 T cells and control of Mtb infection (Figure 1, Table 1).

5 | STRATEGIES TO DEFINE THE PHENOTYPE OF Mtb-SPECIFIC CD4 T CELLS

Flow cytometry is by far the most commonly used technique to interrogate the phenotype of Mtb-specific CD4 T cells. There are several strategies to identify Mtb-specific CD4 T cells from bulk CD4 T cells. The most common strategy is to stimulate cells in vitro with Mtb-derived reagents: either whole preparations (eg, Mtb lysate, PPD), or peptides (such as the ones used for the IGRA assay targeted against the two proteins ESAT-6 and CFP10, or “megapools”, see below). The advantage of peptides is that they are defined synthetic reagents with little variation across batches and thus generate results highly consistent across experiments. They can also be selected based on their MHC binding to target either CD4 or CD8 T cells.26,27 However, they are usually limited to only a few proteins or epitopes of interest. To overcome this limitation, our group has designed a peptide pool that combine 300 primarily MHC class II restricted epitopes that represent more than 80 Mtb proteins (see below).

Regardless of the stimuli used, surrogate markers of antigen-specificity are needed to identify the cells with antigen-specific reactivity following stimulation. For Mtb-specific T cells, the most commonly used measurement is IFNγ. However, as discussed more below, not all Mtb-specific CD4 T cells express IFNγ. Many more cytokines are produced such as IL-2, TNF, and IP-10, as well as other cellular changes occurring in response to Mtb stimuli. To overcome the hurdle of having to specifically select which cytokines to measure, there are assays that measure the expression of surface proteins that are specifically induced upon T cell activation.28 These surface proteins, typically called activation-induced markers, encompasses TNF family receptors OX40, CD137, and CD154, as well as CD69 and PD-L1.

Another strategy to characterize the phenotype of antigen-specific T cells is to use multimeric staining reagents (eg, MHC tetramers), which require precise knowledge of the epitopes’ HLA restriction and HLA expression of the subjects. Multimers captures all T cells capable of binding a given epitope:MHC combination, thus only work in subjects that express the specific MHC allele, and recognize the specific epitope.29 Therefore, multimers are ideally suited for in-depth characterization of a small representative set of

Figure 1: Summary of characteristics of Mtb-specific classical CD4 T cells for differentiation of disease stages and potential correlates of protection. Mtb-specific classical CD4 T cells express different characteristics depending on the disease stage of the individual. There is a higher frequency of Tcm, Th1* and CD153 in LTBI. ATB has higher frequency of Tem and Tscm, HLA-DR expression and increased differentiation. Th1* and CD153 are important for control of Mtb infection. Other cytokines and chemokines include: IL-2, IL-10, IL-17, TNFa, and CXCL9/10/11/12/13.
| Mtb-specific CD4 T cell phenotype | Infection stage | Vaccinated | LTBI | ATB | Severe ATB |
|---------------------------------|----------------|------------|------|-----|------------|
| **Memory phenotype**            |                |            |      |     |            |
| T\(_{\text{cm}}\) (CCR7\(^+\)CD45RA\(^-\)) | Humans\(\uparrow\) T\(_{\text{cm}}\) phenotype in vaccinated newborns\(^{53}\) | Humans\(\uparrow\) T\(_{\text{cm}}\) phenotype compared to ATB\(^{52}\) |            |      |            |
| T\(_{\text{em}}\) (CCR7-CD45RA-) | Humans\(\uparrow\) T\(_{\text{em}}\) compared to LTBI\(^{52,54,55,57,58}\) |            |      |     |            |
| T\(_{\text{scm}}\) (CD45RA\(^+\)CCR7\(^-\)CD27\(^+\)) | Humans\(\uparrow\) T\(_{\text{scm}}\) compared to LTBI\(^{55}\) |            |      |     |            |
| **Th subset**                    |                |            |      |     |            |
| Th1*-Th1/Th17 cells\((\text{CXCR3}^+\text{CCR6}^+\text{CCR4}^-)\) | Humans Re-vaccination with BCG boosted reactivation of polyfunctional Th1/Th17 lymphocytes\(^{49}\) | Humans\(\uparrow\) Th1\(^*\) compared to uninfected controls\(^{64}\) and ATB\(^{48}\) | NHPs\(\uparrow\) CD4 T cells expressing a "hybrid Th1/Th17 immune response" detected using single-cell RNA-seqencing in TB granulomas of NHPs infected with Mtb\(^{73}\) |            | |
| T\(_{\text{fs}}\): RORC, Tbet | NHPs\(\uparrow\) Th1\(^*\) in BCG-vaccinated NHPs\(^{41,205}\) |            |      |     |            |
| **Differentiation markers**      |                |            |      |     |            |
| **Markers Associated With Less Differentiated Phenotype** | | | | | |
| CD27                            | Humans \(\uparrow\) CD27 compared to ATB\(^{78-85}\) |            |      |     |            |
| CD127                           | Humans \(\uparrow\) CD127 in Mtb-specific CD4 T-cells compared to ATB\(^{52}\) |            |      |     |            |
| **Activation markers**           |                |            |      |     |            |
| CD153 (CD30L)                   | Humans \(\uparrow\) CD153 Mtb-specific CD4\(^*\) T-cells compared to ATB\(^{39,41}\) | Mice CD153 deficient mice develop high pulmonary bacterial loads and die early after Mtb infection\(^{41}\) |            |      |            |
|                                 | Mice \(\uparrow\) CD153 expression on Mtb-specific Th1 cells in the lung parenchyma of Mtb-infected mice\(^{41,96}\) |            |      |     |            |
|                                 | NHPs \(\uparrow\) CD153 expression on Ag-specific CD4 T cells in the airways compared to blood in Mtb-infected NHPs that inversely correlates with granuloma bacterial load\(^{18}\) |            |      |     |            |
| HLA-DR                          | Humans \(\uparrow\) HLA-DR in Mtb-specific CD4 T cells compared to LTBI\(^{88,92-95}\) |            |      |     |            |
| CD38                            | Humans \(\uparrow\) CD38 in Mtb-specific CD4 T cells compared to LTBI\(^{84,88,89,97,98}\) |            |      |     |            |

(Continues)
| Mtb-specific CD4 T cell phenotype | Infection stage | Vaccinated | LTBI | ATB | Severe ATB |
|----------------------------------|----------------|------------|------|-----|-----------|
| Ki67                             |                |            |      |     | Humans: ↑ Ki67 in Mtb-specific CD4 T cells compared to LTBI<sup>88,89,97,98</sup> |
| PD-1                             |                |            |      |     | Humans: ↑ PD-1 in Mtb-specific CD4 T cells compared to LTBI<sup>87</sup> |
| **Cell adhesion molecules**      |                |            |      |     |           |
| CD62L (L-selectin)               | Humans         | Mtb-specific CD4+ T cells were confined to the GPA33-CD62L- Th1* subset in IGRA+ individuals<sup>42</sup> |
|                                  | Humans         | Mtb-specific CD4+ T cells from tubercular pleural fluid were effector/memory cells with high CD45RO expression, but low CD62L, CCR7, and CD27 expression<sup>74</sup> |
| **Chemokines and their receptors** |                |            |      |     |           |
| CXCR3                            | Mice           | ↑ CXCR3 on KLGR1- Mtb-specific T cells derived from the lung parenchyma (compared to lung vasculature) of vaccinated mice challenged with Mtb. Adoptive transfer of these parenchymal T cells resulted in greater control of infection compared to more terminally differentiated KLGR1+ T cells localized to the lung vasculature<sup>200-204</sup> |
|                                  | Humans         | ↑ CXCR3 on Mtb-tetramer+ CD4 T cells in LTBI compared to ATB<sup>68</sup> |
| IP-10                            |                |            |      |     | Humans: ↑ IP-10 levels in Mtb-infected individuals (both LTBI and ATB) compared to uninfected individuals<sup>136</sup>. IP-10 expression may be increased in ATB compared to LTBI<sup>137</sup>, but its ability to distinguish ATB from LTBI is controversial<sup>139</sup>. |
| **Cytokines**                    |                |            |      |     |           |
| IFNγ                             | Humans         | ↑ IFNγ response in Mtb-infected individuals compared to uninfected individuals, but IFNγ expression cannot differentiate LTBI from ATB |

(Continues)
epitope specificities. In contrast, stimulation with peptide pools and measurement of cytokines or upregulation of activation markers are possible in most subjects, albeit with some limitations. Mtb-specific T cells that do not express the chosen markers escape detection. In addition, bystander activation can lead to T cells being captured that do not directly react to the epitopes.

**6 | EPITOPE MEGAPOOLS AS A UNIVERSAL TOOL FOR MEASURING CD4 T CELL RESPONSES**

It is often possible to assess T cell responses directly ex vivo by using pools of different epitopes or peptides, so that the overall frequency of responding cells is enhanced. This approach is particularly key to analyze small sample volumes. This "megapool" approach is based on large numbers of peptides pooled and formulated using sequential lyophilization. Specifically, for detection of Mtb-specific responses we have described and validated a comprehensive megapool of 300 Mtb epitopes representing more than 80 Mtb proteins, derived from a proteome-wide screen for epitopes and antigens recognized by IGRA+ individuals. This original pool, named "MTB300", and versions thereof have been used by a number of studies to measure and phenotype Mtb-specific responses. Due to the overlap of epitopes recognized by different species MHC this pool has also been shown to capture T cell reactivity in mice and non-human primates.

These studies have contributed to our understanding of immunity against Mtb and other mycobacteria. For example, peptide megapools contributed to the identification of peptide MHC ligands for TCR groups and the phenotyping of Mtb-specific CD4 T cells. MTB300 was used when CD153 on CD4 T cells was identified as a major mediator of host protections against pulmonary Mtb infection and the subsequent evaluation of the role of CD153 in Mtb infection.

**TABLE 1 (Continued)**

| Mtb-specific CD4 T cell phenotype | Infection stage | Severe ATB |
|----------------------------------|----------------|-----------|
|                                  | Vaccinated     | LTBI      | ATB      | Humans |
| IL-17                            |                |           |          | ↑ IFNγ/IL-17 CD4 T cells in severe ATB compared to less severe disease |
| TNFα                             | Complex role of TNFα in the immune response against Mtb | | | |
| IL-10                            | | | | |
| Polyfunctional cytokine responses | Reports on polyfunctional responses conflicting | | | |
| Dual or Triple-producing (INFγ,IL-2,TNFα) cells | | | | |
| Humans                           | ↑ of double and triple producing T cells in both children and adults post BCG-vaccination, but mainly children | | | |
| NHPs                             | ↑ in CD4 T cells dually producing IFNγ and TNFα following intravenous administration of BCG that were associated with reduced disease pathology | | | |
| Humans                           | ↑ IL2/IFNγ ratio after long-term stimulation with PPD differentiates LTBI from ATB | | | |
| Humans                           | ↑ frequency of triple producing T cells compared to LTBI | | | |

Note: Generally, a marker is included under a specific stage of Mtb infection if it has been described as increased in that stage relative to the other disease stages. Differentially expressed markers that may serve as potential correlate of protection are italicized in red; Dx markers that can distinguish LTBI from ATB are italicized in blue.
infection in humans.\(^{39,43}\) This approach has also been useful in providing evidence for mutations that are detrimental to host immunity against \(Mtb\) and other mycobacteria.\(^{45,47,48}\)

### 7 | MEMORY PHENOTYPE OF \(Mtb\)-SPECIFIC CD4 T CELLS

As mentioned above, understanding the complexity of CD4 T cells responses to \(Mtb\) and their functional attributes is key to developing correlates of protection and informing the design and testing of vaccines and immunodiagnostics. In this and the following sections, we address these issues.

CD4 T cells can be divided into naïve and memory populations based on the expression of CCR7 and CD45RA. Naïve cells have a CD45RA\(^+\)CCR7\(^+\) phenotype. Memory cells can be further partitioned into three different phenotypes: central memory (CCR7\(^+\)CD45RA\(^-\), Tcm), effector memory (CCR7\(^-\)CD45RA\(^+\), Tem), and effector memory re-expressing CD45RA (CCR7\(^-\)CD45RA\(^+\), Temra).\(^{51}\)

In LTBI, \(Mtb\)-specific CD4 T cells have been shown to predominantly express the CCR7\(^-\)CD45RA\(^-\) Tcm phenotype,\(^{52}\) similarly to BCG-specific CD4 T cells after BCG vaccination in newborns.\(^{53}\) In contrast, \(Mtb\)-specific CD4 T cells in ATB have the CCR7\(^-\)CD45RA\(^-\) Tem phenotype.\(^{52,54,55}\) These cells might also represent effector T cells (Teff) since Teff are expected to downregulate CCR7.\(^{56}\) Individuals with ATB have a higher proportion of effector memory cells, likely with less tissue homing capacity but higher effector functions, compared to latently infected individuals.\(^{57,58}\)

Whereas the vast majority of \(Mtb\)-specific CD4 T cells falls into the memory compartment, a small but not negligible fraction of \(Mtb\)-specific CD4 T cells have a naïve CD45RA\(^+\)CCR7\(^-\)CD27\(^+\) phenotype.\(^{36,53,54}\) These cells, initially named naïve-like cells, were subsequently identified as stem cell memory T cells, or Tscm. Tscm are a subset of long-lived memory CD4 T cells that can hold specificity to multiple pathogenic or self-derived antigens in humans and hold enhanced ability for self-renewal and multipotency.\(^{59}\) Transcriptomic analysis of tetramer sorted cells showed that \(Mtb\)-specific Tscm cells have a transcriptomic profile highly similar to bulk Tscm but also share phenotypic and functional properties with both central memory and effector T cells.\(^{60}\) Based on these results, it was suggested that \(Mtb\)-specific Tscm might therefore represent a less differentiated subset of \(Mtb\)-specific T cells.

\(Mtb\)-specific T cells with a Tscm phenotype are induced after primary \(Mtb\) infection\(^{60}\) and BCG vaccination,\(^{61}\) and their blood frequency is increased in ATB compared to LTBI.\(^{55}\) The function of antigen-specific Tscm in the context of TB remain unclear. Adoptive transfer of \(Mtb\)-specific memory T cells with a naïve-like phenotype in mice showed a higher degree of protection compared to \(Mtb\)-specific Tem transfer,\(^{55}\) suggesting they might hold an important protective role in TB.

Taken together, these data suggest a heterogeneity within \(Mtb\)-specific memory CD4 T cell subsets that varies depending on an individual’s position on the spectrum of infection. This is important to consider in progression studies and vaccine efficacy trials.

### 8 | T-HELPER SUBSETS OF \(Mtb\)-SPECIFIC CD4 T CELLS

Different T-helper (Th) subsets can be defined based on their surface expression of chemokine receptors and/or specific transcription factors. The classical Th subsets express different combinations of CXCR3, CCR6, and CCR4, Th1 (CXCR3\(^-\)CCR6\(^-\)CCR4\(^+\)), Th17 (CXCR3\(^-\)CCR6\(^-\)CCR4\(^+\)), and Th2 (CXCR3\(^-\)CCR6\(^-\)CCR4\(^-\)).\(^{62}\)

IFNγ, IL-2, and TNFα producing CD4 T cells (classically called Th1) and IL-17 producing CD4 T cells (Th17) cells are considered to be the main T cell subsets responding to \(Mtb\) infection. Th2 and regulatory T (Treg) cells are also another subset of CD4 T cells that have been found at the site of infection,\(^{63}\) but they play a different role in immunity.\(^{64}\) Previous studies have reported Th2 and Tregs working to impair Th1/Th17 and CD8 cytotoxic T cells (CTLs),\(^{65}\) indicating a suppressive action.

Another Th subset involved in the immune responses against \(Mtb\) infection is Th1* (also called Th1 co-expressing CCR6, Th17.1, Th1Th17, Th17/Th1, and Th1/Th17 cells), which form their own distinct population of CXCR3\(^-\)CCR6\(^-\)CCR4\(^-\) cells co-expressing the transcription factors Tbet and RORC.\(^{62}\)

### 9 | TH1* AS A CRUCIAL SUBSET OF \(Mtb\)-SPECIFIC T CELLS

Our work and others have shown that Th1* contain the majority of \(Mtb\)-specific T cells in IGRA* individuals.\(^{36,62,66}\) We have also found that mycobacteria-specific (including non-tuberculous mycobacteria; NTM) epitopes are also recognized by Th1* cells, in both \(Mtb\)-infected and uninfected individuals.\(^{67}\) Moreover, this specific Th population is present at a higher frequency in IGRA* individuals compared to \(Mtb\)-uninfected controls,\(^{66}\) unlike other Th subsets that were present at similar frequencies in both cohorts. These primary observations suggested a role for these Th1* cells in the containment of \(Mtb\) infection. In a follow-up study, using a DRB5*01:01 tetramer loaded with CFP10\(^{52-66}\), we found that more than 90% of tetramer* T cells were Th1*.\(^{66}\) This finding was confirmed in a study by Strickland et al. who also reported that \(Mtb\)-specific CD4 T cells are predominantly Th1* in HIV-negative IGRA* subjects.\(^{68}\)

Interestingly, they also found that the Th1* subset is contracted in individuals with active TB.\(^{68}\)

Transcriptomic profiling highlighted that Th1* cells have a specific gene signature which is distinct from other Th subsets, and associated with TB susceptibility (CCR2, IL12RB2), augmented cell survival and proliferation (BAFF, MDR1, Kit), and CTL-like cytotoxic cell killing (transcription factor EOMES, granzyme A, granzyme K, perforin), suggesting a role in disease control in LTBI individuals (ie, preventing transition to active disease).\(^{66}\)
Other studies have confirmed an important role for Th1* cells in Mtb infection. Re-vaccination with BCG was shown to boost the reactivation of “polyfunctional Th1/Th17 lymphocytes” (likely Th1*; although not confirmed with cell surface staining) in a cohort of IGRA positive individuals in the absence of isoniazid treatment (INH).

Furthermore, Th1* cells are found in more than one stage of Mtb infection as these cells have also been identified in blood from individuals with active TB, albeit at lower frequency compared to LTBI. In a non-human primate model of latent Mtb infection, both CCR6 and CXCR3 were upregulated on Mtb-specific CD4 T cells in the airways, and CXCR3*CD4 T cells accumulated in the granulomas and their frequency correlated with bacterial burden. CXCR3 is known to be important for T cell migration from the blood to the lung in the context of TB and other respiratory infections. Thus, CXCR3*CCR6+ CD4 T cells might encompass most antigenic reactivity in TB due to their unique ability to circulate between the blood and the site of infection.

10 | FURTHER HETEROGENEITY WITHIN THE TH1* SUBSET

Th1* are capable of producing many different cytokines and effector molecules upon polyclonal stimulation, including IFNγ, IL-2, TNFa, IL-17, CCL3, GZMB, IL-22, and CCL4. However, Mtb-specific Th1* cells produce IFNγ, IL-2, and TNFa, but not IL-17 in IGRA* individuals.

The difference between polyclonal and Mtb-specific stimuli indicated that it is possible to identify cellular heterogeneity within the overall Th1* subset. Applying bulk transcriptomics on sorted memory CD4 T cells in IGRA* individuals, we revealed 74 differentially expressed genes that were able to distinguish IGRA* individuals and Mtb-uninfected controls. In this study, we further refined the Mtb-reactivity was restricted to the GPA33 − CD62L compartment within Th1*, CD62L had also previously been found to be downregulated in Mtb-specific CD4 T cells.

A recent study that used single-cell RNA sequencing to define cellular responses associated with control of Mtb infection (i.e., bacterial killing in granulomas), identified higher proportions of CD4 T cells expressing a “hybrid Th1/Th17 immune response”, thus further strengthening the hypothesis that Th1* have a role in containment of Mtb infection. This may be mediated in part by their expression of CCR6, which mediates cell homing to inflamed tissues, and thus allows peripheral localization. Tissue resident memory cells are also known to co-express CXCR3 and CCR6.

In ATB, several studies also reported that Mtb reactivity within circulating CD4 T cells maps to the Th1* subset. However, another report studying Mtb-tetramer+ CD4 T cells showed a more diverse expression of chemokine receptors, with downregulation of CXCR3 in ATB compared to LTBI.

A caveat in using chemokine receptors for phenotyping Mtb-specific CD4 T cells reside in the fact that most assays identifying Mtb-specific CD4 T cells rely on in vitro stimulation, which impacts the surface expression of chemokine receptors. For such studies, the use of tetramers for identifying Mtb-specific CD4 T cells is thus preferred over in vitro stimulation. Alternatively, pre-sorting of CD4 T cell subsets based on chemokine receptors followed by antigen-specific in vitro stimulation can be utilized. The low stability of chemokine receptor expression on the cell surface might explain the variability observed between some studies, especially in the case of active TB where cells might be already pre-activated in vivo.

Taken together, these studies point toward an important role of Th1* cells in Mtb infection. Future studies using in-depth phenotyping of Mtb-specific T cells in different disease stages will improve our understanding of this particular Th subset, as well as other cells involved in the immune response against Mtb.

11 | THE IMPACT OF T CELL DIFFERENTIATION AND ACTIVATION

Mtb-specific CD4 T cells differ between TB disease states in terms of their differentiation phenotype. Numerous studies have reported a reduction in CD27 expression in Mtb-specific CD4 T cells of ATB infected individuals compared to LTBI. CD27 expression has been further assessed as a potential diagnostic marker for improving active TB diagnosis in children. CD27 was shown to be downregulated in Mtb-specific CD4 T cells of ATB infected individuals compared to LTBI, whereas PD1 was upregulated.

The CD27+CD127+PD1+ phenotype is typically associated with more differentiated T cells (compared to CD27−CD127+PD1− cells), including effector T cells. Thus, in active disease, Mtb-specific CD4 T cells bear a phenotype that reflect enrichment for highly differentiated effector T cells compared to latent infection.

In addition to their differentiation phenotype, another major distinction between Mtb-specific CD4 T cells in ATB vs LTBI lies in the expression of activation markers. In ATB, HLA-DR, CD38 and Ki67 are strongly upregulated in Mtb-specific CD4 T cells compared to LTBI. Uregulation of these three markers in antigen-specific T cells was also reported during acute infection with HIV, EBV, or CMV compared to chronic infection. In particular, HLA-DR expression on Mtb-specific CD4 T cells has shown promising sensitivity and specificity to discriminate between ATB and LTBI, and it was also proposed as a potential prognostic marker for progression to active disease. More recently, our work suggest that HLA-DR marks a subset of Mtb-specific CD4 T cells with an effector phenotype that have recently proliferated upon infection (Tippalagama et al manuscript in preparation).

CD153 (CD30 ligand, encoded by TNFSF8) expressed by CD4 T cells is another cell surface molecule that has been shown to be differentially expressed between LTBI and ATB individuals. In LTBI, the frequency of CD153+ Mtb-specific CD4 T cells is increased compared to ATB. CD153 expression on CD4 T cells was shown to be critical for mounting protective immune responses against Mtb in mice and was associated with lower bacterial load in humans. Recently it was shown that the phenotypic profile of Mtb-specific CD4 T cells, using HLA-DR, CD27, and CD153, can be used to assess severity of TB disease and monitor treatment.
12 | EFFECT OF TB TREATMENT AND HIV CO-INFECTION

Following treatment, Mtb-specific CD4 T cells of individuals with ATB have reduced expression of HLA-DR, CD38, Ki67, PD1 and increased expression of CD27 and CD153 compared to prior to therapy initiation.\(^{39,87,88,97}\) Thus, upon ATB treatment Mtb-specific CD4 T cells shift towards a phenotype similar to LTBI. Moreover, HLA-DR, CD38 and Ki67 expression on Mtb-specific CD4 T cells positively correlated with mycobacterial load upon treatment.\(^{88}\) More recently, Vickers et al\(^{98}\) showed that the pre-treatment frequency of CD27\(^+\)CD38\(^+\)HLA-DR\(^+\) CD4 T cells after in vitro stimulation with PPD can discriminate between slow and fast responders during treatment. Thus, measuring differences in T cell populations pre-treatment can give insights on treatment status of a patient and potentially predict treatment failures. With the increased availability of compact flow cytometers, these findings have the potential to be translated into tools to help diagnose and classify Mtb infections and monitor treatment in less developed and rural endemic areas.

Co-infection with HIV is a major risk factor for developing active TB, and it strongly affects the CD4 T cell compartment. Several studies have focused on determining the effect of HIV co-infection on the phenotype of Mtb-specific CD4 T cells. Mostly, they have found no major differences between HIV seronegative and seropositive TB states. In ATB-HIV co-infected individuals, Mtb-specific CD4 T cells express high levels of HLA-DR and low levels of CD27, compared to LTBI-HIV co-infected subjects.\(^{81,92}\) The frequency of CD153\(^+\)Mtb-specific CD4 T cells is also reduced in ATB compared to LTBI, regardless of HIV seropositive status.\(^{99}\)

13 | Mtb-specific CD4 T Cell Responses Associated with IL-2 Production

As mentioned above, the IGRA tests, which measures Mtb-specific IFN-\(\gamma\) in responses, fails to accurately differentiate between LTBI and ATB. In particular, these tests are often considered ineffective when used to determine a child’s state of infection.\(^{99,100}\) Since Mtb infection is associated with a spectrum of disease manifestations, it is important to develop tests reflecting more closely the spectrum of Mtb infections so that an intervention tailored to the individual patient can be selected.\(^{101,102}\) Hence, parameters other than IFN-\(\gamma\) alone, may be key for future TB diagnostics. This is also relevant for individuals who are highly exposed to Mtb (household contacts of patients with TB) but consistently test negative for both TST and IGRA, that is, so called “resistors.”\(^{103}\)

Early within the immune response mounted by CD4 T cells, rapid short-lived IL-2 secretion is genetically controlled and is key in signaling proliferation and differentiation of other immune cells.\(^{104}\) IL-2 is important for extracellular killing of mycobacteria, as well as granuloma formation. IL-2 has been found to be an accurate indicator for differentiating between ATB and LTBI.\(^{105}\) Particularly, IL-2 in combination with other cytokines makes for useful ratios that accurately distinguish ATBI and LTBI.\(^{106}\) Specific ratios of IL-2 to IFN-\(\gamma\) in long-term stimulation are suggestive of LTBI.\(^{107}\)

Besides being a strong indicator of ATB, IL-2 has also been shown to induce Foxp3\(^+\) Treg growth and response, without impairment of macrophage anti-TB immunity.\(^{108}\) CD4\(^+\)CD25\(^+\)Foxp3\(^+\) Treg cells may be essential to a healthy TB response in humans\(^{109}\) and may actually function to guide Treg cell differentiation of certain CD4 T cells.\(^{110}\) Because CD4\(^+\)CD25\(^+\)Foxp3\(^+\) Treg cells likely have a relevant role within severe Mtb infection, IL-2’s involvement may be important for therapy.\(^{111}\)

14 | IL-17 and TNF Involvement in Mtb-specific CD4 T Cell Responses

One frequently studied cytokine in Mtb infections is IL-17, which works synergistically and cross-regulatorily with IFN-\(\gamma.\)(\(^{112}\)) Stimulation with IL-23 triggers Th17 cells to produce and secrete IL-21, IL-22, and IL-17, the latter of which plays a multifaceted role in TB.\(^{113}\) IL-17 has been shown to induce non-hematopoietic cells to produce CXCL13, an important chemokine required for localization that will be discussed later.\(^{114}\) A further unique role for IL-17 may be the regulation of hypoxic TB granulomas,\(^{115}\) which may be linked to an inverse relationship with IL-17 and TB disease in individuals with active TB. Furthermore, via IL-1/\(\alpha\), and IL-6/STAT3 pathways, IL-17 has been shown to directly—without the involvement of APC or CD8\(^+\) T cells— increase CD4 T cell-resistance to immune regulation.\(^{116}\) These functions contribute to the maintenance of Mtb within the lungs.

While IL-17 does have a strong supporting role in control of Mtb infection, it also has a drawback: IL-17 has been shown to increase antigen load leading to tissue damage.\(^{117}\) This is particularly relevant considering the evidence that multidrug resistant strains of TB induce higher levels of IL-17 producing cells.\(^{118}\) Furthermore, IFN-\(\gamma\)/IL-17 CD4 T cells—increase CD4 T cell-resistance to immune regulation.\(^{116}\) These functions contribute to the maintenance of Mtb within the lungs.

In addition to IL-17, other cytokines that are members of the tumor necrosis factor family also correlate with ATB. As with inflammatory cytokines, TNF-\(\alpha\) works synergistically with IFN-\(\gamma\) in Mtb infection and may be more frequently produced during an active Mtb infection.\(^{112}\) In a study of multiple cytokines after Mtb-antigen stimulation it was found that only TNF-\(\alpha\) was not significantly more abundant in LTBI.\(^{112}\) Furthermore, increased single-positive TNF-\(\alpha\)/Mtb-specific CD4 T cells were also found to be highly predictive of active TB.\(^{122}\)

TNF-\(\alpha\) is primarily produced by macrophages, but can also be secreted by CD4 T cells, and plays a managerial role. TNF-\(\alpha\) activates macrophages via an autocrine/paracrine mechanism, recruits
lymphocytes and monocytes to the infection site via chemokine signaling, restricts mycobacterial growth in granulomas and promotes tissue inflammation and apoptosis, and promotes DC maturation via TNFR1 and DC survival via TNFR2. Furthermore, it may also be involved in controlling Treg responses. Due to the complex role of TNFα in the immune response against Mtb, it is no surprise individuals who receive TNFα-neutralizing medication also have an increased likelihood of developing active TB.

15 | ADDITIONAL PLAYERS IN CD4 FUNCTIONAL RESPONSES: IL-10, CHEMOKINES, AND POLYFUNCTIONALITY

There is also evidence for Mtb-specific IL-10 production in humans with active TB, where IL-10 mediates inhibition of antigen presentation to T cells, and therefore mediates a decreased ability to clear infection contributing to TB pathogenesis. Furthermore, IL-10 is produced after BCG vaccination, and is responsible for a subsequent reduction of Mtb-specific Th immune responses. IL-10 has also been shown to be elevated in serum from active pulmonary TB patients.

Chemokines are significant for their role in signaling the location of infection. For example, Th17 cells are known to express CXCL9, CXCL10, and CXCL11 upon Mtb challenge, which in turn recruit IFNγ producing CD4 T cells to the lung. Interferon gamma-induced protein 10 (CXCL10 or IP-10) has been shown to be at least as effective as IFNγ in diagnostic assays for Mtb infection. When testing for IP-10 levels, the QuantiFERON-TB Gold Plus (QFT-Plus) test revealed that in both individuals with LTBI and ATB IP-10 levels were elevated compared to uninfected subjects, and IP-10 was found to be partially increased in IGRA+ subjects. IP-10 is a promising biomarker for ATB but there is still controversy surrounding IP-10’s ability to accurately distinguish between ATB and LTBI.

Lastly, polyfunctional CD4 T cells are also scrutinized for their role within Mtb infection. In this review, polyfunctional CD4 T cells are defined as dual- or triple-producing CD4 T cells that secrete pro-inflammatory cytokines in response to Mtb. Pertaining to Mtb infection, higher frequency of triple producing T cells (IFNγ, IL-2, and TNFα) was reported during ATB as compared to LTBI; and IL-2/IFNγ are increased during LTBI. During treatment of TB, triple producers are increased in numbers, and after completion of treatment and the clearance of TB, polyfunctional producers decrease.

16 | THE BREADTH OF TARGETS OF CD4 T CELL RESPONSES

Mtb has a large genome which encompasses approximately 4000 open reading frames (ORFs). A relatively small fraction of these ORFs have been described as antigens. Knowledge about the breadth of responses and the specific T cells responding could aid in the design of new diagnostics, therapies, and vaccination strategies. These may include the discovery of antigens recognized both during Mtb infection and following BCG vaccination. Antigens that are exclusively recognized following BCG vaccination, and not after natural Mtb infection, could potentially be removed to improve vaccine efficacy.

Our group performed a proteome-wide screen to detect HLA class II restricted epitopes and antigens recognized in IGRA+ individuals. The proteome-wide screen for Mtb-reactivity was performed in an IGRA+ cohort from San Diego (CA, USA). Subsequent studies of T cell reactivity against the most frequently recognized antigens in cohorts from nine different geographical locations revealed similar response magnitudes and significant correlation between the original cohort and other worldwide locations. Comparison of the reactivity patterns between IGRA+ samples from other locations and the USA cohort revealed a significant correlation both in terms of immunogenicity (magnitude of responses), and immunodominance (eg, relative frequency of recognition). In side-by-side comparisons of antigen-specific T cell reactivity a specific hierarchy of reactivity can be seen.

17 | DISEASE STAGE SPECIFIC ANTIGENS

While IGRA can distinguish prior or current Mtb infection from BCG vaccination and none of the NTM exposure, both the classical TST and IGRA tests are unable to reliably discriminate between active TB and LTBI. Thus, there is a need for a more extensive search for a panel of antigens that can distinguish LTBI and active disease, and better reflect the spectrum of Mtb infections.

Disease stage specific antigens have been described by multiple studies (reviewed in 148). Mtb expresses distinct proteins in the different stages of infection that are expected to give rise to stage-specific immune responses and antigen recognition, which have indeed been reported. In granulomas, Mtb is believed to be in a dormant state, triggered by a range of stress factors including hypoxia, low pH, nitric oxide, nutrient deprivation, and host immune pressure. Under these conditions, genes encoded by the DosR regulon are upregulated and several antigens encoded by this regulon have been described as preferentially recognized by individuals with LTBI reviewed in 148. In addition, some proteins have been described and referred to as “resuscitation antigens”. These are small bacterial proteins that promote proliferation of dormant mycobacteria and are therefore believed to...
be involved in the reactivation of Mtb.\textsuperscript{159} However, these antigens have not been studied in the context of being preferentially expressed or recognized by a certain stage of Mtb infection.

Additionally, a proteome-wide screen for Mtb-reactivity in disease stages other than healthy IGRA\textsuperscript{*}, such as individuals with active TB or BCG vaccinated individuals, has not yet been performed. It would address an important gap in knowledge; as to date most investigations have targeted either the antigens known to be recognized in LTBI, or specific antigen subgroups selected on the basis of a particular hypothesis.

A proteome-wide screen would be of interest for both diagnostic and vaccine development considerations. Boosting immune responses against antigens expressed during the active phase of infection might translate into reduced incidence or reactivation of infection. Conversely, boosting responses against antigens exclusively recognized in the latent phase might be of limited value toward the prevention of infection or reactivation. Interestingly, immunization with Mtb-specific antigens improved BCG-induced protection in mice, whereas boosting with antigens that are also present in BCG did not.\textsuperscript{160} Furthermore, since the spectrum of antigenic specificities associated with different disease stages is incompletely defined, current immunologic tests do not distinguish active disease from LTBI, nor do they quantify the risk of a latently infected individual for progression to active TB. More efficacious predictive diagnostic tests could lead to targeted therapy prior to progression to ATB.

It is also possible that antigens and epitopes recognized during the different stages of Mtb infection might be identical but functionally different in terms of T cell subset, memory phenotype, and cytokine profile elicited. More investigations will establish whether the inclusion of additional or novel antigens in diagnostics and measurement of specific biomarkers can help distinguish individuals with active TB from Mtb-infected healthy individuals. There is a huge diversity between studies not only in subjects and locations, but also regarding which antigens are investigated, the type of antigenic stimuli, the cell types investigated, and how the antigen-specific response is measured,\textsuperscript{148} which contributes to the difficulty to correlate between different studies.

\section*{18 | Mtb-specific immune responses cross-react with non-tuberculous mycobacteria (NTM)}

Mtb-specific reactivity can also be influenced by exposure to non-tuberculous mycobacteria (NTM) and other environmental microbes. Significant reactivity exists in Mtb uninfected individuals that is directed against epitopes conserved among bacteria in the mycobacteria genus, a factor to be considered in diagnostic applications, but also potentially offering an avenue to boost general and widespread reactivity.\textsuperscript{160,67}

Non-tuberculous mycobacteria vary in their ability and the extent to which they cause clinically significant symptoms or disease in humans.\textsuperscript{162} They also vary in other factors such as in vitro growth characteristics and ecological niche, living and multiplying in a variety of human and environmental reservoirs.\textsuperscript{161-163} The majority of NTM are present ubiquitously in the environment including soil, seawater, treated/untreated freshwater and a variety of organic and inorganic surfaces.\textsuperscript{161-163} Exposure to NTM may result in colonization, infection, and/or pathology that is detectable in the skin or respiratory and gastrointestinal tracts of healthy humans.\textsuperscript{161,164}

Mtb-uninfected individuals who have not received BCG are capable of responding to Mtb-derived antigens.\textsuperscript{67} NTM are capable of inducing cross-reactive T-cell responses to Mtb-derived epitopes.\textsuperscript{165-168} Higher baseline positive responses to PPD in Mtb-uninfected adults compared to children might reflect the increased likelihood of NTM exposure with age.\textsuperscript{169}

In our studies we found that control subjects, who were both TB-negative and not immunized with BCG, also responded, albeit to a lower extent, to Mtb-derived T cell epitopes.\textsuperscript{26} This led to a follow up study focusing on Mtb/NTM cross-reactivity at the level of the specific epitopes.\textsuperscript{67} This analysis revealed that their reactivity was likely due to previous NTM exposure since the epitopes they recognize were also conserved in NTM species.\textsuperscript{67}

\section*{19 | Potential functional consequence of environmental exposures on Mtb-specific immune responses}

It is possible that T cell epitopes conserved between Mtb, NTM, and BCG that elicit cross-reactive responses offer protection in the form of heterologous immunity or, to the complete contrary, act deleteriously by preventing the institution of BCG-induced protective responses, creating diagnostic challenges,\textsuperscript{163,170} and/or confounding evaluation of investigative vaccination strategies.\textsuperscript{171} It is hypothesized that exposure to environmental mycobacteria contributes to variable BCG efficacy,\textsuperscript{172,173} with increasing NTM exposure negatively correlated with efficacy. Several intriguing hypotheses have been proposed to explain how environmental NTM exposure may or may not provide protection against Mtb and contribute to variable BCG efficacy. The “masking” and “blocking” hypotheses propose very different mechanisms to explain how NTM cross-reactivity contributes to this variability (recently reviewed in 174).

In addition to being influenced by NTM, we have found evidence that Mtb-specific epitope reactivity is influenced by the microbiome.\textsuperscript{40} We observed differential recognition of Mtb-derived epitopes that was associated with the time period when individuals with active TB undergo treatment. These “treatment sensitive” epitopes are more conserved in the microbiome than “persistent” epitopes. Thus, the strong antibiotic regimen against TB results in the loss of reactivity against a subset of Mtb epitopes, broadly conserved across the microbiome. The influence of
epitope conservation in the microbiome in active TB using longitudinal samples and subject-specific microbiome sequences remains to be determined.

20 | IMMUNE CORRELATES OF PROTECTION

An immune correlate of protection (CoP) is a statistical correlation between a clinical endpoint associated with protection, such as protection against infection, disease, severe disease, or reinfection, and an immune marker, which can or cannot itself play a causative role in the protective response following vaccination or natural infection. A validated CoP, indicative of the ideal immune response an effective vaccine should elicit, could be utilized in the vaccine development pipeline to ascertain or prioritize prospective antigens for inclusion in vaccines or to optimize various aspects of vaccine administration including route, dose, delivery method, adjuvant, and schedule.

Efforts to improve TB vaccination efficacy are obstructed by incomplete understanding of the immune responses affording protection against Mtb. No confirmed CoPs, with ultimate validation determined in a phase III efficacy trial with disease and infection endpoints, for a TB vaccine exist as no TB vaccine trials have been conducted demonstrating both efficacy and sufficient sample number for definitive CoP identification. Live Mtb challenge in humans would be the ideal model for CoP identification, but is an impossibility for obvious ethical and technical reasons. Thus, it is important to mine other sample sets from both human and animals, particularly non-human primates (NHPs), in order to identify potential CoPs that warrant further evaluation. Although other immune cell types (ie, NK cells, CD8 T cells), as well as general features of the broad immunologic landscape (ie, monocyte/T lymphocyte ratio; reviewed in 178) have been suggested as potential correlates of protective immunity, here, we focus on the functional signatures of CD4 T cells as CoPs (Figure 1, Table 1), as this cell subset alone is capable of mediating potent anti-mycobacterial immunity.

21 | CD4 T CELLS SECRETING IFNγ

The long-held paradigm, based largely on animal, but also human studies, is that BCG mediates its protective effects via secretion of IFNγ by Th1 polarized CD4 T cells. Thus, IFNγ is the gold standard biomarker by which to assess protection provided by BCG or other candidate TB vaccines. Defective IFNγ signaling, such as in individuals who develop neutralizing antibodies against IFNγ, increases susceptibility to mycobacterial infection and disease. In addition, in the MVA85A vaccine efficacy trial, while boosting with MVA85A did not improve protection upon primary inoculation with BCG, low BCG-specific IFNγ production by PBMCs from BCG-vaccinated infants associated with increased risk of developing TB disease over the next three years of life.

Despite evidence that IFNγ is needed for host resistance to Mtb, the correlation between IFNγ and protection against TB is notoriously inconsistent between studies. In contrast to the MVA85A efficacy trial, the only other infant CoP study using vaccine samples conducted by Kagina et al found no association between IFNγ-secreting CD4 T cells from BCG-vaccinated South African infants and protection against culture-positive TB two years after vaccination. Moreover, IFNγ positively correlated with symptoms of active pulmonary disease such as fever and weight loss in Mtb-infected individuals. Thus, it is critical to look beyond Th1 at other T cell subpopulations—such as polyfunctional T cells producing multiple cytokines, subsets expressing specific activation markers, and memory subsets at different stages of differentiation and thus equipped with different tissue homing and effector capabilities, for their suitability as CoPs.

22 | POLYFUNCTIONAL CD4 T CELLS AS A POTENTIAL CORRELATE OF PROTECTION

As discussed above, CD4 T cells can be further characterized based on their ability to produce multiple cytokines. Multifunctional/polyfunctional cells had first been associated with protection in other infectious diseases, namely Leishmania and HIV, particularly when antigen load is low. Moreover, studies in mice found an association between IFNγ/TNFα/IL-2 triple-producing or IFNγ/IL-2 double-producing T cells and protection against TB. Intravenous administration of BCG induced polyfunctional CD4 T cells dually producing IFNγ and TNFα that were associated with reduced disease pathology in NHPs. However, the relationship between polyfunctionality and protection against human Mtb infection is less clear. In support of a protective role, some studies report increased polyfunctional T cell responses in patients with LTBI compared to ATB. Moreover, reduced polyfunctional T cell responses in patients with active disease could be recovered with antibiotic treatment for TB. However, on the opposite spectrum, others report an association between increased polyfunctional T cell responses and ATB. Adding to the controversy, the CoP infant vaccine efficacy trial conducted by Kagina et al reported no correlation between polyfunctional BCG-specific CD4 T cells and protection against developing active TB.

Thus, polyfunctional T cells are induced by Mtb infection, but whether these cells are also a correlate of protection is not fully determined. It is likely that the disease stage and bacterial load plays a role in the polyfunctional response and perhaps not protection per se. Alternatively, expression of certain combinations of cytokines may be relevant to protection, but only within the context of a particular subset of Mtb-specific CD4 T cells. This highlights the importance of looking at additional cell surface activation, migration, and memory markers in order to combine a differentiation phenotype with a functional secretory profile.
MEMORY CD4 T CELLS WITH LUNG HOMING CAPACITY

A more discriminative approach combining polyfunctional responses with phenotypic characterization of surface markers, indicative of a T cell’s memory differentiation status and thus effector capabilities, may be critical to separate immunopathology from protective antigen-specific T cell responses that could be used as CoPs. Post-vaccination measurement of multifunctional responses in Mycobacterium tuberculosis (Mtb)-specific, relatively undifferentiated, memory T cell subsets retaining the capacity to traffic to the lung may be more indicative of protective immunity against TB. Specifically, vaccinated mice challenged with Mtb have increased frequencies of KLRG1 Mycobacterium tuberculosis-specific CD4 T cells, derived from activated, replicating PD-1 high cells, that produce IL-2 in the lungs compared to unvaccinated naive mice. KLRG1 is frequently associated with terminal differentiation while IL-2 production is often associated with a central memory phenotype. Intravascular staining of Mtb challenged mice revealed that Mtb-specific CD4 T cells localized to the lung parenchyma highly express the activation marker PD-1 and the chemokine receptor CXCR3, involved in CD4 T cell homing to inflamed tissues, while intravascular Mtb-specific CD4 T cells highly expressed the terminal differentiation marker KLRG1. In support of a role in host protection, adoptive transfer of the Mtb-specific parenchymal CD4 T cells induced much greater control of Mtb infection compared to the intravascular subset. Thus, based on murine studies, migration markers associated with the ability of a CD4 T cells to exit the circulation and enter the lung to interact with Mtb-infected APCs are promising CoP candidates.

OTHER T CELL SUBSETS AND BIOMARKERS

In line with the putative protective role for memory cells with lung tissue homing capacity, antigen-specific Th1 CD4 T cells, expressing the tissue memory marker CCR6 and tissue homing chemokine receptor CXCR3, are a promising CoP candidate. Mtb challenge in BCG-vaccinated macaques, a particularly useful model for CoP identification, also reported an association between protection and Th1, with high levels of antigen-specific Th1/Th17 CD4 T cells found in the bronchoalveolar lavage fluid of macaques that received BCG.

Other cell subsets and cell surface markers involved in activation are being explored as potential CoPs. Activated HLA-DR+CD4 T cells were associated with increased risk of TB in BCG vaccinated infants, implicating a role for T cell activation status in determining the individual response to BCG inoculation.

CD153 is a promising CoP candidate that has been positively correlated with Mtb control. It was recently identified through RNA-sequencing of lung parenchyma residing CD4 T cells that have been shown to be protective in mice. CD153+CD4 T cells increase in the lung tissue of Mtb-infected mice and CD153+ mice exhibit higher bacterial loads. Mycobacterium tuberculosis-specific CD153+CD4 T cells correlated with control of lung granulomas in NHP and, as mentioned above, individuals with active TB had lower expression of CD153 on their CD4 T cells. TNFSF8 (the gene that encodes CD153) was also shown to be differentially expressed in a population of BAL CD4 T cells from IV-BCG vaccinated NHPs who were protected from Mtb challenge. Furthermore, CD153 has been shown to be inversely correlated to bacterial load, suggesting either enhancement of CD4 survival and/or enhancement of NK cell proliferation.

Taken together, these studies highlight the promising value of functional signatures of CD4 T cells as immune CoPs in Mtb infection. The recent encouraging result that IV BCG vaccination prevents or substantially limits Mtb infection in NHP models has important implications for the identification of immune CoPs. However, given the high diversity in results and outcomes across studies and models, these various studies also highlight that a single immune marker is unlikely to predict candidate vaccine-induced protection. Thus, it is necessary to employ a systems biology approach to identify different T cell subsets, cytokine secretory profiles, and specific markers involved in adhesion, migration, activation, and co-simulation that could yield insight into potential new CoPs. Additionally, identified correlates may be highly vaccine specific (eg, only useful to evaluate BCG efficacy) and differ from protective mechanisms at play in natural infection.

MTB INFECTION AND THE COVID-19 PANDEMIC

Direct effect: Relationship between ATB & LTBI and the susceptibility to COVID-19 and disease severity

Several studies have attempted to investigate the risk factors associated with COVID-19 and Mtb infection in an attempt to prioritize treatments for the most vulnerable. A case control study of 36 COVID-19 confirmed patients in China were followed up and categorized based on disease severity. The results suggested a positive correlation between Mtb infection, susceptibility to SARS-CoV-2 and COVID-19 disease severity. Similar studies have emerged from India, Italy, the Philippines, and South Africa, albeit they are all limited by a lack of social determinants and comorbidities that could influence co-infections. Over time as more and larger studies describing COVID-19 and Mtb infections are published there will be more evidence to support the relationship between Mtb infection and COVID-19.

Indirect effects of the COVID-19 pandemic on TB care

A CDC report from 2004, followed up on healthcare workers in a TB moderate to high incidence are in Taiwan. The screening led
to the discovery of 60 suspected ATB cases amongst the healthcare workers. Investigations into the origin of the cluster revealed that an elderly patient was admitted for 12 weeks to the general ward before he was diagnosed with ATB. Between 1998 and 2002, all specialized TB hospitals in Taiwan were closed due to the SARS outbreak and as a result more cases were being managed in a general hospital setting, increasing the nosocomial transmission of Mtb. This meant that Mtb infections were either overlooked or misdiagnosed during the outbreak.

Similarly, during the current COVID-19 pandemic, medical and human resources were being re-directed to the care of COVID-19 patients, while the care of most other diseases was left on the backburner. TB patients were suddenly confronted with the lack of access to diagnosis and treatment facilities. They were also less likely to leave the safety of their homes to obtain necessary treatment. The Hinduja Hospital, a tertiary care hospital in India, observed a drop of 85% out-patient visits in April 2020, following the lockdown. The indirect effects of COVID-19 are not always obvious. For India, the lockdown may result in an around an additional 40,000 TB cases annually for the next five years, and a 5.7% increase in TB deaths.

TB has been around far longer than COVID-19 and the pandemic has caused TB to be left on the sideline, while the world focuses its attention and resources on resolving the current crisis. However, the surge in interest towards COVID-19 can also be beneficial toward developing newer and better diagnostics and therapeutics against TB. For instance, many studies have found that COVID-19 disease and severity correlate with an increased frequency in circulating HLA-DR+ CD4 T cells. As discussed previously in this review, HLA-DR expression on Mtb-specific CD4 T cells has also been repeatedly associated with ATB compared to LTBI. Thus, there seem to be similarities in the immune cell subsets and immune pathways that correlates with protection and/or disease severity against SARS-CoV-2 and Mtb. Understanding the complex relationship between these two pathogens and elucidating the molecular mechanisms behind susceptibility and disease severity in single and co-infections will be fundamental to the development of preventive and treatment strategies for Mtb and Mtb/SARS-CoV-2 infections.

26 | CONCLUSION AND OUTLOOK

Pathogen-specific T cell immunity is a key host mechanism to control Mtb infection. Understanding the complexity of T cell responses is crucial to help with the fight against Mtb. A thorough understanding of the nature of responding classical CD4 T cells and the specific epitopes and antigens they recognize in different Mtb infection states will aid in immunodiagnostics, treatment monitoring and vaccine efficacy trials. For instance, unraveling the heterogeneity and biology of Th1*, the major CD4 T cell subset that contain antigen-specific CD4 T cells in latent Mtb infection might help design better vaccines. Understanding the role of non-IFNγ producing CD4 T cells and identify their alternative secreted cytokines, chemokines and surface markers might improve the sensitivity and specificity of immunodiagnostic tests. The discovery of CD4 T cell antigens that can be recognized in LTBI but not ATB will help better discriminate between Mtb infection states and more closely reflect the spectrum of infection.

There is a large heterogeneity between different studies aiming to identify which immune parameters will be of most use for correlates of protection. The need to have more control for technical variability is crucial, which would allow for comparison between studies. A limitation in comparing results between different studies is the variability in the definition of the different study cohorts included, in particularly for LTBI where there is often considerable heterogeneity. There is also a need for further research defining and characterizing Mtb-specific T cell responses in cohorts representing the entire spectrum of infection, as well as ages and co-morbidities. The so far elusive CoPs are likely not one single immune marker, but instead a combination of secreted and expressed functional molecules acting together.

In addition, it is important to consider the diversity between populations, and the environment they live in, as well as the complex host-pathogen interactions between humans and Mtb, as well as other environmental and commensal bacteria.

Systems biology approaches that combines several molecular levels of information (proteome, genome, transcriptome) and environment (such as microbiomes) and other large-scale endeavors have been and will continue to be very successful in identifying and characterizing the immune response against Mtb.

ACKNOWLEDGEMENTS

This work was supported by the National Institutes of Health grant number 75N93019C00067.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

AUTHOR CONTRIBUTIONS

All authors listed have made substantial, direct and intellectual contribution to the work, and approved it for publication.

DATA AVAILABILITY STATEMENT

Data discussed were all retrieved from published literature as specified in the reference list.

ORCID

Cecilia S. Lindestam Arlehamn https://orcid.org/0000-0001-7302-8002

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**How to cite this article:** Morgan J, Muskat K, Tippalagama R, Sette A, Burel J, Lindestam Arlehamn CS. Classical CD4 T cells as the cornerstone of antimycobacterial immunity. *Immunol Rev*. 2021;301:10–29. https://doi.org/10.1111/imr.12963