B in TB: B Cells as Mediators of Clinically Relevant Immune Responses in Tuberculosis

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The protective role of B cells and humoral immune responses in tuberculosis infection has been regarded as inferior to cellular immunity directed to the intracellular pathogen Mycobacterium tuberculosis. However, B-cell–mediated immune responses in tuberculosis have recently been revisited in the context of B-cell physiology and antigen presentation. We discuss in this review the diverse functions of B cells in tuberculosis, with a focus on their biological and clinical relevance to progression of active disease. We also present the peptide microarray platform as a promising strategy to discover unknown antigenic targets of M. tuberculosis that could contribute to the better understanding of epitope focus of the humoral immune system against M. tuberculosis.

Keywords. tuberculosis; B cells; antibodies; cytokines; host-directed therapy.

THE ROLE OF B CELLS AND ANTIBODY RESPONSES IN TUBERCULOSIS

Tuberculosis is a communicable disease caused by Mycobacterium tuberculosis (Mtib), which is mainly an intracellular pathogen that kills almost 2 million people annually, leaving at least one-third of the world’s population latently infected [1]. The more devastating form of pulmonary tuberculosis disease in adults develops with unspecific and nonproductive inflammation in the lungs, leading to tissue destruction and eventually to organ failure and death [2]. Up to now, protective immune responses in tuberculosis remain poorly understood [3]. While infiltration and activation of CD4+ Th1 cells and CD8+ cytolytic lymphocytes is required for control of human tuberculosis [4], the role of B cells and antituberculosis humoral immune responses remains controversial [5].

Adaptive anti-Mtib immune responses are initiated by effective antigen presentation in secondary lymphoid organs in the upper thoracic region. Upon uptake of live Mtib bacilli or shed antigens, professional antigen-presenting cells (pAPCs), such as dendritic cells and macrophages, traffic from the site of infection in the lungs to the mediastinal lymph nodes. Here, antigen-loaded
pAPCs activate CD4+ and CD8+ T cells, followed by an influx of \textit{Mtb}-specific T cells into infectious foci [6]. Activation of other immune cells at the site of infection includes neutrophils, monocytes/macrophages, and also B cells. This organized orchestration of immune cells leads to the formation of dynamic lymphoid structures (ie, granulomas), which, in most individuals, are able to control further dissemination of \textit{Mtb}. Complete eradication of \textit{Mtb} bacilli is rare; instead, latent tuberculosis is established in the human host [7]. \textit{Mtb} can reside for years within macrophages and monocytes in individuals with latent tuberculosis [6], including CD271+ bone marrow mesenchymal stromal cells [8]. The specific immune responses or factors responsible for progression of active tuberculosis are not well characterized. However, the enrichment of highly specific immune effector cells with potent anti-\textit{Mtb} activity most probably plays a pivotal role to stop progress of tuberculosis infection to clinical disease.

Both naive and memory B cells have been shown to be present in tuberculosis granulomas and lesions in the human lung, which resemble germinal center–like secondary lymphoid structures [9]. The function of B cells in the \textit{Mtb}-infected lung may involve presentation of \textit{Mtb} antigens to T cells and the production of cytokines and \textit{Mtb}-specific antibodies [10]. Inflammatory effector B-cell subsets, including the newly discovered innate B cells [11], can promote development of Th1 responses via production of interleukin (IL) 12, interferon gamma (IFN-\gamma), and tumor necrosis factor alpha (TNF-\alpha) [12]. A Th1-like “milieu” may play a significant role in the development of clinically relevant antmycobacterial immune responses and early control of tuberculosis infection. Conversely, the presence of anti-inflammatory B cells (with regulatory functions and the ability to secrete anti-inflammatory cytokines such as IL-4, IL-33, and transforming growth factor beta [TGF-\beta]) may subvert the inflammatory response orchestrated by Th1 and Th17 cells to reduce tissue damage [10, 13]: A “Th2-like” milieu may help maintain equilibrium between productive and destructive cellular immune responses. \textit{Mtb}-specific antibodies may have clinically relevant effects in adaptive immune responses, in addition to cell-mediated immune response in tuberculosis [5]. Here, various studies in mouse models of tuberculosis over the past decade reveal a potential role for specific antibodies in the host defense against \textit{Mtb} [5]. High-dose administration of intravenous immunoglobulin (IVIG) has shown protective effects in mouse models of tuberculosis by reducing the hyperinflammatory response marked by reduced granulomatous infiltration into the lung, correlating with better control of \textit{Mtb} bacillary load [14]. Induction of humoral immune responses in animal models of tuberculosis as well as humans with active tuberculosis disease [10], along with evidence of antibody reactivity to various \textit{Mtb} antigens primarily found in serum samples from tuberculosis patients, suggests that B cells probably play a significant role in determining the clinical outcome of \textit{Mtb} infection [5]. B-cell epitopes and T-cell epitopes are often closely related because the uptake of the nominal target antigen by the B-cell receptor protects the target epitope from intracellular proteolysis and favors the presentation in the major histocompatibility complex (MHC) class II antigen processing and presentation pathway by MHC class II molecules [15].

**B-CELL ACTIVATION AND EFFECTOR MECHANISMS IN TUBERCULOSIS**

Naive B cells are activated when their surface immunoglobulin-based B-cell receptors bind to antigens presented on MHC class II molecules expressed by antigen-primed CD4+ T cells or pAPCs in addition to maturation signals such as cytokines and CD40–CD40L interactions [16]. Upon activation, some B cells develop into plasma cells, which can produce antibodies and cytokines [12]. \textit{Mtb}-directed antibodies may mediate effector functions such as opsonization of bacterial cells, neutralization of secreted antigens, and antibody-dependent cellular cytotoxicity (ADCC) [16, 17] (summarized in Figure 1). B cells are effective APCs that can readily respond to either cell-free antigens or entire pathogens that can ultimately be presented to CD4+ T cells [16]. B cells can therefore contribute to early protection and the induction of effective CD4+ T cell responses in tuberculosis.

**ANTIBODY RESPONSES IN TUBERCULOSIS: A ROLE IN IMMUNE PROTECTION?**

Several preclinical reports support a protective effect of antibodies in tuberculosis. In mice, immunoglobulin A (IgA) appears to provide early protection against intranasal BCG infection, as IgA-deficient animals succumbed to pulmonary mycobacterial disease as opposed to their wild-type counterparts [18]. Immunoglobulin G (IgG)–mediated opsonization of \textit{Mtb} bacilli leads to enhanced phagocytosis by macrophages via additional binding of complement proteins C3 and C4, and internalization via complement receptors [19]. Both IgG and IgA antibodies can neutralize \textit{Mtb}-derived antigens, and thus potentially block systemic bacterial dissemination [5]. Although unexplored in tuberculosis, IgG-mediated ADCC could be instrumental in early control of \textit{Mtb} infection via opsonization of the infected target cell followed by binding of the IgG Fc<sub>y</sub> region to CD16 (Fc<sub>y</sub>RII) expressed on natural killer [16] and effector memory T cells [20]. CD16 engagement triggers the release of perforin and granzymes from cytolytic lymphocytes, resulting in lysis of the infected target cell, as observed in the elimination of transformed cells [16]. \textit{Mtb}-specific IgG antibodies may promote the depletion of mycobacterial reservoirs in tissue via CD16-mediated ADCC early after infection.
Naturally occurring anti-\textit{Mycobacterium tuberculosis} (\textit{Mtb}) immunoglobulin M (IgM) antibodies may potentially exhibit activity for opsonization and neutralization of secreted toxins [17]. Assessment of antibody-mediated antituberculosis responses upon intranasal immunization of mice with human IgA has been shown to protect animals to subsequent \textit{Mtb} challenge [21], confirming the anti-infective potential of IgA against early \textit{Mtb} infection. These preclinical data have been substantiated in a clinical setting: Ethiopian individuals with latent tuberculosis were found to have higher serum levels of IgA directed against the secreted \textit{Mtb} antigens ESAT-6 and Rv2031c compared with patients with active tuberculosis [22]. Passive administration of human IgG has been shown to promote better control of mycobacterial growth and to reduce pathological inflammation in the lung of \textit{Mtb}-infected mice [5, 14]. This effect apparently requires glycosylation of the Fc\gamma region, as administration of IVIG without Fc region glycosylation does not protect mice against subsequent \textit{Mtb} challenge [14]. In this case, antibodies may bind to the \textit{Mtb} bacilli or to immunodominant \textit{Mtb} antigens, resulting in elimination of bacteria and bacterial products. IgG antibodies may also gain access to the cytosol of the \textit{Mtb}-infected cell and promote growth restriction of intracellular bacteria, as previously shown in the context of \textit{Salmonella} infection [23]. Similarly, antibodies to intracellular nuclear cancer antigens have shown clinical benefit [24], suggesting that the role of antibodies directed against intracellular antigens may be diverse; that is, they may access the cytosol, or, mutually inclusive, they may mediate ADCC and facilitate antigen uptake (from accessible material, ie, after killing of infected macrophages by T cells, or by digested \textit{Mtb} material from neutrophils [25]).

Nevertheless, B-cell responses and antibodies in tuberculosis have also been associated with progressive clinical disease.

\textbf{ANTIBODY RESPONSES IN TUBERCULOSIS AS A RESULT OF PROGRESSIVE DISEASE}

Although the major focus of this review concerns protective B-cell-mediated and antibody-mediated immune responses in tuberculosis, the B-cell compartment may also be involved in disease progression. As \textit{Mtb} is an intracellular pathogen, it is likely that antibody-mediated immune responses become
most effective in the progressive phase of tuberculosis disease, when extracellular bacteria and antigens are released and spread from destructive tissue lesions in the lung (Figure 2). While \textit{Mtb}-specific antibodies may be involved in bacterial clearance and subsequent immune control, it is also possible that an enhanced activation of antibody responses in human tuberculosis reflects a consequence of impaired cellular immunity. Thus, elevated antibody responses in the chronic phase of tuberculosis infection could instead indicate exacerbated disease. The observation that elevated levels of \textit{Mtb}-specific IgG-secreting cells in the peripheral circulation of patients with active tuberculosis are associated with reduced \textit{Mtb}-specific IFN-γ production and more severe forms of tuberculosis disease [26] would support this point. High levels of total and \textit{Mtb}-specific serum antibodies have been shown in patients with advanced tuberculosis disease [27], including cavity forms of pulmonary tuberculosis [28, 29]. Likewise, high levels of purified protein derivative (PPD)–specific IgG antibodies have been reported in rabbits with chronic pulmonary tuberculosis [30]; similar associations between increased antigen-specific antibodies and tuberculosis disease severity have also been found in \textit{Mycobacterium leprae} [31] and helminth infections [32]. Along this line, it was recently shown in mice with B cells lacking the ability to produce antibodies that the \textit{Mtb} load increases in the course of progressive tuberculosis disease due to an excess of IL-10 produced by activated macrophages [33]. IL-10 blockade reduced the bacterial burden in lungs and spleens of these mice to the same level as to wild-type animals, supporting the notion that B cells may modulate cytokine production, macrophage activation, and immunopathology in tuberculosis. Tuberculosis disease may be further exacerbated by cross-reactive antibodies to nontuberculous mycobacteria, which are a common confounder to anti-\textit{Mtb} humoral immune responses in humans [34]. Thus, \textit{Mtb}-specific antibody responses in the sera of patients with active tuberculosis may be useful for diagnostic purposes or considered as biomarkers of active and/or progressive disease, rather than a correlate of protection [26, 27].

**USE OF \textit{Mtb}-SPECIFIC B CELLS FOR DIAGNOSIS OF CLINICAL DISEASE**

Conventional serology [35] involving assessment of serum antibodies [36] shows variable diagnostic results depending on the sputum (smear) result of patients with active tuberculosis as well as their human immunodeficiency virus (HIV) status [37–39]. Instead, assessment of antibody-secreting cells that are temporarily present in the peripheral circulation of patients with active disease may represent a promising alternate way to diagnose tuberculosis [26, 40, 41]. A simple antibody-based, point-of-care test that could provide rapid results of whether or not the patient has active tuberculosis would be very helpful in the clinical management of tuberculosis disease.

**FUNCTIONALITY OF B CELLS IN TISSUE AND NONTISSUE COMPARTMENTS: IMPLICATIONS FOR TUBERCULOSIS**

B cells residing in tissue, as well as antibodies secreted therein, may show functional differences from B cells circulating in the periphery. For example, it was recently shown that production of anti-inflammatory IL-10 by pleural fluid B cells can dampen the IFN-γ-secreting property of Th1 cells following in vitro re-stimulation with irradiated whole \textit{Mtb} bacilli; these B cells were phenotypically different from those found in the patients’ peripheral blood [42]. Furthermore, B-cell–derived IL-10 driven by antigen-specific responses may also be useful in neutralizing chronic, excessive inflammation in the lung at later stages of tuberculosis disease [10].

**Regulatory B-Cell Responses in Tuberculosis**

A subset of regulatory B cells (Breg cells) that controls inflammation and autoimmunity in both mice [43] and humans [44] was recently described: CD19⁺CD24⁺⁺CD38⁺⁺ Breg cells isolated from peripheral blood of healthy individuals have the ability to suppress T-cell functions including the differentiation of IFN-γ– and TNF-α–producing Th1 cells, but also IL-17–producing Th17 cells [45, 46]. Breg cells have also been shown to suppress the production of TNF-α by macrophages [44]. Interestingly, a functional link seems to exist between Breg cells and regulatory
T cells (Treg cells), as Breg cells could promote pulmonary infiltration of Foxp3+ Treg cells that prevent allergic inflammation in ovalbumin-treated mice [47] and also induce Treg cell expansion in a murine lupus model [48]. Likewise, CD19+CD24hiCD38hi Breg cells have been shown to promote the expansion of Foxp3+ Treg cells with suppressive functions in healthy individuals, while Breg cells from patients with different autoimmune conditions have lost their suppressive ability including the capability to maintain Foxp3+ Treg cells [45, 46]. Immune suppression mediated by Breg cells seems to be primarily dependent on IL-10 [43–46]. Patients with tuberculosis have also been found to have elevated levels of a functionally suppressive CD19+CD1d+CD5+ B-cell subset in peripheral blood [13]. Successful antituberculosis treatment reduced the frequency and function of these Breg cells in peripheral blood of patients with pulmonary tuberculosis [49]. Thus, reduced numbers of Breg cells may fail to limit exaggerated inflammatory responses in patients with autoimmune diseases, whereas excess numbers of Breg cells may prevent antimicrobial effector responses in tuberculosis, in part by allowing local expansion of Foxp3+ Treg cells.

**B Cells in Tuberculosis Granulomas**

Tuberculosis granulomas in the lung represent the hallmark of human tuberculosis disease [50]. Tuberculosis granulomas are products of lymphoid neogenesis and therefore support in situ antigen processing and presentation, where B cells constitute a major cellular component [10]. Granuloma-associated B cells have been shown to maintain close contact with CXCR5+ T-cell subsets and Mtb-infected macrophages [10]. These B cells are likely to be involved in IL-10- and IL-21-mediated regression of tuberculosis immunopathology [29], as well as antibody production directed against Mtb-cell envelope components and secreted antigens (reviewed in [5]) that may disrupt bacterial dissemination and delay progression of tuberculosis disease (Figure 2). B cells act also as APCs that can engulf entire Mtb bacilli or antigens and present them to T cells locally in the infected tissue [10]. This process reciprocally promotes the differentiation of B cells to activated plasma cells capable of secreting Mtb-specific antibodies. The APC function of B cells has been postulated as a critical component to enhance targeted and protective CD4+ T-cell recall responses to infection, as may be the case in the microenvironment of the tuberculosis granuloma [5, 10]. Therefore, B cells in granulomas are likely to express a vast repertoire of Mtb-antigen specificities and participate in curbing Mtb infection at an early stage. Novel insights into tuberculosis granuloma-associated B-cell populations and their capacity to eliminate Mtb reservoirs and/or control pathological inflammation in the human host promise important clinical significance. Here, solid latent tuberculosis granulomas in surgical resections from patients with lung abnormalities [51] represent an ideal source to study lung-resident effector and memory B-cell subsets in humans. Alternatively, resected tissue from patients with lung cancer who recovered from an episode of clinical pulmonary tuberculosis [52] may contain calcified/healed granulomas harboring memory B cells with therapeutic potential in tuberculosis.

**B Cells in Bone Marrow**

The maintenance of long-lived memory B cells in the bone marrow (BM) and their regulation by antigen availability is well established [53]. Homing of mycobacterial antigen-experienced, high-affinity memory B cells to the BM following contraction of the primary immune response has been documented [54]. In addition, BM-derived B cells can process and present cognate antigen ex vivo, and readily transform into IgG- and cytokine-producing plasma cells upon antigen rechallenge [54]. Furthermore, allogeneic BM transplant has also been shown to enrich the Haemophilus influenzae type b–specific IgG repertoire in recipients via transfer of memory B cells [55]. There is also evidence for qualitative editing of memory B cells prior to their repopulation of the BM, a process that obliterates several poly- and autoreactive subsets [56]. Thus, one may assume that memory B cells in the BM are highly selective and functional in nature—with a potential impact on productive and/or protective immune responses, also in tuberculosis.

**B Cells in Sputum**

Microscopic confirmation of Mtb bacilli present in patient sputum is a routine test used in clinical tuberculosis diagnostics [1]. Sputum contains macrophages and keratinocytes as well as lymphocytes (B cells to a lesser extent than T cells) [57]. Sputum-associated B cells have been described to express HLA-DR and CD40, both features of activated B cells [16]. Interestingly, antibodies generated in the airways or lungs have been shown to be present in the sputum of patients with different diseases. For example, high levels of IgA, IgM, and IgG autoantibodies have been reported in the sputum of patients at risk for or diagnosed with early rheumatoid arthritis [58]. Also, IgA antibodies against Pseudomonas aeruginosa found in nasal secretions/sputum of cystic fibrosis patients were shown to be able to distinguish between the various clinical manifestations of lung infection with the pathogen [59]. Presence of anti-Mtb antibodies in sputum are yet to be demonstrated in clinical tuberculosis but present a viable avenue to explore, largely for diagnostic and potentially for therapeutic applications.

**THE PEPTIDE MICROARRAY PLATFORM AS A PROMISING TOOL TO DISCOVER NOVEL Mtb ANTIGENS WITH CLINICAL RELEVANCE**

Although protein antigens induce antibody production by plasma cells, it is the presence of specific peptides or epitopes within these cognate antigens that evoke this immune response in an organism or individual. Peptide- or epitope-specific responses
Table 1. Ranking of 63 Mycobacterium tuberculosis Proteins Derived From Peptide Microarray Data According to Immunological Significance

| Rank | Accession | Protein Name | Rv No. |
|------|-----------|--------------|--------|
| Secreted proteins | | | |
| 1 | P0A564 | 6 kDa early secretory antigenic target (ESAT-6) | Rv3875 |
| 2 | P0A566 | ESAT-6-like protein esxB (10 kDa culture filtrate antigen CFP-10) | Rv3874 |
| 3 | P0CS89 | Antigen 85B (30 kDa extracellular protein) (Ag85B) | Rv1886c |
| 4 | P0A8B7 | Alpha-crystallin (Acr) (14 kDa antigen) | Rv2031c |
| 5 | P0A5Q2 | Immunogenic protein MPT63 (antigen MPT63) | Rv1926c |
| 6 | P0A5Q4 | Immunogenic protein MPT64 (antigen MPT64) | Rv1980c |
| 7 | P0A668 | Immunogenic protein MPT70 | Rv2875 |
| 8 | O50430 | Low molecular weight T-cell antigen TB8.4 | Rv1174c |
| 9 | P96213 | ESX-1 secretion-associated protein EspE | Rv3864 |
| 10 | Q7U2C8 | Esat-6 like protein EsxG (conserved protein TB9.8) | Rv0287 |
| 11 | Q933K8 | ESX-1 secretion-associated protein EspB (antigen MTB48) | Rv3881c |
| 12 | P0A570 | ESAT-6-like protein EsxN | Rv1793 |
| 13 | P64091 | ESAT-6-like protein EsxQ | Rv3017c |
| 14 | P64093 | ESAT-6-like protein EsxR | Rv3019c |
| 15 | P0A568 | ESAT-6-like protein EsxH (10 kDa antigen CFP7) (CFP-7) | Rv0288 |
| 16 | P63879 | Probable cutinase Rv1984c/MT2037 (EC 3.1.1.74) | Rv1884c |
| Proteins involved in cell wall maintenance | | | |
| 17 | P0A670 | Cell surface lipoprotein MPT83 (lipoprotein p23) | Rv2873 |
| 18 | Q79FV1 | Uncharacterized PPE family protein PPE14 | Rv0915c |
| 19 | L7N675 | PPE family protein (PPE family protein PPE18) | Rv1196 |
| 20 | Q79FE1 | PPE family protein PPE41 | Rv2430c |
| 21 | Q79FC6 | Uncharacterized PPE family protein PPE42 | Rv2608 |
| 22 | Q6MVX9 | PPE family protein PPE55 | Rv3347c |
| 23 | I6Y936 | PE family protein (PE family protein PE7) | Rv0916c |
| 24 | P95130 | PGL/p-HBAD biosynthesis rhamnosyltransferase (EC 2.4.1.-) | Rv2962 |
| 25 | P95134 | PGL/p-HBAD biosynthesis glycosyltransferase/MT3034 (EC 2.4.1.-) | Rv2958c |
| 26 | Q7U1Z4 | Probable cyclopropane-fatty-acyl-phospholipid synthase UfaA1 (cyclopropane fatty acid synthase) | Rv0447c |
| 27 | P0A599 | PGL/HBAD biosynthesis glycosyltransferase/MT3031 (EC 2.4.1.-) | Rv2957 |
| 28 | P0A5J0 | Lipoprotein lipH (19 kDa lipoprotein antigen) | Rv3763 |
| 29 | P30234 | Alanine dehydrogenase (EC 1.4.1.1) (40 kDa antigen) (TB43) | Rv2780 |
| 30 | P02251 | Mycocerosic acid synthase (EC 2.3.1.111) | Rv2940c |
| 31 | P67157 | UPF0073 membrane protein Rv1085c/MT1117 | Rv1085c |
| 32 | Q79F29 | MCE family protein 1A (MCE-family protein Mce1A) | Rv0169 |
| 33 | P67300 | Putative membrane protein insertion efficiency factor | Rv3922c |
| 34 | O33192 | Lipoprotein LprJ (probable lipoprotein LprJ) | Rv1690 |
| 35 | P0A521 | 60 kDa chaperonin 2 (65 kDa antigen) (cell wall protein A) | Rv0440 |
| Proteins involved in central biochemistry of Mtb | | | |
| 36 | O05870 | Phosphate-binding protein pstS 2 (PBP 2) (PstS-2) | Rv0932c |
| 37 | P0A5Y2 | Phosphate-binding protein pstS 3 (PBP3) (PstS-3) (antigen Ag88) | Rv0928 |
| 38 | P15712 | Phosphate-binding protein pstS 1 (PBP1) (PstS-1) (antigen Ag78) (protein antigen B) | Rv0934 |
| 39 | O07157 | Probable serine protease PepA (serine proteinase) (MTB32A) | Rv0125 |
| 40 | O05871 | Serine/threonine-protein kinase pknD (EC 2.7.11.1) | Rv0931c |
| 41 | O06186 | Hypoxic response protein 1 (HRP1) | Rv2626c |
| 42 | O53811 | Isocitrate dehydrogenase, NADP-dependent, monomeric type (EC 1.1.1.42) | Rv0066c |
| 43 | P65097 | Isocitrate dehydrogenase [NADP] (EC 1.1.1.42) | Rv0339c |
| 44 | P09621 | 10 kDa chaperonin (10 kDa antigen) (BCG-A heat shock protein) (GroES protein) (protein Cpn10) | Rv3418c |
| 45 | P0A5J4 | Malate synthase G (EC 2.3.3.9) | Rv1387c |
| 46 | P71495 | Acyl-CoA synthase | Rv2941 |
| 47 | O53896 | probable serine protease pepd (serine proteinase) (mtb32b) (EC 3.4.21) | Rv0983 |
Table 1 continued.

| Rank | Accession | Protein Name | Rv No. |
|------|-----------|--------------|--------|
| 48   | P63456    | 3-oxoacyl-[acyl-carrier-protein] synthase 2 (EC 2.3.1.41) (β-ketoacyl-ACP synthase 2) (KAS 2) | Rv2246 |
| 49   | P65402    | Probable molybdemen cofactor guanylyltransferase | Rv2453c |
| 50   | P64897    | NAD-specific glutamate dehydrogenase (NAD-GDH) (EC 1.4.1.2) | Rv2476c |
| 51   | O53673    | Heat shock protein (heat-stress-induced ribosome-binding protein A) | Rv0251c |
| 52   | P0A510    | Biotinylated protein TB7.3 | Rv3221c |

Proteins involved in transcriptional regulation of Mtb

| Rank | Accession | Protein Name | Rv No. |
|------|-----------|--------------|--------|
| 53   | O06153    | Universal stress protein Rv1636/MT1672 (USP Rv1636) | Rv1636 |
| 54   | O06189    | Universal stress protein Rv2623/MT2698 (USP Rv2623) | Rv2623 |
| 55   | P95193    | Transcriptional regulatory protein DevR (DosR) | Rv3133c |
| 56   | P0A674    | DNA-directed RNA polymerase subunit β (EC 2.7.7.6) | Rv0668 |
| 57   | P0A680    | DNA-directed RNA polymerase subunit β (EC 2.7.7.6) | Rv0667 |
| 58   | Q7D52     | RNA polymerase sigma factor SigF (Sigma factor SigF) | Rv3286c |
| 59   | P0A652    | 50S ribosomal protein L7/L12 | Rv0652 |

Uncharacterized/unknown proteins

| Rank | Accession | Protein Name | Rv No. |
|------|-----------|--------------|--------|
| 60   | O06183    | Uncharacterized protein Rv2629/MT2704 | Rv2629 |
| 61   | Q7U2P4    | Conserved protein tb18.5 | Rv0164 |
| 62   | Q7YY1     | Conserved protein tb16.3 | Rv2185c |
| 63   | O50383    | Putative uncharacterized protein | Rv3354 |

Serum from 34 patients with pulmonary tuberculosis from Armenia, 6 patients from Stockholm, and 35 healthy individuals from the United States were used for differential Mtb-epitope recognition analysis using the peptide microarray platform [63]. The peptide microarray technology picked up epitopes within relevant Mtb targets that have been described previously (eg, Ag85, ESAT-6), yet also (intracellular) targets associated with Mtb biochemistry. The appreciation of the presence/absence of certain antibody reactivity profiles, which may be biologically pertinent to antituberculosis humoral responses in different tissue compartments. For example, we have previously used the 3D regression surface model to profile and graphically display the human antibody response against 63 Mtb proteins (listed in Table 1) [63]. This method allows for description of differences between immunoglobulin target recognition in various anatomical compartments and sources (ie, serum, lung, bone marrow, sputum), longitudinally, in a single individual or a group of people who harbor latent tuberculosis or who have active tuberculosis disease (Figure 3A and 3B). Alternatively, this model can also show functional differences in immunoglobulin G recognition patterns in tuberculosis-positive individuals vs tuberculosis-negative individuals (Figure 3C) spanning the entire Mtb proteome. Thus, results from peptide microarray studies can directly contribute to the discovery of previously unknown antigenic Mtb targets with significant clinical relevance.

**APPLICATION OF Mtb-SPECIFIC IMMUNOGLOBULIN G FOR ANTITUBERCULOSIS IVIG**

Polyspecific IVIG contains a broad array of multiple IgG antibodies and represents an established treatment to suppress inflammation by blocking the interaction between the Fcγ receptor and proinflammatory ligands [65] while reducing antigen-specific T-cell...
proliferation without inducing apoptosis [66]. IVIG administration has been clinically used for patients with low antibody production due to pathologies arising from immunodeficiency [67], autoimmunity, immune-mediated and inflammatory diseases, or as adjunctive treatment in clinical manifestations such as neurological disorders [68], bacterial sepsis [69], HIV infection [70], and allogeneic stem cell transplant–associated cytomegalovirus infection [71]. IgG directed against specific *Mtb* antigens, present in individuals with latent tuberculosis but not in patients with active tuberculosis, could be tailored for intravenous administration, following in vitro confirmation of antituberculosis activity. However, a more precise definition of the nominal *Mtb* target antigens may be needed to select the best IVIG profile.

**CONCLUSIONS**

Our current knowledge of the role of B cells in tuberculosis is limited, yet current data suggest an active role in tuberculosis protection, as well as in tuberculosis progression, associated with the nature of antibody specificities and with the cytokine profiles elaborated by B cells. Evaluation of B-cell–mediated antituberculosis immune responses in mice and nonhuman primates suggests a clinically relevant role at the early stages of *Mtb* infection. Conversely, abundance of antibodies in sera of patients as well as in various animal models (rabbits and mice) suggests that B cells are involved in immunopathology during active disease. In addition, the regulatory function of B cells, concomitant with that of T cells, may actively participate in determining the outcome of *Mtb* infection in humans. Further insights into tissue-specific anti-*Mtb* immune reactivity using novel, cutting-edge technology may uncover novel mechanisms by which B cells orchestrate productive, clinically relevant immune responses in tuberculosis and whether the nature of the target antigen, in addition to the milieu interne, determines the cytokine production pattern of antigen-specific B lymphocytes in tuberculosis infection.
Notes

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