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Human Immunology of Tuberculosis

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ABSTRACT Immunology is a central theme when it comes to tuberculosis (TB). The outcome of human infection with *Mycobacterium tuberculosis* is dependent on the ability of the immune response to clear or contain the infection. In cases where this fails, the bacterium replicates, disseminates within the host, and elicits a pathologic inflammatory response, and disease ensues. Clinical presentation of TB disease is remarkably heterogeneous, and the disease phenotype is largely dependent on host immune status. Onward transmission of *M. tuberculosis* to new susceptible hosts is thought to depend on an excessive inflammatory response causing a breakdown of the lung matrix and formation of lung cavities. But this varies in cases of underlying immunological dysfunction: for example, HIV-1 infection is associated with less cavitation, while diabetes mellitus comorbidity is associated with increased cavitation and risk of transmission. In compliance with the central theme of immunology in tuberculosis, we rely on detection of an adaptive immune response, in the form of interferon-gamma release assays or tuberculin skin tests, to diagnose infection with *M. tuberculosis*. Here we review the immunology of TB in the human host, focusing on cellular and humoral adaptive immunity as well as key features of innate immune responses and the underlying immunological dysfunction which associates with human TB risk factors. Our review is restricted to human immunology, and we highlight distinctions from the immunological dogma originating from animal models of TB, which pervade the field.

Immunity to *Mycobacterium tuberculosis* is an interplay between the innate and adaptive immune response, both cellular and humoral. This interplay is not static but changes over time as we grow, age, and respond to our environment. Animal models enable examination of individual components of the immune response at distinct time points during the course of infection. This has enabled identification and understanding of key immune mechanisms for *M. tuberculosis* control. However, rational development of interventions, such as more effective vaccines and other host-directed therapies, has to take into consideration the enormous heterogeneity of the interactions between *M. tuberculosis* with human innate and adaptive immune responses, which are profoundly influenced by genetic variation, environment, and comorbidities.

Recent technological advances now being applied to the field of tuberculosis (TB) have pushed the boundaries of our understanding of the host-pathogen interactions. These include the use of highly sensitive imaging such as positron emission tomography/computed tomography (PET/CT; ^{18}fluorodeoxyglucose positron emission and computerized axial tomographic scanning) to identify subclinical TB lesions in asymptomatic individuals to study early stages of human infection; the

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explosion of high-throughput "omics" technologies for unbiased transcriptomic, genomic, proteomic, and metabolomic investigation of blood and tissues isolated from the site of disease; and the ability to isolate human *M. tuberculosis*-specific T cell populations by the use of *M. tuberculosis* peptide-specific tetramers and flow cytometry. Moreover, rigorous design of clinical studies, improved and standardized clinical definitions, and extensive collection of clinical data and appropriate specimens for immunological studies have significantly advanced our understanding of human immunology. Together, these advances have led to a revolution in how we understand the different stages of *M. tuberculosis* infection and the interplay of innate and adaptive immunity in humans (Fig. 1).

ACQUISITION OF *M. TUBERCULOSIS* INFECTION

The host-pathogen interaction between *M. tuberculosis* and humans has been honed by thousands of years of coevolution (1). The estimation that a third of the global population is sensitized to *M. tuberculosis* (2) bears testament to the supreme success with which the bacterium infects, survives, and spreads within its human host. Billions of humans have experienced acquisition of *M. tuberculosis* infection. Despite this, our knowledge of the immunological events that occur during exposure and acute infection in humans is very limited. This is primarily due to the lack of diagnostic tests that directly identify *M. tuberculosis* in those with infection and to our limited ability to study early disease processes at the site of disease, such that the majority of human studies investigate immune responses *ex vivo* in peripheral blood or after *in vitro* infection of primary cells or cell lines.

Primary Response to *M. tuberculosis* Infection

Meticulous clinical observation of TB contacts, combined with serial tuberculin skin testing (TST) to detect the onset of hypersensitivty to *M. tuberculosis* antigens, allowed Arvid Wallgren to document the symptomology of incident *M. tuberculosis* infection in 1948 (3). He reported that most people who converted from a negative to a positive TST presented with erythema nodosum and/or fever, while many also had elevated erythrocyte sedimentation rates (3). This suggests that acute infection is associated with a systemic innate inflammatory response that precedes the induction of a detectable adaptive immune response. Erythema nodosum is still a symptomatic trigger that may lead to investigation and diagnosis of human infection with *M. tuberculosis* (4–6) or *Mycobacterium bovis* (7).

The inflammatory processes that underlie erythema nodosum, febrile illness, and erythrocyte sedimentation are thought to be causally linked to the delayed hypersensitivity reaction that underlies priming of the *M. tuberculosis*-specific T cell response. However, at least in people without prior sensitization to mycobacteria, it is likely that innate immune responses to the infecting pathogen precede these T cell-driven reactions. It is thought that the first event that occurs upon inhalation of *M. tuberculosis*-containing microdroplets is that the bacilli are taken up by alveolar macrophages (AMs). A number of important barriers and antimicrobial hurdles must be negotiated by aerosolized *M. tuberculosis* particles to reach the alveoli, most of which are poorly understood in humans and are often neglected. However, it is likely that the pathogen is particularly susceptible to mechanical and immunological attack during its journey through the upper airways. A better understanding of these events, and of the cellular and humoral components that frequent the mucosal surfaces, could lead to interventions that prevent infection at the port of entry. In fact, a sizable proportion of people who are heavily exposed to *M. tuberculosis* do not develop any evidence for immune sensitization (8, 9), suggesting that prevention of infection is possible (10).

Alveolar macrophages

AMs are regarded as the sentinels of *M. tuberculosis* infection. Their role in initial *M. tuberculosis* phagocytosis is unquestionable, according to animal models of infection. Defining their role in the human response to *M. tuberculosis* infection has been more problematic, and our knowledge can only be inferred from studies of cells collected by invasive bronchoalveolar lavage (BAL), investigation of tissue sections from autopsies, or lung resections (generally only indicated due to severe disease pathology). As a consequence, our picture of macrophage responses to *in vitro M. tuberculosis* infection is confounded by their removal from their tissue matrix and surrounding immunological milieu, including activated cytokines and other interacting cell populations. Although reductionist, the latter has provided enormous insight into differences between human AMs and peripheral monocytes.

A number of studies of BAL-isolated AMs from healthy donors have compared responses to *in vitro* infection with virulent (H37Rv) or avirulent (H37Ra) laboratory strains of *M. tuberculosis* (11–14). In comparison to mouse studies indicating that tumor necrosis factor

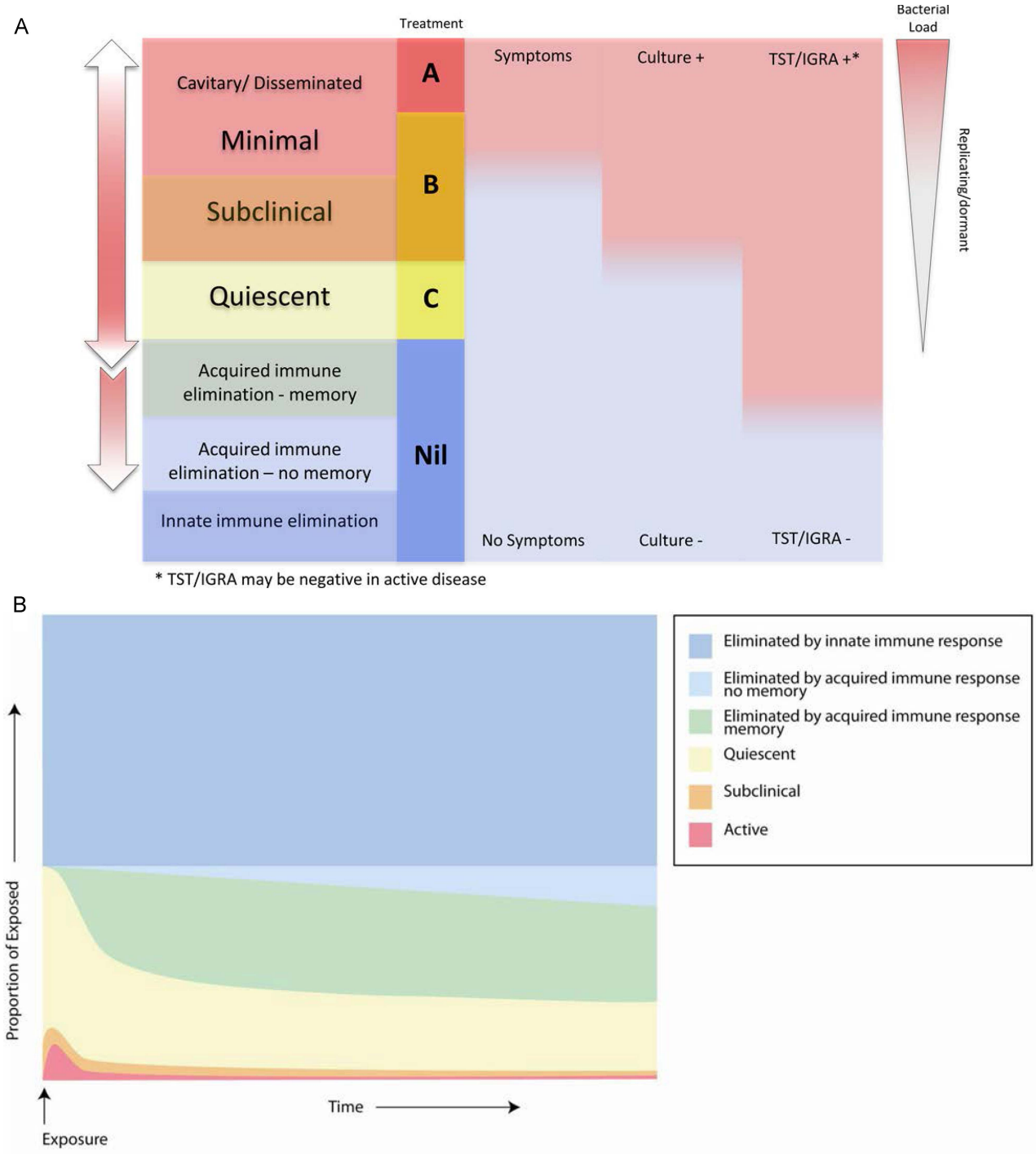

FIGURE 1 **(A)** Hypothesized stages of response to *M. tuberculosis* infection, beginning with elimination mediated by innate immune cells without induction of a long-lasting memory response; further stages of elimination may be mediated via acquired immune mechanisms. If antigen-specific effector memory persists, this can be measured via IFN-γ release assays (IGRA) or tuberculin skin test (TST) and may provide protection from infection for a variable period of time. If the acquired immunity does not eliminate the bacteria, then infection will persist over a range of bacterial states. Increasing bacterial load is hypothesized to correlate with progression to active TB. **(B)** For all exposed individuals, the risk of developing TB is highest immediately following exposure and changes over time. The longitudinal risk of developing TB, predicted in the exposed individual, is presented (adapted from references 204 and 205).

(TNF) provides a protective response against *M. tuberculosis* infection and that it is vital for granuloma formation, these studies showed that virulent *M. tuberculosis* induces higher levels of TNF secretion from AMs than avirulent *M. tuberculosis*, that TNF levels correlated with increased *M. tuberculosis* growth, that TNF induces apoptotic cell death in culture, that cytoxicity can be inhibited by anti-TNF treatment, and that exogenous application of TNF increases both the intracellular bacterial load and the number of infected AMs (13, 14). It is further hypothesized that increased apoptosis may spread the infection to neighboring AMs via efferocytosis, and extensive apoptosis has been demonstrated within caseating granulomas of lung tissue samples from TB patients (12).

Phagocytosis by AMs is mediated primarily by complement receptor 4 (CR4), whereas blood monocytes utilize CR1, CR3, and CR4. As such, uptake of *M. tuberculosis* can be enhanced by increasing concentrations of serum and decreased by heat inactivation of serum (15). AMs are also more efficient than monocytes at limiting intracellular growth of *M. tuberculosis*, and they produce high levels of TNF (15). Interestingly, phagocytosis alone is not responsible for TNF production, as uninfected AMs within the same culture also produce TNF. However, this does not occur if uninfected AMs are separated from infected AMs via a 0.4-μm transwell, indicating that cell-cell interaction or a soluble factor larger than 0.4 μm is required for TNF production in uninfected AMs (13).

AMs are highly heterogeneous in *M. tuberculosis* phagocytic potential, despite homogeneity in phagocytosis of latex beads, such that up to only 20% of AMs in culture become infected with *M. tuberculosis*, even with excessive infection (multiplicity of infection of 10:1 for 18 h) (13). This may be mediated by variable surface expression levels of CR, while differential cytokine response can be linked to expression of pattern recognition receptor (PRR) expression. Nucleotide-binding oligomerization domain-containing protein 2 (NOD2), Toll-like receptor 2 (TLR2), and TLR4 expression on AMs is highly correlated and variable between each AM (16). The expression of PPR also changes on AMs from TB patients following treatment, indicating that the phenotype of AM changes during infection (16). The differential expression of these PPRs may be important for primary restriction of *M. tuberculosis* replication because NOD2 activation in AMs by muramyldipeptide (MDP) induces expression of interleukin-1β (IL-1β), IL-6, and TNF; the antimicrobial peptide cathelicidin (LL-37); and the autophagy enzyme IRGM, and it restricts intracellular growth of *M. tuberculosis* (17). Interestingly, LL-37 is not detected in AMs in tuberculous granulomas, suggesting that LL-37 participates only during early infection or that defects in LL-37 production can lead to *M. tuberculosis* growth and progression to disease (18).

When comparing BAL from TB patients versus healthy controls, TB AMs express higher concentrations of IL-1β, IL-6, and TNF, and this correlates with higher protein levels in BAL fluid and with IL-6 and TNF in serum (19). AMs from TB patients also show higher levels of chemokines CXCL10 (IP-10), CXCL8 (IL-8), and nuclear factor-kappa B (NF-κB) repressing factor (NRF), and these levels correlate with higher bacillary loads in the AMs. Interestingly, peripheral blood mononuclear cells (PBMCs) from patients with high bacillary load also have high expression of CXCL10 and CXCL8, while NRF levels are higher in AMs than in PBMCs (20).

The hyperreactivity of AMs in TB patients may be either due to an innate defect leading to susceptibility to TB or because *M. tuberculosis* infection changes the phenotype of AMs. The observation that the AM phenotype changes during therapy (16) supports the finding that the infection is modifying AM function. Recent evidence of a shift in the metabolic state of AMs following infection also supports this hypothesis. Macrophages can be classified as classical (M1) or alternatively activated (M2), with pro- and anti-inflammatory properties, respectively. M1s derive ATP via aerobic glycolysis and M2s via oxidative phosphorylation. *M. tuberculosis* infection of healthy donor AMs induces a shift from oxidative phosphorylation to aerobic glycolysis, leading to increased IL-1β and prostaglandin synthase PTGS2 and decreased IL-10, while blocking this shift to aerobic glycolysis leads to increased intracellular *M. tuberculosis* survival (21), suggesting that AM polarization to M1 activates antimicrobial activity.

Neutrophils

Peripheral neutrophilia is a hallmark of TB disease in humans and a predictor of poor outcome and morbidity (22, 23). The lack of neutrophil involvement in murine TB and the difficulties associated with studying neutrophils *in vitro* have led to limited investigation of their role in human TB. A resurgence of interest in neutrophils occurred after the first whole-blood microarray study of TB patients compared with healthy controls, which showed profound neutrophil involvement in the gene expression signature that differentiated between TB patients and controls (24). While it seems clear that neutrophils promote pathology during disease development, an understanding of their role in initial infection is more

difficult to acquire. Recent TB contacts show increased peripheral blood neutrophil counts compared to healthy controls, and risk of *M. tuberculosis* infection has been shown to be inversely associated with neutrophil count (25). Neutrophil depletion from whole blood also decreases *M. tuberculosis* killing, which is primarily mediated through phagocytosis and the respiratory burst. In addition, neutrophils can kill through the release of antimicrobial peptides including human neutrophil peptides (HNPs) 1–3, LL-37, and lipocalin 2 (25). Neutrophils can also capture mycobacteria in neutrophil extracellular traps (NETs) composed of DNA coated with antimicrobial peptides (26). Interestingly, individuals of African ancestry have lower circulating neutrophil numbers and lower serum levels of HNP1–3 and lipocalin 2 compared to Caucasian individuals (25, 27). CXCL8, one of the chemokines most highly expressed by *M. tuberculosis*-infected AMs, with neutrophil recruiting activity, has recently been shown to bind *M. tuberculosis* directly and enhance phagocytosis and killing by neutrophils (28). These data suggest that neutrophils may have an early protective effect against *M. tuberculosis* infection.

Innate T cells

Interest in lung-resident and germline-encoded lymphocyte populations has recently been growing, with the rationale that these cells may act rapidly upon *M. tuberculosis* infection. These cells naturally reside at mucosal sites in the airways and are thus ideally located to respond to invading pathogens (reviewed in reference 29). This is an important advantage over conventional T cell responses that require priming in primary lymphoid tissue and subsequent differentiation into effector cells before trafficking to the site of infection. Tissue-resident T cells, such as mucosal-associated invariant T (MAIT) cells, also possess immediate effector functions, including cytokine expression and cytotoxicity, which further enable immediate antimicrobial activity. It is currently not known whether airway-resident lymphocytes play a key role in resistance to infection with *M. tuberculosis* in humans.

Most individuals who are exposed to *M. tuberculosis* do appear to acquire an established infection and develop readily detectable CD4 T cell responses to protein components of *M. tuberculosis*. This immune response, which typically persists for years and even decades, forms the basis for the diagnosis of human infection with *M. tuberculosis*, using TST or interferon gamma (IFN-γ) release assays (IGRAs). The utility of these diagnostic methods has been extensively reviewed (30).

The Granuloma

The structure of the granuloma is formed primarily through the coalescence of recruited macrophages around *M. tuberculosis*-infected macrophages, of which some differentiate into epithelioid cells and some can fuse to become multinucleated giant cells. In the typical granuloma structure these macrophages are interspersed with recruited neutrophils and are surrounded by a lymphocyte cuff, including T cells and B cells. A recent review of the historical literature has shown that granulomata are highly diverse, displaying a wide spectrum of structures and sizes and cell composition, and that this diversity can be observed even within a single host (Fig. 2; reviewed in references 31 and 32). It is thought that the granuloma functions to contain the spread of *M. tuberculosis*, although it can also act as a physical barrier, preventing the penetration of TB drugs and protecting the organism from the adaptive immune response. The phenotype of macrophages within the granuloma can affect the likelihood that the granuloma will contain *M. tuberculosis*, break down and transmit *M. tuberculosis*, and initiate an inflammatory response (33).

Adaptive Responses and the Spectrum of *M. tuberculosis* Infection

B cells

The dominance of T cell responses and the concealment of *M. tuberculosis* within the infected macrophage suggest that antibodies would play a minor role in possible prevention of infection with *M. tuberculosis* during exposure. However, it has recently been recognized that B cells and antibodies have a variety of mechanisms for the modulation of the immune response to intracellular bacteria that are likely to be important in the control of *M. tuberculosis* (reviewed in references 34–37) (Fig. 3). B cells are a major cellular component of the lung granuloma, where they can process and present antigen to T cells, secrete antibodies, and modulate inflammation through the production of IL-10 (reviewed in reference 37). Although likely to be important, few clinical studies have examined the B cell response in *M. tuberculosis* infection. Plasmablasts and memory B cells are elevated in *M. tuberculosis*-infected compared to uninfected controls (38), and *in vitro* human B cells have been shown to ingest mycobacteria, produce IgM, and upregulate the expression of the costimulatory molecules CD80 and CD86 and the chemokine CXCL10 (39, 40). Further studies of the role of B cells in *M. tuberculosis* infection are required.

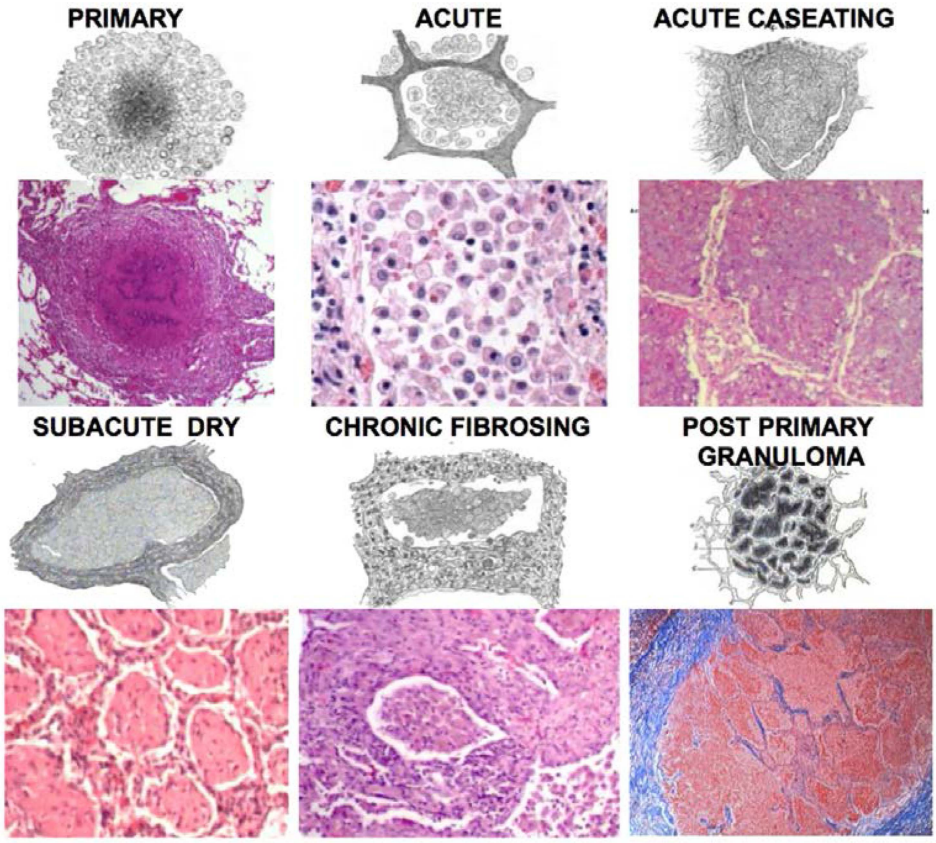

FIGURE 2 The spectrum of pulmonary TB lesions that can be found in the same host and that represent different stages of disease. Primary TB is characterized by the hallmark circular granuloma with caseating necrosis which forms within the center, surrounded by a lymphocytic cuff. Conversely, post-primary TB is typically represented by a diverse range of pathologies. Acute post-primary lesions are composed of paucibacillary lobular pneumonia; these may either resolve (subacute dry), fibrose (chronic fibrosing) or necrose (acute caseating). Caseating granulomas in post-primary TB are distinct from the granulomas of primary TB in that they form around and in response to caseous necrosis of pneumonic lesions (post-primary granuloma) rather than necrosis occurring in the center of preformed lesions as occurs in primary TB. Cavities are formed from the dissolution of these caseating pneumonic lesions. Six stages are represented by a 19th century drawing and a 21st century photomicrograph of sections stained with hematoxylin and eosin or trichrome, imaged at 40 to 400×. (Reproduced from references 31, 220, and 221).

T cells

A lot of emphasis has been placed on the *M. tuberculosis* antigens targeted by T cell responses. Early literature has focused on T cell responses that recognize a relatively small set of immunodominant antigens, including early secretory antigenic target-6 (ESAT-6), 10-kDa culture filtrate protein antigen (CFP-10), TB10.4, and antigen 85A (Ag85A) and Ag85B (41–45). These were the first to be incorporated as antigens into subunit vaccines (46, 47). However, a recent unbiased, genome-wide analysis of CD4 T cell responses to *M. tuberculosis* antigens in adults with latent *M. tuberculosis* infection (LTBI) revealed that the human CD4 T cell response targets a very broad array of more than 80 antigens (48). These responses were predominantly restricted to CD4 T cells and highly enriched for a CXCR3+CCR6+ subset that exhibits Th1-response characteristics (48). Nearly half of the epitopes identified in this study were derived from proteins that had not previously been identified as T cell antigens. This study and subsequent others demonstrate that the human immune response to *M. tuberculosis* is very heterogeneous and as yet poorly defined (48–50). An intriguing question is whether T cell recognition of distinct *M. tuberculosis* antigens is associated with TB disease risk.

Functional and phenotypic characteristics of *M. tuberculosis*-specific T cell responses have received par-

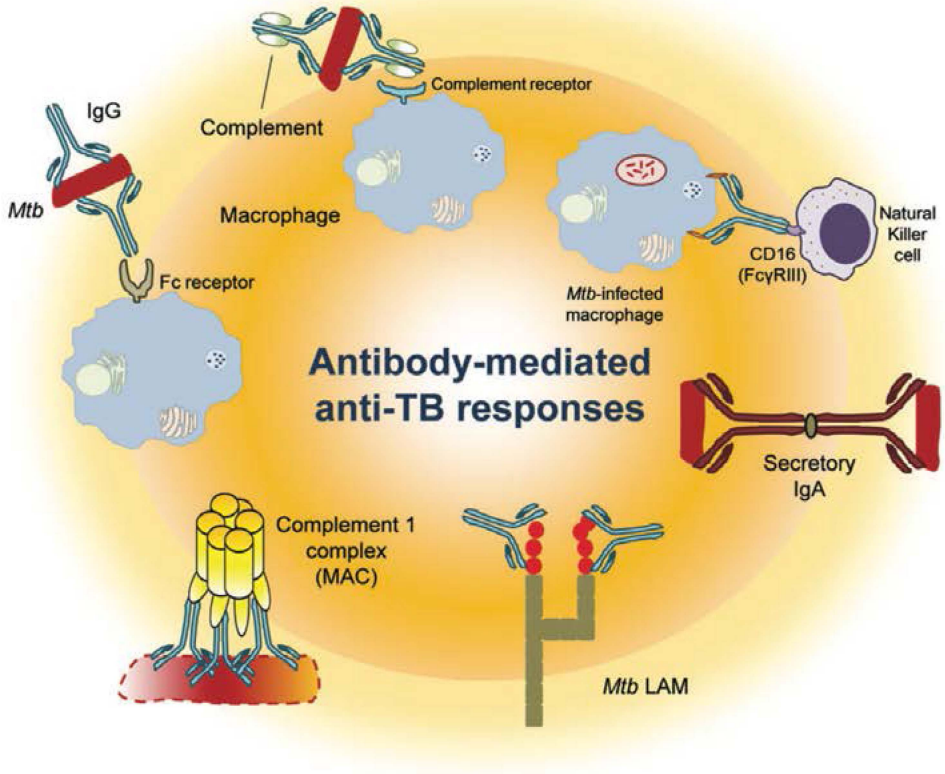

FIGURE 3 Role of antibodies in anti-*M. tuberculosis* (*Mtb*) infection. Antibodies may directly bind to mycobacteria, triggering complement deposition and lysis of *M. tuberculosis*, or complement may mediate opsonophagocytosis of the organism. Alternatively, *M. tuberculosis*-bound antibody may enhance macrophage uptake through Fc receptor binding or activate NK cell activity through Fc receptor engagement. It is also possible for immune complexes to form between mycobacterial antigen and antibody. Abbreviations: FcγRIII, Fc gamma receptor III; IgA, immunoglobulin A; IgG, immunoglobulin G; LAM, lipoarabinomannan; MAC, membrane-attack complex. (From reference 37 with permission.)

ticular attention in recent times, and interesting associations with the presentation of *M. tuberculosis* infection have been described. While the *M. tuberculosis*-specific T cell response in healthy people is dominated by CD4 T cells (48), a number of studies have revealed an increased contribution by CD8 T cells in patients with TB disease (51–53). The mechanism for this finding is currently not clear, but this pattern appears robust and has been proposed as a diagnostic approach for TB disease (53).

A prominent theme has been the pattern of Th1 cytokine coexpression, shown to be associated with the degree of T cell differentiation in viral infections (54). Comparative studies of patients with TB disease and latently infected people have reported elevated frequencies of *M. tuberculosis*-specific CD4 T cells expressing only TNF or TNF+IFN-γ+ CD4 T cells in TB patients, while those with latent infection have higher frequencies of polyfunctional TNF+IFN-γ+IL-2+ *M. tuberculosis*-specific CD4+ T cell responses (51, 55, 56). Furthermore, successful TB treatment appears to reverse this functional pattern, because CD4 T cells coexpress IFN-γ, TNF, and IL-2 to a greater degree after cure (51, 56). However, other studies have reported the opposite: active TB disease was accompanied by greater frequencies of polyfunctional TNF+IFN-γ+IL-2+ CD4 T cells than was LTBI (57–59). An immune correlates study in bacillus Calmette-Guérin (BCG)-vaccinated infants aimed to determine whether frequencies or cytokine coexpression patterns of mycobacteria-specific Th1-cytokine-expressing CD4 or CD8 cells, measured at 10 weeks of age, were associated with subsequent risk of TB disease (60). The study reported no association between frequencies or cytokine expression patterns in BCG-specific CD4 and CD8 T cells (61). However, more recently, BCG-specific IFN-γ-secreting T cells measured by enzyme-linked immunosorbent spot assay (ELIspot)

were found to be associated with reduced risk of developing TB disease in a South African infant cohort from the same population (62).

Such functional differences in T cell cytokine expression may simply reflect differential levels of T cell exposure to *M. tuberculosis* antigens, indicating *in vivo* bacterial load (51). This hypothesis is supported by phenotypic analyses of *M. tuberculosis*-specific T cells, which suggest that higher bacterial load in active disease is associated with greater T cell activation.

Activation of antigen-specific CD4 T cells, measured by HLA-DR, CD38, or Ki67 expression, was significantly higher in patients with active TB compared to controls with LTBI (63). These activation markers seemed to track antigen load well, as expression levels gradually decreased during treatment of active disease, suggesting that T cell expression of these activation markers can be useful as treatment response markers (63). A recent study investigated T cell activation as a biomarker of risk of TB (see section on progression to TB disease).

B cells and antibody responses

Many studies have assessed the ability of antibodies to accurately diagnose active TB, and these studies will be discussed below. In some studies the ability of antibodies to differentiate *M. tuberculosis*-infected subjects (defined as either TST+ or IGRA+) and uninfected controls has also been assessed (Table 1). It is estimated that a third of the world's population is latently infected with *M. tuberculosis*, although this varies greatly from region to region (2) and latency is likely to represent a spectrum from transient exposure to subclinical TB disease (64). Analysis of antibodies in those with *M. tuberculosis* infection, approximately 90% of whom are able to contain infection, versus uninfected controls enables the identification of antibody responses that may be important in the control of *M. tuberculosis* infection. *M. tuberculosis* infection induces mycobacteria-specific antibodies against a broad range of antigens, with no single antigen or group of antigens emerging as a preferential target for an antibody response (Table 1). Mycobacterial antigen-specific IgG, IgA, and IgM have all been reported in *M. tuberculosis* infection (Table 1). Perley et al. report approximately equal ratios of IgG and IgM in response to live cell surface, whole cell lysate, lipoarabinomannan (LAM), and cell wall and secreted mycobacterial proteins in *M. tuberculosis*-infected and uninfected controls (65). There are few studies in HIV-infected populations and little evidence for elevation of mycobacterial antibodies in HIV-infected, *M. tuberculosis*-infected versus HIV-1-infected, *M. tuberculosis*-uninfected populations (66, 67). All studies agree that antibody levels in *M. tuberculosis* infection are highly variable, with a high degree of overlap between infected individuals and uninfected controls. The greatest separation between *M. tuberculosis*-infected and uninfected control populations was reported by Baumann et al. (68), who found discrimination between *M. tuberculosis*-infected (defined as IGRA+ or TST+) and uninfected controls with 80% sensitivity and 93% specificity using AlaDH (Rv2780)-specific IgA, and 84.2% sensitivity and 93% specificity using NARL (Rv0844c)-specific IgA. In a separate study, they reported 74% sensitivity and 83% specificity using a combination of IgA and IgG specific for LAM and PE35 (Rv3872) (68). Perley et al. found better discrimination when measuring antibodies directed against the live cell surface of mycobacteria when compared to cell wall, LAM, or secreted proteins from *M. tuberculosis* (65).

T cell responses to *M. tuberculosis*-specific antigens, including ESAT-6 and CFP-10, are used as the basis for IGRAs to discriminate between *M. tuberculosis*-infected and uninfected individuals (30). However, antibody responses against *M. tuberculosis*-specific antigens are generally poor at discriminating between infected and uninfected individuals, although Hoff et al. found that they performed better in low-burden settings (69–72). It is important to note the potential bias because *M. tuberculosis* infection is defined by a cellular immune response measured by either TST reaction or IGRA response to an *M. tuberculosis*-specific antigen. There is currently no method that does not depend upon detection of a cell-mediated immune response for the detection of *M. tuberculosis* infection. While antibody responses are higher in those with a positive TST or IGRA, several studies have described high levels of *M. tuberculosis* antibodies in individuals with TST anergy, suggesting that antibodies can be elevated following *M. tuberculosis* exposure in the absence of a cell-mediated immune response (73, 74).

BCG vaccination and antibodies

A detectable increase in mycobacterial specific antibody is not always observed following BCG immunization (75), most likely due to pre-existing high-titer antibody induced by exposure to environmental mycobacteria (76, 77). BCG, however, has been found to induce modest levels of mycobacterial antigen-specific antibodies in several studies (78–81). Higher levels of Ag85A IgG antibodies in 4- to 6-month-old South African infants vaccinated with BCG at birth were found to be

TABLE 1 Antibodies in *M. tuberculosis* infection

Author	Study design	HIV-1 pos/neg	Class of antibody	Antigen	Detected difference[a] (% sensitivity; % specificity)
Chen J et al., 2010 (70)	LTBI versus controls (China)	Neg	IgG	Rv1985c	62; 97
Baumann R et al., 2015 (68)	LTBI versus controls (South Africa)	Neg	IgA	NARL (Rv0844c)	84.2; 93
			IgA	MPT83 (Rv2873)	63.2; 93
			IgA	19 kDa (Rv3763)	78.9; 93
			IgA,	AlaDH (Rv2780)	89.5; 93
			IgG	AlaDH (Rv2780)	26.3; 93
					NS
			IgA	PstS3 (Rv0928)	57.9
Baumann R et al., 2014 (222)	LTBI versus controls (South Africa)	Neg	IgM, IgA	MPT32 (Rv1860)	49; 100
					IgA
			IgA, IgG	PE35 (Rv3872)	PE35 + LAM
			IgA, IgG	LAM	74; 83
					IgA and IgG combined
			IgA, IgG	Tpx (Rv1932)	NS
			IgA, IgG	16 kDa (Rv2031c)	NS
			IgA	HSP20 (Rv251c)	NS
Hur Y et al., 2015 (71)	LTBI versus controls, TST+ and IGRA+ (South Korea)	Neg	IgG	38 kDa (Rv0934)	NS
			IgG	16 kDa (Rv2031c)	NS
			IgG	ESAT-6 (Rv3875)	NS
			IgG	CFP-10 (Rv3874)	NS
			IgG	LAM	NS
Niki M et al., 2015 (72)	LTBI versus controls, IGRA+ (Tokyo)	Neg	IgG/IgA	HrpA (Rv0251c/hsp)	IgG, $P < 0.01$
			IgG/IgA	MDP1 (Rv2986c)	IgA, $P < 0.05$[b]
			IgG/IgA	ESAT-6 (Rv3875)	NS
			IgG/IgA	CFP-10 (Rv3874)	NS
			IgG/IgA	Ag85A (Rv3804c)	NS
			IgG/IgA	16 kDa (Rv2031c)	NS
			IgG/IgA	HBHA (Rv0475)	NS
Hoff S et al., 2007 (69)	Control versus LTBI (Denmark, Brazil, Ethiopia, Tanzania)	Neg	IgG	ESAT-6-CFP10 fusion	$P < 0.01$
			IgG	Denmark	$P = 0.043$
			IgG	Brazil, Ethiopia	$P = 0.038$
Siev M et al., 2014 (67)	Control versus TST+ (U.S.)	Pos	IgG	MPT51 (Rv3803c)	NS
			IgG	echA1 (Rv0222)	NS
			IgG	MS (Rv1837c)	NS
			IgG	38 kDa (Rv0934)	NS
Yu X et al., 2012 (66)	Control versus TST+ (U.S.)	Pos	IgG	Arabinomannan	NS
			IgM	Arabinomannan	NS
			IgA	Arabinomannan	NS
			Neg	IgG	Arabinomannan
			IgM	Arabinomannan	NS
			IgA	Arabinomannan	NS
Perley CC et al., 2014 (65)	Control versus IGRA+ (U.S.)	Neg	IgG	Live cell surface	$P < 0.001$
			IgG	Whole cell lysate	$P < 0.01$
			IgG	IgG avidity, live cell surface	$P < 0.05$[b]
			IgG	IgG avidity, whole cell lysate	NS
			IgG	LAM	NS
			IgG	Cell wall	NS
			IgG	Secreted proteins	NS
			IgG	IgG avidity, LAM	$P < 0.05$
			IgG	IgG avidity, cell wall	NS
			IgG	IgG avidity, secreted proteins	$P < 0.001$

[a]NS, not significant.
[b]Decreased in LTBI.

associated with reduced risk of developing TB disease over the next 3 years of life (62). BCG-induced antibodies may contribute toward a protective immune response through mechanisms including the opsonization of mycobacterial cells for uptake by phagocytes (82).

Mechanisms of antibody action

Kumar et al. found that treatment with sera from *M. tuberculosis*-infected, healthy subjects enhanced uptake and intracellular killing of mycobacteria by donor myeloid-derived macrophages (MDM). Interestingly, not all mycobacterial antigens opsonized mycobacteria, and two antigens with approximate molecular weights of 48 and 80 kDa (possibly *M. tuberculosis* 48 and *M. tuberculosis* 81) were absent from opsonizing antibody extracts (83). Opsonized mycobacteria were killed more rapidly with enhanced IFN-γ and IL-6 production, enhanced phagosome acidification, and increased inducible nitric oxide synthase and nitric oxide production (83). The enhanced uptake of serum-coated mycobacteria by neutrophils and monocyte/macrophages was found to be IgG dependent in a separate study (82).

Antibody-inducing vaccines

There are no TB vaccine candidates currently in clinical development that are designed for the specific enhancement of a B cell or antibody response, although whole-cell mycobacterial vaccines such as VPM1002 (recombinant BCG) (84) and VAC (attenuated *M. tuberculosis*) (85) will induce a broad-spectrum response including antibodies. It is possible to enhance antibody responses to subunit TB vaccines through the use of specific adjuvants (86). Alum is widely used for the induction of antibodies, although it also skews toward a Th2 type immune response and does not protect against *M. tuberculosis* (86). There are, however, adjuvants such as MF59 which induce both a Th1 type cellular response and antibody and have been shown to enhance protection in mice challenged with *M. tuberculosis* (86).

PROGRESSION FROM *M. TUBERCULOSIS* INFECTION TO TB DISEASE

Although most individuals who become infected with *M. tuberculosis* remain asymptomatic, in some the immune response fails to contain the infection and clinical symptoms develop, including fevers, night sweats, weight loss, and chronic coughing, among many others. Definitive diagnosis of TB disease is based on detection of acid-fast bacilli, most often in sputum from the patient. The risk of progression to disease is greatest immediately following infection (87, 88); however, *M. tuberculosis* can persist for years in asymptomatic individuals. Long-term persistence of viable bacilli was reported in 1927, when *M. tuberculosis* was cultured from apparently healthy tissues of individuals with no pathological evidence of TB who died from other causes (89). Progression to active disease is possible even decades after exposure (88) and is typically triggered by immune compromise. This was elegantly demonstrated by reactivation of LTBI in rheumatoid arthritis patients who received anti-TNF blocking antibodies or other immunotherapies (90, 91). Many factors, including the magnitude of the infectious dose, the bacterial strain, time since exposure, and a multitude of other risk factors have been associated with risk of TB. Innate and adaptive immune mechanisms are clearly very important for successful control of *M. tuberculosis*, since impairment of immunity through steroids, chemotherapy, biologics, and HIV coinfection predisposes to TB disease (reviewed in reference 92).

Immune Mediators of TB Risk

TB susceptibility is driven by immune dysfunction, whether during acute or chronic latent stages of *M. tuberculosis* infection. The control of infection requires a precise balance between immune-mediated eradication of *M. tuberculosis* and limitation of inflammation to prevent immunopathology. As such, it is thought that any immune dysfunction which tips the balance in either direction can lead to disease progression. Among the greatest risk factors for TB are HIV-1 infection, malnutrition, diabetes mellitus, smoking, vitamin D deficiency, drug/alcohol abuse, male gender, age, and anti-TNF therapy (93–97). These risk factors are not mutually exclusive and can exacerbate each other (98–102). However, the phenotype of immunodeficiency induced by each is different, and therefore the interrelationship between comorbidities and disease susceptibility is complex. Studies of the underlying causes of each of these risk factors and their effects on TB risk can provide important insights into the mechanisms of protective immunity against *M. tuberculosis* in humans.

HIV

The resurgence of TB in sub-Saharan Africa is linked to the onset of the HIV-1 pandemic (103). Coinfection with HIV-1 is thought to increase susceptibility to TB via a number of mechanisms, primarily through dysfunctional and decreased numbers of CD4 T cells and impaired activation of T cell responses by phagocytes

(100, 104–106). However, increased risk of TB typically occurs in HIV-infected individuals prior to significant T cell depletion (107), suggesting that HIV may alter cellular responses to *M. tuberculosis* infection. HIV-1 and *M. tuberculosis* coinfection of PBMCs or macrophages has been shown to synergistically increase replication of both pathogens *in vitro* (108, 109). *M. tuberculosis* infection induces HIV-1 replication via a number of mechanisms, including upregulating the transcription factors, NF-κB (108), nuclear factor of activated T cells-5 (NFAT5), positive transcription elongation factor (P-TEFb), and loss of an inhibitory C/EBPβ (110–112). Induction of chemokines during *M. tuberculosis* infection also increases cellular recruitment of CCR5-positive monocytes and CD4 T cells into the site of infection, increasing the pool of cells that can be infected by HIV-1 (113). Conversely, the effect of HIV-1 on the macrophage response is variable and subtle, modifying cytokine and chemokine production required for T cell recruitment and activation (109, 114). In a large multicenter study in India, Lagrange et al. found higher levels and greater sensitivity for antibody-based TB diagnostic tests among HIV-positive compared to HIV-negative TB patients (115). In HIV-positive TB, secretion of BCG-specific IgG antibodies from peripheral plasmablasts was higher than in HIV-negative TB, and it increased further as CD4 T cell counts declined (116). The higher levels of antibody in HIV-positive TB likely reflect increased systemic mycobacterial load. The interaction of HIV with *M. tuberculosis* susceptibility is discussed in detail in reference 117.

Diabetes mellitus

The link between type 2 diabetes mellitus (T2DM) and increased TB risk has long been recognized (118), but the immunological mechanisms are poorly understood. Up to 22% of TB cases are attributed to T2DM in countries where both conditions are endemic (119). Recent systematic reviews have shown that individuals with T2DM have a 3-fold greater risk of TB and increased risk of mortality, delayed sputum conversion, treatment failure, and relapse, as well as developing drug resistance due to T2DM, interfering with rifampin metabolism (120–124). TB patients with T2DM are also more likely to have cavitary TB (124), while HIV-1 decreases this risk (125). Therefore, while HIV-1 increases TB risk up to 50-fold (126), the increasing prevalence of T2DM could have a relatively greater impact on TB control in the future (127).

Two mechanisms underlying the T2DM-associated risk for TB have been hypothesized: (i) dysregulated glucose metabolism results in hyperglycemia and insulin resistance, enhancing *M. tuberculosis* replication, and (ii) increased inflammation by adipose-resident monocytes activated by free fatty acids and lipid intermediates, associated with insulin resistance, promotes a generalized proinflammatory environment that favors progression to TB disease (118, 128). In support of the second hypothesis, it has recently been shown that TB patients with T2DM have increased circulating Th1 (IFN-γ, TNF, IL-2), Th17 (IL-17A), and other proinflammatory (IL-1β, IL-6, IL-18) cytokines, hyperreactive T-helper cells, and reduced frequencies of regulatory T cells (Tregs) (129–131). How T2DM-associated inflammation impacts TB susceptibility, TB immunopathology, and *M. tuberculosis* killing is unknown, but longitudinal studies investigating HbA1c levels in TB patients during TB treatment have shown that glucose intolerance decreases following successful TB treatment (132, 133). This suggests that in some cases T2DM may result from infection-induced impaired glucose metabolism, rather than prior T2DM increasing TB risk. Irrespective of the sequence of attainment, screening and treatment for glucose intolerance during TB are likely to improve treatment outcome.

Vitamin D

Vitamin D deficiency is common in active TB patients (102, 134), is exacerbated in TB patients with HIV-1, and is more prevalent in people with LTBI who progress to active TB (135). Furthermore, individuals who carry a vitamin D receptor (VDR) polymorphism at the Taq1 locus (rs731236) or the vitamin D binding protein Gc2 haplotype (T420K amino acid change) and are vitamin D deficient are more susceptible to TB (134, 136). The effects of vitamin D on the immune system are pleiotropic (137). Consequently, the exact mechanisms by which vitamin D may help prevent TB remain a subject of contention. Moreover, the unique antimicrobial effect of vitamin D metabolites, mediated by expression of cathelicidin antimicrobial peptide (*CAMP*), is unique to humans (and other primates), who have evolved three vitamin D response elements in the *CAMP* promoter. These promoter elements are missing from rodents and cattle (138), species commonly studied as models of human TB.

Vitamin D has two modes of action. One is fast-activating via membrane VDR, increasing reactive oxygen species (139), nitric oxide (140), and phagolysosome fusion during mycobacterial infection (141). The other occurs via binding to the nuclear VDR, forming a transcription factor complex which targets more than 900

promoters (142). VDR activation induces expression of cathelicidin (proteolytically cleaved into LL-37), which has direct antimycobacterial effects and also induces autophagy (25, 101, 143). Vitamin D treatment also reduces matrix metalloproteinase (MMP) activity, which is linked to lung matrix degradation and chemokine processing (144, 145). Conversely, vitamin D drives the adaptive response toward an anti-inflammatory state, increasing IL-10 production and regulatory T cell differentiation and inhibiting proinflammatory cytokines (99, 146, 147). While a decrease in proinflammatory responses during initial infection is counterintuitive to a protective response, the anti-inflammatory effects of vitamin D are likely to enhance resolution of pathologic inflammation during TB treatment (148). The same may occur during initial infection, limiting excessive inflammation while enhancing antimycobacterial activity.

The antimicrobial effects of vitamin D metabolites have also been shown to be crucial for the protective activity of IFN-γ. We and others have shown that stimulating human monocytes and MDM with IFN-γ in vitamin D-sufficient media prior to infection increased *CAMP* expression and autophagolysosomal fusion and reduced intracellular *M. tuberculosis* growth (149, 150). Conversely, pre- and postinfection treatment of MDM with IFN-γ had no effect on vitamin D-mediated *M. tuberculosis* growth restriction when vitamin D metabolites were added postinfection (149). This suggests that maintaining vitamin D sufficiency prior to infection will enhance macrophage and T cell-mediated innate cell responses during *M. tuberculosis* infection.

Malnutrition

Malnutrition has historically been associated with peaks in TB incidence, but the direct effect of malnutrition on TB risk is ill-defined (151). Body mass index (BMI) and TB incidence have been demonstrated to have an inverse relationship, with a 13.8% reduction in TB per unit increase in BMI (152). Malnutrition can encompass both macronutrient and micronutrient deficiencies; however, the underlying interaction of each with host immunity to increase TB risk is poorly understood. Studies have shown that TB patients from various populations have deficiencies of vitamins A, C, D, and E, zinc, and iron (153). Vitamin D, being the most studied, has been described above. Recent evidence suggests that vitamin C has direct anti-*M. tuberculosis* activity, dependent on high ferrous ion levels and reactive oxygen species production (154). Vitamin A (retinol) deficiency is also associated with TB and may synergize with vitamin D, as the retinol X receptor RXR forms a heterodimer with the VDR to form a transcription factor complex, and cotreatment with vitamin D_3 plus retinoic acid inhibits *M. tuberculosis* entry and survival within macrophages, possibly through rescue of phagosome maturation arrest (155). Vitamin A, via its active metabolite all-trans retinoic acid, has recently been shown to induce myeloid cells to express *NPC2*, which helps the cell effectively remove cholesterol from the lysosomes so *M. tuberculosis* bacteria cannot access it. This increases lysosome acidification and *M. tuberculosis* killing (156). Moreover, vitamin A adjunct therapy during intensive-phase TB treatment enhances sputum smear conversion (157).

Inflammation and Progression to TB

TB disease is a chronic inflammatory condition, and the pathology of the disease is a consequence of the host immune response to the mycobacterium, rather than direct destruction of tissue by *M. tuberculosis* itself. The balance of sufficient inflammation for containment of infection and immune pathology as a result of excessive inflammation is critical to our understanding of human TB disease. In 1891 Robert Koch reported results of a study in which he repeatedly injected TB patients with tuberculin (158). This treatment did not cure TB but, rather, induced inflammation, swollen lymph nodes, and tissue necrosis and in some patients resulted in death (158). In addition to the magnitude of the inflammatory response, the timing and location of the response are also likely to be key for the balance between control of infection and progression to active disease (reviewed in reference 33). While animal models have revealed the importance of individual cell types such as neutrophils, classically and alternatively activated macrophages, and specific cytokines such as IL-10 and TNF (159) in a balanced inflammatory response, it has been harder to understand these processes in human populations. Genetics can influence the inflammatory response, but the strongest driver of variability in the human inflammatory response appears to be our environment. In an immune phenotyping study, differences in immune cell populations were largely associated with environmental and not genetic factors, with cytomegalovirus identified as the major microbial driver of immune variation (160).

Type I interferons in TB

The type I interferon response is classically a response to viral infection, and yet human biomarker studies have identified IFN-α/β proinflammatory immune signatures as key components of active TB disease (24, 161–164). This response is likely driven by mycobacterial load

because it associates with disease pathology and declines in response to TB treatment (164, 165). *In vitro* experiments show that type I interferons reduce the expression of IFN-γ and the ability of macrophages to respond to IFN-γ and control intracellular growth of *M. tuberculosis* (166). IL-1 can limit excessive type 1 interferon activity in mice, suggesting that this pathway could provide a target for host-directed therapy in TB (167).

Monocytes in TB disease

Monocytes are the primary target of mycobacterial growth among PBMCs infected *in vitro*, and in peripheral blood, monocyte numbers expand during active TB disease (168). In the 1930s it was recognized that the ratio of monocytes to lymphocytes in peripheral blood may be important for the resistance or susceptibility to TB disease. During healing of lesions, an increase of lymphocytes around the granuloma has also been detected, and this correlated with an increase in lymphocyte:monocytes in the periphery (169).

Monocytes can be phenotypically and functionally distinct and can differentiate into M1 or M2 macrophages with pro- and anti-inflammatory properties, respectively, although this bipolar nomenclature is becoming more contentious with the increasing emergence of more polarization states which are relative to the activation agent (170). CD16+ "inflammatory" monocytes have recently been shown to modulate immunity to mycobacteria through the production of IL-10 (171). Monocytes can also modulate immunity through amino acid catabolism, in particular tryptophan and arginine, through the induction of indoleamine 2,3-dioxygenase and arginase (reviewed in reference 172). T cells are sensitive to amino acid levels in the microenvironment, and depletion of arginine and tryptophan can result in T cell anergy. Increased ratios of monocytes in peripheral blood are associated with increased type I interferon-related transcript signatures and a reduction in ability to inhibit mycobacterial growth (173). The frequency of monocytes relative to lymphocytes has also been associated with risk of progression to TB disease (174–177). Typically, M1 macrophages are associated with killing of mycobacteria, whereas M2 macrophages are associated with tissue repair and bacterial persistence (178, 179). Therefore, in addition to monocyte quantity, the polarization state of monocytes is likely important for maintenance of balance in the inflammatory response in TB disease.

Tissue Remodeling

The ability of *M. tuberculosis* to induce degradation of pulmonary extracellular matrix (ECM) contributes to its success as a pathogen. Induction of lung cavitation allows bacilli to replicate in an immunologically privileged site, promoting persistence and transmission, while penetration of the alveolar basement membrane allows extrapulmonary dissemination of infection. MMPs, a family of zinc- and calcium-dependent endopeptidases, are capable of degrading all components of the pulmonary ECM. Moreover, MMPs also regulate the innate immune response by controlling cytokine and chemokine processing, apoptosis, and antimicrobial peptide activation (for review, see reference 180). These potent enzymes are expressed by a wide variety of cells, including lymphocytes, resting monocytes, and activated macrophages. MMP-1 (interstitial collagenase) and MMP-9 (92-kDa gelatinase B) are the major secreted MMPs of human monocytes and alveolar macrophages under basal conditions (181). *M. tuberculosis* induces expression of *MMP-1, MMP-7*, and *MMP-10* in infected human macrophages (144, 182), and increased expression of *MMP-1, MMP-7*, and *MMP-9* has also been demonstrated in cells isolated from the lungs of TB patients. MMP-1 and MMP-7 have been shown to colocalize to macrophages around the central area of necrosis in tuberculous granulomata (182, 183). *M. tuberculosis*-induced MMP-1, MMP-7, and MMP-9 secretion by mononuclear phagocytes has been shown to be prostaglandin E2 (PGE2) dependent (182, 184, 185), and IL-4 and IL-10 can inhibit monocyte secretion of MMP-1, MMP-7, and MMP-9 (184, 186, 187). Together this suggests that inhibiting MMP production though reduced PGE2 signaling may limit cavitation and potentially resolve pulmonary pathology during treatment.

T Cell Responses during TB

A recent analysis of immune correlates in infants who participated in the recent Phase IIb efficacy trial of MVA85A (188) suggests that elevated CD4 T cell activation is associated with risk of TB. Infants who developed TB disease during follow-up in the trial had significantly higher levels of total CD4 T cells expressing HLA-DR at study baseline than infants who remained healthy (62). Importantly, this association was replicated in an independent cohort, *M. tuberculosis*-infected adolescents, in whom elevated CD4 T cell activation was also found to correlate with risk of TB (62).

Positive TST or IGRA tests can spontaneously revert to negative (reviewed in reference 10). Reversion has been reported in many studies throughout the last century at rates of 10 to 50% (189–194). The mechanisms of reversion are not understood, and immune suppres-

sion, egress of *M. tuberculosis*-specific T cells from the peripheral blood to sites of infection, or decreases in bacillary load are possible underlying causes. However, TST or IGRA reversion may also be indicative of clearance of *M. tuberculosis* infection. The most comprehensive study of TST reversion was performed in the 1920s in household contacts of TB cases (193). Among household contacts with at least two TSTs, 11.1% reverted from positive to negative. These TST reverters had a very low risk of active TB over the subsequent 5 years (0.72%). By contrast, 23.3% of the entire cohort developed disease. The largest study of Quantiferon Gold In-Tube (QFT) reversion was performed in South African adolescents, in whom annual reversion rates of 5.1% were reported (189). Although the number of TB cases was too low for robust stratification of disease risk in this study, incident TB was 8-fold higher among QFT reverters than among individuals with consistently negative QFT results (1.47 versus 0.18 cases/100 person-years) (189). Additional studies are necessary to establish the clinical significance of TST and IGRA reversion.

B Cells and TB Antibody Responses

Although antibodies are induced against a broad range of protein and nonprotein antigens in active TB disease, they are not useful for diagnosis due to a lack of sensitivity and specificity. There is evidence for reduced antibody avidity in active TB disease and for perturbation in Fc receptor expression, suggesting that phagocytosis and antibody-mediated cellular cytotoxicity could be dysregulated. Transcriptomic signatures for B cells are also depressed in TB, suggesting downregulation or exhaustion of the B cell response. B cells and antibodies are involved in the immune response to TB, and the interaction of antibodies with phagocytic cells through Fc receptor engagement is emerging as a key area for research.

The quantity of antibody produced during *M. tuberculosis* infection is related to bacterial load, and higher antibody responses are observed in those at risk of disease and are correlated with mycobacterial load during disease (195). This suggests that antibodies are important in the control of active TB disease and has also led to the development of antibody-based assays for TB diagnosis. Antibody-based diagnostic assays are cheap to produce and amenable to development as point-of-care tests which can be used in remote settings because they do not require specialist equipment. Much effort has been invested in developing an antibody-based assay for TB diagnosis, with limited success (196). In a systematic review and meta-analysis of the literature, which included 67 studies amounting to 5,147 participants, the sensitivity was 0 to 100% and specificity was 31 to 100% (196). It was concluded that antibody assays produce inconsistent and inaccurate results, and these data were used to inform a World Health Organization policy statement against the use of serological tests (http://whqlibdoc.who.int/publications/2011/9789241502054_eng.pdf).

However, poor performance of antibodies as a diagnostic test does not translate to lack of importance in immune control of TB disease. This section discusses evidence from human studies of a role for antibodies in the control of TB disease.

Antibody quality

The primary purpose of antibody measurement in human studies has been the diagnosis of TB disease, and most studies focus on the quantity of antibody detected and not antibody quality. Antibody avidity is variable in TB patients (197) and shortly following TB treatment there is an increase in antibody quantity and a decrease in avidity, suggesting exhaustion of the B cell response (198). Perley et al. found a decrease in avidity of antibody specific for the live cell surface of mycobacteria in TB patients, suggesting conversion of antibodies to low-avidity IgG or B cell exhaustion (65). Antibody avidity was also higher in those previously immunized with BCG, which raises the possibility of using vaccination to improve antibody avidity as a potential mechanism for protection against TB.

Antibody function

Given the extensive literature on antibody quantity in TB, there are few studies assessing antibody function using clinical samples. It is known that complement binds efficiently to the surface of mycobacteria and that the classical, lectin, and alternative complement pathways are activated (15, 199). Preincubation with human serum containing mycobacterial specific IgG and IgM further enhances complement binding to mycobacteria (200). The ability of human sera to enhance uptake of mycobacteria into the macrophage is retained after heat treatment to inactivate complement, suggesting that uptake of mycobacteria also occurs via engagement of Fc gamma receptors on the phagocyte cell surface (83). Fc gamma receptor expression has consistently been identified in active TB disease biomarker studies with decreased expression in TB disease, indicating that downregulation of Fc gamma receptor may play a role in pathogenesis of TB disease (161, 162, 201, 202).

BIOMARKERS IN HUMAN TB

A major limitation of IGRA tests and TST is their inability to differentiate between LTBI and active TB disease (reviewed in reference 203). These tests cannot predict risk for progression to TB disease (30). Moreover, whole-blood transcriptomic signatures comparing TB, LTBI, and healthy controls support the recent change in dogma that LTBI represents a spectrum of disease states (Fig. 1), rather than a single clinical stage (204, 205). An important finding from whole-blood transcriptional profiling is that the gene expression signatures of some individuals clinically classified as LTBI cluster with the signature of active TB patients. These data suggest that these asymptomatic individuals may have subclinical TB disease (24, 206).

A biomarker that accurately identifies those at high risk for progression would allow targeted preventive antimicrobial therapy to prevent TB disease. This would be especially useful in settings of TB endemicity, where treating all latently infected people for 6 to 9 months is not feasible. Many investigators are therefore engaged in projects aimed at identifying correlates of risk of TB. The first study to report an association between gene expression in peripheral blood cells and risk of TB disease compared 15 HIV-infected drug users who ultimately progressed to active TB with 16 who remained disease free. Expression of two transcripts, IL-13 and AIRE, was found to correlate with risk of TB (207).

Transcriptomic Profiling

The unprecedented increase in our understanding of the human immune response to *M. tuberculosis* in the past decade is largely attributed to studies using whole-genome transcriptional profiling during various stages of pathogenesis and treatment (reviewed in reference 208). In general, these studies have characterized gene expression in whole blood. While these studies have been able to identify biomarkers with impressive diagnostic sensitivity for active TB, it is not possible to infer correlates of protection against disease from such cross-sectional study designs. To learn about mechanisms that underlie protective responses or progression from infection to disease, longitudinal studies in which individuals transition from LTBI to active TB are necessary. Three recent studies with such longitudinal designs have been completed. The first two were in BCG-vaccinated infants, and the results are discussed in the T cell and antibody sections above. The third was a large cohort study of 6,363 adolescents, half of whom were *M. tuberculosis* infected, who were followed up for 2 years. Incident TB disease was diagnosed during follow-up in 47 adolescents (209). A prominent IFN response signature distinguished asymptomatic, HIV-uninfected persons who progressed to TB disease from those who remained asymptomatic. An additional fourth study adopted an alternate study design using PET/CT imaging of HIV-infected individuals with *M. tuberculosis* infection to identify those with sublinical TB and those at risk of TB (210). In those with underlying HIV infection, IFN response signatures did not readily discriminate between persons with subclinical TB and controls, most likely because HIV infection leads to increased expression of interferon transcripts in peripheral blood (H. Esmail, personal communication). This suggests that discriminating interferon signatures for TB risk may perform with reduced accuracy as predictive biomarkers of disease risk in HIV-infected individuals. However, additional signatures implicating myeloid inflammation and complement components were also identified as correlates of TB risk in both studies. It is clear that more such studies are required to delve deeper into the processes that underlie the transitions between the stages of *M. tuberculosis* infection in HIV-infected and uninfected individuals.

Treatment Response

A large number of studies across various populations have investigated serum biomarkers of TB and their response during therapy. In addition to the classical acute-phase markers C-reactive protein (CRP), serum amyloid A (SAA), and albumin, other highly regulated proteins in TB which change during TB therapy include CXCL9, CXCL10, S100A9, MMP1, MMP9, D-dimer, PGE-2, HGF, VEGF, and sIL-2R (148, 211, 212). Serum markers that can predict fast versus slow response to therapy have also been identified in multiple studies. However, performance of these biomarkers varies depending on the cohort and the cut-off used to define fast response. Some of the most consistent markers include CRP, SAA, sTNF-R1, sIL-2R, and neutrophil-associated proteins, including granzyme B and MMP1 (27, 213–216). Ethnic genetic variation has been identified as a key variable affecting the performance of biomarkers of TB response (149), a finding that should be considered if protein biomarkers are to be used in TB diagnosis and the monitoring of therapy.

The measurement of antibody levels during TB drug treatment has been inconsistent, with some studies reporting a decline in antibody over time of treatment and others finding that antibody levels rise or do not change (217, 218). As described above, biomarker studies have identified changes in Fc gamma receptor expression in active TB disease (164, 219).

Transcriptomic signatures associated with B cells and humoral immunity have also been identified in biomarker studies focused on the response to TB treatment (164). Gene signatures associated with B cells are initially depressed and rise through therapy. This suggests that B cells are depleted or less functional during active disease, which is consistent with the observation of reduced antibody avidity in active TB (65).

CONCLUSION

Remarkable advances in our understanding of the immune response have been made since the advent of molecular biology and modern immunology. Among the themes are the incredible heterogeneity in infection states, disease presentation, and the complexity of the host response to *M. tuberculosis*. However, the exact immune mechanisms that underlie protective immunity against *M. tuberculosis* in humans remain unknown. Continued concerted research efforts and application of modern technologies are likely to enhance our understanding of the immunopathogenesis of TB in humans and facilitate rational development of highly effective interventions.

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