SUPPLEMENT ARTICLE

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.

For this review, we performed a literature search on PubMed, PubMedCentral, and Google using the following terms: *tuberculosis*, *B cells*, *humoral immunity*, *antibodies*, *tumor-specific B-cell responses*, *cytokines*, and *immunotherapy*. Results were filtered based on relevance to the respective subsections presented in this review—immunological background of B cells in tuberculosis, intratumor B-cell responses, and significance of antibodies in clinical tuberculosis. We have also incorporated some data generated in-house to illustrate the applicability of our peptide microarray platform for gauging specific antituberculosis antibody responses.

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THE ROLE OF B CELLS AND ANTIBODY RESPONSES IN TUBERCULOSIS

Tuberculosis is a communicable disease caused by *Mycobacterium tuberculosis* (*Mtb*), 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-*Mtb* immune responses are initiated by effective antigen presentation in secondary lymphoid organs in the upper thoracic region. Upon uptake of live *Mtb* 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 *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 *Mtb*. Complete eradication of *Mtb* bacilli is rare; instead, latent tuberculosis is established in the human host [7]. *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-*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 *Mtb*-infected lung may involve presentation of *Mtb* antigens to T cells and the production of cytokines and *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-γ), and tumor necrosis factor alpha (TNF-α) [12]. A Th1-like "milieu" may play a significant role in the development of clinically relevant antimycobacterial 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-β]) 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. *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 *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 *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 *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 *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]. *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 *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 *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 *Mtb* infection via opsonization of the infected target cell followed by binding of the IgG Fcγ region to CD16 (FcγRIII) 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]. *Mtb*-specific IgG antibodies may promote the depletion of mycobacterial reservoirs in tissue via CD16-mediated ADCC early after infection.

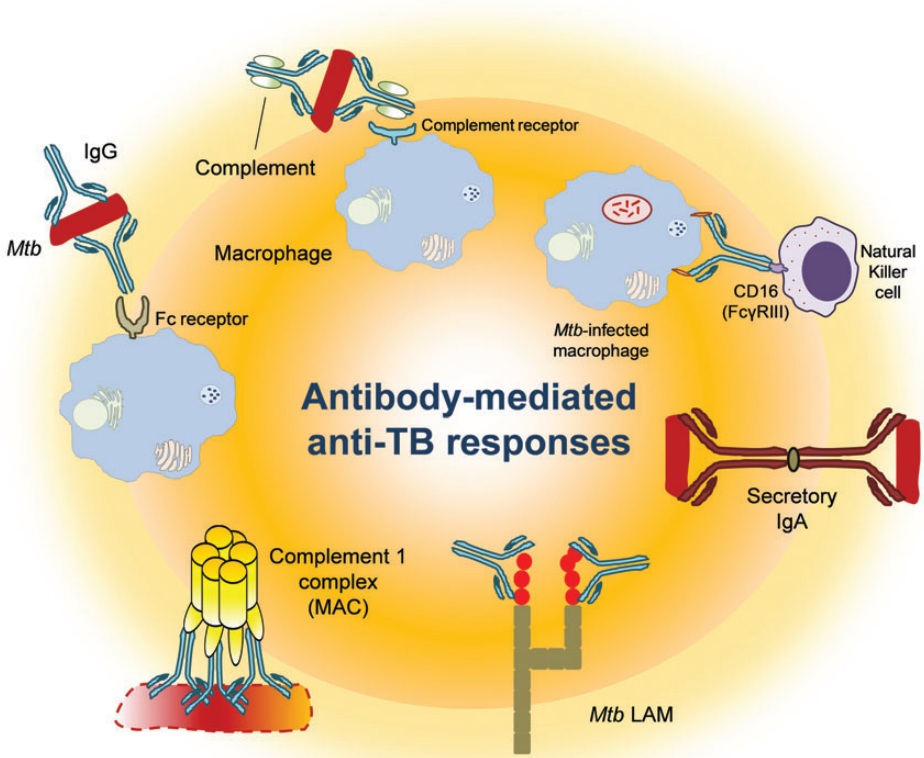

Figure 1. Role of antibodies in anti–*Mycobacterium tuberculosis* (*Mtb*) infection. Abbreviations: FcγRIII, Fc gamma receptor III; IgA, immunoglobulin A; IgG, immunoglobulin G; LAM, lipoarabinomannan; MAC, membrane-attack complex; TB, tuberculosis.

Naturally occurring anti-*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 *Mtb* challenge [21], confirming the anti-infective potential of IgA against early *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 *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 *Mtb*-infected mice [5, 14]. This effect apparently requires glycosylation of the Fcγ region, as administration of IVIG without Fc region glycosylation does not protect mice against subsequent *Mtb* challenge [14]. In this case, antibodies may bind to the *Mtb* bacilli or to immunodominant *Mtb* antigens, resulting in elimination of bacteria and bacterial products. IgG antibodies may also gain access to the cytosol of the *Mtb*-infected cell and promote growth restriction of intracellular bacteria, as previously shown in the context of *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 *Mtb* material from neutrophils [25]).

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

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 *Mtb* is an intracellular pathogen, is it likely that antibody-mediated immune responses become

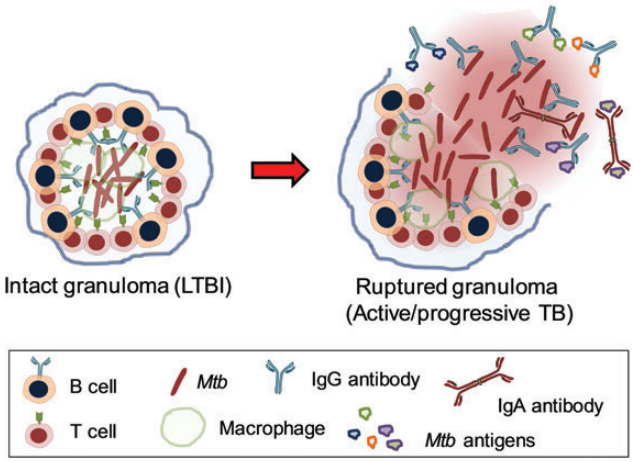

Figure 2. In situ activity of anti–*Mycobacterium tuberculosis* (*Mtb*) antibodies in lung granulomas. In addition to secreted *Mtb* antigens, immunoglobulin G and dimeric immunoglobulin A antibodies recognize *Mtb* surface structures such as lipoarabinomannan, heparin-binding hemagglutinin, and lipoproteins leading to antigen neutralization. Abbreviations: IgA, immunoglobulin A; IgG, immunoglobulin G; LTBI, latent tuberculosis; TB, tuberculosis.

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 *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 *Mtb*-specific IgG-secreting cells in the peripheral circulation of patients with active tuberculosis are associated with reduced *Mtb*-specific IFN-γ production and more severe forms of tuberculosis disease [26] would support this point. High levels of total and *Mtb*-specific serum antibodies have previously been shown in patients with advanced tuberculosis disease [27], including cavitary forms of pulmonary tuberculosis [28, 29]. Likewise, high levels of purified protein derivative (PPD)–specific serum 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 *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 *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-*Mtb* humoral immune responses in humans [34]. Thus, *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 *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 restimulation with irradiated whole *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: $CD19^{+}CD24^{hi}CD38^{hi}$ 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$^+$CD24^{hi}CD38^{hi} 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	P0C5B9	Antigen 85B (30 kDa extracellular protein) (Ag85B)	Rv1886c
4	P0A5B7	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)	Rv1984c
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	Q6MWX9	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/p-HBAD biosynthesis glycosyltransferase/MT3031 (EC 2.4.1.-)	Rv2957
28	P0A5J0	Lipoprotein lpqH (19 kDa lipoprotein antigen)	Rv3763
29	P30234	Alanine dehydrogenase (EC 1.4.1.1) (40 kDa antigen) (TB43)	Rv2780
30	Q02251	Mycocerosic acid synthase (EC 2.3.1.111)	Rv2940c
31	P67157	UPF0073 membrane protein Rv1085c/MT1117	Rv1085c
32	Q79FZ9	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	O07175	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	O53611	Isocitrate dehydrogenase, NADP-dependent, monomeric type (EC 1.1.1.42)	Rv0066c
43	P65097	Isocitrate dehydrogenase [NADP] (EC 1.1.1.42)	Rv3339c
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)	Rv1837c
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 molybdenum 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*			
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	Q7D5S2	RNA polymerase sigma factor SigF (Sigma factor SigF)	Rv3286c
59	P0A5V2	50S ribosomal protein L7/L12	Rv0652
Uncharacterized/unknown proteins			
60	O06183	Uncharacterized protein Rv2629/MT2704	Rv2629
61	Q7U2P4	Conserved protein tb18.5	Rv0164
62	Q7TYY1	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.

Abbreviation: *Mtb*, *Mycobacterium tuberculosis*.

reflect epitope-antibody complexes targeting the nominal protein, or cross-reacting antibodies that recognize structurally related targets derived from "self" or "nonself" antigens [60]. Peptide microarray technology allows the objective and simultaneous testing of several thousand unique epitopes displayed as stretched linear peptides on glass slides. We have recently used protein microarrays with linear peptides to profile specific antibody responses in autoimmunity [61] as well as to influenza virus [62] and *Mtb* [63] epitopes. These studies have revealed an unbiased, global view of the human immune response without preselecting target proteins. In the above-mentioned context, a peptide might be important not only because it resides in a particularly biologically relevant protein, but also due to its specific location within the protein. In addition, most of the current analyses focus on the "positive recognition" of targets, yet important information may be missed if they are tagged as "negative events." This absence of recognition of target structures is not appreciated, as the absence of certain naturally occurring antibodies can nevertheless be biologically relevant [64]. The demonstration of the presence or absence of immunoglobulin reactivity may therefore be clinically relevant in tuberculosis, uncovering potential therapeutic immunoglobulin subclasses.

The peptide response can be visualized using a high-content peptide microarray [60], whereas immunoglobulin reactivity may be graphically represented using a 3D regression surface model. The global analysis of immunoglobulin reactivity will allow 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 3*A* and 3*B*). Alternatively, this model can also show functional differences in immunoglobulin G recognition patterns in tuberculosis-positive individuals vs tuberculosis-negative individuals (Figure 3*C*) 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

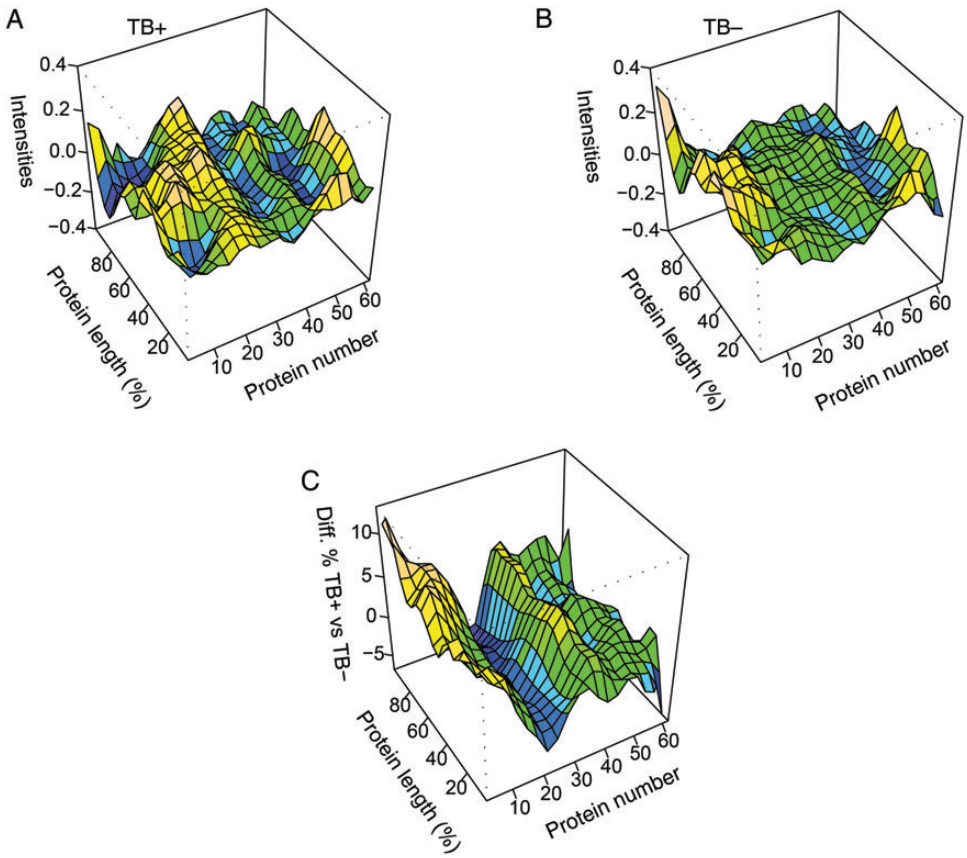

Figure 3. Three-dimensional regression model for visualizing the 63 *Mycobacterium tuberculosis* (*Mtb*) antigens listed in Table 1 captured via the peptide microarray platform. Intensity of antibody responses to the peptides constituting a particular protein is aligned against the length of the entire molecule, to indicate possible immunogenic "hotspots" of recognition among patients with tuberculosis (*A*) and healthy individuals (*B*). *C,* Differences in recognition between patients with tuberculosis and healthy individuals are plotted against protein length. Protein number refers to the sequential order of the antigens in Table 1. Abbreviations: Diff., difference; TB, tuberculosis.

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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