The Immunological Footprint of *Mycobacterium tuberculosis* T-cell Epitope Recognition

Rebecca Axelsson-Robertson, Isabelle Magalhaes, Shreemanta K. Parida, Alimuddin Zumla, and Markus Maeurer

Department of Microbiology, Tumor and Cell Biology, Karolinska Institutet, Center for Allogeneic Stem Cell Transplantation, Karolinska Hospital, Stockholm, Sweden; Vaccine Grand Challenge Program, Department of Biotechnology, Ministry of Science and Technology, Government of India, New Delhi; Division of Infection and Immunity, University College London Medical School, United Kingdom; and Department of Laboratory Medicine, Karolinska Institutet, Stockholm, Sweden

Aerosols containing *Mycobacterium tuberculosis* (MTB) generated from the cough of patients with active pulmonary tuberculosis are the source of MTB infection. About 70% of individuals exposed to infected aerosols do not get infected, depending on the intensity and duration of MTB exposure. Only 40% of the rest of the individuals (about 10% of those originally exposed) develop primary tuberculosis, whereas the remaining 60% contain the infection with generation of a robust immune response leading to latent tuberculosis, which is regarded as a spectrum rather than a single entity. The mechanisms involved in this natural protection are not yet well understood. There is an increasing need to integrate all disparate observations into a coherent systems biology approach for a comprehensive understanding: we need to decipher the nature of success and failure in MTB infection in humans. New advances in cellular immunology will aid in achieving that goal. We review here the nature of MTB peptide generation, antigen presentation, and detection of major histocompatibility complex class I and II–presented T-cell epitopes. Cross-sectional thinking from lessons learned in the context of the major efforts to develop vaccines will help to dissect biologically relevant mechanisms that need to be translated into the clinical context of MTB infection with the aim to (1) better understand clinically relevant T-cell responses in individuals protected from tuberculosis disease and develop markers of immune protection and vaccine take, (2) characterize the nature of the immune response in individuals who are not able to contain MTB infection, and ultimately (3) characterize markers to gauge response to therapy.

Tuberculosis, caused by infection with *Mycobacterium tuberculosis* (MTB), is currently a global health catastrophe. There are nearly 10 million new cases of active tuberculosis annually, causing an estimated 1.7 million deaths every year. This makes tuberculosis one of the most important causes of death from an infectious disease worldwide. Several outcomes of infection with MTB exist: MTB infection can progress to active clinical disease [1], it can be eliminated by the host immune response, or it can be contained as latent infection. The interactions of MTB with the human host are complex, and much remains unknown. Our understanding of pathogenesis and protective innate and adaptive immune responses is changing considerably with advances in technology. It is estimated that only up to 10% of humans infected with MTB will progress to develop active clinical disease, with the remainder retaining immune control throughout their lifetime, which is indicative of an effective immune response in the majority of people. MTB has evolved elaborate survival mechanisms in humans, allowing it to remain in a clinically latent state, constantly engaging the human immune system. Latent tuberculosis is a clinical condition that occurs after an individual is infected with MTB; the infection is established, and a host immune system response is generated that controls the MTB bacilli in an apparent quiescent
clinical phase, prevents active MTB replication, and induces tissue damage. Thus, MTB bacilli are present in the host tissue and yet there are no clinical symptoms or signs of active tuberculosis. In areas where human immunodeficiency virus (HIV) infection/AIDS and tuberculosis are endemic, particularly sub-Saharan Africa, a high proportion of HIV-infected patients develops secondary tuberculosis disease owing to reactivation of latent MTB infection. This situation leads to the breakdown of tuberculosis control programs and the sustained increase in the numbers of active tuberculosis cases and deaths. The high rates of clinical tuberculosis among persons infected with HIV or among persons with various inherited defects of the interferon γ (IFN-γ) signaling pathway and those receiving immunosuppressive therapy (steroids, tumor necrosis factor α [TNF-α] antagonists, and cytotoxic drugs) indirectly indicate a role for the adaptive immune system. Conventional dogma about tuberculosis immunity, supported by an extensive body of research, has focused on cell-mediated immunity, with T lymphocytes and macrophages as major players in protective immunity. However, the final effector pathway and mechanisms of protection remain unclear [2]. Better understanding of the interplay between the host immune system and MTB through study of the immunological footprint of MTB T-cell epitope recognition may provide a platform for the identification of strategies to select suitable vaccine candidate antigens and for identifying biomarkers that provide correlates of risk for, or protection against, active tuberculosis disease [3].

**ADAPTIVE CELLULAR IMMUNE RESPONSES**

Anti-MTB–specific CD4+ and CD8+ T cells as well as unconventional T cells are part of the cell-mediated adaptive immune response in humans; a concerted action of these T-cell populations appears to be crucial for optimal control of MTB [4, 5]. Professional antigen-presenting cells (APCs) such as alveolar macrophages, monocyte-derived macrophages, dendritic cells (DCs) [6], and B cells phagocytose MTB. DCs travel to draining lymph nodes and present MTB antigens to CD4+ and CD8+ T cells. Activated lymphocytes that are home to infected lung tissue are orchestrating the formation of the granulomatous structure that in most cases contains MTB. The structure of the human granuloma is composed of layers of macrophages, epitheloid cells, multinucleated Langerhans giant T cells, and lymphocytes surrounding a central necrotic core. Very little is known of antigen presentation in the actual human granuloma, but CD68+ APCs (macrophages) have been identified in the inner layer of the granuloma surrounding central necrosis. Lymphocyte tracking is possible, and MTB is able to enter already established granuloma structures; this notion is crucial in the context of identifying the nature and specificity of immune cells able to contain MTB infection

[7]. Spots of proliferating lymphocytes surrounding APCs have also been detected within peripheral lymphocyte infiltrates in the granuloma. These APCs have also been shown to contain mycobacteria; therefore, it is likely that the cross-talk between host and pathogen occurs at these locations [8]. Some studies using in vitro models of human granuloma structures have, in addition to DCs and macrophages, identified antigen-presenting capacity in macrophage-derived multinucleated giant Langerhans cells [9]. MTB is able to interact with other nonphagocytic cells as well, such as alveolar epithelial cells, at least in vitro [10], or adipocytes [11]. The ability of these nonprofessional APCs to present MTB antigens remains to be examined; they may contribute to the production of cytokines and other immune mediators. In particular, the interface of adipocytes with the immune system has more recently sparked attention, and future studies will debunk the role of infected adipocytes in MTB infection [12–14]. Hernandez-Pando and colleagues revisited the work of Opie and Aronson (performed in 1927), using both conventional polymerase chain reaction (PCR) as well as in situ PCR on small blocks of grossly normal-appearing lung tissues from 47 Ethiopian and Mexican patients who had died of causes other than tuberculosis (rather than using guinea pig passage) and demonstrating the presence of mycobacterial DNA in cells other than macrophages, such as type 2 pneumocytes, endothelial cells, and fibroblasts. These cells might offer a plausible protected site for persistent bacilli aside from the granuloma, because they do not process and present foreign antigens [15–17].

**IDENTIFYING MTB EPITOPE TARGETS FOR T-CELL RESPONSES**

Different approaches exist to identify epitopes provided from protein targets that can be presented through major histocompatibility complex (MHC) class I and II antigens on APCs to CD4+ and CD8+ T cells. Novel identification of biologically relevant epitopes from MTB proteins is a vital field in tuberculosis immunology and vaccinology because epitope information can be useful in the identification and design of new vaccine candidates, diagnostics (including companion diagnostics to gauge vaccine take), and markers to follow response to therapy. Variables to consider during T-cell epitope recognition studies are listed in Table 1.

In the past, the focus has been on peptide-derived MTB epitopes from strongly expressed early phase proteins [31, 32]; however, the sequencing of the entire MTB genome [33] opened new possibilities to identify molecularly defined protein targets for CD4+ and CD8+ T cells. Several strategies have been applied, such as the classical approach using overlapping peptide pools covering a specific protein [34–36] and reverse immunology based on bioinformatics tools followed by in vitro confirmation by demonstration of peptide-epitope–expanded
Table 1. Critical Variables to Consider During T-Cell Epitope Recognition

<table>
<thead>
<tr>
<th>Variable</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nature and activation status of APCs</td>
<td>[6, 18]</td>
</tr>
<tr>
<td>Affinity and off-rate between MHC and peptide</td>
<td>[19, 20]</td>
</tr>
<tr>
<td>Epitope variability</td>
<td>[21, 22]</td>
</tr>
<tr>
<td>Quality of interaction between peptide-MHC and individual TCR (affinity and efficacy)</td>
<td>[23, 24, 25]</td>
</tr>
<tr>
<td>Competition between different T-cell precursor clones recognizing the same epitope with different avidity</td>
<td>[26, 27]</td>
</tr>
<tr>
<td>Need or no need for CD8 and/or CD4 costimulation</td>
<td>[28, 29]</td>
</tr>
<tr>
<td>Cytokine environment</td>
<td>[30]</td>
</tr>
<tr>
<td>Different compartments or localized immune response</td>
<td>[8]</td>
</tr>
</tbody>
</table>

Abbreviations: APC, antigen-presenting cell; MHC, major histocompatibility complex; TCR, T-cell receptor.

T cells [37, 38]. Today, approximately 800 human T-cell peptide epitopes originating from a total of 170 MTB proteins have been published in the immune epitope database [39]. Because MTB may express approximately 4000 proteins [40], the coverage of the entire MTB proteome is limited: human cell-mediated immune-recognized epitopes have only been identified in approximately 4% of the entire MTB proteome, leaving the majority of the MTB proteome unexplored. For >100 MTB proteins, only a single epitope has been identified, whereas for other proteins, such as the early expressed virulence factors 6-kDa early secretory antigenic target (ESAT-6), 10-kDa culture filtrate antigen (CFP-10), TB10.4, and the Ag85-complex [35, 41–44], as well as the very abundant PE and PPE family proteins [45, 46], >20 different epitopes have been identified per protein (Figure 1). This bias was also seen in a previous compilation of MTB epitopes by Blythe et al [47]. A large number of overlapping MTB peptide epitopes have been identified per single protein for some proteins such as ESAT-6. A detailed analysis of these T-cell epitopes reveals that very different immunological readouts have been used to identify them (Figure 2). It is biologically and clinically relevant whether a T-cell epitope has been identified by (1) gauging cytokine production, gauging T-cell proliferation, or using soluble MHC class I/II peptide complexes (Figure 2); or (2) using blood samples from individuals exposed to MTB and/or mycobacteria other than MTB (MOTT), individuals vaccinated with BCG, or individuals with latent or active tuberculosis. In addition, the functions of some MTB virulence factors are still hypothetical or poorly characterized, based on the classification of the tuberculosis database (Figure 1) [48]. Another problem is that the protein expression at different disease or infection stages is often unknown. The majority of the MTB epitopes have been identified in blood samples from individuals with active tuberculosis, whereas a smaller number of T-cell epitopes associated with exposed household contacts or with purified protein derivative (PPD)–positive individuals have been reported [47].

Several of the highly immunogenic proteins such as ESAT-6, CFP-10, and PPE68 belong to the region of deletion 1 region present in MTB but are absent in the BCG vaccine strains as well as in many of the environmental mycobacteria. This factor makes this well-studied region attractive for diagnostics and vaccine development. It is also an interesting part of the tuberculosis genome because it has been shown to be important for MTB pathogenicity by means of ex vivo or animal models. The mechanisms may involve immune modulatory mechanisms such as cytokine suppression impairment of DC and T-cell functions [49] or macrophage deactivation [50].

The cellular immune system is able to control MTB, which is associated with a latent stage in the majority of individuals infected with MTB. Therefore, identifying antigenic proteins associated with latency has been considered to be a research priority. MTB proteins expressed mainly under hypoxic conditions (eg, the dormancy-related proteins regulated by the DosR regulon such as HSpX [51, 52]) have in some studies been linked to strong T-cell responses in individuals with latent tuberculosis, but other studies failed to detect a direct correlation [53].

Although different immunogenic proteins and epitopes have recently been identified, we still lack information in many areas. First, the restricting MHC allele and subsequently the type of the antigen-specific T cells are unknown for a majority of the epitopes. Second, a clear bias toward epitopes presented by the most common human leukocyte antigen (HLA) types in a Caucasian population has been observed [39, 54]. This is problematic because the highest tuberculosis disease burden exists in sub-Saharan Africa and Southeast Asia [1]. Therefore, it is of crucial importance to identify immunogenic epitopes recognized by individuals with the genetic background of these areas for future use in diagnostic tests or for testing of MTB vaccine candidates. Third, there is also a bias toward CD4+ T-cell [39] epitopes, based on the estimation that usually epitopes of 8–10 amino acids in length are presented via MHC class II complex to CD8+ T cells and longer epitopes are presented via MHC class I molecules to CD4+ T cells. This could be explained by the most widely used immunological readouts, such as detection of cytokine production [44, 55] or cell proliferation [56], as well as the established view that CD4+ T cells are crucial for anti-MTB immune responses. Fourth, information related to the specific MTB strains from which the epitopes are derived is usually missing, or a laboratory MTB strain such as H37Rv is used. Clinical MTB isolates would need to be used in order to elucidate relevant T-cell epitopes. This is crucial given the fact that...
variations in MTB genomes may contribute not only to drug resistance but also to changes in epitopes presented by antigen-specific T cells [57]. Previous exposure to MTB may induce T cells that react differently as they encounter variant MTB T-cell epitopes. Variations in single amino acid residues have been reported to switch subsequent T-cell effector function responses, including cytokine profile, in infectious diseases such as HIV infection or infection with hepatitis B virus [21, 22, 58]; a similar situation may be operational if MHC class I–specific and epitope-specific T cells encounter variant peptides provided by mutant MTB strains. Formally, several mechanism are possible: the variant peptide epitopes may serve as (1) agonists, (2) partial agonists or partial antagonists (it has previously been shown that epitope-associated amino acid substitutions affecting the affinity of the peptide-MHC T-cell receptor (TCR) complex could affect the phosphorylation of the CD3 ζ chain and thereby effector functions of the T cells such as cytokine production), or (3) superagonists leading to increased immune effector functions [23]. The impact of MTB proteome variation on T-cell responses needs to be addressed in this context, because amino acid substitutions in the nominal T-cell epitopes may lead to abrogated or increased binding to the MHC class I or II molecules. Furthermore, biological T-cell readouts (ie, cytokine production and tetramer analysis) reflect the impact of the MHC-peptide complex on the adaptive immune responses, yet it does not address whether mutant epitopes are indeed presented.

It has been shown in one study that individuals of African descent have a lower resistance to MTB while living under the same socioeconomic conditions compared with individuals of non-African decent [59]. Although this study has limitations, such as consideration of environmental factors

![Figure 1](image_url)

**Figure 1.** A, Number of epitopes present in the 170 different immunogenic tuberculosis proteins identified in the immune epitope database and classification of function of the individual proteins. Dark blue, hypothetical proteins; red, proteins with poorly characterized function; green, proteins involved in *Mycobacterium tuberculosis* metabolism; purple, proteins involved in cellular processing and signaling; light blue, proteins involved in information storage and processing. B, The 12 proteins for which >20 different epitopes have been identified and individual functional classification. Gray shading indicates proteins coded by genes belonging to the region of deletion 1 region absent in the BCG vaccine.
and the heterogeneity of the African population, it also raises interesting questions regarding the importance of genetic influences in susceptibility to MTB. Although environmental factors such as stress, nutrition, and helminth infections are important factors that shape the individual immunological status and the subsequent ability to control tuberculosis [60–62], there has lately been increasing evidence of the association of host gene polymorphism with decreased or increased susceptibility of tuberculosis. Examples of genes that have been validated by genetic association studies are those for IFN-γ, dendritic cell-specific intercellular adhesion molecule-3-grabbing ion-integrin (DC-SIGN), vitamin D receptor (VDR), some Toll-like receptors, nitric oxide synthase [63], and several MHC class II molecules. Regarding beneficial gene variants, a single-nucleotide polymorphism (SNP) in the IFN-γ gene (+874T/A) has been shown to be associated with protection from pulmonary tuberculosis in a meta-study based on 11 separate studies [64]. The opposite is true regarding 1 SNP in the DC-SIGN gene and 1 SNP in the VDR gene; these genes were associated with an increased disease risk of tuberculosis in a population from Guinea-Bissau [65]. Toll-like receptors have been shown to be important in the mouse model of tuberculosis but also in humans. Different variants have been associated with recognition of MTB antigens and initiation of the human immune response [66]. Several different polymorphisms in these genes (eg, TLR1-248S, TLR1-602I, and TLR6-249S) have been associated with a significantly increased MTB disease risk among African Americans [67].

Figure 2. Epitopes that have been reported in the immune epitope database from the tuberculosis protein ESAT-6 and locations compared with the stretched amino acid sequence of the protein. Colors indicate the immunological readouts: orange, cell proliferation; purple, enzyme-linked immunosorbent assay (ELISPOT); light blue, enzyme-linked immunosorbent assay (ELISA); dark blue, cell toxicity assay; red, major histocompatibility complex or tetramer staining; green, intracellular cytokine staining (ICS). Note the diversity of the immunological readouts, which leads to conflicting interpretations yet also provides a unique opportunity to capture the entire spectrum of immune readouts in a more comprehensive data set analysis.

and the heterogeneity of the African population, it also raises interesting questions regarding the importance of genetic influences in susceptibility to MTB. Although environmental factors such as stress, nutrition, and helminth infections are important factors that shape the individual immunological status and the subsequent ability to control tuberculosis [60–62], there has lately been increasing evidence of the association of host gene polymorphism with decreased or increased susceptibility of tuberculosis. Examples of genes that have been validated by genetic association studies are those for IFN-γ, dendritic cell-specific intercellular adhesion molecule-3-grabbing ion-integrin (DC-SIGN), vitamin D receptor (VDR), some Toll-like receptors, nitric oxide synthase [63], and several MHC class II molecules. Regarding beneficial gene variants, a single-nucleotide polymorphism (SNP) in the IFN-γ gene (+874T/A) has been shown to be associated with protection from pulmonary tuberculosis in a meta-study based on 11 separate studies [64]. The opposite is true regarding 1 SNP in the DC-SIGN gene and 1 SNP in the VDR gene; these genes were associated with an increased disease risk of tuberculosis in a population from Guinea-Bissau [65]. Toll-like receptors have been shown to be important in the mouse model of tuberculosis but also in humans. Different variants have been associated with recognition of MTB antigens and initiation of the human immune response [66]. Several different polymorphisms in these genes (eg, TLR1-248S, TLR1-602I, and TLR6-249S) have been associated with a significantly increased MTB disease risk among African Americans [67].

Regarding the MHC class II molecules, HLA-DR2 has, for example, repeatedly been associated with increased risk of disease in populations with different genetic background such as Indians and Russians [68, 69]. HLA-DR4 has, in some studies, been shown to be associated with an increased risk of contracting clinical disease [70], whereas HLA-DR3 appears to play a protective role in the Russian population [69]. Furthermore, studies focusing on the MHC class I molecules have been...
undertaken but no single significant allele variant has been identified, although some have been associated with an increased disease risk together with certain MHC class II molecules such as HLA-B14 and HLA-DR4 [70].

Although several genes have been found to be associated with significantly increased or decreased susceptibility to tuberculosis in different studies, it is important to examine in greater detail the penetration of these individual genes in different population groups; it is also crucial to understand that susceptibility to tuberculosis seems to be polygenetic and highly affected by environmental influences.

Although there is no evidence of an association between certain MHC class I alleles and increased or decreased risk of MTB infection, some antigen-specific T-cell responses directed against epitopes presented via certain alleles have been shown to be more dominant. It has been shown that HLA-B–restricted epitopes elicit stronger CD8+ T-cell response in MTB infection [35, 43, 71] as well as in viral diseases (eg, HIV infection [72] and cytomegalovirus infection [73]) as compared with HLA-A–presented epitopes. This dominance of HLA-B–restricted responses has also been shown to be true if a promiscuous epitope is able to bind to both HLA-A and HLA-B alleles [35].

For a limited number of MTB proteins, both CD4+ and CD8+ T-cell epitopes have been identified throughout the amino acid sequence or clustered to certain immunological hotspots (see Figure 2). In these hotspots, the same or very similar epitopes are restricted via several different MHC class I and II molecules [35, 43]. This promiscuity of epitopes is not unique for MTB antigens; it has also been seen in HIV infection [74] and in other diseases such as cancer [75]. The biological implications are still unknown, but beneficial and detrimental effects of hotspot recognition may arise: it could be favorable in vaccinology because identification of hotspots will aid in designing vaccines that could be presented by MHC molecules of a majority of people. There might also be a danger by narrowing the focus of the immune response to a few epitopes or a few regions of a protein, which could lead to immune exhaustion or escape mutations. Variant epitopes have already been seen in some MTB proteins such as TB10.4 (Rv0288) and periplasmic phosphate-binding lipo-protein pstS1 (Rv0934) [57].

WHAT ARE THE PRINCIPAL MODES OF T-CELL EPITOPE GENERATION?

MTB resides in the phagosome, a component of the MHC class II pathway, and despite the different mechanisms used by MTB to inhibit antigen presentation [76, 77], MTB epitopes are presented in the context of MHC classes I and II as well as by nonclassical MHC molecules. Thus far, immunodominant T-cell epitopes have been reported to be predominantly derived from secreted MTB proteins [78, 79], suggesting the leakage of those proteins from the phagosome into the cytosol. Secreted proteins may act as decoy antigens and represent a strategy to divert the immune responses from recognizing nonsecreted MTB proteins, and this should be taken into account in the choice of target antigens (secreted and/or nonsecreted) for new vaccine candidates [77]. In the cytosol, HLA-B–restricted epitopes derived from MTB proteins (CFP-10, EsxJ, DPV, and Ag85B) have been shown to require endoplasmic reticulum–Golgi transport, the proteasome, and transporter associated with antigen processing (TAP)-dependent transport [80]. The relevance of MTB translocation described by van der Wel and colleagues [81] in epitope generation remains to be elucidated. DCs cross-present MTB targets to T cells in the context of MHC class I and CD1b molecules: MTB antigens derive from apoptotic MTB-infected macrophages in a proteasome-independent way but require acidification of the late endosomal and lysosomal compartment [82]. Divangahi and colleagues have shown that macrophages prone to apoptosis, but not to necrosis, induced increased MTB-specific T-cell responses [83] by setting the stage in which anti-MTB responses develop. Although DC antigen presentation has been extensively studied in vitro, the in vivo contributions of MTB-infected DCs remain to be studied in greater detail. A recent report showed an association with a polymorphism (LMP7-145 Gln/Lys and Lys/Lys genotypes) in the proteasome subunit low molecular mass protein 7 in 168 Chinese patients with intestinal MTB infection, but not pulmonary MTB infection. However, it is important to note that the number of patients with active pulmonary tuberculosis was lower (n = 57) [84], suggesting that differences in antigen processing and presentation may orchestrate epitope generation and subsequent T-cell activation dependent on genetic differences and modulation of subunits of the antigen-processing machinery, for example, elaborated by interleukin-10 (IL-10) on human macrophages [85].

UNCONVENTIONAL ANTIGEN PRESENTATION

In addition to conventional CD8+ or CD4+ T cells, which recognize the majority of their nominal target epitopes via the MHC class I and II molecules, other antigen presentation pathways and T-cell compartments have been shown to play an important role in cellular anti-MTB immune reactivity. Alternative presentation pathways are the MHC class Ib molecules such as HLA-E or CD1. HLA-E has been reported to present peptides from a wide range of antigens including Rv1047 (transposase), Rv2030c, and CysN to CD8+ T cells [39]. CD1 presents lipid-derived antigens to different T-cell subsets using both the γδ-TCR and the γδ-TCR with or without help from the CD4 and CD8 coreceptors. CD1-restricted CD8+ T cells recognize lipids such as mycolic acids and lipoarabinomannan from the bacterial cell wall [86]. Certain
\(\gamma\delta\)-T-cell subsets play a role in the early establishment of anti-MTB specific immune responses and in chronic tuberculosis. T cells with the V\(\gamma\)2V\(\delta\)2 receptor (V\(\gamma\)9V\(\delta\)2 T cells) respond to nonpeptidic phosphoantigens [87] such as (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate [88] and exercise effector functions similar to those of CD8\(^+\) T cells, including IFN-\(\gamma\) [89] production and cytotoxic killing of MTB-infected cells [90]. Antigen processing and presentation of prenyl pyrophosphates to V\(\gamma\)2V\(\delta\)2 T cells is an active field of investigation [91].

**OTHER IMMUNE CELLS**

The role of other cell types of the innate immune system, neutrophils and natural killer (NK) cells, has recently been elucidated in tuberculosis immunopathology. NK cells contribute to anti-MTB directed immune responses by directly lysing infected macrophages via their receptors NKP46 and NKG2D [92]; they also set the stage of the adaptive immune response by cytokine production (ie, IFN-\(\gamma\) and interleukin 22), which help to increase phagolysosomal fusion of MTB-infected macrophages, resulting in decreased MTB growth. [93, 94]. NK cell–derived IFN-\(\gamma\) may also impact on immune effector functions, particularly in CD8\(^+\) T cells [95].

Neutrophils are the predominantly infected cells early on during MTB infection [96]. They are recruited to the site of inflammation by cytokines and chemokines produced in response to interleukin 17 (IL-17), a cytokine that may also be important in the nature of neutrophil activation and subsequent role in immunopathology [97, 98]. The role of neutrophils is multifaceted. They contribute to tuberculosis pathology by causing local inflammation; they are responsible for overexpression of the programmed death ligand 1, which may lead to the suppression of protective adaptive immune responses and subsequent less efficient control of the MTB infection. [99]. Neutrophils are involved in granuloma formation and cellular recruitment [100], as well as in situ cytokine production; type I IFN and IFN-\(\gamma\); transcripts of these genes were overexpressed in neutrophils from patients with active tuberculosis [101]. A different, strategic role of neutrophils in tuberculosis is the initiation of adaptive immune responses by supporting DC trafficking of MTB and migration to local lymphoid tissues [102]. This has been shown in murine models and needs to be tested in relevant ex vivo human material.

**IMMUNE READOUTS—A BIASED PICTURE: THE ANSWER IS DEPENDENT ON THE EXPERIMENTAL QUESTION**

Most MTB peptide recognition has been achieved via T-cell readouts. This is an indirect approach and reflects the presence of antigen-specific T cells (and their immune effector function) and not necessarily \textit{bona fide} antigen presentation: MTB epitopes may be presented, yet the experimental readouts may simply not able to detect relevant T-cell responses directed against these targets. The limitations concerning the use of T cells to demonstrate MTB epitopes are as follows: (1) IFN-\(\gamma\) or TNF-\(\alpha\) production is usually used as a readout to gauge for T-cell reactivity. If other immune effector functions (ie, other than IFN-\(\gamma\) or TNF-\(\alpha\)) are operational (eg, IL-17a), we may miss demonstrating T-cell reactivity. (2) Differences may exist between T cells from different patients depending on active or latent tuberculosis, exposure to MOTT, or duration of the infection with MTB; whether T cells are analyzed directly ex vivo or expanded in vitro; or whether T-cell responses can be achieved from healthy nonexposed individuals. (3) Most of the assays employ, due to accessibility, T cells from the peripheral circulation, which may not reflect the situation in situ [103].

**MORE DETAILED KNOWLEDGE OF IMMUNOLOGY MAY HELP TO MAKE BETTER DIAGNOSTICS AND IDENTIFY MARKERS OF IMMUNE PROTECTION**

The establishment of immune readouts for the diagnosis of latent and active tuberculosis is an ongoing field of intensive research and development because the tuberculin skin test (TST), based on the injection of tuberculin developed by Robert Koch in 1890 [104], is still used for the diagnosis of latent tuberculosis. The diagnosis of active tuberculosis relies on microbiology, molecular tests, and clinical and radiological assessments. New advances in immunological techniques helped to better define the immunological components of the TST, which is based on the measurement of the induration in the hypersensitivity reaction reflecting the recruitment of mycobacterial antigen-specific memory T cells, predominantly CD4\(^+\) CD45RA\(^-\) CD62L\(^-\) CD28\(^-\) T cells is an active field of investigation [91].

(IGRAs) rely on the detection of IFN-\(\gamma\) production by memory T cells specifically directed against the MTB-specific antigens ESAT-6, CFP-10, and TB7.7 that are absent in BCG strains as well in most of the environmental mycobacteria (except \textit{Mycobacterium flavescens}, \textit{Mycobacterium marinum}, \textit{Mycobacterium kansasi}, and \textit{Mycobacterium szulgai}) [109, 110]. On the basis of a limited number of studies, IGRAs may be superior to TST for the diagnosis of latent tuberculosis [111],
Table 2. Studies Reporting the Detection of *Mycobacterium tuberculosis*–Specific T Cells Using Multimers

<table>
<thead>
<tr>
<th>Subjects (No.) [Reference]</th>
<th>HLA Allele</th>
<th>Antigen&lt;sub&gt;Epitope&lt;/sub&gt;</th>
<th>Type of Sample</th>
<th>Frequency of Antigen-Specific T Cells Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>BCG-vaccinated donors</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>Ag85A&lt;sub&gt;GLPVEYLQV&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Mean, 0.03%</td>
</tr>
<tr>
<td>BCG-vaccinated donors</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>Ag85A&lt;sub&gt;GLPVEYLQV&lt;/sub&gt;</td>
<td>PBMCs with peptide restimulation (10 days)</td>
<td>~ 5%</td>
</tr>
<tr>
<td>BCG-vaccinated donors</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>Ag85A&lt;sub&gt;KLIAANTRV&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Mean, 0.026%</td>
</tr>
<tr>
<td>BCG-vaccinated donors</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>Ag85A&lt;sub&gt;KLIAANTRV&lt;/sub&gt;</td>
<td>PBMCs with peptide restimulation (10 days)</td>
<td>~ 5%</td>
</tr>
<tr>
<td>Children with tuberculosis</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>Ag85A&lt;sub&gt;GLPVEYLQV&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Mean, 0.14%</td>
</tr>
<tr>
<td>PPD-positive children</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>Ag85A&lt;sub&gt;GLPVEYLQV&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Mean, 0.76%</td>
</tr>
<tr>
<td>PPD-negative children</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>Ag85A&lt;sub&gt;GLPVEYLQV&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Mean, 0.001%</td>
</tr>
<tr>
<td>BCG-vaccinated donor</td>
<td>HLA-B&lt;sup&gt;*&lt;/sup&gt;35:01</td>
<td>Ag85C&lt;sub&gt;WPTLIGLAM&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>No significant staining</td>
</tr>
<tr>
<td>BCG-vaccinated donor</td>
<td>HLA-B&lt;sup&gt;*&lt;/sup&gt;35:01</td>
<td>Ag85C&lt;sub&gt;WPTLIGLAM&lt;/sub&gt;</td>
<td>PBMCs with peptide restimulation (short-term bulk culture)</td>
<td>3.48%</td>
</tr>
<tr>
<td>BCG-responsive donors</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>Ag85B&lt;sub&gt;KLIVANNTRL&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>No significant staining</td>
</tr>
<tr>
<td>BCG-responsive donors</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>Ag85B&lt;sub&gt;KLIVANNTRL&lt;/sub&gt;</td>
<td>PBMCs with BCG stimulation (short-term culture)</td>
<td>6%–23%</td>
</tr>
<tr>
<td>Adults with latent tuberculosis</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>Ag85B&lt;sub&gt;GLPVEYLQV&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Median, 0.8% (IQR, 0.56%–0.94%)</td>
</tr>
<tr>
<td>Adults with tuberculosis disease</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>Ag85B&lt;sub&gt;GLPVEYLQV&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Median, 0.6% (IQR, 0.31%–0.92%)</td>
</tr>
<tr>
<td>Patients with tuberculosis disease</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>Ag85B&lt;sub&gt;KLIVANNTRL&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>3% and 5%</td>
</tr>
<tr>
<td>Patients with tuberculosis disease</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>Ag85B&lt;sub&gt;KLIVANNTRL&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Mean, 1.47% (range, 0.5%–3%)</td>
</tr>
<tr>
<td>Patients with tuberculosis disease</td>
<td>HLA-DR4</td>
<td>Ag85B&lt;sub&gt;PEYLOYPSMGRO&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Mean, 1.31% (range, 0.4%–2.25%)</td>
</tr>
<tr>
<td>BCG-vaccinated donor</td>
<td>HLA-B&lt;sup&gt;*&lt;/sup&gt;35:01</td>
<td>Ag85B&lt;sub&gt;PAEFLLENF&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>No significant staining</td>
</tr>
<tr>
<td>Adults with latent tuberculosis</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>ESAT-6&lt;sub&gt;AMASTEGNV&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Median, 0.56% (IQR, 0.33%–0.96%)</td>
</tr>
<tr>
<td>Adults with tuberculosis disease</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>ESAT-6&lt;sub&gt;AMASTEGNV&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Median, 0.29% (IQR, 0.17%–0.66%)</td>
</tr>
<tr>
<td>Patients with tuberculosis disease</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>ESAT-6&lt;sub&gt;LLEDEGKOSL&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Mean, 3.97% (range, 1%–9.4%)</td>
</tr>
<tr>
<td>Patients with tuberculosis disease</td>
<td>HLA-DR4</td>
<td>ESAT-6&lt;sub&gt;FAGIEAAASAIQGNV&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Mean, 0.66% (range, 0.2%–1.5%)</td>
</tr>
<tr>
<td>Patients with tuberculosis disease</td>
<td>HLA-DR&lt;sup&gt;*&lt;/sup&gt;08:32</td>
<td>ESAT-6&lt;sub&gt;HSSLLDEGKOSL&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Median, 0.67% (IQR, 0.29%–1.10%)</td>
</tr>
<tr>
<td>Patients with tuberculosis disease</td>
<td>HLA-DR&lt;sup&gt;*&lt;/sup&gt;08:18</td>
<td>ESAT-6&lt;sub&gt;HSSLLDEGKOSL&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Median, 0.5% (IQR, 0.3%–1%)</td>
</tr>
<tr>
<td>Adults with latent tuberculosis</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>16kDa&lt;sub&gt;GILTVSVAV&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Median, 0.3% (IQR, 0.19%–0.55%)</td>
</tr>
<tr>
<td>Adults with tuberculosis disease</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>16kDa&lt;sub&gt;GILTVSVAV&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Median, 0.13% (IQR, 0.05%–0.34%)</td>
</tr>
<tr>
<td>Adults with latent tuberculosis</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>Hsp65&lt;sub&gt;KLQERLAKL&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Median, 0.57% (IQR, 0.42%–0.8%)</td>
</tr>
<tr>
<td>Adults with tuberculosis disease</td>
<td>HLA-A&lt;sup&gt;*&lt;/sup&gt;02:01</td>
<td>Hsp65&lt;sub&gt;KLQERLAKL&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Median, 0.41% (IQR, 0.31%–0.74%)</td>
</tr>
<tr>
<td>Treated patients with tuberculosis (5), BCG vaccinees (3), and PPD converters (2)</td>
<td>HLA-DR3</td>
<td>Hsp65&lt;sub&gt;MAKTAYDEEARR&lt;/sub&gt;</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Median, 0.19% (range, 0.03%–0.62%)</td>
</tr>
</tbody>
</table>
but they do not discriminate latent from active tuberculosis. Therefore, IGRAs cannot replace TST, but they may be used as an adjunct for the diagnosis of active tuberculosis in patients for whom diagnosis is difficult (eg, those with negative sputum culture) [112].

**THE ROLE OF MHC CLASS I AND II RESTRICTED T CELLS—THE EFFECTOR FUNCTIONS**

Reports simultaneously measuring different cytokine levels (IFN-γ, interleukin 2 [IL-2], and TNF-α) by intracellular cytokine staining (ICS) suggest that the profile of cytokines produced by MTB-specific T cells differs in peripheral blood samples between individuals with latent tuberculosis and those with active tuberculosis. In a recent study (performed with a total of 124 individuals with latent tuberculosis and 26 individuals with active tuberculosis), the use of polychromatic flow cytometric analysis identified single-positive TNF-α ESAT-6–specific and CFP-10–specific CD4+ T cells as the strongest predictor of active versus latent tuberculosis [113]. A similar observation of increased PPD-specific TNF-α single-positive CD4+ T cells, and a loss of ESAT-6–specific IL-2–producing CD4+ T cells was reported in individuals with smear-positive tuberculosis (23 out of 38 individuals) as compared with individuals with latent tuberculosis (23 of 30 individuals) and smear-negative tuberculosis (12 of 16 individuals), suggesting a correlation between antigen load and the profile of cytokine production [114]. Of note, no significant difference in single-positive TNF-α ESAT-6–specific CD4+ T cells was observed in response to ESAT-6 peptide pools [114]. A shift to single-positive IFN-γ PPD-specific CD4+ T cells was observed in patients with active tuberculosis (n = 24) as compared with patients with successfully treated tuberculosis (as a surrogate for latent tuberculosis infection; n = 28) [115]. In contrast, other studies reported increased frequencies of polyfunctional T cells: IFN-γ IL-2–TNF-α+ PPD, ESAT-6/CFP-10 fusion protein [116], Ag85B, ESAT-6, and MTB 16 kDa–specific CD4+ T cells [117] in individuals with active tuberculosis. The frequency and/or profile of cytokine production by CD4+ T cells in response to maximal stimulation (with staphylococcal enterotoxin fragment B or anti-CD3/CD28) was not found to differ between patients with active tuberculosis and control individuals [114, 116, 117] or was not reported [113, 115]. Stimulation with positive controls but also with control antigens derived from highly prevalent pathogens such as Epstein-Barr virus or cytomegalovirus should be included and reported because cytokine production patterns may not be antigen-specific but rather or also due to differential general immune reactivity patterns.

A limited number of studies have reported the use of MHC multimers (in most cases MHC class I tetramers) to gauge MTB-specific CD4+ and CD8+ T cells in peripheral blood mononuclear cells (PBMCs) (Table 2). In most cases MHC class I tetramers (mostly HLA-A*02:01 molecules) have been used, with a limited number of antigens and epitopes and usually with a limited number of individuals. More studies that gauge new MTB antigens and epitopes [127] and multimers constructed with a broader panel of HLA molecules [35, 43, 128] are needed to visualize the complexity of MHC-restricted T-cell responses. Ideally, this should be done using standard protocols and reagents when possible [129]. Interestingly, the frequency of MTB-specific CD4+ T cells in peripheral blood or pleural fluid samples [125] was shown to be increased in patients with active tuberculosis compared with healthy donors or controls (individuals without active tuberculosis) but

---

**Table 2 continued.**

<table>
<thead>
<tr>
<th>Subjects (No.) [Reference]</th>
<th>HLA Allele</th>
<th>Antigen Epitope</th>
<th>Type of Sample</th>
<th>Frequency of Antigen-Specific T Cells Reported</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients with tuberculosis disease (10) [58]</td>
<td>HLA-A*02:01</td>
<td>19kDaVLTDGNPPEV</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Median, 1.25% (range, 0.1%–2%)</td>
</tr>
<tr>
<td>Patients with tuberculosis disease (4) [124]</td>
<td>HLA-A*02:01</td>
<td>19kDaVLTDGNPPEV</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Mean, 1.7% (range, 0.3%–5%)</td>
</tr>
<tr>
<td>Patients with tuberculosis disease (6) [124]</td>
<td>HLA-DR4</td>
<td>19kDaQMPYQPOVQPOVEA</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Mean, 0.97% (range, 0.3%–1.5%)</td>
</tr>
<tr>
<td>Adults with latent tuberculosis (9) [122]</td>
<td>HLA-A*02:01</td>
<td>Rv1490FLLGLFFV</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Median, 0.54% (IQR, 0.16%–0.8%)</td>
</tr>
<tr>
<td>Adults with tuberculosis disease (13) [122]</td>
<td>HLA-A*02:01</td>
<td>Rv1490FLLGLFFV</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Mean, 0.19% (IQR, 0.11%–0.65%)</td>
</tr>
<tr>
<td>Adults with latent tuberculosis (9) [122]</td>
<td>HLA-A*02:01</td>
<td>Rv1614FLYELIWINV</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Median, 0.65% (IQR, 0.58%–0.85%)</td>
</tr>
<tr>
<td>Adults with tuberculosis disease (13) [122]</td>
<td>HLA-A*02:01</td>
<td>Rv1614FLYELIWINV</td>
<td>Fresh/unstimulated PBMCs</td>
<td>Median, 0.3% (IQR, 0.11%–0.59%)</td>
</tr>
</tbody>
</table>

Abbreviations: HLA, human leukocyte antigen; IQR, interquartile range; PBMC, peripheral blood mononuclear cell; PPD, purified protein derivative.
decreased compared with individuals with latent tuberculosis [122]. Different populations (with different HLA allele frequencies) and different HLA restriction and MTB antigen epitopes were studied, and direct comparison of the reports is not possible at this point. More recent reports underlined the need to detect ex vivo T cells from individuals with African descent, by examining the most frequent African alleles HLA-A*30:01 and HLA-A*30:02. Detailed analysis of T-cell epitope mapping showed that HLA-A*30:01 is very selective in peptide binding and HLA-A*30:02 is promiscuous, which calls for a more detailed analysis of the biological consequence of a more restricted peptide repertoire displayed for some MHC class I molecules [128]. Although reports and patient numbers are limited concerning the MHC class I/II MTB peptide complex, it appears that upon antituberculosis therapy, the number of MHC-peptide reactive T cells decreases as MTB is eliminated [58, 123, 124, 125]. In contrast, some studies reported a trend of increased frequency of MTB-specific CD8+ T cells in PBMCs from adults [122] and children [119]. Because flow-guided analysis is fast and objective, the enumeration of antigen-specific T cells could help to guide antituberculosis therapy, yet more comprehensive studies are needed in order to establish whether the association between MTB load and the number of circulating T cells is viable and robust enough to develop into clinically meaningful tests. Tetramers can detect MTB-specific T cells in PBMCs and also directly in situ, that is, in granuloma tissue [124, 130]. This finding has also been combined with IFN-γ and TNF-α detection showing that at least some antigen-specific MTB-specific T cells produced IFN-γ and/or TNF-α [125] and suggests that these tetramer-positive cells may be functional [126].

Of note, CD4+ T cells specifically directed against glucose monomycolate in the context of the nonclassical MHC molecule CD1b have recently been identified in patients with active and latent tuberculosis by means of tetramers [131]. The combinatorial use of ICS and multimeric MHC-peptide complexes may also aid in learning more about the nature of the T-cell response, including homing characteristics, T-cell maturation and differentiation, and subsequent immune effector functions such as those defined by CD45R/CCR7 [132] as a result of vaccination and immune cell memory formation. Examination of T-cell homing markers such as CCR5 may reveal clinically relevant immune responses. CCR5 has recently been shown to be instrumental not only in tissue-homing patterns but also in CD8+ T-cell memory formation whereby tissue inflammation regulates access to antigen presentation and subsequent CD8+ T-cell activation [133]. We can also learn from other disease models: CCR5−/− *Schistosoma mansoni*–infected mice show increased liver granuloma size and interleukin 13 production, changes in collagen deposition, and decreased recruitment of FoxP3-positive (potentially T regulatory) cells to liver granulomas compared with infected wild-type mice [134].

Thus, a more concerted view of immune cell analysis is required in order to understand the physiological events in anti-MTB directed T-cell responses, effector functions, and long-term memory formation.

**MECHANISMS OF ANTIGEN PRESENTATION**

Multicentric studies that include standardized assays and larger numbers of clinically well-defined study participants are needed to gauge and discriminate the profile (ie, cytokine production and phenotype) and frequencies of MTB-specific CD4+ and CD8+ T cells, between individuals with latent tuberculosis and those with active tuberculosis. Here we can learn lessons from the cancer field. Most of the assays use surrogate readouts (ie, enzyme-linked immunospot assays or tetramers), yet they do not address whether the nominal T cells are indeed recognizing the naturally processed and presented corresponding epitopes on autologous or MHC class I/II matched target T cells. This is of course a caveat, because antigen processing and presentation depend on a number of factors including LMP2/7 processing and TAP1/2 function. Proinflammatory cytokines (TNF and IFN) induce the immunoproteasome, and other cytokines such as IL-10 may have adverse effects and affect whether potential T-cell epitopes can be processed and ultimately presented [135, 136]. Downregulation of MHC class I or II molecules upon MTB infection, mediated by the MTB 19-kDa antigen, may also shape the nature of the subsequent T-cell response [137–139]. Although a large number of peptide epitopes could be presented, they may never reach the cell surface because either they are not generated or specific MHC class I/II epitope complexes may never reach the cell surface. The demonstration whether T cells, present in situ, are indeed able to detect MTB-infected cells, their function, and their defined MHC-epitope specificity may indeed be clinically relevant. Again, a glance from the cancer field may be helpful: very detailed studies showed that clinically relevant tumor-rejection antigens (ie, T cells which are *bona fide* able to eradicate tumor cells) are directed against private antigens and not at all related to the immunizing vaccine; immunization leads to induction of antigen-specific T cells, which may help to refocus the immune response (for review see [140–143]). We need to learn whether a similar situation is true in tuberculosis and which T-cell specificities and immune effector functions are most likely to mediate clinically relevant immune responses in MTB infection.

The study of innate immune responses may also help to provide diagnostic tools. A blood transcriptional profiling analysis identified a tuberculosis-specific 86-gene whole blood signature, dominated by a neutrophil-driven IFN-γ and type I IFN-α inducible gene profile, that segregated patients with
active tuberculosis from individuals with latent tuberculosis or healthy donors, with a sensitivity of 90% and 92% in the United Kingdom and South Africa, respectively, and a pooled specificity of 83% [101]. This may help to set the stage for a successful anti-MTB reactive cellular immune response capable of eradicating or containing viable MTB bacilli.

THE WAY FORWARD: MARKERS OF IMMUNE PROTECTION, THE BEST-CASE SCENARIO, AND A WISH LIST OF MTB IMMUNOLOGY AND CLINICAL RELEVANCE

Aerosols containing MTB generated from the cough of patients with pulmonary tuberculosis are the source of infection. About 70% of individuals exposed to infected aerosols do not get infected, depending on the intensity and duration of exposure, and the pathogen fails to establish a foothold in the human host [144]. Only 40% of the rest of the individuals (about 10% of those originally exposed) develop primary tuberculosis disease, whereas the remaining 60% contain the infection with generation of a robust immune response. This leads to latent tuberculosis infection, which is regarded as a spectrum and not as a single entity [145]. The mechanisms involved in natural protection have not been well understood. With the advancement of technologies, most researchers have alluded to protective mechanisms without going deeper into basic questions—which were well-articulated by many scientists in past centuries, such as Kristian Feyer Andvord, Wade Hampton Frost, Georges Canetti, and Walsh McDermott. Speculations of the role of innate immunity go back to 1932, as well as the concepts of longitudinal cohort studies and index cases in public health. Histobacteriological studies of larger varieties of tuberculous lesions in humans have attempted to elucidate the mechanisms of microbial persistence. In the past 5 years, we have started to obtain some new directions to look beyond the black box to decipher the immunopathogenesis of MTB infection with the aim to design better interventions. This needs concerted efforts from all the disciplines and cohesive interactions from the clinics to the laboratories and back.

In the present decade, several reports have clearly demonstrated the diversities among the pathogen clinical strains of MTB as well as the variable host responses across different geographic locations and genetic diversities [146–148]. These issues have been grossly underestimated in our global understanding of the disease. As an immunologists’ strategy, most of the studies are conducted in an isolated phenotype, whereas in vivo studies represent multiple concurrent events and interactions in a dynamic state; hence the need of integrating all disparate observations into a coherent systems biology approach for a comprehensive understanding. We need to decipher the nature of success and failure in MTB infection in humans; new advances in cellular immunology will help to achieve that goal.

The best way to address this effort is to organize multiple longitudinal cohort studies with very basic questions on the disease epidemiologic pattern in real time (disease occurrence among the TST-positive individuals, single-strain infection versus multiple-strain infection, and cross-protection between and pathology of prevailing circulating clinical strains). Many of these would require the involvement of multiple stakeholders through national tuberculosis control programs to which laboratory research could be superadded to make meaningful outcome of these efforts and to unravel the finer details of the immunoprotective mechanisms. Validations of these early results among several of these cohorts are essential to establish the biosignatures in specific context. Cross-sectional thinking, from lessons learned in the context of the major efforts to develop anticancer vaccines will also help to dissect biologically relevant mechanisms which need to be translated into the clinical context of MTB infection. Biomarkers are defined as characteristics that are objectively measured and evaluated as indicators of normal biological processes, pathological processes, or physiological or pharmacological responses to an intervention [149]. There is a need to establish a better and more detailed understanding of T-cell biology through a concerted and collaborative effort to decipher immune mechanisms that control MTB infection. These efforts will help to identify novel ways for clinically meaningful intervention in tuberculosis and will provide more information about the nature of successful cellular immune responses in tuberculosis and their failure in immunosuppressed individuals.

Notes

Financial support. M. M. receives grant support from Vetenskapsrådet (VR), Vinnova, Hjärt-lungfonden (HLF), and European and Developing Countries Clinical Trials Partnership (EDCTP) (TB Neat). A. Z. receives grant support from the UK Medical Research Council (MRC), EuropeAID Active Detection of Active TB (ADAT), EDCTP (TB Neat, Remox, and Pan-African Consortium for Evaluation of Anti-tuberculosis Antibiotics [PANACEA]), the UK National Institute for Health Research (NIHR) University College London Hospitals Biomedical Research Centre (UCLH-CBBC), and the UBS Optimus Foundation.

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

References


64. Middleton D, Menchaca L, Rood H, Komerofsky R. New allele fre-
60. Spence DP, Hotchkiss J, Williams CS, Davies PD. Tuberculosis and
59. Stead WW, Senner JW, Reddick WT, Lofgren JP. Racial differences
57. Comas I, Chakravartti J, Small PM, et al. Human T cell epitopes of
56. Mustafa AS, Shaban F. Mapping of Th1-cell epitope regions of
55. Kumar M, Sundaramurthi JC, Mehra NK, Kaur G, Raja A. Cellular
51. Houghton EG. Investigation of chromosome 17 candidate genes in susceptibility:

44. Reddy TB, Riley R, Wymore F, et al. TB database: an integrated plat-
43. Natarajan K, Latchumanan VK, Singh B, Singh S, Sharma P. Down-
39. Khomenko AG, Litvinov VI, Chukanova VP, Pospelov LE. Tubercu-
38. Stead WW, Senner JW, Reddick WT, Lofgren JP. Racial differences
37. Comas I, Chakravartti J, Small PM, et al. Human T cell epitopes of
36. Mustafa AS, Shaban F. Mapping of Th1-cell epitope regions of
35. Kumar M, Sundaramurthi JC, Mehra NK, Kaur G, Raja A. Cellular
34. Reddy TB, Riley R, Wymore F, et al. TB database: an integrated plat-
27. Khomenko AG, Litvinov VI, Chukanova VP, Pospelov LE. Tubercu-
26. Stead WW, Senner JW, Reddick WT, Lofgren JP. Racial differences
25. Comas I, Chakravartti J, Small PM, et al. Human T cell epitopes of
19. Khomenko AG, Litvinov VI, Chukanova VP, Pospelov LE. Tubercu-
18. Stead WW, Senner JW, Reddick WT, Lofgren JP. Racial differences
17. Comas I, Chakravartti J, Small PM, et al. Human T cell epitopes of
16. Mustafa AS, Shaban F. Mapping of Th1-cell epitope regions of
15. Kumar M, Sundaramurthi JC, Mehra NK, Kaur G, Raja A. Cellular
7. Khomenko AG, Litvinov VI, Chukanova VP, Pospelov LE. Tubercu-
5. Comas I, Chakravartti J, Small PM, et al. Human T cell epitopes of


96. Eum SY, Kong JH, Hong MS, et al. Neutrophils are the predominant infected phagocytic cells in the airways of patients with active pulmonary TB. Chest 2010; 137:122–8.


