Latently and uninfected healthcare workers exposed to TB make protective antibodies against *Mycobacterium tuberculosis*

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The role of Igs in natural protection against infection by *Mycobacterium tuberculosis* (Mtb), the causative agent of TB, is controversial. Although passive immunization with mAbs generated against mycobacterial antigens has shown protective efficacy in murine models of infection, studies in B cell-depleted animals only showed modest phenotypes. We do not know if humans make protective antibody responses. Here, we investigated whether healthcare workers in a Beijing TB hospital—who, although exposed to supra-infectious doses of pathogenic Mtb, remain healthy—make antibody responses that are effective in protecting against infection by Mtb. We tested antibodies isolated from 48 healthcare workers and compared these with 12 patients with active TB. We found that antibodies from 7 of 48 healthcare workers but none from active TB patients showed moderate protection against Mtb in an aerosol mouse challenge model. Intriguingly, three of seven healthcare workers who made protective antibody responses had no evidence of prior TB infection by IFN-γ release assay. There was also good correlation between protection observed in vivo and neutralization of Mtb in an in vitro human whole-blood assay. Antibodies mediating protection were directed against the surface of Mtb and depended on both immune complexes and CD4+ T cells for efficacy. Our results indicate that certain individuals make protective antibodies against Mtb and challenge paradigms about the nature of an effective immune response to TB.

TB | antibodies | immune complex | humoral immunity | TB restrictors

TB is the world's most deadly infectious disease with 9 million active cases, resulting in 1.5 million deaths in 2015. The only licensed TB vaccine, bacillus Calmette–Guérin, has variable efficacy, protecting children from disseminated TB but offering little protection for pulmonary TB in young people and adults—which is the primary transmitted form of the disease (1). Hundreds of preclinical and a dozen clinical vaccine candidates have been proposed (2), almost all focused on eliciting cell-mediated immunity. The costly failure of the most advanced clinical candidate, MVA85A, in a phase IIb clinical trial (3) has led to a reevaluation of what may constitute protective immunity to TB.

The role of humoral immunity to TB is controversial. Mice lacking B cells have increased bacterial burden in lungs when infected by *Mycobacterium tuberculosis* (Mtb) (4), but this defect is dramatically less than mice lacking CD4+ T cells or IFN-γ (5). Furthermore, as an intracellular pathogen, it has been argued that Mtb would not be within the therapeutic reach of antibodies. Nonetheless, multiple studies have shown the efficacy of mAbs in ameliorating murine models of TB infection (6–12), and antibody-mediated immunity to several intracellular pathogens, including *Cryptococcus neoformans* and typhoid *Salmonella*, has been documented (13), raising the possibility that antibody-mediated immunity may also exist for TB. Pooled Igs from human donors have been shown to protect mice against Mtb (14), but it was not known if this was caused by a generalized immunomodulatory effect (15) targeting the inflammatory component of the disease or whether protective antibodies directly targeting critical mycobacterial antigens could be isolated from individuals.

To address this question, we studied a population that had known high exposure to pathogenic Mtb but nonetheless, remained healthy. We hypothesized that this population may be enriched with individuals who made protective humoral responses against Mtb. Healthcare workers (HCWs) in developing world settings have an extremely high exposure to tubercule bacilli because of the lack of appropriate barrier protection and a subsequent raised risk of infection (16, 17). We specifically chose to investigate individual responses as opposed to cohort effects because of the experience in the HIV field that showed that protective antibodies were made by only a subset of infected individuals (18). We found that a significant minority of HCWs—both those with and without evidence of prior exposure to Mtb by IFN-γ release assay—made antibody responses that mediated directly protective effects against Mtb in both murine challenge and in vitro human whole-blood assays (WBAs).

Results

To investigate whether individuals highly exposed to pathogenic Mtb make protective antibody responses, we decided to study workers in a busy specialist TB hospital in Beijing, China. Hospital standard infection procedures do not mandate particulate filter

Significance

It is not known whether natural immunity to TB exists or whether antibodies play an important role in immunity against infection by *Mycobacterium tuberculosis* (Mtb), the causative agent of TB. Here, we identify that a significant minority of healthcare workers who are exposed to high doses of Mtb make protective antibodies against infection as measured in both mouse infection and in vitro models. Some of these individuals had no prior evidence of latent TB infection, suggesting that they may represent a subset of "restrictors" who can control infection by Mtb. These results have important implications in the rational development of both protective and therapeutic vaccines against TB.

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masking for HCWs, and they are seldom used. Subjects were categorized as either "latent" if they had evidence of latent TB infection (LTBI) based on ELISPOT testing or "highly exposed but uninfected" (HEBUI) if there was no evidence of LTBI. We also selected 12 patients who were being treated for active TB (Table S1) to compare potentially protective antibody responses from individuals who had active TB with those of individuals with no active TB.

We initially tested whether all groups made detectable antibody responses to Mtb antigens. As expected, Ig isolated from active TB patients had high titers of reactive antibody to Mtb antigens, and this titer was significantly higher than those from healthy HCWs—regardless of classification as LTBI or HEBUI (Fig. S1). Our data suggest that the majority of subjects who are highly exposed to Mtb—through active infection, latent disease, or work-related exposure—make detectable antibody responses to Mtb-derived antigens.

Individual HCWs Make Protective Antibody Responses Against TB in a Mouse Infection Model. Although it was known that both active TB patients and healthy subjects make detectable antibody responses to Mtb (19–21), it was not known whether any of these antibodies afford direct protection against infection. We decided to test total antibody from our subjects in a mouse protection assay (Fig. 1*A*). We tested each donor's antibody response separately to increase sensitivity of identifying individuals who make protective antibodies. Briefly, purified Ig from each donor was injected into three mice. Five hours later, mice were simultaneously infected by aerosol infection with a locally isolated fully drug-susceptible strain of Mtb. The antibodies were tested in four batches, with three donors from each of three groups (active, LTBI, and HEBUI) per batch. Fourteen days after infection, the mice were killed, and Mtb burden in the lungs was determined (Fig. 1*B*). In total, antibodies from three donors [donors 21, 23 (LTBI), and 28 (HEBUI)] offered significant (2.0- to 3.3-fold) protection against Mtb infection. Antibodies from all other donors, including all of the active TB patients, did not differ from the PBS control (Fig. 1*B*).

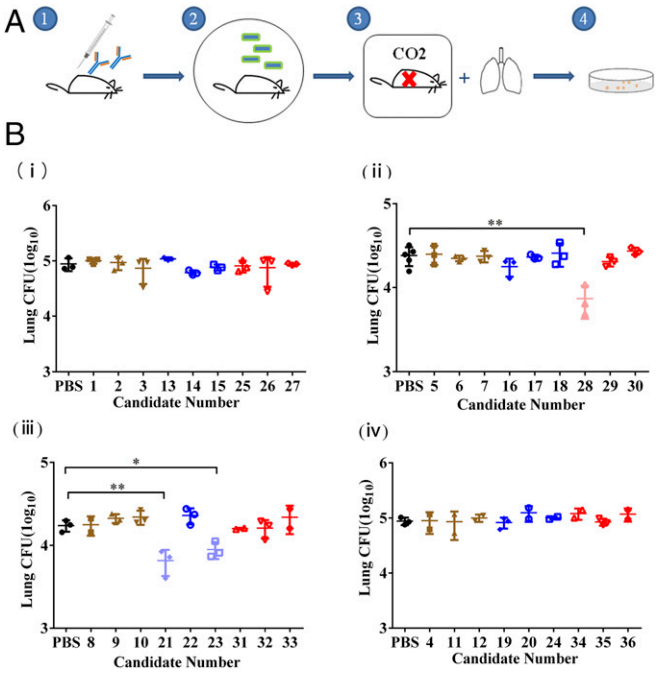

Fig. 1. Antibody isolated from some individual HCWs is protective against Mtb infection. (*A*) Schematic illustrating the mouse protection assay. (1) Mice were injected with 20 mg purified Ig or PBS via the i.p. route. (2) After 5 h, they were infected by aerosol with ∼100–200 cfu Mtb per mouse. (3) After 14 d, mice were killed, and total lung homogenates were plated for cfu enumeration. (4) Plates were read after 3–4 wk (*Methods*). (*B*) Mice were infected in four batches (*i–iv*). Antibodies from three donors (21, 23, and 28) showed protection against TB infection. All three positive donors and two negative donors (24 and 30) were rebled and retested with similar results. Brown represents active TB patients; blue represents LTBI HCWs; and red represents HEBUI HCWs. HCWs making protective responses are represented by paler shades (pale blue/pink). $*P < 0.05$, $**P < 0.01$ by Student's *t* test.

To exclude the possibility that the protective effects seen with antibodies from these three donors were caused by increased persistence of antibodies specifically from these three individuals, we injected 5 mg of polyclonal antibody into mice from donors 21, 23, and 28 and also nonprotective donor 26 and then measured individual Ig subsets at five time points to day 14 (Fig. S2*A*). Although there were significant differences between the four groups, the only obvious difference between the protective and nonprotective (26) antibody groups was the abundance of IgG3 at days 1 and 3. Antibodies have many functions, only one of which is recognition of specific antigens by the variable portion of the molecule (20, 22–24), and a recent report suggested that protective effects of anti-TB antibodies may depend on IgA (24). We, therefore, tested the relative abundance of Ig subclasses from the HCWs (Fig. S2*B*). There was no clear pattern of isotype abundance in antibodies isolated from individuals making protective vs. nonprotective responses.

To investigate whether the protective antibody response required elements of host immunity for protection, we tested antibodies from one of the protective donors (21) along with a nonprotective donor (26) for efficacy in athymic (nude) mice, which lack T-cell responses. Total bacterial counts were higher, as expected, in the nude mice, but antibody-mediated protection was abrogated (Fig. S3), raising the possibility that antibody-mediated protection may also require an element of functional T-cell activity for full efficacy.

Antibody-Mediated Protection in an in Vitro WBA Requires CD4+ T Cells. The data from the mouse infection protection assay suggested that polyclonal antibodies from a minority of exposed HCWs could limit mycobacterial growth in vivo. Because only some individuals made protective responses, sera/antibodies could not be pooled. The mouse experiments also required a large amount of antibody; therefore, we decided to investigate whether an in vitro assay could be used that would allow us to perform more mechanistic experiments. Although most human–mouse antibody Fc Receptor (FcR) interactions do cross-talk (25), this human-only system would eliminate potential between-species confounders. To measure Mtb growth restriction in vitro, we modified an in vitro WBA (26). Antibodies from protective donors but not from nonprotective donors were able to enhance mycobacterial growth control (Fig. 2*A*), verifying the specificity of the assay. Two of three protective donors showed a significant protective response at 48 h, and all three showed a significant protective response by 120 h of incubation (Fig. 2*A*). To determine whether protection afforded by antibody followed a dose–response, we tested two doses, 50 and 500 μg/ml, of purified antibody in the WBA. Again, antibodies from the protective donor exhibited a clear dose–response, which was absent in antibodies from the nonprotective donor (Fig. 2*B*).

It is not clear how antibodies may enhance protection against Mtb. Some recent studies have suggested that Mtb-specific antibodies may enhance phagocytosis of mycobacteria by macrophages (27). We, therefore, tested whether addition of antibodies from some of the HCWs enhanced mycobacterial phagocytosis. None of five antibodies tested—from three protective and two nonprotective donors—enhanced phagocytosis by THP-1 cells (Fig. S4*A*), in keeping with other recent findings that opsonophagocytosis is not associated with protection (20).

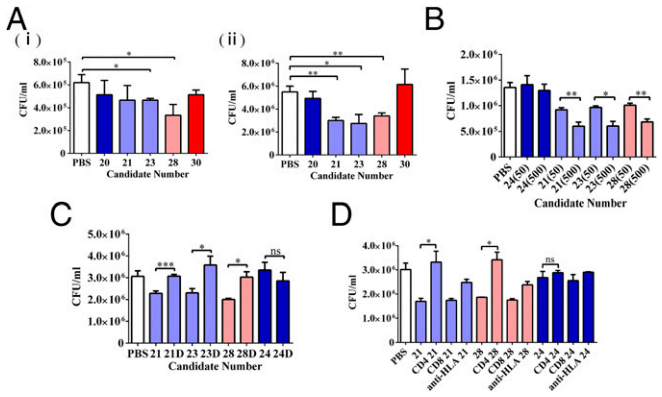

Fig. 2. Antibody from protective donors requires CD4+ T cells for efficacy in a WBA. (*A*) Antibody from protective donors but not from nonprotective donors increased growth control of Mtb in whole blood after (*i*) 48 and (*ii*) 120 h of incubation. (*B*) Higher antibody dose (500 μg/mL compared with 50 μg/mL) showed increased protection only from protective donors. (*C*) Depletion of total T cells using anti-CD3 antibodies (*D*) abrogates the growth restriction effect of antibodies specifically from protective donors in the WBA. (*D*) Depletion of CD4+ but not CD8+ T cells or blocking of MHC class II ("anti-HLA") abrogates the efficacy of antibodies from protective donors in the WBA. Blue represents LTBI HCWs, and red represents HEBUI HCWs. HCWs making protective responses are represented by paler shades (pale blue/pink). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ by Student's *t* test. ns, not significant.

Given that nude mice do not have a simple T-cell immune defect, the WBA also allowed us to determine whether T cells played a role in antibody-mediated protection in vitro. We first depleted all T cells from the whole blood using anti-CD3 antibodies. Growth restriction of Mtb by antibodies from protective donors was completely abrogated in the absence of T cells (Fig. 2*C*). Importantly, there was no increased growth of Mtb with T-cell depletion when antibodies from a nonprotective donor were used in the WBA (Fig. 2*C*). To further determine which class of T cells may play a role, we depleted CD4+ and CD8+ T cells separately as well as blocked MHC class II molecules. Depletion of CD4+ T cells but not CD8+ T cells abrogated antibody-mediated protection (Fig. 2*D*). Although blocking of MHC class II molecules decreased Mtb growth restriction slightly, the results failed to reach significance ($P = 0.1$).

Protective Antibodies Target Antigens on the Mycobacterial Surface. We sought to determine the class of mycobacterial antigens against which protective antibodies arose. Antibodies from all three of the protective (and most nonprotective) donors made detectable responses to soluble Mtb protein antigens by ELISA (Fig. S1). We, therefore, decided to test whether depletion of antibody directed against soluble Mtb antigens would abrogate the protective effect in the WBA. As shown in Fig. 3 *A*, *i*, depletion of antibody against soluble TB antigens had no effect on the protective response. However, depletion against intact Mtb completely abrogated protection (Fig. 3 *A*, *ii*), verifying that this was mediated against Mtb antigens expressed on the bacterial surface. These results might also explain why the magnitude of antibody titer against soluble Mtb lysate (Fig. S1) was not correlated with protection.

If the target of protective antibodies was expressed on the mycobacterial surface, we reasoned that standard culture of mycobacteria in our experiments, which includes axenic growth in media containing the mild detergent Tween-80, might strip away some of the mycobacterial capsule components (28). We, therefore, grew Mtb either on 7H10 agar plated in the absence of Tween-80 or in axenic culture in supplemented 7H9 medium, which includes Tween-80, and then used the bacteria in the WBA. Mtb grown in the absence of Tween-80 was significantly more growth restricted than bacteria grown with Tween-80 in the WBA but only in the presence of antibodies from protective donors (Fig. 3*B*), further suggesting that the antigen targets for protective antibodies are surface expressed.

To determine if the antigen targets of the protective antibodies were proteinaceous in nature, we panned antibodies from three protective donors and two nonprotective donors (24 and 26) against an Mtb proteome chip (29). The five pools of antibodies made multiple detectable binding responses against a variety of recombinantly expressed Mtb antigens (Dataset S1). To identify potential antigens to which all three protective antibodies make responses in common, we identified 13 antigens to which antibodies from all three protective donors bound but antibodies from the two nonprotective donors did not bind (Table S2). Of these 13 proteins, 6 were described in an online database (tuberculist.epfl.ch) as being surface expressed. We, therefore, depleted antibodies against these six pooled recombinant proteins before testing their protective efficacy in the WBA. Depletion did not alter protection (Fig. S4*B*), suggesting that the targets of the protective antibodies were not proteins, proteins with post-translational modifications not represented in our recombinant proteins, or other proteins that we did not test.

The protective effect of the polyclonal antibodies may be caused by direct antibody–antigen binding or other antibody-mediated functions, such as immune complex formation. To examine the potential role of immune complexes in antibody-mediated

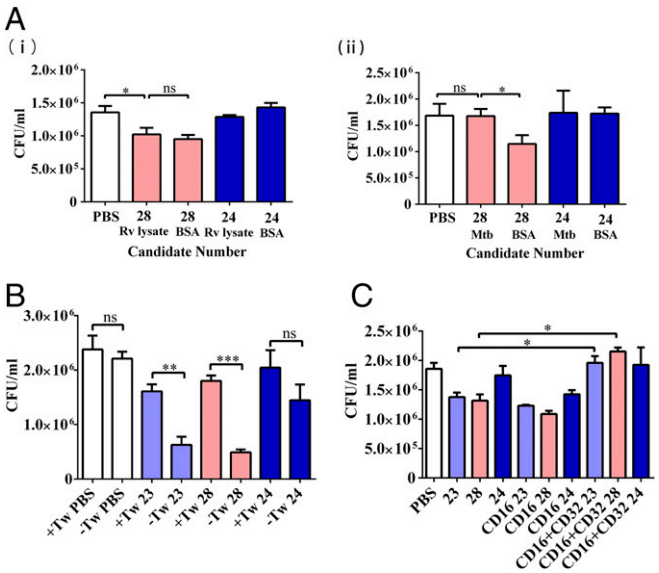

Fig. 3. Protective antibodies are directed at the Mtb surface, and immune complexes are critical for efficacy. (*A*) Depletion of antibody directed against the Mtb surface but not directed against soluble Mtb antigens abrogates their protective effect. Antibody from donors 24 (nonprotective) and 28 (protective) was incubated either (*i*) with Mtb soluble lysate (Rv lysate) or BSA-coated wells overnight or (*ii*) against intact Mtb vs. BSA and then used in the WBA. No difference in protective effect was observed when antibody was depleted against soluble Mtb antigens, but protection was abrogated when antibody was depleted against whole Mtb bacteria. (*B*) Use of Mtb grown in the absence of Tween-80 (–Tw) results in greater Mtb growth restriction when antibodies from protective donors are used in the WBA compared with use of Mtb grown in the presence of Tween-80 (+Tw). (*C*) Blockade of CD16 and CD32A together but not CD16 alone results in abrogation of Mtb growth restriction from antibodies from protective donors in the WBA. Blue represents LTBI HCWs, and red represents HEBUI HCWs. HCWs making protective responses are represented by paler shades (pale blue/pink). $*P < 0.05$, $**P < 0.01$, $***P < 0.001$ by Student's *t* test. ns, not significant.

protection, we blocked FcγRIII (CD16) alone and with FcγRIIa (CD32A). Blockade of CD16 had no effect on antibody-mediated protection, but blocking CD16 and CD32A together abrogated antibody-mediated immunity, suggesting that immune complexes play an important role in protection against Mtb by antibodies—perhaps by enhancing antigen presentation or other mechanisms.

The WBA Acts as an in Vitro Correlate of Murine Antibody-Mediated Protection and Can Be Used to Identify Additional Protective Donors. If more potent protective antibody-mediated responses against Mtb are to be identified, it is likely that a large number of donors will need to be screened. However, the murine protection assay is both costly and cumbersome and requires a large amount of antibody. In vitro assays using macrophage-mediated killing might identify some protective antibodies (20, 24, 27), but given the dependence of T-cell immunity for full protection of the antibodies identified by our studies, they may not identify all donors making protective antibodies. We wanted to determine whether the WBA could be used to identify donors who make antibodies that are also protective in the murine assay. We, therefore, consented and bled another 24 HCWs (12 LTBI and 12 HEBUI), and we tested them as before but this time, first in the WBA and then in the murine assay. The WBA identified another four donors who restricted Mtb growth (Fig. S5*A*)—two from the LTBI group and two from the HEBUI group. Importantly, antibodies isolated from all four of these donors and not from any other donors were also protective in the murine assay (Fig. S5*B*). These results suggest that the WBA assay might be used as a screening tool to identify individuals who make protective antibody responses against TB and that such individuals are present as a substantial minority (7 of 48 in our study) among Chinese HCWs.

Discussion

The evidence for and against antibody-mediated immunity to TB is a cause of ongoing debate. Mice lacking elements of cell-mediated immunity, such as CD4+ T cells, IFN-γ, or TNF-α, quickly succumb to primary infection by Mtb, whereas mice or nonhuman primates lacking B cells have a much more modest phenotype (4, 30). Passive immunization of mice with either human or murine polyclonal sera showed a protective role against infection (14, 31). However, a concern about the protective effect seen with polyclonal sera is the known immunomodulatory effects of i.v. Ig (15). Was the protection seen because the sera contained antibodies directed against mycobacterial antigens or because of their general effect on the murine immune system? The abrogation of immunity when antibodies against the mycobacterial surface were depleted (Fig. 3) strongly argues against the observed phenotype being because of the potential immunomodulatory effect of passive immunization with pooled Ig that had been previously reported (14). The Ig subclass may have important implications for the nature of immune function (23, 32). The abundance of IgG3 from protective donors, which is associated with increased activity (32), was higher at days 1 and 3 than that from a nonprotective donor when testing for decay kinetics in the mouse model (Fig. S2*A*). However, because abundance of subclasses was not overtly different in antibodies isolated from protective or nonprotective donors (Fig. S2*B*) and because the half-life of IgG3 from all four donors tested at ∼4 d was in keeping with previous studies (32, 33), it is not clear if this finding was relevant to the protection in the in vivo model.

We had hypothesized that the HCWs that would make protective antibody responses would be more likely to be among the HEBUI group. Surprisingly, of seven individuals identified in our study as making protective antibody responses, four had evidence of LTBI. A meta-analysis of risk of active TB found a substantially decreased risk in those with prior LTBI (34). Additional recent work also verified that antibodies from LTBI individuals offered superior protection in a macrophage infection model compared with those isolated from active TB patients (20). Therefore, it may be that some individuals exposed to Mtb with evidence of LTBI may be able to mount protective antibody responses to prevent additional active infection.

Recent reports suggest that antimycobacterial antibodies may act by increasing the rate of opsonophagocytosis and phagolysosomal fusion in infected macrophages (27). Nonetheless, efficacy for antibody from our protective donors required intact T-cell immunity (Fig. 2 and Fig. S3) and immune complex formation (Fig. 3), suggesting that protective antibodies isolated from our study are part of a complex interplay between the pathogen and host immune system and highlighting a protective role beyond the macrophage. This scenario would be similar to the requirement for both antibody- and CD4+-mediated immunity to protect mice against challenge with virulent *Salmonella* after immunization (35). However, the precise role for CD4+ T cells in antibody-mediated protection remains to be characterized. Surprisingly, T cells were important even in HEBUI donors, who by definition, make no detectable T-cell responses against two conserved Mtb antigens—the 6-kDa early antigen (ESAT-6) and the 10-kDa culture-filtrate protein (CFP-10). It is, therefore, likely that the CD4+ T cells that are required in our system either are not specific for those two antigens or do not secrete IFN-γ in response to antigen.

The magnitude of the protective response that we observed was modest: at most, 3.3-fold over PBS or nonprotective antibody. The in vivo mouse protection model that we tested used a "low-dose aerosol" infection method: delivering ∼100–200 viable bacilli per mouse on average to ensure that every animal was infected. Nonetheless, this low-dose challenge represents 2 logs more than the actual infectious dose: although the precise infectious dose of Mtb is not known, it is thought that most sufficiently infectious microdroplets will harbor just one bacillus (36, 37). Furthermore, we showed that protection was enhanced in vitro when the Mtb surface was not disrupted by detergents (Fig. 3*B*), suggesting that higher levels of protection may have been observed if the mouse assay was performed under similar conditions and that the protection that we witnessed might be an underestimate of what occurs physiologically (38).

Testing efficacy in a natural transmission model, perhaps using guinea pigs (39), would be a more physiological test of protection. Because of limited quantities of antibody from our HCW donors, these experiments may only be possible after isolation and recombinant expression of protective mAbs from our donors.

Our study addresses an important point of controversy in the TB field: whether antibodies play a role in immunity against Mtb (20, 24), and it also identifies components of this immunity, such as the role for CD4+ T cells. A mechanistic understanding of these issues is critical for the rational as opposed to purely empiric approach for the development of effective preventative and therapeutic TB vaccines.

Methods

Ethics Statement. This study was approved by the institutional review board of the Beijing Tuberculosis and Thoracic Tumor Institute (no. 2014–2-25), and all subjects (HCWs, patients, and healthy donors) gave written informed consent before blood was drawn. Mice were cared for according to the institutional protocols of the Beijing Tuberculosis and Thoracic Tumor Institute (Beijing Chest Hospital). Standard safety procedures for biosafety level-3 (BSL-3) work according to institutional protocols were used throughout.

Study Design and Participants. Patients were selected as having active TB according to standard medical criteria and under treatment at time of blood draw. Healthy HCWs who gave informed consent to participate in the study were scored by ELISPOT (see below) as LTBI or HEBUI. Subjects had to have worked at the Beijing Chest Hospital for at least 3 years to be eligible for the study. Exclusion criteria for both patients and HCWs included HIV+ status (routinely tested as part of both care and occupational screening), history of other immune deficiency or hematopoietic disorder, or symptoms suggestive of active TB (in HCWs). Active TB patients were diagnosed according to the

standard China 2001 diagnostic and treatment protocol. Patient and subject characteristics can be found in Table S1.

Mice. Specific pathogen-free female BALB/c and nude BALB/c (BALB/c nu/nu) mice ages 6–8 wk old were purchased from the Institute of Laboratory Animal Sciences, Chinese Academy of Sciences or Peking Union Medical College and housed under standard conditions in the institutional BSL-3 laboratory.

Mtb Strains and Culture. Mtb H37Rv (Rv) and a locally isolated, fully drug-susceptible clinical isolate [strain 165 (40)] were used in the experiments as described. For the latter, drug sensitivity to first-line antibiotics was verified according to standard diagnostic laboratory procedures and strain-verified as Beijing type by multiplex PCR of the RD105 region as previously described (41). Both strains were grown at 37 °C in Middlebrook 7H9 medium (Difco) supplemented with 10% (vol/vol) oleic acid-albumin-dextrose-catalase (OADC; BD Biosciences), 0.5% glycerol, and 0.1% Tween-80 or on 7H10 plates (Difco) supplemented with 10% OADC and 0.5% glycerol for 3 wk before counting cfus.

ELISPOT. HCWs were tested by commercially available ELISPOT (T.SPOT^{TB}; Oxford Immunotec) according the manufacturer's instructions. Spots were counted using a 166 CTL ELISPOT Counter (CTL-Immunospot S5 Versa Analyzer). Results were scored as positive (LTBI) according to the manufacturer's instructions as ≥24 spots for ESAT-6, CFP-10, or both.

Ig Isolation. Blood was collected by sterile technique into a vacutainer CPT tube (BD Diagnostics). The tube was centrifuged at 250 × *g* for 10 min to separate plasma from cells. Plasma was diluted 1:1 with sterile PBS and then filtered through a 0.25-μm filter. A cartridge containing 4 mL Protein A/G Plus agarose (ThermoFisher) was equilibrated with PBS, and the plasma/PBS mixture was passed over the column. The column was washed with 20 mL 1× PBS. Ig was eluted with 12 mL 0.1 M glycine (pH 2.5) into 12 1-mL fractions containing 0.1 mL 1 M Tris·Cl (pH 8.5). The eluted antibody was placed into a Sartorius viva-spin 10-kDa cutoff column to concentrate it and buffer exchange into PBS. Briefly, the tubes were spun at 7,430 × *g* for 30 min at 4 °C and washed three times with 12 mL PBS. Antibody was quantified by Bradford assay using standard curves of Ig, aliquoted, and stored at −80 °C.

TB Lysate Preparation. Mtb Rv was inoculated into a 50-mL culture of 7H9 and grown to OD = 1. Cells were pelleted and washed five times with PBS. After washing, bacteria were killed by incubation in a heat block at 85 °C for 1 h. The cells were then transferred to 2-mL impact resistant tubes containing 0.5 mL of 0.1-mm beads and lysed using an MP FastPrep-24 bead-beating machine. The samples were then centrifuged for 5 min at 3,300 × *g* at 4 °C, and the supernatant was aspirated, which was used as lysate for subsequent experiments. The total protein concentration was determined using Bradford assay, and the sample was aliquoted and frozen at −80 °C.

Human Antibody Titers Using ELISA. The concentration of Ig from 36 donors was normalized to 1 mg/mL for ELISA experiments. Mtb Rv lysate was diluted to 5 μg/mL in 0.05 M carbonate bicarbonate (CBC) buffer (pH 9.6), lysate pipetted into an ELISA 120-well microtiter plate (Costar 3509) at a concentration of 500 ng per well, and incubated at 4 °C overnight. The following day, wells were washed with 1× PBS with 0.05% Tween-20 (PBST) three times and then blocked with 5% nonfat milk powder in CBC buffer overnight. Wells were washed three times with PBST. Total Ig in 5% milk/PBST solution was added to each well in serial dilutions and left overnight, with 5% milk/PBST without Ig as the negative control. After washing, HRP-conjugated goat anti-human (IgG; Abcam) was added to the wells for 1 h at 37 °C, aspirated, and washed, and the wells were developed with 0.1 mL tetramethylbenzidine for 15 min at room temperature (RT). Reaction was stopped with 50 μL 1 M phosphoric acid. The plates were read at 450 nm with a Multiscan Go microplate plate reader (ThermoFisher). Reactive titers were defined as the maximum dilution that gave an OD value at least twice the background and determined by exponential interpolation using Graphpad Prism software.

Mouse Protection Assay. Purified total Ig was injected via the i.p. route into BALB/c or BALC/c nu/nu mice (20 mg per mouse) 5 h before infection, and PBS was used as a negative control. The locally isolated WT Beijing strain was used for infection—this culture was diluted to 1 × 10^6 cfu per 1 mL for aerosol infection. All mice per batch experiment were infected at the same time using a Glas-col inhalation exposure system; 24 h after infection, three of the PBS control mice were killed, and lungs were removed, homogenized using MP Fastprep, and plated on 7H10 agar plates for cfu enumeration of the infecting dose, which was verified as between 100 and 200 cfu per animal for all batch experiments and the repeats. The remaining mice were killed after 14 d, and both lungs were homogenized and plated with dilutions for cfu enumeration. Colonies were counted after 3–4 wk.

WBA. Blood from a healthy volunteer donor was drawn into a CPT vacutainer tube. The same donor was used for any one set of experiments to rule out donor-mediated variability. Blood was diluted 1:1 with RPMI-1640 (Gibco); 0.1 mL of Beijing Mtb isolate (at 10^5 cfu) was added to 0.9 mL of diluted blood in 15-mL sterile falcon tubes. Antibody (or PBS) was added to each tube in concentrations as indicated in triplicates. The tubes were incubated for the indicated time points at 37 °C in a shaking incubator at 20 rpm. At each time point, tubes were centrifuged for 10 min at 680 × *g*; then, 8 mL sterile water was added per tube, and the tubes were incubated for 10 min at RT. After blood lysis, tubes were spun at 680 × *g* for 10 min, supernatant was discarded, and the pellet was resuspended in 1 mL PBS. The samples were serially diluted, plated onto 7H10 agar plates, and incubated for 3 wk at 37 °C. After the initial experiment, subsequent WBA experiments were assessed at the 120-h time point. For the "depletion" experiments, Rv lysate (10 μg), recombinant proteins (12 μg total), or BSA (10 μg) as a negative control was pipetted into wells of an ELISA microplate and incubated at 4 °C overnight to coat the wells. Antibody was added to the wells and incubated overnight at 4 °C to deplete for antibody specific for Rv lysate proteins. For depletion against live Mtb, 1 × 10^8 cfu Mtb (Rv) was placed in an Eppendorf tube, pelleted, and washed three times with cold PBS. For control depletion against BSA, 10 μg of BSA was coated to each tube as before. Then, 50 μg of antibody was added to each of the tubes. The tubes were shaken overnight at 4 °C; then, bacteria were pelleted by centrifugation, and the supernatant was used in the WBA as above.

T-Cell Depletion Assay. Blood was drawn from a volunteer into a CPT vacutainer tube and diluted 1:1 with RPMI-1640 (Gibco) as before for the WBA. Then, 50 μL of Human CD3 MicroBeads, CD4 Microbeads, or CD8 MicroBeads (Miltenyi Biotec) were added separately into each 2-mL diluted blood sample and incubated for 30 min at 4°C. The LS columns (Miltenyi Biotec) were attached to the Midimacs and primed with 3 mL PBS and 3 mL RPMI-1640 sequentially; 6 mL of diluted samples with the beads as above were added to the column. Control blood samples without CD3, CD4, or CD8 microbeads incubation were prepared under the same conditions as above. The depleted and control samples were used in the WBA described above.

Fc Receptor Blocking Assay. The Fc receptors antibodies anti-human CD32A (clone: 6C4; eBioscience) and anti-human CD16 (clone: 3G8; BioLegend) were used as the blocking antibodies for this assay; 1 μg CD32, 2 μg CD16, or both were used in the designated samples. The blood treatment and infection are the same as described in the WBA.

Phagocytosis by Flow Cytometry. The phagocytosis assays were carried out as previously described (42). THP-1–activated macrophages were infected with bacillus Calmette–Guérin (multiplicity of infection of 20:1) for 3 h at 37 °C with 5% CO_2. Antibodies were used in this assay at the concentration of 50 μg/mL. The samples were analyzed by a BD C6 flow cytometer. Data were analyzed using FlowJo software.

Human Antibodies Isotyping Assay. The blood samples were centrifuged at 1,000 × *g* for 10 min at 4 °C, and the upper-layer plasma was collected. The seven antibodies subclasses (IgG1, IgG2, IgG3, IgG4, IgA, IgM, and IgE) levels were measured by the ProcartaPlex Multiplex Immunoassay kit (eBioscience). The plasma samples were diluted at 1:20,000 with the Universal Assay Buffer provided; 50 μL magnetic beads were added into designated wells of the 96-Well Flat Bottom Plate. The plates were securely inserted into the Hand-Held Magnetic Plate Washer for 2 min. The liquid in the wells was removed by inverting the washer quickly, and then, 150 μL of Wash Buffer added into each well for 30 s. The liquid was removed quickly, and then, the plates were removed from the washer; 25 μL standards and diluted plasma were added into dedicated wells and incubated in a shaker at 500 rpm for 2 h at RT. The plates were washed twice, and then, Detection Antibody was added into each well and incubated in a shaker at 500 rpm for 30 min at RT. Then, the plates were washed twice, and streptavidin, R-phycoerythrin conjugate solution was added into each well and incubated at 500 rpm for 30 min at RT. The plate was washed twice, and Reading buffer was added into each well and incubated at 500 rpm for 5 min at RT. The plate was run on the Luminex 200 system, and the output data were analyzed by the ProcartaPlex Analyst 1.0 software (eBioscience).

Antibodies Persistence Calculation. Five milligrams of each designated purified antibody was injected into BALB/c mice via the i.p. route. The mouse blood was collected at indicated time points by the retrobulbar venous plexus puncture method. The blood was allowed to clot for 30 min at RT and centrifuged at 1,000 × *g* for 10 min at RT. The serum fraction was collected and stored at −80 °C. The thawed sera were diluted at 1:2,500 and measured by Luminex 200 according to the method for the human antibodies isotyping assay described above.

Mtb Proteome Binding Array. Antibodies from five donors from this study were shipped to TB Healthcare (China), which commercialized the assay. The assay was run as previously described (29). Briefly, the Mtb proteome microarrays were blocked for 1 h at RT with blocking buffer (3% BSA in 1× PBS, 0.1% Tween-20, pH 7.4) in a shaker. After blocking, the blocking buffer was aspirated, and the purified total Ig at the final concentration of 0.1 μg/μL in blocking buffer was loaded onto the microarray; the microarray was incubated at 4 °C overnight. After incubation, the arrays were washed in Tris-buffered saline with 0.1% Tween-20 (TBST) buffer three times. The blocking buffer was aspirated, and blocking buffer with the secondary antibodies cy3 anti-human IgG, cy5 anti-human IgM, and FITC anti-human IgA at dilutions of 1:1,000 were added. The arrays were incubated at RT for 1 h. After washing three times in TBST, arrays were dried in a Slide Washer (CapitalBio) and then scanned with a GenePix 4200A microarray scanner (Molecular Devices). Data were analyzed with GenePix Pro-6.0 (Molecular Devices). Data are presented in Dataset S1.

Statistical Analysis. Data are expressed as mean ± SD unless indicated otherwise. Means were compared using an unpaired Student's *t* test (Graphpad Prism) and $P < 0.05$, $P < 0.01$, and $P < 0.001$ were considered statistically significant. For the mouse protection assay, donors of three positive results and two negative donors were consented and rebled, and the results were verified in a second experiment. The experiment was not repeated for the other 31 donors. All other experiments were repeated at least once on a separate occasion, and representative results are shown except for the data presented in Fig. S5, which were performed once.

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