

Immunological mechanisms of human resistance to persistent *Mycobacterium tuberculosis* infection

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Abstract | *Mycobacterium tuberculosis* is a leading cause of mortality worldwide and establishes a long-lived latent infection in a substantial proportion of the human population. Multiple lines of evidence suggest that some individuals are resistant to latent *M. tuberculosis* infection despite long-term and intense exposure, and we term these individuals ‘resisters’. In this Review, we discuss the epidemiological and genetic data that support the existence of resisters and propose criteria to optimally define and characterize the resister phenotype. We review recent insights into the immune mechanisms of *M. tuberculosis* clearance, including responses mediated by macrophages, T cells and B cells. Understanding the cellular mechanisms that underlie resistance to *M. tuberculosis* infection may reveal immune correlates of protection that could be utilized for improved diagnostics, vaccine development and novel host-directed therapeutic strategies.

Purified protein derivative (PPD) skin reactivity test

A delayed-type hypersensitivity test that measures induration at the site of an intradermal injection of peptide extract from mycobacterial culture filtrate. A positive result reflects a pre-specified minimal diameter of skin induration and suggests the presence of latent *Mycobacterium tuberculosis* infection but may also result from non-*M. tuberculosis* sensitization, including prior vaccination with *Mycobacterium bovis* Bacillus Calmette–Guérin. Also known as the Mantoux tuberculin skin test (TST).

Bacillus Calmette–Guérin (BCG)

A culture-adapted, attenuated *Mycobacterium bovis* strain that is used for vaccination against *Mycobacterium tuberculosis*.

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Through a long coevolutionary history with humans, *Mycobacterium tuberculosis* has developed into a highly successful pathogen that infects 23–32% of the world population^{1,2} and tuberculosis (TB) is the leading infectious cause of death³. Following aerosol exposure to *M. tuberculosis*, three potential clinical outcomes are possible, namely, resistance or early clearance of the bacillus, asymptomatic or latent *M. tuberculosis* infection (LTBI) that can persist for decades, or symptomatic ‘active tuberculosis’, which includes pulmonary disease that can result in further transmission. Recent whole-blood transcriptomic profiling^{4,5} and advanced lung imaging modalities⁶ have provided new insight into the transition from subclinical to active TB^{7,8}. However, why some heavily exposed individuals never acquire, or perhaps immediately eliminate, infection is poorly understood.

No reliable test exists that directly detects the presence or absence of *M. tuberculosis* in asymptomatic individuals. The purified protein derivative (PPD) skin reactivity test measures delayed-type hypersensitivity to mycobacterial antigens and has been the gold standard for the diagnosis of LTBI for >100 years^{9,10}. As PPD is enriched for protein antigens¹¹ that are not necessarily specific to *M. tuberculosis*, prior immunization with *Mycobacterium bovis* Bacillus Calmette–Guérin (BCG) or exposure to non-tuberculous mycobacteria can yield false positive results. IFN γ release assays (IGRAs) were developed as a whole-blood diagnostic test that measures IFN γ release from *M. tuberculosis*-antigen-specific CD4⁺ T cells after

stimulation with two to three specific *M. tuberculosis* antigens and thus avoids false positive results from prior BCG vaccination⁸. In the absence of a direct measure of infection, the best surrogates for initial infection are the PPD skin reactivity test and/or IGRA responses that convert from negative to positive (that is, incident positive) after *M. tuberculosis* exposure.

In endemic TB settings, some adults who are heavily and repeatedly exposed to *M. tuberculosis* remain negative for reactivity in the PPD test and the IGRA. These individuals, whom we term resisters, can be defined clinically as resistant to infection (FIG. 1). However, as this designation includes several assumptions about exposure and the stability of the results of these diagnostic tests, we propose the following enrolment criteria for studies of resisters (BOX 1). First, a high level of exposure is crucial and should include indices of exposure intensity (for example, high bacillary load of a known index case and close proximity of contact, such as sharing a room or bed) as well as indices of exposure duration that capture cumulative exposure (for example, repeated household exposures or employment in settings of documented high *M. tuberculosis* transmission), all of which can be assessed using validated exposure risk scores^{12,13}. Second, diagnosis of a resister requires a negative result for both the PPD skin reactivity test and the IGRA to avoid misclassification. Third, these diagnostic tests should be carried out serially following documented exposures to capture any conversions to LTBI (or TB).

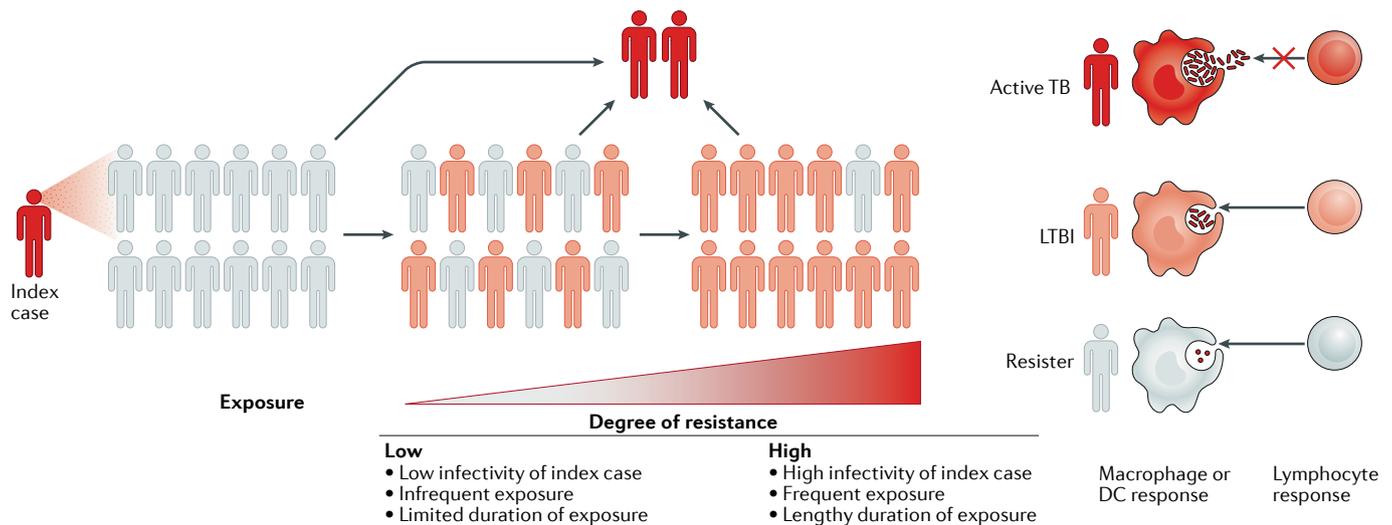


Fig. 1 | The spectrum of human resistance to infection by *Mycobacterium tuberculosis*. The extent of resistance to *Mycobacterium tuberculosis* infection is proportional to the duration and the intensity of exposure to *M. tuberculosis*. Individuals who resist infection despite heavy exposure to *M. tuberculosis* are more likely to have immunogenetic mechanisms of resistance than those who resist infection after a lower extent of exposure. Individuals with resistance to *M. tuberculosis* infection after intense exposure are termed 'resisters' (white), whereas individuals who test positive in the purified protein derivative (PPD) skin reactivity test and/or the IFN γ release assay (IGRA) can either be asymptomatic (latent *M. tuberculosis* infection (LTBI); pink) or be symptomatic with tuberculosis (TB; red). DC, dendritic cell.

IFN γ release assays (IGRAs). Whole-blood immune assays in which a patient's blood is cultured in the presence of select antigens specific to *Mycobacterium tuberculosis* and the secretion of IFN γ is quantified. Positive results are independent of prior Bacillus Calmette–Guérin vaccination and negative results ensure a sufficient response to a mitogen positive control.

Resister
An individual who remains negative for the purified protein derivative skin reactivity test and the IFN γ release assay throughout serial testing despite heavy exposure to *Mycobacterium tuberculosis*.

The resister phenotype is probably heterogeneous and might involve innate immunological mechanisms of early clearance of *M. tuberculosis* (innate resisters). Alternatively, protective immunity might also occur through adaptive immune responses (adaptive resisters), including IFN γ -independent responses that have been measured in preclinical models^{14–16}, and thus a potential role for B cell or unconventional T cell responses in early clearance of *M. tuberculosis* should be considered. Longitudinal, rigorous epidemiological studies of resisters, together with data from genetic and immunological platforms, are providing new insights into human resistance to *M. tuberculosis* infection.

In this Review, we describe the epidemiology of the resister phenotype and discuss several caveats about the definition of a resister. Next, we review the genetic determinants of the resister phenotype and discuss potential effector mechanisms in macrophages, unconventional T cells and B cells. We examine immune factors that prevent or limit *M. tuberculosis* infection in innate resisters or adaptive resisters, which could be harnessed for the

development of host-directed therapies or more effective vaccines and adjuvants.

Epidemiology

Despite the absence of a direct microbiological measure of *M. tuberculosis* in latent infection, historical and rigorous modern epidemiological studies suggest that resisters exist, although their estimated frequency varies in different studies.

Historical case–contact studies. Strong epidemiological evidence suggests that some individuals are naturally resistant to infection, even after heavy exposure to *M. tuberculosis* in closed environments. In 1966, all enlisted personnel and officers aboard the destroyer U.S.S. Richard E. Byrd were enrolled in a study after a 5 cm pulmonary cavity was detected (by radiography) in a crew member who suffered progressive respiratory symptoms for the prior 6 months aboard the ship¹⁷. Of the 308 at-risk enlisted crew members, 7 developed active TB, 6 of whom shared the same berthing compartment as the index case. Most crew members in this 'high-burden' compartment showed evidence of new infection (either active TB or an incident positive PPD), whereas seven crew members (~10%) were negative for the PPD skin test during the initial study and remained so at follow-up. Other studies, including an evaluation of nursing students in the pre-antibiotic era, also showed evidence of PPD-negative responses despite intense exposure to patients with TB (TABLE 1). A systematic review of household contact studies detected heterogeneity in resistance to infection, with an average of 50% of close contacts remaining uninfected¹⁸. However, these studies reveal substantial variability in PPD-conversion rates, which is consistent with unmeasured exposure variables.

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Innate resisters

Conceptually, resisters who immediately clear *Mycobacterium tuberculosis* following exposure to the bacterium and before priming of any adaptive immune responses.

Adaptive resisters

Conceptually, resisters who clear or contain *Mycobacterium tuberculosis* infection via T cell and B cell mechanisms but who remain negative for the purified protein derivative skin reactivity test and the IFN γ release assay.

Household contact

An individual who resides within the same domicile as an index case (patient with tuberculosis) for a pre-specified amount of time.

Hyperendemic conditions

Regions in which ongoing *Mycobacterium tuberculosis* transmission is unusually high owing to an elevated prevalence of tuberculosis.

Silicosis

An occupational pulmonary disease that is prevalent among gold miners owing to protracted inhalation of silicate dust, which leads to scarring and increased *Mycobacterium tuberculosis* infection.

Not surprisingly, proximity and duration of contact with the index case as well as the infectivity of the index case (that is, bacillary burden) were predictors of PPD conversion.

The results of most early studies in hyperendemic conditions suggest that a minority of the population (5–50%) has the resister phenotype, and this variability probably reflects exposure intensity and the duration of follow-up^{17,19,20}. However, the results of some studies suggest that rates of resistance are higher (for example, up to 70% of exposed individuals are resisters), even after periods of high exposure (that is, sleeping in the same bed or room) to the index case^{21–24}. Importantly, most of these studies either had a short duration of follow-up or contained data that were difficult to accurately review by modern epidemiological standards. Finally, the strain of *M. tuberculosis* probably differed among the various studies and thus might affect transmission rates. For example, increased virulence and transmission might be one of several factors that are involved in the increased range and incidence of the lineage 2/Beijing strains of *M. tuberculosis*^{25–27}.

The results of these early studies suggest that a resister phenotype exists, but the epidemiological methods that were used could not ensure high exposure and adequate follow-up to confirm the presence of clinical resistance to *M. tuberculosis* infection.

Contemporary cohort studies. Population studies of individuals in hyperendemic TB settings in the past 5 years have provided opportunities to examine resistance to *M. tuberculosis* infection. For example, among South African gold miners, exposure to silica dust and the congregate working, living and social conditions that are associated with mining contribute to the high level of ongoing *M. tuberculosis* transmission^{28,29}. Mathematical modelling suggests that the annual risk of infection (that is, conversion in the PPD skin test) among gold miners is as high as 20%³⁰. The prevalence of LTBI in these gold miners was estimated at 89%, and 13% (15/115) of HIV-negative participants showed no evidence of

M. tuberculosis infection (that is, induration = 0 mm in the PPD skin test)³¹. In this extremely high-burden environment, any gold miners who remain persistently uninfected are likely resisters. According to mathematical models, the likelihood that a 40-year-old gold miner without evidence of *M. tuberculosis* infection (induration = 0 mm in the PPD skin test) is a resister is 93%, compared with only 22% among the general community³². Two prospective studies in South Africa are currently being carried out to identify HIV-uninfected or HIV-infected resisters who have been mining for ≥ 15 years, are 33–60 years of age, do not have evidence of past or active TB or silicosis and have neither a history of treatment for LTBI nor any non-HIV immunosuppressive conditions.

Few longitudinal studies of conversion in the PPD skin reactivity test or the IGRA in hyperendemic settings have incorporated extended follow-up periods. Uganda is a high-burden TB setting with a 3% annual risk of *M. tuberculosis* infection, as determined by PPD skin test and/or IGRA conversion³³. LTBI is considerably more prevalent among household contacts than among community members, and consequently, there is a risk difference of 30% among adults³⁴. In Kampala, Uganda, household contacts of index cases with culture-confirmed TB were evaluated for active TB and underwent PPD skin testing³⁵. The annual incidence of new cases of culture-positive TB in this study was 740 per 100,000 individuals, compared with country-wide estimates of 159 per 100,000 individuals³³. If an individual was negative in the PPD skin reactivity test at baseline (<10 mm induration for HIV-negative individuals >5 years old, <5 mm induration for HIV-negative individuals ≤ 5 years old or <5 mm induration for all HIV-positive individuals), repeated PPD skin tests were carried out at 3, 6, 12 and 24 months after enrolment. Among the entire study cohort, 11.7% of adult close household contacts remained persistently negative in the PPD skin test throughout the two year follow-up^{12,36}. Both the South African and Ugandan cohorts described here provide an opportunity to rigorously define the resister phenotype and explore its underlying cellular mechanisms.

Box 1 | Proposed criteria to define the resister phenotype

Resisters (individuals who show resistance to *Mycobacterium tuberculosis* infection despite long-term, intense exposure to the bacillus) are exemplified by the following characteristics:

Extent of exposure to *Mycobacterium tuberculosis*

- Intensity: high bacillary burden of index case (for example, sputum-positive), closed environment (household, mine shafts and so on) and use of validated epidemiological risk scoring^{12,13}
- Duration: cumulative exposures (that is, health-care workers, miners, household contacts or adults with community exposure in hyperendemic areas)

Result of diagnostic tests

- Negative result in both the purified protein derivative (PPD) skin reactivity test and the IFN γ release assay (IGRA)

Durability of responses

- Multiple negative results for the PPD skin reactivity test and the IGRA in the first year following exposure
- Additional longitudinal testing, when available and if exposures persist, to minimize false negative test results and enrich for conversions after cumulative exposure

Complexities of the resister phenotype. Given that the definition of a resister relies on epidemiological data and utilizes an indirect, immunological measurement of *M. tuberculosis* infection, it is likely that some resisters are misclassified. Several alternative hypotheses may explain the resister phenotype.

Despite meeting enrolment criteria as a household contact, an individual with a negative result in the PPD skin reactivity test may simply have had insufficient exposure, either from a low intensity of exposure (that is, a low bacillary burden in the index case, minimal contact with the index case or good ventilation within the household) or a short duration of contact with an index case. In the Ugandan study, a published modified epidemiological exposure risk score¹³, consisting of characteristics of the TB index case and the intensity of the contact with the index case, did not differ between resisters and individuals with LTBI¹². This result suggested that all household contacts were highly exposed to *M. tuberculosis*. Participants in the Ugandan household

Table 1 | Prevalence of the resister phenotype

Exposed population	Length of follow-up	Cumulative resister prevalence (percentage) ^a	Refs
<i>Historical purified protein derivative studies</i>			
Sailors sharing berthing compartment with an individual with active TB	>6 months	9/65 (13.8)	17
Sailors with frequent contact with two individuals with incident TB aboard ship	6–12 months	76/226 (33.7)	173
Young nursing students working in heavy-burden TB hospital in Norway	3 years	0/1,453	44
Nursing students at Philadelphia General Hospital (USA) in 1935–1939	3 years	0/637	45
Nurses training at Boston City Hospital (USA) in 1932–1936	3 years	6/126 (4.8)	174
University of Minnesota nursing classes graduating in 1932–1935 (before removal of TB ward and installation of infection control)	3 years	16/184 (8.7)	175
Professional students of Wisconsin General Hospital and Wisconsin State Sanatorium in 1934–1944 (RNs) and 1934–1943 (MDs)	RNs: 3 years MDs: 4 years	RNs: 30/112 (26.8) MDs: 50/283 (17.7)	176
Close family contacts of index pulmonary TB cases in India	5 years following diagnosis of index case	110/851 (12.9)	22
Close family contacts of individuals with pulmonary TB in Pakistan	6 months following diagnosis of index case	103/292 (35.3)	21
Household contacts of index cases with sputum positive for the AFB-smear test	12 months following diagnosis of index case	83/253 (32.8)	23
<i>Contemporary studies</i>			
Gold miners in South Africa	Variable	15/115 (13.0)	31,32
Close household contacts of individuals with pulmonary TB in Uganda	2 years	142/1,210 (11.7)	12,177

AFB, acid fast bacilli; MDs, medical students; RNs, nursing students; TB, tuberculosis.
^aThe number of contacts persistently negative for reactivity in the purified protein derivative (PPD) skin test (and the IFN γ release assay, if available) as a proportion of all contacts enrolled in the study (that is, PPD-positive contacts, PPD-negative contacts and PPD converters).

contact study were re-contacted 8–10 years after the original study and were tested multiple times with IGRAs (3 assays over 3 years) and once with a repeat PPD skin reactivity test. Although they were not necessarily re-exposed within the same household in the interval since their initial enrolment as a household contact, all these individuals continued to live in areas of Kampala with high TB prevalence in which community exposure to *M. tuberculosis* might be substantial^{37–39}. These data will further clarify the durability of the resister phenotype.

An alternative explanation for the resister phenotype is that persistently negative results in PPD skin reactivity tests or IGRAs are false negative results

owing to the inherent limitations of an immunological definition of a clinical phenotype. Both PPD skin test and IGRA responses are incomplete measures of anti-mycobacterial immunity for a number of reasons. As discussed later, these LTBI diagnostic tests fail to detect the response of T cells that are specific for non-protein mycobacterial antigens via MHC-independent pathways, and IGRAs do not detect the response of antigen-specific T cells with alternative (that is, IFN γ -independent) cytokine-producing profiles^{40–42}. T cell anergy could also cause false negative results in the PPD skin test. In the Uganda study, HIV-negative individuals were not more likely to be PPD-negative than HIV-positive individuals, for whom a positive PPD test was defined as induration >5 mm (REF. 12). Furthermore, in recent follow-up studies, all resisters demonstrated reactivity to the mitogen positive control that was included in the IGRA methodology (W.H.B. and C.M.S., unpublished observation), suggesting that generalized CD4⁺ T cell anergy or depletion alone does not explain the resister phenotype. This does not rule out a contribution of pathogen-specific anergy, which has been observed in human lepromatous leprosy⁴³.

Resistance to *M. tuberculosis* probably has a threshold, above which relative resistance can be overcome by a sufficiently high exposure. For example, in two landmark studies in the 1930s and 1940s in Norway and at the Philadelphia General Hospital, USA, the prevalence of LTBI among nursing students at matriculation was 45.3% (668/1,453)⁴⁴ and 57% (366/643)⁴⁵, respectively. At 3-year follow-up, however, 100% of students that were initially negative for the PPD skin test in both studies converted, and the majority of these negative-to-positive conversions occurred during the early stages of training. Considering these data, together with those from the contemporary studies discussed earlier, it is possible that repeated testing (for example, serial testing every 4 months with 2 doses⁴⁵) and the use of tuberculin of non-standardized purity (for example, the two early studies were carried out before large-scale standardized preparations of tuberculin, such as PPD-S, became available in 1941) effectively sensitized some individuals in the absence of natural infection. Non-tuberculous mycobacterial infections are also known to produce false positive results in the PPD skin test in some populations, a response that could potentially be boosted by serial PPD testing, as was seen with *M. tuberculosis* infection⁴⁶. Furthermore, the induration thresholds that were used for each method (Mantoux or von Pirquet) were not described in these studies. Nonetheless, the results of these pre-antibiotic era studies suggest that absolute *M. tuberculosis* resistance occurred at a low frequency in these populations.

Relative resistance to *M. tuberculosis* disease might also be a function of the infectious dose. The classical work of Lange and Chaussé showed that small droplets carrying one bacillus or a few bacilli are the effectors of aerosol transmission and that the vast majority of individuals with TB show only a single Ghon complex^{47,48}. In natural infections, the frequency of exposure to *M. tuberculosis* is the most parsimonious surrogate for infectious dose, but how consistency of exposure is linked to innate resistance mechanisms is not known.

Relative resistance

A threshold phenomenon in which mechanisms of host resistance to *Mycobacterium tuberculosis* infection may be overcome owing to frequent or lengthy exposure to contacts with a high bacillary burden.

The epidemiological studies discussed above suggest that some individuals are resistant to *M. tuberculosis* infection, as defined by a persistently negative result in the PPD skin test and/or the IGRA, despite sustained high-level exposures over extended periods of time. Furthermore, these individuals fail to develop active TB over an extended follow-up period. Future research to uncover the mechanisms that are involved in resistance to *M. tuberculosis* infection will require detailed assessments of exposure to the bacillus to define the resister phenotype (Box 1). The true prevalence of resisters among individuals who have been exposed to *M. tuberculosis* is not known, but evidence from studies that assessed exposure variables and included longitudinal sampling suggest that the prevalence is <10%.

Genetics

The cellular mechanisms that mediate *M. tuberculosis* resistance are unknown, although multiple susceptibility loci have been linked to or associated with the resister phenotype in genome-wide analyses.

Genetic basis of resistance to other pathogens. Genetic studies indicate that polymorphisms and mutations exist that are associated with resistance to some infections. For example, a 32 bp deletion in CC-chemokine receptor 5 (CCR5) in CD4⁺ T cells results in relative resistance to infection with R5-tropic HIV-1 and delays disease progression⁴⁹. Similarly, mutations in the gene encoding fucosyltransferase 2 (FUT2), which is required for the cell surface expression of histo-blood group antigens (HBGAs; the receptors for norovirus), results in decreased HBGA levels on mucosal surfaces and thus prevents virus uptake and entry into epithelial cells. Consequently, individuals with these *FUT2* mutations are completely (100% penetrant) resistant to Norwalk and other norovirus genogroups^{50,51}. Atypical chemokine receptor 1 (ACKR1; also known as DARC and CD234) is the receptor on erythrocytes for *Plasmodium vivax*. Most west African individuals are homozygous for a promoter mutation that ablates ACKR1 expression in erythrocytes and, consequently, are resistant to *P. vivax* infection⁵². Although these examples show Mendelian inheritance (for example, single-gene-inactivating mutations that result in highly penetrant resistance), it is likely that complex polygenic inheritance patterns are important for resistance to other pathogens, including *M. tuberculosis*.

Genetic studies of purified protein derivative skin reactivity. Numerous lines of evidence, including results from twin^{53,54}, Mendelian primary immunodeficiency⁵⁵, genome-wide linkage^{56–58} and candidate-gene studies^{59,60}, suggest that host genetics influences susceptibility to TB. Mendelian Susceptibility to Mycobacterial Disease (MSMD) is an immunodeficiency disorder in young children who are susceptible to disseminated BCG and non-tuberculous mycobacteria and who have mutations in the IFN γ and IL-12 signalling pathways⁵⁵. Several studies also suggest that host genetic factors modulate resistance to *M. tuberculosis* infection. For example, the intensity of tuberculin reactivity after exposure to

household contacts is correlated among siblings but not among unrelated children of the same household⁶¹.

In a genome-wide linkage analysis of the Ugandan cohort, suggestive evidence for a linkage of regions on chromosome 2q21–2q24 and chromosome 5p13–5q22 to the resister phenotype was detected⁵⁷. These regions are distinct from the regions that are linked to protection from TB, suggesting that the genetic underpinnings of these two ends of the TB spectrum (that is, asymptomatic infection and clinical disease) are unique. In a low endemicity setting, a candidate-gene association study of individuals with or without LTBI who had close contact with an index pulmonary TB case reported that polymorphisms in Toll-interacting protein (TOLLIP) and Unc51-like kinase 1 (ULK1) were associated with LTBI susceptibility^{62,63}. The specific roles of these proteins in the resister phenotype are discussed later.

Another genome-wide analysis assessed PPD skin test reactivity in *M. tuberculosis*-exposed South African individuals. This cross-sectional study measured rates of PPD-positivity both as a binary trait (that is, PPD = 0 mm versus >0 mm) and as a quantitative trait by measurement of skin induration (in millimetres)⁶⁴. As a binary trait, a significant linkage signal that met the stringent genome-wide linkage cut-off was found at the *TST1* locus on chromosome 11p14. Strikingly, in a later study of the same families, the *TST1* locus was found to be genetically indistinguishable (at a resolution of 2–14 Mb) from a major quantitative trait locus (QTL) that controls tumour necrosis factor (TNF) production following stimulation of whole blood with either BCG alone or BCG plus IFN γ ⁶⁵. These findings indicate that a genetic determinant linked to TNF secretion in response to BCG and IFN γ might also determine PPD negativity, perhaps through TNF-induced microbicidal pathways. However, the genomic resolution around the *TST1* locus in these studies was low (2–14 Mb), and this locus includes other genes besides *TNF*. When reactivity in the PPD skin test was measured in the same study as a quantitative trait (that is, the size of skin induration in millimetres), significant linkage was found with a second locus, *TST2*, on chromosome 5p15 (REF.⁶⁴). This region of chromosome 5 overlaps with the region that was associated with the resister phenotype in the Ugandan cohort⁵⁷. Although the *TST1* locus was strictly linked to resistance to *M. tuberculosis* infection and invokes mechanisms that precede T cell priming, the *TST2* locus might modulate T cell responses that govern the intensity of the response in the PPD skin test.

HIV-infected resisters, who have an increased likelihood of having inadequate adaptive immune responses owing to CD4⁺ T cell depletion, might be particularly dependent on innate immune mechanisms for resistance to *M. tuberculosis*. As HIV and TB are co-prevalent in Uganda and Tanzania, this hypothesis was tested in a genome-wide association study of PPD reactivity in HIV-infected individuals, which found that a locus on chromosome 5q31.1 was significantly associated with both binary (positive or negative in the PPD skin test; $P = 1.22 \times 10^{-8}$) and quantitative (induration in millimetres; $P = 1.45 \times 10^{-8}$) responses in the PPD skin test⁶⁶. This region contains four genes, including the gene

Ghon complex

The focus of primary *Mycobacterium tuberculosis* infection in the lung parenchyma that histologically corresponds to a granuloma and radiographically is recognized by its associated lymphadenopathy and calcification.

Quantitative trait locus

(QTL). A genomic region containing polymorphisms that are associated with a quantifiable phenotype, such as the extent of induration (in millimetres) in the purified protein derivative skin reactivity test. When variation at these loci affect the expression levels of specific genes, they are referred to as expression QTLs.

encoding IL-9, which is expressed and secreted by mast cells and T helper 2 (T_H2) cells and was previously associated with bronchial hyper-responsiveness. Interestingly, an inverse relationship between the incidence of asthma and TB was reported^{67,68}, suggesting that IL-9 and its pleiotropic effects on lung inflammatory responses protect against *M. tuberculosis* infection. Although *IL9* is an intriguing possible resistance gene, the other genes in the 5q31.1 region may also have a role in resistance to *M. tuberculosis*. The study also replicated associations in the regions previously identified in HIV-negative resistors (on chromosomes 2, 5 and 11; discussed previously), including susceptibility loci near *TST1*.

Together, these results suggest that the resister phenotype has polygenic variance; that is, many individual genetic variants each with small individual effects contribute additively to the phenotype. Although evidence to support broad epistatic effects on infectious disease susceptibility is lacking, gene pair interactions have been described in genome-wide analyses of susceptibility to TB^{69,70} and leprosy⁷¹. Nonetheless, the substantial overlap in the loci that are associated with the resister phenotype or in the results of PPD skin reactivity tests that was detected in independent analyses argues that evolutionary pressure has selected variants that protect against the initial *M. tuberculosis* infection, at least in geographical regions that are hyperendemic for TB.

Macrophage-mediated resistance

After inhalation, aerosolized *M. tuberculosis* must navigate multiple intrinsic barriers in the upper airways, including airway mucins and ciliated epithelial cells. The bacterium then encounters resident alveolar macrophages, which exhibit pro-inflammatory and anti-inflammatory responses and are uniquely positioned to have a key role in mediating early clearance of *M. tuberculosis*^{72–74}. The effector functions of alveolar macrophages might be modulated by neutrophils through, for example, their formation of neutrophil extracellular traps; however, the role of neutrophils in both microbicidal and immunopathological activities is complex and is reviewed elsewhere^{75,76}. *M. tuberculosis* is recognized by multiple pattern recognition receptors (PRRs) at the cell surface or within the phagosome or by cytosolic PRRs after phagosomal rupture^{77,78}. Subsequently, the outcome of infection depends on effective phagosome acidification, phagosome–lysosome fusion, 6 kDa early secretory antigenic target (ESAT6) secretion system 1 (ESX1)-dependent escape of *M. tuberculosis* from the phagosome⁷⁹, activation of the inflammasome (resulting in maturation of pro-IL-1 β and pro-IL-18)⁷⁸, upregulation of host genes that are either required for intracellular killing mechanisms (for example, reactive oxygen species, nitric oxide (produced by inducible nitric oxide synthase), cathelicidin antimicrobial peptide (CAMP)⁸⁰ and autophagy) or detrimental to the host (that is, IFN β production following intracellular sensing of *M. tuberculosis* DNA by cyclic GMP–AMP synthase (cGAS)^{81,82}) and, ultimately, the activation of host cell death pathways (for example, pyroptosis, apoptosis and necroptosis)^{83,84}. Resistance alleles could modulate a number of these airway clearance functions or macrophage antimicrobial mechanisms (FIG. 2).

Inflammatory signalling pathways. Genetic regulation of inflammatory pathways is well described^{85–88}, and some studies have associated susceptibility to mycobacterial infections with polymorphisms in Toll-like receptor (TLR) pathway components^{89–96}, regulators of TLR function^{97,98}, C-type lectin receptors⁹⁹, autophagy proteins^{100,101} and other pathways for pro-inflammatory cytokine production¹⁰². However, only some of these studies define functional polymorphisms (that affect TLR expression or signalling, for example), and the identified associations with clinical phenotypes require replication in other populations. Interestingly, patients with Mendelian primary immunodeficiency of myeloid differentiation primary response 88 (MYD88) or IL-1 receptor-associated kinase 4 (IRAK4), which are central to most TLR signalling pathways, do not develop mycobacterial diseases¹⁰³. Furthermore, few human genetic studies have found an association of resistance to *M. tuberculosis* infection (as opposed to susceptibility to disease) with polymorphisms in genes involved in any of these cellular pathways. A recently discovered single nucleotide polymorphism (rs5743854) in the promoter of the gene encoding TOLLIP is an expression QTL for which the G/G allele is associated with lower TOLLIP expression in monocytes and with increased risk of LTBI, pulmonary TB and meningeal TB⁶³. Although the specific mechanisms through which high TOLLIP expression contributes to *M. tuberculosis* resistance as well as to protection from disease are not known, the known role of TOLLIP in directing autophagy and its negative regulation of TLR2, TLR4 and IL-1 receptor (IL-1R) suggest that these pathways are mechanisms of resistance. Although replication of the genetic association findings in independent cohorts is needed, the studies discussed above suggest that resistance alleles exist and may be relevant to the resister phenotype.

Autophagy. Autophagy limits intracellular survival of *M. tuberculosis* and maturation of the mycobacterial phagosome^{104,105}. An in vitro genome-wide screen identified autophagy factors as the host factors that were most influential in restricting the intracellular growth of *M. tuberculosis*¹⁰⁶. The *M. tuberculosis* ESX1 secretion system is required for autophagy initiation through cGAS–stimulator of interferon genes (STING)-triggered recruitment of microtubule-associated proteins 1A/1B light chain 3 (MAP1LC3) to autophagosomes^{82,107,108}, but ESX1 may also be involved in the impairment of autophagosome maturation¹⁰⁹. Whether autophagy is involved in host protection versus virulence in the mouse model is controversial. Genetic deletion of the key autophagy factor *Atg5* in mice results in more severe disease that correlates with both uncontrolled *M. tuberculosis* growth and exacerbated lung immunopathology¹¹⁰. However, this severe disease was later suggested to result from increased neutrophil recruitment in the autophagy protein 5 (ATG5)-deficient animals and not from defective autophagy¹¹¹. Genetic variants within the autophagy pathway have been associated with TB in candidate-gene association studies^{100,101}, but whether similar variants control LTBI risk is poorly studied. A recent candidate-gene association study found that

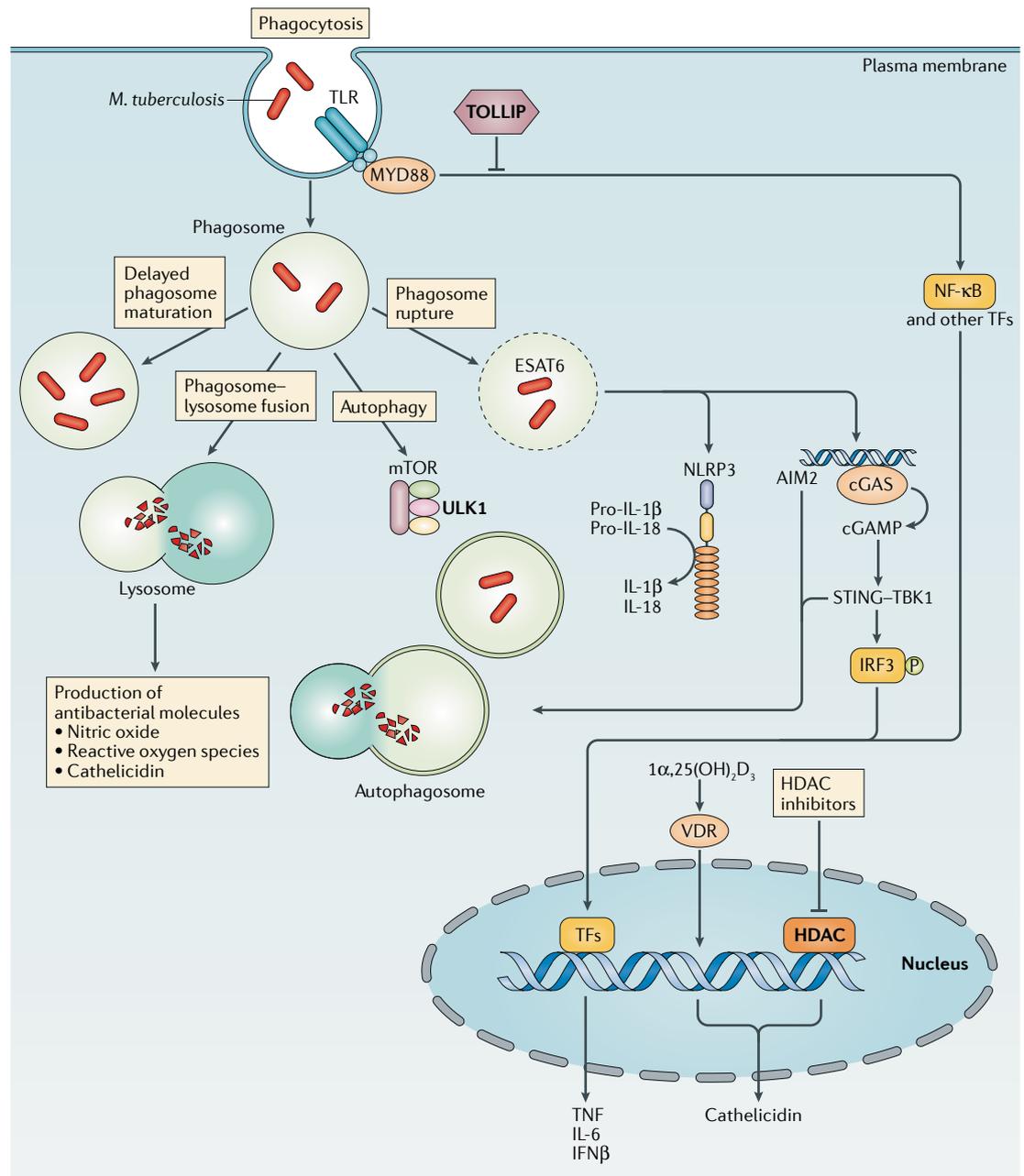


Fig. 2 | Macrophage-mediated resistance to *Mycobacterium tuberculosis*. Macrophages can provide resistance to *Mycobacterium tuberculosis* infection at multiple points in the infection process, including the initial uptake of bacilli into phagosomes and through any of the possible fates of these phagosomes. These fates include effective (versus delayed) phagosome maturation (resulting in the generation of microbicidal products, such as nitric oxide, reactive oxygen species and cathelicidin), fusion with the lysosome (resulting in degradation of the phagosomal contents), recruitment of autophagy factors (resulting ultimately in autophagosome–lysosome fusion) or phagosomal escape of bacteria into the cytosol. Pro-inflammatory cytokine production may result from Toll-like receptor (TLR) ligation or the recognition of signals following phagosomal rupture, including inflammasome-triggered maturation of pro-IL-1 β or pro-IL-18 and recognition of cytosolic *M. tuberculosis* DNA by cyclic GMP–AMP synthase (cGAS) and the resultant stimulator of interferon genes (STING)-dependent interferon regulatory factor 3 (IRF3)-driven transcriptional response. Vitamin D₃ receptor (VDR)-dependent transcription, which requires prior activation of vitamin D to 1 α ,25(OH)₂D₃ through TLR or IFN γ signalling events, results in cathelicidin expression. Genetic and cellular evidence exists for a role for the negative TLR regulator Toll-interacting protein (TOLLIP), the autophagy factor Unc51-like kinase 1 (ULK1) and histone deacetylases (HDACs; which are master transcriptional regulators) in resistance. AIM2, absent in melanoma 2; cGAMP, cyclic GMP–AMP; ESAT6, 6 kDa early secretory antigenic target; mTOR, mechanistic target of rapamycin; MYD88, myeloid differentiation primary response protein MYD88; NF- κ B, nuclear factor- κ B; NLRP3, NOD-, LRR- and pyrin domain-containing 3; TBK1, TANK-binding kinase 1; TFs, transcription factors; TNF, tumour necrosis factor.

two polymorphisms in *ULK1*, which encodes a component of an upstream protein complex that transduces signals to central autophagy effectors, were associated with LTBI in Asian contacts of index cases⁵². After adjustment for clinical risk factors for LTBI, each of these *ULK1* minor alleles was estimated to confer an 80% reduction in LTBI risk. This result might be due to an improvement in selective autophagy, decreased *M. tuberculosis* replication or increased TNF secretion in response to TLR ligands, as suggested from mechanistic studies using *ULK1*-deficient cells⁶².

Monocyte gene expression and histone deacetylase pathways. One hypothesis to explain the resistance to *M. tuberculosis* infection in the Uganda study¹² is that following infection, the expression profiles of blood monocytes from individuals with LTBI and resisters are distinct. In a comparative transcriptional profiling study, monocytes from each clinical group were infected *ex vivo* with *M. tuberculosis*¹¹², and the microarray data were analysed with Gene Set Enrichment Analysis (GSEA)¹¹³ to identify host pathways that are associated with each clinical phenotype (resister and LTBI) according to curated gene sets from transcriptional databases. The gene set that was most significantly associated with the resister group was from a previous study of sodium butyrate-stimulated cells¹¹⁴. Sodium butyrate is a short chain fatty acid that inhibits histone deacetylases (HDACs), which modify chromatin and regulate cellular function, including signalling pathways^{115,116}. Interestingly, phenylbutyrate, a class I HDAC inhibitor that is known to synergize with vitamin D to induce CAMP production and inhibit *M. tuberculosis* growth in macrophages¹¹⁷, has been tested as a potential host-directed therapy for pulmonary TB¹¹⁸. Activated vitamin D ($1\alpha,25(\text{OH})_2\text{D}_3$) binds to the vitamin D₃ receptor (VDR), which translocates to the nucleus to induce expression of CAMP and drive other host processes, including autophagy and autophagosome–lysosome fusion⁸⁰. CAMP has direct antimicrobial activity against *M. tuberculosis* in the phagosomal compartment⁸⁰. Together, these data suggest that monocytes from resisters and individuals with LTBI respond differently to *ex vivo* *M. tuberculosis* infection. Targeting HDACs with inhibitors might provide a novel therapeutic option for *M. tuberculosis* infections; however, the specific mechanisms by which HDAC-dependent pathways are altered in resisters, and whether epigenetic mechanisms or ‘trained immunity’ are involved, are unknown.

T cell-mediated resistance

Several lines of evidence from human studies and animal challenge models underscore the importance of T cells in controlling *M. tuberculosis* infection^{119–121}. HIV-infected individuals with low CD4⁺ T cell counts have increased risk of progressing from LTBI to TB. In addition, patients with MSMD have defects in the IL-12–IFN γ pathway and increased susceptibility to disseminated mycobacterial infections⁵⁵. Furthermore, mouse models of acute *M. tuberculosis* infection have demonstrated the importance of IFN γ production in controlling bacterial replication and survival¹²². However, the source of IFN γ remains

controversial, as its production by CD4⁺ T cells may be dispensable for control of *M. tuberculosis* infection in some mouse models^{14–16}, whereas IFN γ production is essential in other mouse models¹²³. Furthermore, IFN γ -producing CD4⁺ T cells have not emerged as an immunological correlate of protection from infection in humans, which suggests that this profile is important but is not sufficient for protection against *M. tuberculosis* infection^{124,125}. In individuals with LTBI, MHC-restricted $\alpha\beta$ T cells recognize a broad repertoire of MHC class I-restricted and MHC class II-restricted *M. tuberculosis* peptides and exemplify a classical T_H1 cell-like response. Implicit in their definition, resisters lack reactivity in the PPD skin test or secretion of IFN γ by T cells following antigen stimulation, although, hypothetically, these individuals might have IFN γ -independent T cell responses that mediate resistance to *M. tuberculosis* or its clearance.

IFN γ -independent T cell responses. IFN γ -independent T cell responses to mycobacterial antigens have been described (FIG. 3). For example, Cambodian patients with pulmonary TB who remained anergic to PPD following treatment completion displayed tuberculin antigen-specific T cell responses *in vitro* that were characterized by the production of IL-10 rather than IFN γ ^{126,127}. IL-10, which is known for its anti-inflammatory role in mitigating T_H1 cell responses to minimize the potentially deleterious effects of TNF and IFN γ , is mostly implicated in the immune response to TB, and no evidence exists for a protective role of IL-10 in the early stages of *M. tuberculosis* infection¹²⁸. However, a genotyping study in Ghana found that individuals with the *IL10* promoter haplotype with the highest association with low circulating levels of IL-10 were more likely to have TB or be PPD-positive than to be PPD-negative¹²⁹. Whether high IL-10 levels in these individuals truly confer resistance to infection, as opposed to PPD-specific anergy in the presence of LTBI, remains to be determined.

Additional CD4⁺ T cell subsets, including T_H17 cells, could contribute to the resister phenotype through IFN γ -independent mechanisms. In mice, the production of IL-17A, one of several T_H17-related cytokines, recruits neutrophils and other inflammatory cells to the lung and is involved in granuloma maturation¹³⁰. Adoptive transfer of BCG-specific T_H17 cells harvested from immunized IFN γ -deficient mice into recipient V(D)J recombination-activating protein (RAG)-deficient mice gave these recipient mice a survival advantage when challenged with *M. tuberculosis*, which was comparable to that seen with transfer of T_H1 cells from IFN γ -competent mice¹³¹. This IFN γ -independent partial protection provided by T_H17 cells against *M. tuberculosis* was also seen in another adoptive transfer model¹³². Unlike IL-10, IL-17 is not known to directly interfere with IFN γ production, and whether these IFN γ -independent T_H17 activities are relevant to the resister phenotype is unknown. However, taken together, these examples illustrate that despite having persistent negative responses in PPD skin reactivity tests and IGRAs, it is possible that resisters have other mycobacteria-specific MHC class I-restricted or MHC class II-restricted T cell responses.

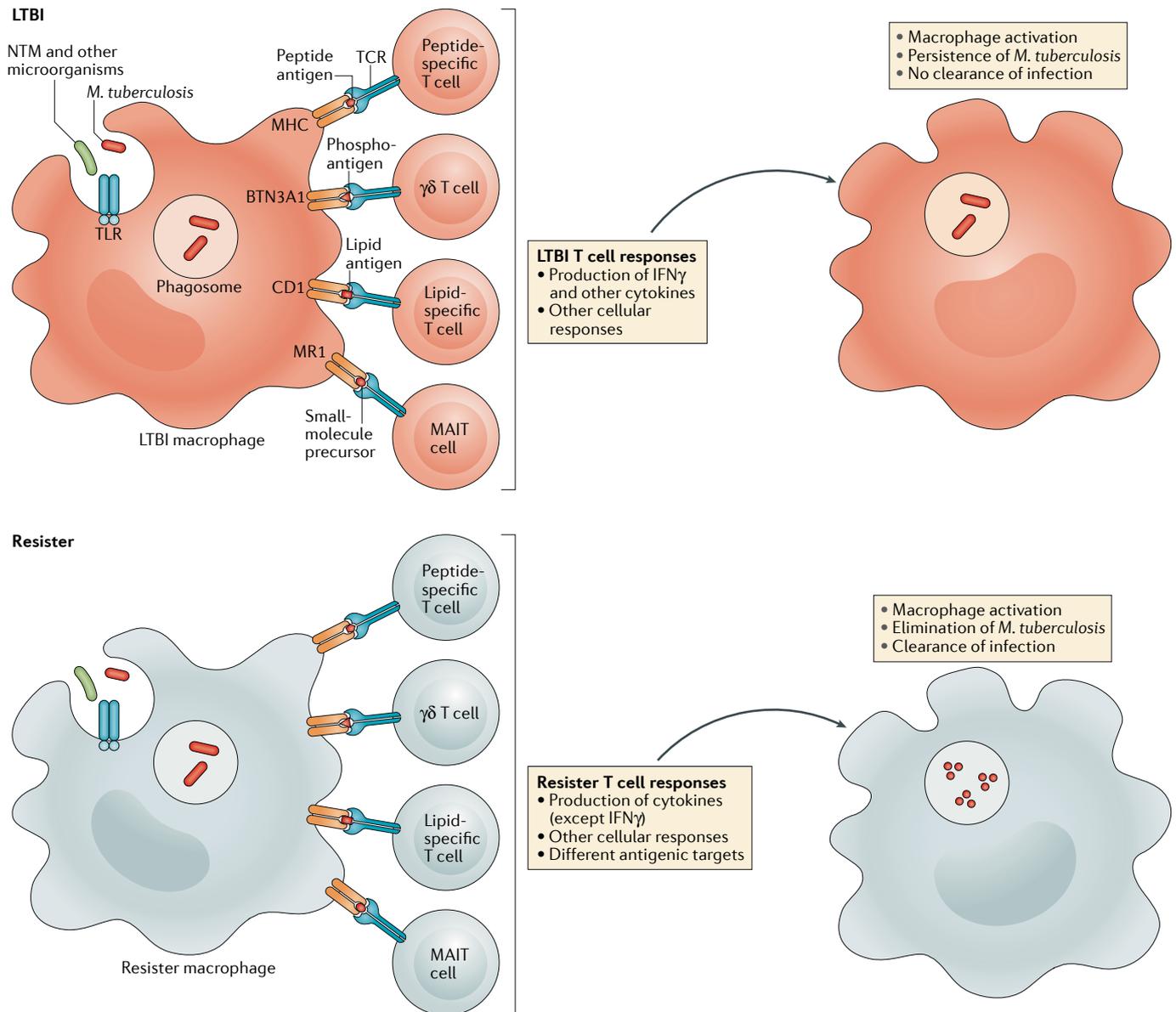


Fig. 3 | T cell-mediated resistance to *Mycobacterium tuberculosis*. After phagocytosis of *Mycobacterium tuberculosis* by macrophages and dendritic cells (not shown), various T cell responses are stimulated, including conventional MHC-restricted responses (peptide-specific T cells) and MHC-independent responses ($\gamma\delta$ T cells, which detect phosphorylated prenyl metabolites (also known as phosphoantigens)), CD1-restricted (lipid-specific) T cells and MHC class I-related gene protein (MR1)-restricted T cells (also known as mucosal-associated invariant T (MAIT) cells); MR1 presents small molecules, such as vitamin B₂ derivatives. Production of IFN γ by CD4⁺ T cells is a hallmark of the T cell response in individuals with latent *M. tuberculosis* infection (LTBI), whereas by definition, ‘resisters’ lack this IFN γ response. Qualitative differences in T cell responses between resisters and individuals with LTBI lead to the production of different macrophage-activating cytokines and chemokines, resulting in either the elimination of *M. tuberculosis* by resisters or persistence of *M. tuberculosis* in individuals with LTBI. BTN3A1, butyrophilin subfamily 3 member A1; NTM, non-tuberculous mycobacteria; TCR, T cell receptor; TLR, Toll-like receptor.

Unconventional T cell responses. In addition to mycobacterial protein antigens, T cells also recognize non-protein mycobacterial antigens (FIG. 3). For example, T cells are activated by mycobacterial cell wall lipids that are bound to CD1 proteins, which are homologous to MHC class I molecules but are functionally non-polymorphic^{40,133,134}. The human CD1 locus encodes four proteins (CD1A–CD1D) that are localized at the cell surface and present lipid antigens to T cells. In a

cross-sectional study of South African adolescents with or without LTBI, there were low intra-donor correlations between T cell responses to protein and lipid antigens¹³⁵, indicating that T cell responses to lipid antigens are not redundant with those to protein antigens and that the responses to the different antigens could be complementary. Immunity to lipid antigens could be primed by exposure in early life to non-tuberculous mycobacteria, which could facilitate clearance of *M. tuberculosis*

following infection, thus limiting the initiation of peptide-specific T cell responses that are mostly responsible for producing a positive result in the PPD skin test. In addition, lipid-specific T cells could also be primed by vaccination with BCG. Functionally, T cells specific for mycobacterial lipids have been shown to have a T_H1 cell cytokine-producing profile *ex vivo*, supporting their role in the clearance of *M. tuberculosis*-infected cells¹³⁶.

The MHC class I-related gene protein (MR1) antigen-presenting molecule presents small molecules instead of peptides or lipids to T cells¹³⁷ and is important for the development of mucosal-associated invariant T (MAIT) cells^{138–140}. MAIT cells can kill *M. tuberculosis*-infected cells in an MR1-dependent manner⁴¹, and *Mr1*-knockout mice have a higher lung burden of *M. bovis* BCG than wild-type mice¹⁴¹. Furthermore, in candidate-gene association studies, *MR1* and *CD1* polymorphisms were associated with susceptibility to TB^{142,143}. Finally, $\gamma\delta$ T cells are a T cell lineage that express a heterodimeric T cell receptor and are activated by phosphorylated prenyl metabolites^{144,145}. V γ 9V δ 2 T cells are activated by BCG-infected cells and can lyse BCG-infected target cells¹⁴⁶.

Thus, in PPD-negative individuals, the CD1-specific, MR1-specific and $\gamma\delta$ T cell populations might provide cellular immunity against *M. tuberculosis* that is independent of the IFN γ production by PPD-specific CD4⁺ T cells that defines LTBI. Further study is required to determine whether these other populations of T cells protect against *M. tuberculosis* infection in resisters or, alternatively, whether these T cells prevent progression

of LTBI in individuals who are unable to produce IFN γ in response to *M. tuberculosis* antigens.

B cell-mediated resistance

Antibodies are powerful mediators of protective immunity against many infectious diseases, and stimulating their production is the goal of vaccination strategies^{147,148}. In *M. tuberculosis* infection and disease, however, the role of humoral immunity is probably complex and remains unclear^{149,150}. Passive transfer of antibodies has not consistently conferred protection^{151–154}, and immunoglobulin G (IgG)-deficient individuals do not exhibit reduced control of bacterial infections¹⁵⁵. By contrast, a post hoc analysis of the MVA85A vaccine trial found that elevated Ag85A-specific IgG titres correlated with protection against TB¹⁵⁶. In addition, depletion of B cells in a non-human primate model of *M. tuberculosis* infection resulted in increased lesional bacterial burden¹⁵⁷. Furthermore, in patients with active TB, B cell function is initially abnormal but returns to normal after successful treatment^{158,159}. These observations highlight the immunoregulatory role of B cells in controlling *M. tuberculosis* infections, which extends beyond their direct antibody-mediated effector functions (FIG. 4).

Antibodies and latent *Mycobacterium tuberculosis* infection. Antibodies, plasma cells and antibody-responsive innate immune cells that express crystallizable fragment (Fc) receptors are abundant in *M. tuberculosis* granulomas^{160,161}, indicating that these

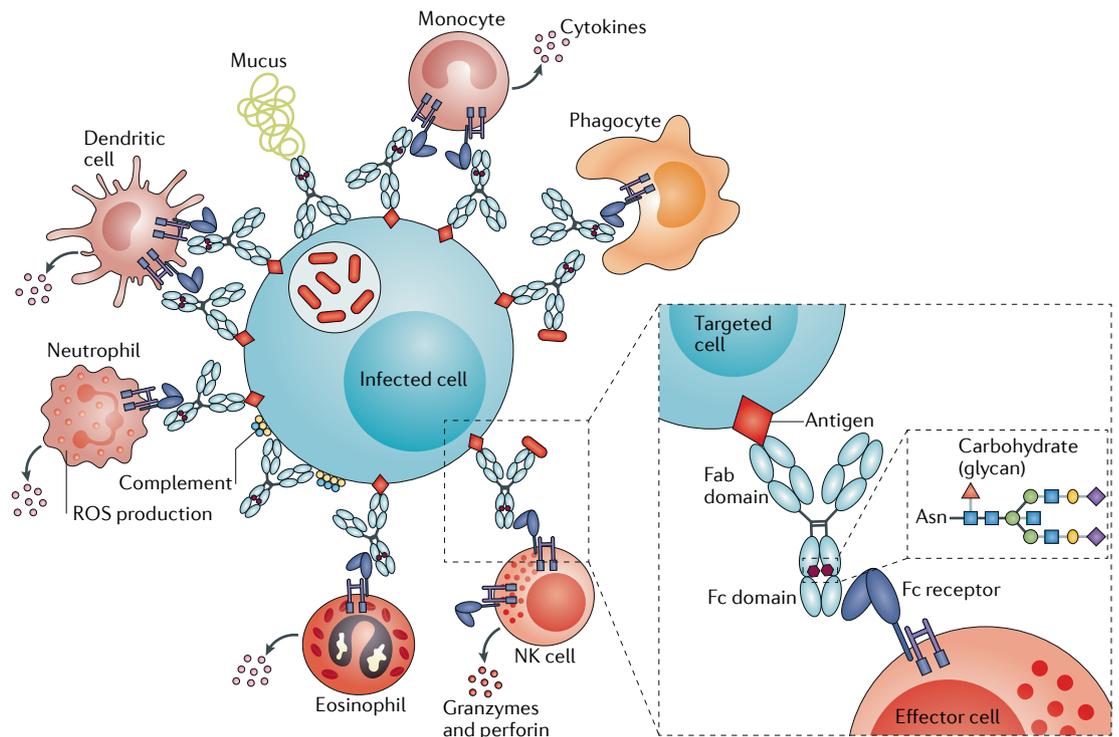


Fig. 4 | **Antibody-mediated resistance to *Mycobacterium tuberculosis*.** Beyond their role in clearing pathogens, antibodies also direct the rapid destruction of infected cells via the recruitment of innate immune cells (such as phagocytes) that express crystallizable fragment (Fc) receptors. Two modifications to the Fc domain of an antibody control its affinity for Fc receptors, namely, changes in the antibody subclass or isotype and its glycosylation. Fab, antigen-binding fragment; NK cell, natural killer cell; ROS, reactive oxygen species.

innate immune cells are actively recruited and might potentially contribute to antimicrobial activity^{162,163}. Distinct Fc effector profiles were observed in individuals with LTBI versus those with active disease. Individuals with LTBI had improved antibody functionality that was linked to elevated binding to the low-affinity immunoglobulin- γ Fc region receptor III-A (Fc γ R3A)¹⁶⁴, which is highly expressed by natural killer cells, neutrophils, mature macrophages and dendritic cells¹⁶³. Furthermore, these antibodies not only improved innate immune cell activity but also contributed to the restriction of intracellular survival of *M. tuberculosis* in previously infected primary macrophages. Interestingly, this differential functional activity was not related to altered subclass selection but was instead related to substantial changes in antibody glycosylation among the *M. tuberculosis*-infected individuals¹⁶⁴.

Antibodies and the resister phenotype. It is unclear whether antibodies contribute to the resister phenotype, but it is plausible that at the time of repeated *M. tuberculosis* exposure that pre-existing antibodies might direct the antimicrobial activity of macrophages, neutrophils or dendritic cells to eliminate the bacteria more effectively. Although in vitro studies indicate a limited role for antibody-mediated opsonophagocytic prevention of macrophage infection by *M. tuberculosis*, the addition of pooled IgG derived from individuals with LTBI to previously infected healthy donor primary macrophages can drive bacterial elimination by a process that probably involves activation of the inflammasome¹⁶⁴. These results suggest that, in addition to their minor role in limiting initial infection through opsonization, antibodies ‘cure’ infected macrophages if they are present at the right time and place. Although the majority of healthy donor macrophages restricted *M. tuberculosis* survival in the presence of pooled IgG from individuals with LTBI¹⁶⁴, the level of restriction varied by macrophage donor, which suggests that antibody-mediated killing of *M. tuberculosis* is also dependent on factors that are intrinsic to innate immune cells.

Thus, whether natural or affinity-matured humoral immunity exists among resisters is unknown, but humoral immunity might provide an exciting novel immunological axis that links the adaptive and innate immune responses to target and eradicate *M. tuberculosis* after exposure and warrants further exploration.

Conclusions and outlook

Historically, significant breakthroughs in treating infectious diseases have been made by studying mechanisms of resistance rather than susceptibility to infections. The most notable historical example is the observation that milkmaids exposed to cowpox were resistant to smallpox¹⁶⁵. A more contemporary example is the HIV resistance of individuals with a variant of CCR5, CCR5 Δ 32 (REFS^{166,167}). Both these observations led to transformative therapeutic interventions — the smallpox vaccine and CCR5 inhibitors, respectively¹⁶⁸.

For much of the past century, many investigators have postulated that some individuals are naturally resistant to *M. tuberculosis* infection in the face of

intense exposure. However, a number of challenges have impeded the elucidation of potential resistance mechanisms, including the limitations of using an indirect immunological test to define the resister phenotype and the need to incorporate measurements of exposure intensity in study designs. Despite this, contemporary epidemiological evidence supports the existence of resisters. Genome-wide association analysis of data from independent studies has identified loci that are associated with resistance to *M. tuberculosis* infection. The elucidation of immunological and genetic mechanisms of the resister phenotype is at an early stage, but these studies might reveal heterogeneous protective responses that are relevant to different types of resisters. For example, macrophage-dependent pathways that prevent bacterial uptake or rapidly clear *M. tuberculosis* before the development of an adaptive immune response define innate resisters. Alternatively, adaptive resisters are individuals in which T cell and B cell effector functions eliminate or restrict *M. tuberculosis* infections, either independently of IFN γ production or through priming by non-protein antigens. Further study is required to clarify whether adaptive resisters have greater resistance to progression to active TB than individuals with traditionally defined LTBI; however, these studies could identify novel correlates of immune protection or aid current prognostic efforts to stratify those individuals who are most at risk of disease progression^{4,5}.

Looking ahead, integrated research approaches using advanced epidemiological, genetic and immunological tools will be required to dissect mechanisms of resistance. In future studies, features of an optimally defined resister should incorporate several variables outlined in BOX 1, including, first, indices of exposure (both intensity and duration) using, at a minimum, validated epidemiological risk scoring^{12,13}; second, the use of both the PPD skin reactivity test and the IGRA to avoid misclassification bias resulting from discordant immunological tests; and third, longitudinal sampling for serial LTBI testing.

Epidemiological risk scores must include an assessment of the nature of contact between the index case or cases and the exposed individual (that is, whether the index case is the primary caregiver and the frequency and proximity of contact) and some assessment of infectivity of the exposure environment (for example, whether the index case is coughing, whether their sputum is smear-positive for *M. tuberculosis* and whether there are multiple index cases within a household or employment setting). Although not yet ready for practical implementation, more advanced assessments of infectious risk, such as aerobiological environmental sampling where transmission risk has been correlated with the degree of ventilation and with the number of viable bacteria sampled from cough aerosols, could be incorporated in future studies^{37,169,170}.

Even in areas of high TB endemicity, some level of discordance exists between the results of PPD skin reactivity tests and IGRAs¹⁷¹. Consequently, individuals with discordant results in the PPD skin reactivity test and the IGRA are not categorized as resisters, although retaining clinical samples from these individuals is important to facilitate studies to better understand the mechanistic

basis of this discordance. As the resister category might encompass multiple biological phenotypes, at this early stage in their characterization, we favour more stringent criteria to avoid misclassification, which justifies the increased expense and logistical complexity of implementing multiple testing modalities (PPD skin reactivity tests and IGRAs) and serial testing. Longitudinal sampling should include, at a minimum, multiple LTBI diagnostic tests in the year following initial exposure, as most but not all conversions occur in the first 3 months following exposure³⁶. In circumstances in which resisters remain in regions of high *M. tuberculosis* transmission, follow-up testing may be warranted to ensure that any new conversions owing to community exposures are detected.

Advances in the past decade have transformed genetic methods and platforms, which now include affordable and standardized genome-wide detection of polymorphisms. These tools provide assessment of polymorphisms that are annotated in public databases. Whole-genome sequencing is also more affordable and can be used to examine resisters for unidentified polymorphisms that are not annotated in public databases. To address limitations in the availability of patient samples,

new advances in stem cell work (for example, induced pluripotent stem cells derived from peripheral blood mononuclear cells) have made possible the creation of permanent cell lines from individuals, which can be differentiated into different cell types that are more relevant for studying *M. tuberculosis* pathogenesis (for example, alveolar macrophages). Finally, if *M. tuberculosis* cultures from index cases are available, comparisons of strain type, host phenotype (resister versus TB) and host genotype, as well as determination of whether transmission events are sympatric or allopatric¹⁷², are exciting avenues of investigation.

The integration of rigorous epidemiological phenotyping with mechanistic studies that investigate global immune pathways in macrophages, T cells and B cells, and how these pathways might differ between resisters and individuals with LTBI, should provide further biological insight into the mechanisms of early clearance or prevention of infection by *M. tuberculosis*. These potential biological mechanisms should provide targets for drugs and vaccines that could improve specific immune functions and improve therapeutic options for treating TB.

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Author contributions

J.D.S., C.S. and T.R.H. contributed to the drafting and editing of all sections. Focused contributions were made to the Epidemiology and Genetics sections (C.M.S., E.S. and G.C.), the B cell-mediated resistance section (G.A.) and the Macrophage-mediated resistance section (M.C.). Initial figure drafts were provided by J.D.S., M.C. and G.A. Detailed review and editing were additionally provided by S.F., R.S.W., W.H.B. and H.M.-K.

Competing interests

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