The role of antibodies in tuberculosis diagnosis, prophylaxis and therapy: a review from the ESGMYC study group

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Shareable abstract (@ERSpublications)
Despite being controversial, antibodies could play a role in the diagnosis of active TB, LTBI and treatment response monitoring. Antibodies could also help when administered as passive transfer and in the protection generated by new TB vaccine candidates. https://bit.ly/3tgEKeP

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Abstract

Tuberculosis (TB) is still responsible for the deaths of >1 million people yearly worldwide, and therefore its correct diagnosis is one of the key components of any TB eradication programme. However, current TB diagnostic tests have many limitations, and improved diagnostic accuracy is urgently needed. To improve the diagnostic performance of traditional serology, a combination of different *Mycobacterium tuberculosis* (MTB) antigens and different antibody isotypes has been suggested, with some showing promising performance for the diagnosis of active TB. Given the incomplete protection conferred by bacille Calmette–Guérin (BCG) vaccination against adult pulmonary TB, efforts to discover novel TB vaccines are ongoing. Efficacy studies from advanced TB vaccines designed to stimulate cell-mediated immunity failed to show protection, suggesting that they may not be sufficient and warranting the need for other types of immunity. The role of antibodies as tools for TB therapy, TB diagnosis and TB vaccine design is discussed. Finally, we propose that the inclusion of antibody-based TB vaccines in current clinical trials may be advisable to improve protection.

Introduction

The World Health Organization (WHO) has estimated a worldwide total of 10 million cases of tuberculosis (TB) and 1.5 million deaths in 2020 [1] Currently approved diagnostic tools for both TB and latent tuberculosis infection (LTBI) have limitations [2]. Smear microscopy has variable sensitivity [2, 3], sputum culture is time-consuming and requires biosafety level three facilities [2], and GeneXpert requires trained professionals and has high equipment costs [3]. The tuberculin skin test has been used for many decades to diagnose LTBI but has false-positive results due to cross-reactivity with bacille Calmette–Guérin vaccine (BCG) and the need for follow-up visits to interpret the result limits its use [4]. Further, even though commercial interferon (IFN)-γ release assay (IGRA) tests have been approved by the Food and Drug Administration (FDA) and have better specificity for diagnosing LTBI, they are unable to differentiate between LTBI and active TB, are poor predictors for TB progression and are expensive [4]. In light of the above, alternative rapid and cost-effective diagnostic markers are urgently needed for both active TB and LTBI.

Monitoring of treatment outcomes in TB is also essential due to the long duration of TB treatment [5, 6] and the possibility of recurrence of TB afterwards [7]. Sputum culture conversion (SCC) is used to monitor

treatment outcomes in TB [8, 9], even though it has shown modest sensitivity and specificity for predicting treatment relapse and failure [10]. As such, better biomarkers to predict TB treatment outcomes are needed because of their potential to impact the clinical TB management [8].

Numerous efforts are ongoing to develop new TB vaccines as the BCG is known to be effective in preventing severe forms of TB in infants but fails to protect against pulmonary TB (PTB) in young people and adults [11]. Almost all candidate TB vaccines currently in clinical trials are designed to induce cell-mediated immunity (CMI). However, evidence suggests the need for other types of immunity to achieve complete protection and consideration of the potential use of antibodies is emerging [12, 13].

Immune-based biomarkers detected from easily accessible samples such as blood and urine are considered to be ideal alternatives for the diagnosis of TB where sputum culture is either challenging or inefficient [2, 14–16]. In this regard, a growing body of literature suggests that mycobacterial-specific antibodies may be promising markers for the diagnosis of active TB and LTBI [4, 5, 17–19].

In this review, we discuss the role of antibodies in the diagnosis of active TB and LTBI, and highlight their use for treatment response monitoring, as well as their protective roles in passive transfer, and in current TB vaccine candidates.

The role of antibodies in TB diagnosis and treatment monitoring

Although the antibody-based assay for diagnosis of TB displays variable sensitivity and specificity, further research to develop new serological tests is underway [20]. Their potential advantages (cost-effectiveness, easy implementation and fast test performance) favour their use in low-resource TB high-endemic settings [21].

Antibody responses for diagnosis of active TB

Recently the WHO has developed target product profiles (TPPs) to define the end-user requirements that non-sputum-based rapid biomarkers should meet for acceptable performance of the test, namely a sensitivity and specificity of at least 98% for adult pulmonary TB [22]. Different strategies have been used to improve the accuracy of the serodiagnosis of TB (supplementary figure S1). Use of multiple mycobacterial antigens instead of a single antigen is one of the promising strategies for enhancing the performance of existing serological tests (tables 1 and 2) [18]. The failure of a serological assay based on several antigens may be due to the complex nature of the humoral immune response to *Mycobacterium tuberculosis* (MTB), with differential antigen expression at different stages of infection [18, 19].

Khaliq *et al.* [18] (working in Pakistan) reported an antibody response in a multiplex serological assay comprising 11 recombinant MTB antigens, showing an IgG response with a sensitivity of 95% in sputum smear-positive and 88% in smear-negative individuals and allowing the diagnosis of up to 360 patients per day, thus suggesting the scalability of this assay for the rapid diagnosis of active TB in endemic settings. In Uganda, the median IgG levels for eight MTB antigens showed good sensitivity and specificity for the screening of active TB, meeting the TPP targets set by the WHO for a TB screening test [19]. However, further studies including LTBI and HIV-infected individuals should be conducted.

IgG response against six MTB antigens and fusion proteins from these antigens was used to discriminate TB patients from non-TB controls in a Brazilian cohort. The use of a fusion polyprotein improved the diagnostic accuracy up to 90%, suggesting the need to design a multiantigen cocktail/fusion protein to improve the diagnostic accuracy [23]. A related study demonstrated the antibody response to a panel of 24 MTB antigens using serum samples from Africa, Brazil and USA. The overall antibody response to a mixture of seven MTB antigens amounted to 90% sensitivity and 100% specificity for the diagnosis of PTB [24]. In a Chinese cohort, ELISA-based serodiagnosis of active PTB using new recombinant MTB antigens achieved an accuracy similar to the targets set by the WHO [25]. Overall, antibody-based diagnosis of active TB from blood samples using appropriate tools that allow antibody detection with multiple antigens could offer an exciting approach for the diagnosis of active TB if validated by further research, especially in TB-endemic countries [18, 19].

Another approach suggested to improve the accuracy of serological tests is combining different antibodies, such as IgA, IgM and IgG, instead of a single MTB-specific IgG [5, 21] (table 1). In this regard, anti-16 kDa IgA and anti-MPT64 IgA were found the ideal candidates for the diagnosis of active TB and LTBI, with a sensitivity and specificity > 90% in both cases. The combined antibodies improved the performance and accuracy, the authors concluding that individual and combined markers of IgG and IgA were a potential marker for the diagnosis of active TB [5]. Similarly, Baumann *et al.* [21] demonstrated

TABLE 1 List of antibodies for the diagnosis of active tuberculosis (TB), latent TB infection (LTBI) and treatment monitoring

Antibodies and MTB antigen	Results and relevance		References
	Sen and Sp	TPP status	
Antibodies for diagnosis of active TB			
IgG against 11 antigens (Rv3881, Rv0934, Rv2031c, Rv1886c, Rv1860, Rv3874, Rv2875, Rv3841, Rv1926c, MEMH37Rv and Rv1984)[#]	Sen: for dx of smear positive PTB, 95% Sen: for dx of smear negative PTB, 88% Sp: in COPD patients 96% and healthy controls 91%	Moderately comparable to the target (⩾98%) Met the optimal value needed (⩾68%) Moderately comparable to the target (>98%)	[18]
IgG against 8 antigens (Ag85B, Ag85A, Ag85C, Rv0934-P38, Rv3881, BfrB, Rv3873 and Rv2878c)[#]	Sen: for screening active TB: 90.6% Sp: 88.6%	Met the minimal value for TB screening (>90%) Met the optimal target for TB screening test (>70%)	[19]
IgG against 6 antigens and fusion proteins from these antigens (Rv0934, Rv3874, Rv2875, Rv2031, Rv2032 and Rv0831)[¶]	Sen: for dx of smear positive PTB, 93% Sp: 96%	Moderately comparable to the target (⩾98%) Moderately met the optimal value needed (>98%)	[23]
IgG against 7 antigens (PstS1, Rv0831c, FbpA, EspB, bfrB, HspX and ssb)[+]	Sen: for dx of PTB, 74–90% Sp: 96–100%	Slightly comparable to target (>98%) Met the optimal value (>98%)	[24]
IgG against Rv0220, Rv2958c, Rv2994 and Rv3347c[§]	Sen: 80.4–91.3% Sp: 91.7–98.3%	Slightly comparable to the optimal value (⩾98%) Moderately comparable	[25]
Anti-TB-LTBI IgG, anti-Tpx IgG and anti-MPT64 IgA. TB-LTBI: (antigen cocktail composed of Tpx and L16)[f]	Sen: for dx of active TB, 95.2% Sp: 97.6%	Moderately comparable to the optimal value (⩾98%) Comparable to the target (⩾98%)	[5]
Anti-LAM IgA, anti-LAM IgG, anti-Tpx IgA, anti-HSP16.3 IgG and anti-HSP20 IgA[##]	Sen: 81% Sp: for dx of non-TB cases, 94%	Slightly comparable to the optimal value (⩾98%) Moderately comparable to the target (⩾98%)	[21]
IgG against A60 (commercial test)[¶¶]	Sen: 91.2% Sp: 92%	Moderately comparable Moderately comparable	[26]
IgG against 38-kDa, 16-kDa, and 6-kDa (commercial test)[#]	Sen: 78.1% Specificity: 100%	Slightly comparable to the optimal value (⩾98%) Met the optimal value (>98%)	[29]
Antibodies for diagnosis of LTBI			
IgG against ESAT6 and MDP1		IgG against ESAT6 and MDP1 were significantly higher in recent LTBI compared to remote LTBI (Sen and Sp data not available)	[17]
IgG against PPE17		Sen for dx of LTBI 87%, for dx of active TB (95%) (Sp data not available)	[4]
IgG against PPD		Sera from LTBI has less fucose and more galactose and sialic acid compared to active TB patient	[13]
Antibodies for treatment monitoring			
IgG against 14 kDa		Increased following treatment, and decreased after 3 years	[33]
IgG against 38 kDa and LAM		Increased at month 2 and month 8 following treatment	[34]
IgG against Rv2626c and ESAT6		Decreased following treatment	
Anti-16 kDa IgA and IgM		Decreased following treatment	[5]
IgG against LAM and TB – LTBI		Increased at month 6 of anti-TB treatment completion	
Anti-Tpx IgG and anti-ESAT-6 IgA		Predicated slow responders with an accuracy of 90.5%	[32]
Anti-16 kDa IgG		Increased following TB treatment	[6]
Anti-ESAT-6 and CFP-10 IgG		Higher before treatment, and increased following treatment	

Recent LTBI: <2 years since infection; remote LTBI: >2 years since infection. MTB: *Mycobacterium tuberculosis*; Sen: sensitivity; Sp: specificity; TTP: targeted product profiles; PTB: pulmonary TB; LAM: Lipoarabinomannan; dx: diagnosis. [#]: IgG against multiple antigens (measured individually, analysed in combination); [¶]: IgG against multiple antigens and fusion protein (measured individually, analysed in combination); [+]: IgG against multiple antigens and antigen cocktails (measured individually, analysed in combination); [§]: IgG against novel single or multiple antigens; [f]: multiple antibodies (IgG, IgM, IgA) against multiple antigens and antigen cocktail (measured individually, analysed in combination); [##]: multiple antibodies against multiple antigens (measured individually, analysed in combination); [¶¶]: IgG against single protein (multiantigen complex).

that single anti-lipoarabinomannan (LAM) IgG showed a sensitivity of 71.4% and specificity of 86.6%, while a combination of other MTB antigens and antibodies improved the accuracy for discriminating between TB and LTBI to 86.5%. In another study, anti-A60 IgG showed a sensitivity and specificity of >90%, the combination of IgG with IgA or IgM not improving the diagnostic accuracy. This may be because of a lower immune response to the other Ig subclass and suggests that more than one antigen may be required to improve the accuracy [26]. Also, A60 antigen is a complex protein that is highly conserved in typical and atypical mycobacteria which could explain why, in a related study, anti-A60 IgG detection failed to discriminate active TB with a better performance by anti-45 kDa IgG [27].

An ELISA based on the detection of antibodies against natural lipid antigens contained in the cell wall has been described as accurate for TB diagnosis in a cohort of 349 subjects coming from high TB countries whose samples were shared by WHO–TDR [28]. Using a single lipid antigen, the lipid-based test provided a sensitivity and specificity of 85% and 88%, respectively, for smear- and culture-positive PTB detection; this increased to 96% and 95%, respectively, by a statistical combination of the results with seven antigens. In the validation study, a sensitivity and specificity of 87% and 83% was reached with a single antigen and increased up to an area under curve of 0.95 by combination of different antigens.

A commercial immunochromatographic test has been evaluated in Tunisia for the diagnosis of active TB by detecting serum IgG and IgM against three mycobacterial antigens. IgG against 38-kDa, 16-kDa, and 6-kDa antigens showed a sensitivity of 78.1% and specificity of 100% for discriminating active TB from non-TB. Further studies in larger samples are required to confirm the result and clinical use of the test [29].

In summary, antibodies play a role in TB diagnosis and show promising potential for rapid, point-of-care diagnostic tests for the diagnosis of active TB that allow early treatment and reduce the transmission of TB in the community.

Antibody response in the diagnosis of LTBI

Identification of patients at risk of progressing to active TB is critical for the proper and timely management of active TB patients [17, 30].

Significantly higher anti-ESAT-6 and anti-MDP1IgG levels were found in recent LTBI compared with non-infected and remotely infected individuals in Japan. The authors pointed out that the antibody responses to both growth- and dormant-stage antigens are critical and could help the diagnosis of recent LTBI, which has been associated with a high risk of developing active TB [17]. In another study conducted in India, a significantly higher IgG level was observed for PPE17 MTB antigen compared to ESAT-6, CFP-10 and PPD, and it discriminated between LTBI and healthy individuals, suggesting that antibodies could also be used in conjunction with the current QuantiFERON test to diagnose LTBI [4]. Similarly, Lu *et al.* [13] compared the anti-PPD IgG profiles of LTBI and active TB sera from patients from South Africa, USA and Mexico and found distinct glycosylation patterns of the immunoglobulin Fc portion in LTBI and active TB, antibodies from LTBI serum showed less fucose. In addition, LTBI serum, but not active TB, was found to contain more sialic acid and galactose, which have been associated with an anti-inflammation status [13]. Similarly, a study in Italy and USA showed di-galactosylated glycan structures found on IgG-Fc were associated with LTBI and with TB cure [31]. This suggests that differences in the glycosylation pattern of mycobacteria-specific antibodies could be used to differentiate between LTBI and active TB, which the currently approved IGRAs are unable to do; however, validation studies are needed. Furthermore, given the lack of published data, the assessment of the prognostic value of all of these biomarkers is also required, although this has not yet been done in a published study.

Antibody response in TB treatment response monitoring

Efforts to find prognostic markers are ongoing [32]. Detection of MTB-specific antibodies pattern at different timepoints during treatment, and their association with treatment outcome, could provide help [5, 32–34]. It is known that the levels of antibody response during treatment vary depending on the MTB antigens used [5, 33, 34]. For example, TB-specific IgG4 were recently shown to be increased in active TB, as compared to LTBI, and significantly decreased after the treatment in a study involving Italian and US patients [31]. The levels of IgG anti-14 kDa, anti-LAM or anti-38 kDa increase following treatment subsequently decreasing months and even years after its completion [5, 33, 34]. Moreover, not all patients respond in the same way, possibly due to differences in their immune status [33].

The detection of an antibody response at the time of diagnosis could identify TB patients at risk of poor treatment outcomes in a South African study [32]. The level of anti-Tpx IgG and anti-ESAT-6 IgA measured before the start of TB treatment has been found to predict slow responders with an accuracy of

90.5%, suggesting its potential prognostic value [32]. Similarly, Mattos *et al*. [6] found that the IgG response to 16 kDa was elevated after 1 month of treatment, whereas anti-ESAT-6 and CFP-10 IgG levels were higher before the initiation of treatment and elevated following anti-TB treatment, suggesting that the antibody response to cytosolic and secreted MTB antigens is different during chemotherapy.

An increase in antibody response during treatment could be due to a strong humoral response to MTB antigens released from dead bacilli, the disappearance of MTB antigens, which results in the release of antibodies from immune complexes, and the absence of inhibitory factors that stimulate the immune responses [33, 34]. In contrast, the levels of some antibodies decreased during TB treatment. As suggested by the authors, some MTB antigens correlate with the bacterial load better than others and could therefore be used to monitor treatment failure or success by serving as indirect biomarkers of reduced bacterial load following chemotherapy [34].

The role of antibodies in TB prophylaxis and therapy

It was initially thought that antibodies could not reach the inside of cells and therefore intracellular bacteria [35]. However, intracellular pathogens can be found in the extracellular space, suggesting that they could potentially be targeted by antibodies [36], and in fact several studies support this [37, 38]. Although the results published to date are inconsistent, the growing scientific literature supports the protective role of passive transfer of certain antibodies against TB [11]. If proven useful, passive antibody transfer could help shortening anti-TB treatment and reducing the rate of disease progression [39]. Although we did not find any evidence on human studies, neither in PubMed nor in the clinicaltrials.gov database, multiple reports are available on the TB protection achieved by the passive transfer of antibodies against TB in animal models [40].

The administration of human intravenous immunoglobulins (IVIg) to mice has been shown to protect against TB, mainly due to antibody responses against the BCG vaccine, LTBI or exposure to environmental mycobacteria (table 3) [41–43]. In pooled IVIg treatment of MTB-infected mice, a significant reduction in bacterial load in tissues was demonstrated [43]. In another study, passive transfer of intact but not de-glycosylated IVIg reduced lung bacillary load and pneumonia in a murine model, suggesting the importance of Fc glycosylation in protection against MTB [42].

The protective effect of Ig has prophylactic as well as therapeutic advantages. Intranasal administration of human IgG to mice prior to MTB challenge resulted in a significant reduction in pulmonary bacterial load which lasted up to 2 months; the authors suggested that a certain immunomodulation of the T-cell immunity contributed to this protection [41]. Similarly, Guirado *et al*. [44] demonstrated in a severe combined immunodeficiency mouse model that hyperimmune sera, obtained from mice vaccinated with the RUTI vaccine, significantly reduced both the bacillary load and lung pathology compared to the control sera, and noted that MTB-specific antibodies protect against post-chemotherapy relapse independently of CMI.

Some antibodies found in LTBI subjects have also shown protection against MTB in animal models [13, 40]. Passive transfer of antibodies from healthcare workers with either confirmed LTBI or high MTB exposure showed a degree of protection in mice infected with MTB, and this protection correlated with the *in vitro* neutralisation of MTB [40]. Additional evidence shows that anti-PPD IgG from LTBI individuals exhibits a distinct glycosylation pattern, including higher sialic acid and galactose (with anti-inflammatory role) and less fucose (known to improve binding to FcγRIII) [31]. In support of the study by Olivares *et al*. [42], glycosylation differences have been shown to have a potential impact on the observed protection, which may allow for the development of effective monoclonal MTB-specific antibody-based immunotherapies in TB [31].

Monoclonal antibodies (mAbs) therapies available target cytokines (*i.e.* tumour necrosis factor or interleukin (IL)-1 or IL-6) that are actually crucial for TB containment and that if neutralised, may reactivate TB in those with LTBI. However, the use of certain mAbs has been shown to confer some degree of protection in mice. Intranasal inoculations with human mAb (2E9IgA1) against Acr antigen significantly reduced the bacterial burden in transgenic mice depending on FcαRI, suggesting the involvement of Fc receptors in antibody-mediated protection [39]. In another study, a 10-fold transient reduction in lung bacillary load was observed in mice after intranasal inoculation of monoclonal IgA (TBA61) against Acr [45]. Furthermore, coating BCG with mAbs against heparin-binding haemagglutinin adhesin (HBHA, an adhesin associated with extrapulmonary dissemination of MTB [46]) reduced bacterial dissemination in a mouse model [47]. Despite its promising potential as a vaccine candidate, passive immunisation of mAbs against HBHA failed to provide protection in an MTB challenge mouse model,

TABLE 2 Descriptions of the *Mycobacterium tuberculosis* (MTB) antigens included in this review

Antigens	Synonyms	Descriptions and biological activities	References
esxA	Rv3875, ESAT-6	6 kDa early secretory antigenic target of unknown function. Elicits high level of INF-γ from memory effector cells during the first phase of a protective immune response. Component of QuantiFERON test, and absent in BCG strain.	[17, 77]
esxB	Rv3874, CFP10	10 kDa culture filtrate antigen of unknown function.	[77]
esxW	Rv3620	Putative ESAT-6 like protein 10 of unknown function.	[77]
esxV	Rv3619	Putative ESAT-6 like protein 1 of unknown function.	[77]
Rv1813c	Rv1813c	A conserved hypothetical protein of unknown function.	[78]
LAM		Components of MTB-cell wall. Inhibit IFN-γ mediated macrophage activation; T-cell proliferation, IL-12 production, neutrophil recruitment, DC activation.	[79]
fbpA	Rv3804c, Ag85a	Fibronectin binding protein A and components of Ag85 complex (Ag85a), possesses a mycolyltransferase activity (85A) required for the biogenesis of trehalose dimycolate (cord factor), main component for maintaining cell wall integrity.	[77]
fbpB	Rv1886c, Ag85b	Fibronectin binding protein B and components of Ag85 complex (Ag85b). Secreted antigen acts as mycolyltransferase (85B), which is involved in cell wall mycoloylation.	[77, 80]
fbpC	Rv0129c, Ag85c	Fibronectin binding protein C and components of Ag85 complex (Ag85c). Secreted antigen acts as mycolyltransferase (85C), which is involved in cell wall mycoloylation.	[77]
espB	Rv3881c	Secreted ESX-1 substrate protein B, EspB. A conserved alanine and glycine-rich protein of unknown function.	[77]
mpt70	Rv2875	Major secreted immunogenic protein of unknown function.	[77]
Acg	Rv2032	Acg (acr-coregulated gene). Conserved protein of unknown function.	[77]
Rv0831	Rv0831	A conserved protein of unknown function.	[77]
bfrB	Rv3841	Bacterioferritin. Intracellular molecule involved in iron storage.	[77]
Rv0831c	Rv0831c	A conserved protein of unknown role.	[77]
HspX	Rv2031c, 16/14 kDa	Heat shock protein (alpha-crystallin homolog). Stress protein induced by anoxia. Proposed role in long-term survival of the bacilli during latent infections.	[77, 79]
PstS1	Rv0934, 38kDa	Periplasmic phosphate-binding lipoprotein (PBP-1). Immunodominant antigen involved in active transport of inorganic phosphate across the membrane.	[34, 77]
Ssb	Rv0054	Helix-destabilising protein involved in DNA replication, recombination and repair.	[77]
lipC	Rv0220	Probable esterase LipC with unknown function. A lipolytic enzyme probably active in cellular metabolism.	[77]
Rv2958c	Rv2958c	Possible glycosyl transferase with unknown function. Probably involved in cellular metabolism and has a role in resistance to killing by human macrophages.	[77]
Rv2994	Rv2994	Probable conserved integral membrane protein with unknown function; could be involved in efflux system.	[77]
Rv1813c	Rv1813c	A conserved hypothetical protein of unknown function.	[81, 82]
A60		Located in the cytosol, macromolecular antigen complex, conserved in typical and atypical mycobacteria, main component of commercial ELISA kit (Anda Biologicals, Strasbourg, France).	[26]
HbhA	Rv0475	Protein regulated by iron that promotes extrapulmonary dissemination and facilitates adherence to epithelial cells. Induces mycobacterial aggregation.	[79]
Tpx	Rv1932	Probable thiol peroxidase with antioxidant activity. Could remove peroxides or H_2O_2.	[77]
Apa	Rv1860, MPT-32 45/47kDa	Alanine- and proline-rich secreted protein (fibronectin attachment protein). Could mediate bacterial attachment to host cells.	[77]
AcrA1	Rv3391	Multifunctional enzyme with acyl-CoA-reductase activity. Possibly involved in cellular metabolism.	[77]
hrp1	Rv2626c	Hypoxic response protein 1 of unknown function.	[77]
PPE17	Rv1168c	PPE of unknown function. Induces strong B-cell responses.	[4, 77]
PPE42	Rv2608	PPE family protein of unknown function.	[77]
PPE55	Rv3347c	PPE family protein of unknown function.	[77]
MDP1		A histone-like nucleoid associated protein expressed by many mycobacterial species. Could play a role in long-term survival of mycobacteria.	[17]

INF: interferon; BCG: bacille Calmette–Guérin; LAM: lipoarabinomannan; IL: interleukin; DC: dendritic cell; MDP1: mycobacterial DNA-binding protein 1; HbhA: heparin binding haemagglutinin (adhesin); Ssb: single-strand binding protein; H_2O_2: hydrogen peroxide; PPE: proline-proline-glutamic acid.

suggesting that antibodies alone may not be sufficient to provide complete protection [46]. A mAb (SMITB14) directed against LAM also showed protection: anti-LAM IgG1 mAb inoculations resulted in increased survival, reduced weight loss and less bacillary load in tissues [48].

Several groups have identified different murine isotypes (IgM, IgG1, IgG3, and IgA) that show protection against MTB [37]. As such, antibody isotype appears to play a role in determining the protective function in antibodies in TB [49]. Specifically, a monoclonal anti-LAM and anti-HBHA IgA, but not IgG, reduced

TABLE 3 The protective antibodies in tuberculosis (TB) upon passive transfer of polyclonal and monoclonal antibodies

Antibody	Description	Protective effects	References
Polyclonal antibodies in TB			
Human IVIg	Treatment in MTB-infected mice	Significant reduction in bacterial load in spleen and lung	[43]
Human IVIg	Intact *versus* deglycosylated IVIg in murine models of progressive TB	Intact IVIg reduced bacillary load in the lung	[42]
Human IgG	Intranasal inoculation 2 h before MTB infection in mice	Significant reduction in pulmonary bacterial load lasting for 2 months	[41]
Hyperimmune sera (RUTI)	Protection against post-chemotherapy relapse TB infection in SCID mice	Significant reduction in bacillary load, less granuloma and less pneumonia compared to the control sera	[44]
Human sera	Antibodies from LTBI and active TB in aerosol MTB challenge in mice	Antibodies from LTBI showed moderate protection	[40]
Human IgG against PPD	IgG from LTBI showed higher sialic acid and galactose	Anti-inflammatory activity	[13]
Monoclonal antibodies in TB			
Human mAb (2E9IgA1) against Acr	Intranasal inoculation in transgenic mice	Reduced the bacterial burden Dependant on CD98 (FcαRI)	[39]
mAbs IgA (TBA61) against Acr	Intranasal inoculation in mice	10-fold reduction in CFU	[45]
Coating of BCG mAbs against HBHA	Intranasal infection in mice	Prevents extrapulmonary dissemination	[47]
mAbs (SMITB14) anti-LAM IgG1	Intranasal administration in mice	Improved survival, reduced CFU, reduced weight loss	[48]

IVIg: intravenous immunoglobulin; MTB: *Mycobacterium tuberculosis*; SCID: severe combined immunodeficiency; LTBI: latent TB infection; mAb: monoclonal antibody; Acr: alpha crystalline; LAM: lipoarabinomannan; CFU: colony-forming unit; HBHA: heparin-binding haemagglutinin adhesin.

bacterial uptake by human lung epithelial cells (A549). Similarly, purified serum IgA from TB patients reduced the mycobacterial load in A549 cells, whereas purified polyclonal IgG increased MTB uptake by lung epithelial cells, thus indicating the superiority of IgA over IgG in mediating MTB protection. However, human lung epithelial cells (A549) only express neonatal Fc receptors rather than the conventional Fcγ receptors [49]. Therefore, the observed lack of protection by IgG might be due to the type of cells used, implying the need for a validation study with other types of cells expressing conventional Fc receptors. Another study supports the reduced performance of IgG in intranasal inoculation in mice, suggesting that the protection conferred by IgA is better than that for IgG upon internasal inoculation in a mouse model [45].

Antibody response in TB vaccines

The current TB vaccines under clinical development are designed to target CMI rather than humoral immunity [50]. However, a growing body of studies suggest that antibodies could be induced by some TB vaccines.

Antibody response in BCG vaccine

Several studies have shown that MTB-specific antibodies are induced by BCG (the only approved vaccine against TB), which could result in protection against MTB infection [51, 52]. Chen *et al*. [51] demonstrated the protective role of antibodies by using human sera collected pre- and post-BCG vaccination from UK and US patients, showing enhanced phagocytosis, phagolysosome fusion, and intracellular growth inhibition with post-vaccination sera but not with pre-vaccination sera. The observed effect correlated with reactive IgG titres to a few arabinomannan (AM) epitopes rich in mannose residues, and the authors suggested that these could play a protective role against mycobacterial infection [51]. Levels of mycobacterial cell lysate-specific IgG1, IgG2, and IgG3 were found to be significantly increased (but not IgE and IgG4) at month 2 after vaccination in the BCG-vaccinated group compared to the controls, suggesting Type one cytokine biased antibodies [53]. In another study, LAM-specific IgG were associated with enhanced phagocytosis, phagolysosome fusion, bacterial growth inhibition by neutrophils and macrophages, and enhanced mycobacteria-specific IFN-γ production. The authors pointed out that LAM-specific antibodies improve the quality of both innate immunity and CMI in the protection against mycobacterial infection [52]. However, large population studies containing different BCG strains are needed to support the observed protective role of antibodies in BCG.

Antibody response in TB vaccine candidates

There are currently 22 TB vaccine candidates in preclinical and clinical development [54], based on different strategies. Due to the lack of sufficient protection from induction of CMI alone, evidence supporting the role of antibodies in TB is attracting increasing attention [11]. Table 4 shows the current evidence for humoral immunity induced by the current TB vaccine candidates.

M72/AS01$_E$, a subunit vaccine, has shown a 54% efficacy in preventing active PTB in South African adolescents [55] and induces M72-specific antibodies, which are sustained for 3 years [56]. However, larger sample size studies involving other populations, such as healthy non-exposed individuals, HIV-infected individuals, household contacts, and people from different origins, were recommended [57]. Moreover, the protection against PTB observed in the M72/AS01$_E$ vaccine may be due to antigen-specific antibodies but also might be enhanced by the adjuvant AS01$_E$ [57, 58].

A phase I trial conducted in BCG-primed South African individuals to evaluate the safety and immunogenicity of three vaccines (H4:IC31, H56:IC31, and BCG revaccination) showed that all of them induced cellular and humoral immune responses [59]. H4:IC31 and H56:IC31 induced H4- and H56-specific IgGs. Anti-H4 IgG was observed on day 14 and significantly increased at day 70, whereas H56-specific IgG was detected at day 70. IgG1 and IgG3 were the predominant IgG subclass in both groups, and H4 and H56 antibody responses were not observed in the BCG revaccinated and placebo groups [59]. Nemes *et al.* [60] evaluated the efficacy of H4:IC31 and BCG revaccination in a phase II study in South Africa. Although both vaccines failed to prevent primary infection, sustained MTB infection was reduced by 45.4% and 30% for BCG and H4:IC31, respectively [60], suggesting that antigen-specific antibodies may play a role in the observed protection. Similar evidence has been obtained in a phase I clinical trial of the H56:IC31 vaccine also in South Africa. Primary vaccination induced anti-H56 IgG, and the levels thereof increased significantly after a subsequent booster dose [61]. In summary, H56- and H4-specific antibodies could play, together with CD4$^+$ T-cells, a protective role in MTB infection, which promotes further studies to evaluate the protective efficacy of these vaccines.

Another novel subunit vaccine designed to prevent PTB in adults, ID93/GLA-SE, was evaluated in a phase I trial in South Africa, with ID93-specific IgG being detected even though the vaccine was designed to stimulate a T-helper cell (Th)1 response [62]. However, the detection of antigen-specific antibodies alone

TABLE 4 Antibody responses in current tuberculosis (TB) vaccine candidates

TB vaccine	Current stage[#]	Evidence of humoral immunity	References
Antibodies in BCG vaccines			
BCG		IgG against AM enhanced phagolysosome fusion and intracellular growth inhibition	[51]
BCG		IgG against LAM enhanced cell and innate immunity, enhanced phagocytosis	[52]
BCG		IgG against mycobacterial cell lysate elevated post-BCG	[53]
Antibodies in subunit TB vaccines			
M72/AS01$_E$	IIb	Anti-M72 IgG in humans correlated with protection and persisted for 3 years	[56]
H56:IC31	IIb	Induced H56-specific IgG in phase I trial	[61]
(H4:IC31, H56:IC31 and BCG revaccination)	I	Induced anti-H4 and anti-H56 IgG1 and IgG3	[59]
ID93/GLA-SE	IIa	ID93-specific IgG induced in phase I trials	[62]
AM-Ag85A		Protective anti-AM and anti-Ag85A antibodies in preclinical study	[63]
Antibodies in viral vector-based TB vaccines			
MVA85A	II	Ag85A-specific CD4 T-cell failed to show protection while anti-Ag85A IgG was protective	[12]
MVA85A-IMX313 and MVA85A		Induced IgG specific to Ag85A in phase I trials	[66]
ChAdOx185A/MVA85A	I	Prime vaccination induced anti-Ag85A IgG, and IgG levels increased by a booster vaccine	[67]
Antibodies in whole-cell TB vaccines			
VPM1002 (rBCG)	Live/II	Higher anti-PPD antibodies in human in phase II compared to BCG	[68]
DAR-901	Killed/IIb	Induced humoral immunity in mouse model of infection	[69]
RUTI	Killed/IIa	Mixed Th1 and Th2 response with antigen-specific antibodies	[70]

BCG: bacille Calmette–Guérin; AM: arabinomannan; LAM: lipoarabinomannan; Th: T-helper cell. [#]: the stages of the current TB vaccines included in the table refer to clinical development stages and are based on the TuBerculosis Vaccine Initiative [54].

is no guarantee of protection. Two polysaccharide conjugate vaccines designed to induce humoral immunity, namely AM conjugated with Ag85B or with a protective antigen (PA) from *Bacillus anthracis*, elicited antibodies to AM conjugates with different specificity [63]. Mice vaccinated with AM-Ag85b and AM-PA conjugate showed a significant reduction in tissue bacterial load and longer survival when compared to the unvaccinated controls, suggesting that AM-specific antibodies could help to reduce bacterial dissemination and increase survival in infected mice [63]. COSTELLO *et al*. [64] also demonstrated the pattern of anti-LAM IgG responses using serum samples obtained from children from the UK and India with or without local or disseminated TB. Low levels of anti-LAM IgG were associated with a disseminated form of mycobacterial disease, suggesting that anti-LAM and AM-specific IgG may play a role in protecting against dissemination, which has implications for the design of improved TB vaccines [64].

In summary, antibodies targeting structural components of MTB, such as AM or LAM, may be of interest for the development of new TB vaccines.

A different type of viral vector expressing one or more immunodominant mycobacterial antigens has been developed as vaccines (table 4). In a phase IIb study conducted in Gambia, MVA85A, designed to induce a T-cell response, failed to provide protection [65]. However, anti-Ag85A-specific IgG was associated with protection in terms of risk of TB in BCG-vaccinated infants in MVA85A vaccine trials [12]. In a phase I study, the novel vaccines MVA85A-IMX313 and MVA85A induced Ag85A-specific IgG [66]. Moreover, a ChAdOx185A prime–MVA85A booster vaccine trial was conducted to assess safety and immunogenicity in healthy adults in the UK. Vaccination with ChAdOx185A induced IgG and vaccination with MVA85A vaccine boosted the level of IgG response, thus suggesting that vector-based TB vaccines could induce antibodies with the potential to contribute to the observed protection against TB [67].

Although whole-cell TB vaccines have been designed to stimulate CMI, few of them also induce an antibody response. VPM1002, a recombinant live BCG vaccine, has proved to induce an antigen-specific antibody response (anti-PPD) in a study conducted in South Africa with significantly higher antibodies levels compared to unvaccinated subjects. This is likely due to the apoptosis induced by modification of the antigens in BCG, which results in the release of BCG-derived antigens and increases the possibility of the maturation of B-cells into antibody-producing plasma cells [68]. The gene expression related to the differentiated B-cell compartment was significantly overexpressed at a later time point, confirming their involvement in the vaccine-induced immune response, even if the B-cells signature did not seem to be influenced [68]. Similarly, DAR-901, which was designed to boost BCG vaccination and which has shown significant protection against TB in a phase III trial, induced antibodies specific to DAR-901 in mice and showed better protection against MTB than mice given BCG booster doses. Together with the observed IFN-γ response, antibodies may be involved in the protection of mice challenged with MTB [69]. Furthermore, in an immunogenicity study of the RUTI vaccine, a mixed Th1 and Th2 response with antigen-specific antibodies (IgG1, IgG2a, and IgG3) was observed [70].

Mechanism of antibody-mediated protection

Different protection mechanisms have been proposed in passive-transfer, vaccine, and *in vitro* studies (figure 1), with these mechanisms varying depending on MTB antigens, the glycosylation pattern, and route of inoculation [11, 71]. Anti-LAM IgG derived from post-BCG-vaccinated sera showed enhancement of MTB phagocytosis in macrophages and neutrophils. Mycobacteria-specific antibodies enhance the fusion of the phagolysosome and inhibit bacterial growth inside the phagocytic cells [51, 52]. In another study, extrapulmonary dissemination was reduced when mAbs against HBHA were used to coat wild-type BCG [47], suggesting that antibodies play a role by blocking the binding of the HBHA adhesin to the epithelial cells, thereby preventing bacterial dissemination. Moreover, antibodies also enhance CMI, as in the example of anti-LAM IgG, which enhances the proliferation and secretion of IFN-γ by MTB-specific $CD4^+$ T-cells and the degranulation of $CD8^+$ T-cells [52]. Furthermore, *in vitro* neutralisation of the bacilli was demonstrated using sera from LTBI and healthcare worker subjects [40]. Finally, antibodies have been shown to enhance antibody-dependant cellular cytotoxicity (ADCC) by activating natural killer cells. Anti-PPD Ig from LTBI, but not active TB, enhanced ADCC and increased the co-localisation of MTB with lysosomes [13].

Studies not showing the protective functions of antibodies

Not all studies support the protective role of antibodies in TB [71]. In a recent study, a rhesus cytomegalovirus-based TB vaccine failed to induce a significant antibody response in rhesus macaques, while protection was associated with the activation of tissue-resident memory $CD4^+$ and $CD8^+$ T-cells [72]. Moreover, the role of B-cells and antibody depletion is another ongoing debate in TB [71]. In a study by KHERA *et al*. [73], there was no difference in vaccine-induced T-cell protection between B-cell knock-out

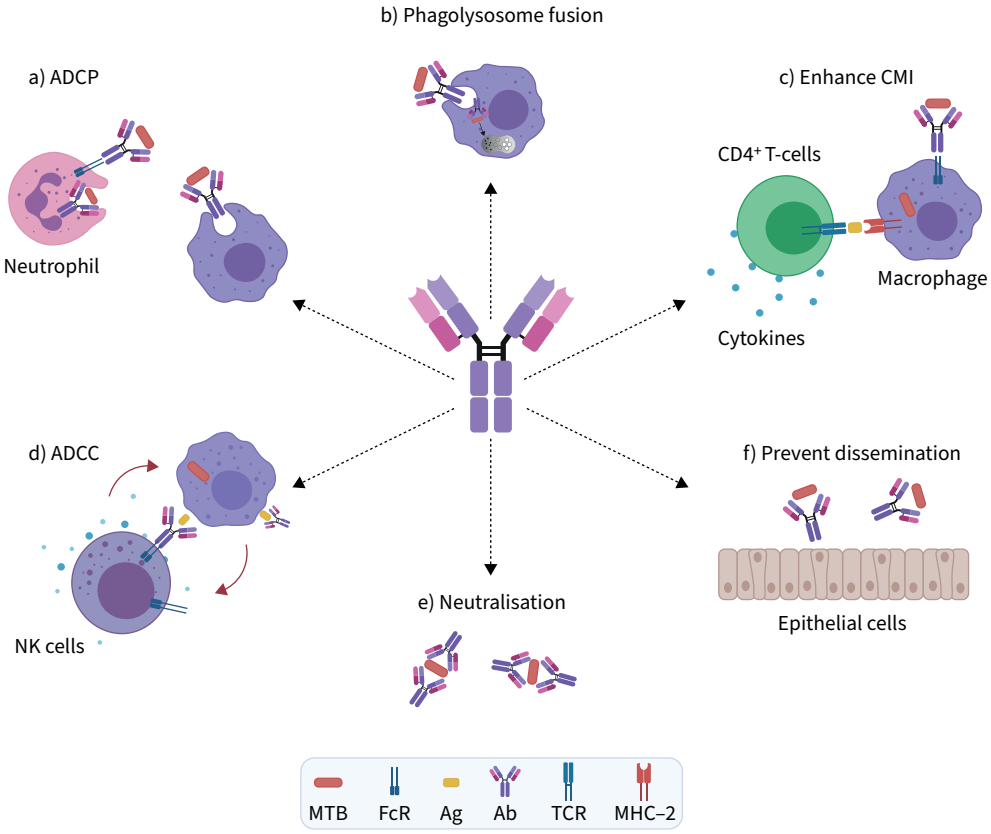

FIGURE 1 Potential mechanisms of action of antibodies in tuberculosis: a) antibodies enhance phagocytosis of *Mycobacterium tuberculosis* (MTB) by neutrophils and macrophage *via* FcR binding; b) antibodies also enhance the fusion of phagosome and lysosome; c) antibodies activate $CD4^+$ T-cells; d) antibodies enhance killing of infected macrophages by activating natural killer (NK) cells; e) antibodies facilitate direct neutralisation of the bacilli; f) antibodies block the binding of MTB to epithelial cells and prevent extrapulmonary dissemination of the bacteria. ADCP: antibody-dependent cellular phagocytosis; CMI: cell-mediated immunity; ADCC: antibody-dependant cellular cytotoxicity; Ag: antigen; Ab: antibody; TCR: T-cell receptor; MHC: major histocompatibility complex.

and B-cell competent mice, with the authors highlighting the minimal role of B-cells in mucosal vaccine-induced T-cell protection against PTB. In another study showing the vaccine potential of HBHA, although serum antibodies specific to HBHA were elicited, passive immunisation of mAbs IgG against HBHA failed to protect against MTB challenge in mice. The lack of protection upon passive transfer might be due to the mAbs used being insufficient for protection; therefore, the authors suggested further studies using polyclonal anti-HBHA sera and IgA antibodies [46]. Moreover, passive-transfer studies of immune sera from different animals have shown variable efficacy in protection against TB [11]. However, due to a lack of information regarding the optimal antibody concentration required for protection and the heterogeneity of the humoral immunity to TB, any conclusion that the antibody response is not protective should be made with caution unless studied extensively [37]. On the other hand, literature on TB in patients with common variable immunodeficiency (CVID) is scarce, even though CVID is a rare type of primary immunodeficiency affecting one or more types of immunoglobulins (IgG, IgA, IgM) that makes the patients more prone to infections [74]. Also, there is no evidence linking the use of immunosuppressive drugs, such CD20 inhibitors blocking CD20 protein (widely expressed on B-cells), to an increased risk of TB progression [75, 76]. In our opinion, studies taking into account all these data could provide valuable information about the role of antibodies in TB and generate research questions to be answered in further studies.

Conclusion and perspectives

At present and despite of the existence of commercial kits, antibodies have no role in routine clinical diagnosis and therapy and still have very little role in TB vaccines in TB field. Further research is needed

to address this topic. The humoral immune response against TB is heterogeneous, mainly due to the differential antigen expression at different stages of infection, and the use of multiple MTB antigens and different antibody isotypes for diagnosis has improved the accuracy of commercial serology. Most of the studies reviewed in this paper showed comparable sensitivity and specificity to the target set by the WHO for non-sputum culture-based TB biomarkers and might be used together with existing T-cell-based IGRA tests for both the screening and diagnosis of active TB. However, several limitations of currently published data have been encountered: the need for validation studies before routine use in clinical practice, lack of appropriate controls for comparison, exclusion of immunocompromised subjects, and lack of blinding to reduce unwanted bias. Future studies should consider improving these limitations. Unlike IGRAs, the differences in the antibody pattern for LTBI and active TB suggest the potential role of antibodies in identifying those LTBI cases at risk of developing active TB. Currently, there is no validated prognostic marker for monitoring treatment outcome in TB, and antibody response during intensive treatment has not been well investigated. As such, it would be of interest to evaluate further the potential use of antibodies against MTB antigens generated during the active and latent periods to assess anti-TB treatment response and identify slow and fast responders earlier, thus ensuring better TB management.

Despite the controversies concerning the protective role of the humoral immune response in TB, a growing body of literature suggests that antibodies could play a role. However, further research in different animal models is required. Evidence from clinical trials with TB vaccine candidates suggests that both cellular immunity alone and antibodies alone may not be sufficient to prevent TB infection and disease. Since antibodies have been shown to enhance cellular immunity, the combination of antibodies with T-cell immunity could improve the efficacy of TB vaccines. Finally, glycosylation seems to play a role in the protective function of antibodies in TB. As such, the development of mAbs by targeting the different glycosylation patterns may be of interest for TB immunotherapy. The development of antibody-based TB vaccine candidates would also be of interest, although further studies are needed to demonstrate the protective efficacy of antibodies in current TB vaccine candidates. Since the evidence generated with the mouse model is not always validated in human TB, studies investigating the mechanism of action and protective efficacy in other experimental animal models, such as guinea pigs and non-human primates, are recommended.

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