OPINION

In search of a new paradigm for protective immunity to TB

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Abstract | Clinical trials of vaccines against *Mycobacterium tuberculosis* are well under way and results are starting to come in. Some of these results are not so encouraging, as exemplified by the latest Aeras-422 and MVA85A trials. Other than empirically determining whether a vaccine reduces the number of cases of active tuberculosis, which is a daunting prospect given the chronic nature of the disease, we have no way of assessing vaccine efficacy. Therefore, investigators seek to identify biomarkers that predict vaccine efficacy. Historically, focus has been on the production of interferon-γ by $CD4^+$ T cells, but this has not been a useful correlate of vaccine-induced protection. In this Opinion article, we discuss recent advances in our understanding of the immune control of *M. tuberculosis* and how this knowledge could be used for vaccine design and evaluation.

Tuberculosis (TB) is caused by the pathogenic bacterium *Mycobacterium tuberculosis*, which is transmitted between people via aerosol droplets that contain bacteria. The droplets are inhaled and deposited in distal lung alveoli[1] (FIG. 1). *M. tuberculosis* is an intracellular bacterium and, although it can infect different cell types, alveolar macrophages are its 'favourite' niche. The initial stages of infection are characterized by innate immune responses that involve the recruitment of inflammatory cells to the lungs[2]; induction of an adaptive immune response occurs only later, after the dissemination of *M. tuberculosis* to draining lymph nodes[3–5]. In the lymph nodes, presentation of bacterial antigens by dendritic cells leads to priming and expansion of antigen-specific T cells, which differentiate from naïve T cells into effector T cells. The effector T cells then migrate to the infected lung and, in combination with other leukocytes, stimulate the formation of granulomas. Granulomas are organized structures that contain macrophages, lymphocytes and fibroblasts[6]. Within the granuloma, macrophages are activated — for example, by interferon-γ (IFNγ) secreted by $CD4^+$ T cells (that is, T_H1 cells) — which is thought to restrict the dispersal and replication of *M. tuberculosis*.

Although the human immune system can control the infection, control does not invariably lead to sterilization. In fact, most people who are infected with *M. tuberculosis* are clinically asymptomatic, which is a state that is referred to as latent TB[7]. These latently infected people — who are estimated to make up one-third of the global population — represent an enormous reservoir of potential disease. Epidemiological studies find that 5–10% of people with latent TB will develop active disease sometime during their lives[8]. Individuals with active TB cough and generate infectious droplets that propagate the infection (FIG. 1).

An effective vaccine is needed to stop the ongoing pandemic. Bacille Calmette–Guérin (BCG), which is an attenuated form of *Mycobacterium bovis*, was introduced nearly a century ago as a vaccine against *M. tuberculosis*, but it has had little effect on eliminating TB. In part, this is because BCG efficacy against active pulmonary TB is extremely variable between populations and BCG-induced protection is significantly lower in the developing world[9]. Remarkable progress has been made in the development of new vaccine candidates and several are now in clinical trials (BOX 1). Although there is some pessimism about whether a vaccine that averts infection can be developed, the general consensus is that a vaccine that prevents progression to active disease could reduce the prevalence of pulmonary TB and ultimately break the cycle of transmission.

Most antiviral vaccines that have proven to be effective are based on antibody-mediated immunity. As is the case for many intracellular bacteria, *M. tuberculosis* is able to avoid most of the antibacterial effects that are mediated by antibodies by living and growing inside macrophages. Thus, on the basis of the substantial experimental foundation that T cell immunity is required to control primary *M. tuberculosis* infection, the consensus among vaccine specialists is that vaccine-induced T cell-mediated immunity will be required to prevent clinical TB. However, despite considerable advances in defining how the immune system responds to *M. tuberculosis*, our understanding of protective immunity following infection (that is, natural immunity) is incomplete. Furthermore, little is known about the mechanisms of vaccine-induced immunity and whether it differs from natural immunity, and studies to answer these questions have not kept pace with the speed with which new vaccines are entering clinical trials. It is unknown which immunological parameters or biomarkers predict who will control the infection and who will develop clinical disease, both in the setting of natural immunity and in the setting of vaccine-induced immunity. Such knowledge would revolutionize our approach to the surveillance, control and treatment of TB and would greatly accelerate vaccine design and evaluation. However, identifying biomarkers of vaccine protection is difficult; until there is a successful vaccine that induces protective immunity, how can such a biomarker be identified? As it stands, any success or failure of TB vaccines will mostly be empiric and difficult to predict.

In this Opinion article, we discuss immune defences against *M. tuberculosis* infection. T cells predominantly mediate protective immunity, and recent results

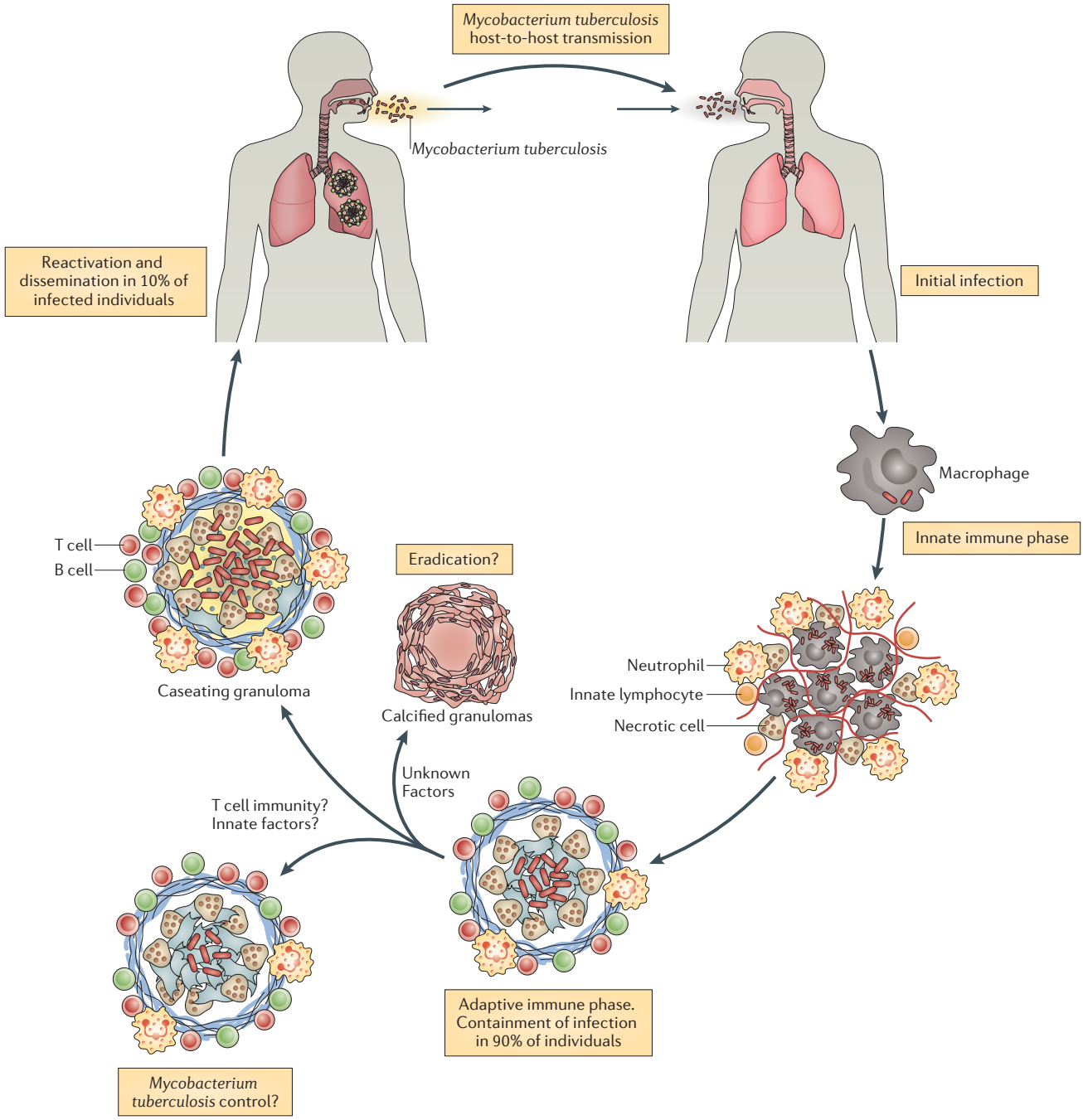


Figure 1 | **TB pathogenesis.** Infection is initiated by the inhalation of aerosol droplets that contain bacteria. The initial stages of infection are characterized by innate immune responses that involve the recruitment of inflammatory cells to the lung. Following bacterial dissemination to the draining lymph node, dendritic cell presentation of bacterial antigens leads to T cell priming and triggers an expansion of antigen-specific T cells, which are recruited to the lung. The recruitment of T cells, B cells, activated macrophages and other leukocytes leads to the establishment of granulomas, which can contain *Mycobacterium tuberculosis*. Most infected individuals will remain in a 'latent' state of infection, in which no clinical symptoms are present. A small percentage of these people will eventually progress and develop active disease, which can lead to the release of *M. tuberculosis* from granulomas that have eroded into the airways. When individuals with active tuberculosis (TB) cough, they can generate infectious droplets that transmit the infection.

begin to clarify how different T cell subsets and functions restrict bacterial growth. Finally, we discuss how one might use knowledge about these different mechanisms to develop new vaccine strategies for the prevention of TB.

The 'central dogma' of protective immunity

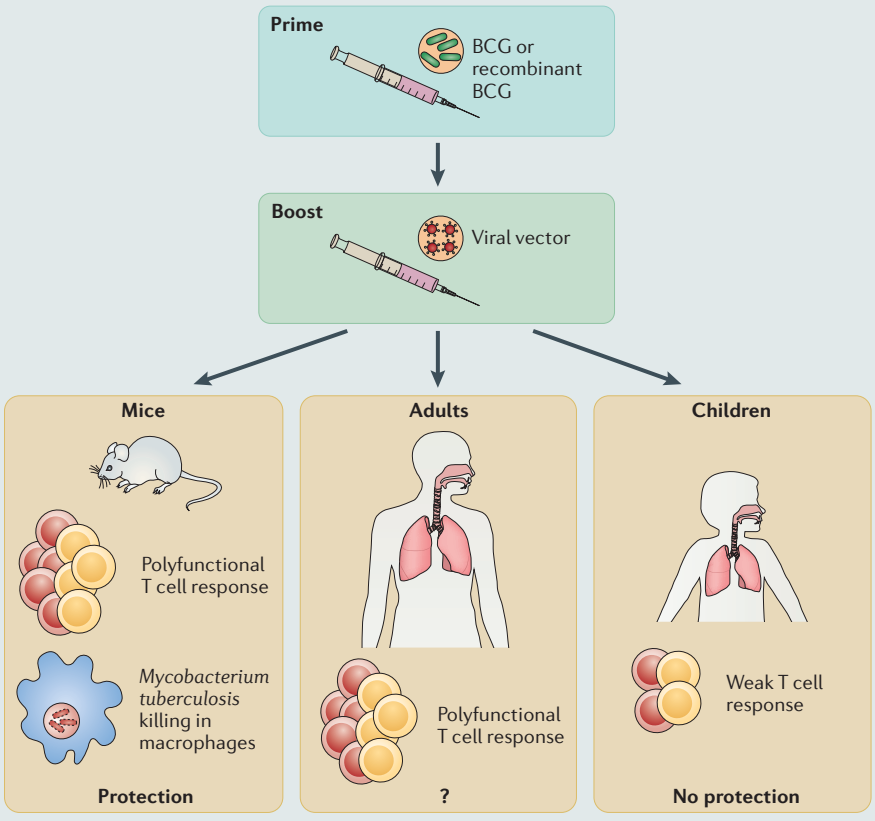
Establishing the importance of IFNγ. During the past four decades, the predominant paradigm in both basic and clinical research has been that the production of IFNγ by $CD4^+$ T cells is the major driver of immunity to TB. Research in the 1970s found that T cells, and not antibodies, are required for host resistance to TB and established the mouse as a useful model of tuberculosis[10]. The T cell hypothesis was further refined in the 1980s with the identification

Box 1 | **Tuberculosis vaccines**

Owing to the shortcomings of bacille Calmette–Guérin (BCG) vaccination in preventing tuberculosis (TB), considerable effort has been put into developing new vaccines. More than 12 candidate vaccines are currently being tested in clinical trials[136,137]. These candidate vaccines aim to replace BCG or to function as booster vaccines following BCG. The vaccines include viral vectors that express *Mycobacterium tuberculosis* antigens, *M. tuberculosis* proteins with improved adjuvants, recombinant BCG strains and live attenuated *M. tuberculosis* vaccines. Unfortunately, some preliminary results have been disappointing: Aeras-422 — which is a recombinant BCG strain — failed owing to safety concerns[136], and MVA85A — which is a new vaccine that consists of modified vaccinia ankara virus (MVA; a replicative-defective variant of vaccinia virus) that expresses the *M. tuberculosis* antigen 85A — showed no efficacy at enhancing BCG-induced protection in a Phase IIb trial[138].

MVA85A has been extensively investigated as a booster following BCG vaccination, in what has become known as the 'prime-boost' strategy (see the figure). MVA85A is effective in boosting BCG vaccination in a variety of *M. tuberculosis* animal challenge models. Initial studies with MVA85A in people showed promise, as significantly more antigen-specific T cells from the boosted group secreted interferon-γ (IFNγ) and were polyfunctional compared with those from people who were vaccinated with BCG alone[139,140]. These effects were durable and lasted at least 24 weeks after the MVA85A boost[139]. However, the recent results of the Phase IIb clinical trial indicate that MVA85A is not effective at preventing *M. tuberculosis* infection or tuberculosis[138]. Administered to infants aged 4–6 months as a booster to the BCG vaccination that is given at birth, MVA85A elicited overall small numbers of $CD4^+$ T cells that secrete IFNγ, interleukin-2 (IL-2) and tumour necrosis factor (TNF) at 28 days after vaccination. Although slightly greater T cell responses were noted in the vaccinated group, no differences in protection from TB were observed in a 2 year follow-up[138].

A recurring question is whether the cytokines that were measured in these studies are useful predictors of vaccine protection or whether there are specific markers that could have predicted a lack of protection. Another issue is whether the immature immune systems of infants compromise potential vaccine efficacy. These findings raise the question of whether MVA85A should be evaluated in adults.



of $CD4^+$ T cells that produce IFNγ (that is, T_H1 cells) as the dominant T cell subset that participates in the immune response to *M. tuberculosis*[11,12]. The use of knockout mice in the 1990s established a crucial role for $CD4^+$ T cells, with additional roles for $CD8^+$ T cells, invariant natural killer T (iNKT) cells and γδ T cells[13,14]. The discovery that AIDS — a condition that is often associated with TB — was caused by HIV, which is a virus that infects and kills $CD4^+$ T cells, supported a key role for $CD4^+$ T cells in immunity against *M. tuberculosis* in people[15].

A central role for IFNγ — a cytokine that is involved in the response against viruses and intracellular bacteria — in antimycobacterial immunity is based on the extreme susceptibility of mice that lack IFNγ[16,17]. IFNγ activates macrophages to kill intracellular bacteria by activating downstream antimicrobial effector pathways, including inducible nitric oxide synthase (iNOS), IFNγ-inducible GTPases, phagosomal maturation and acidification, autophagy and vitamin D receptor signalling[18–23]. Genetic studies confirm a role for IFNγ in people: families with mutations in the interleukin-12 (IL-12)–IFNγ–signal transducer and activator of transcription 1 (STAT1) axis develop disseminated infections caused by BCG and non-tuberculosis mycobacteria (NTM) species. This inherited susceptibility, which is called Mendelian susceptibility to mycobacterial disease (MSMD), reveals the crucial nature of this signalling pathway, which was first described in mice[16,17,24,25].

These discoveries helped to define the 'central dogma' of TB immunity, namely, that the production of IFNγ by T cells activates macrophages to kill intracellular *M. tuberculosis* (FIG. 2a). Indeed, detection of IFNγ produced by T cells is the most widely used method for monitoring immune responses following infection or vaccination.

Shortcomings of the central dogma. Although IFNγ and $CD4^+$ T cells are key components of the immune responses against mycobacteria, the intricacies of immunity to *M. tuberculosis* require that we reassess their roles. For example, the risk of active TB significantly increases during the first year after HIV infection, despite normal $CD4^+$ T cell counts[26], and progression to AIDS — which is characterized by a substantial loss of $CD4^+$ T cells — does not correlate with the development of active TB[26,27]. HIV infection induces several immunological abnormalities, some of which are apparent even before $CD4^+$ T cell numbers decline[28]. It is possible that alterations in $CD4^+$ T cell function that are secondary to HIV infection increase TB susceptibility even before $CD4^+$ T cell numbers decrease. However, this pattern of susceptibility is clearly different from other opportunistic infections, in which incidence correlates with the peripheral blood $CD4^+$ T cell count in HIV patients[29].

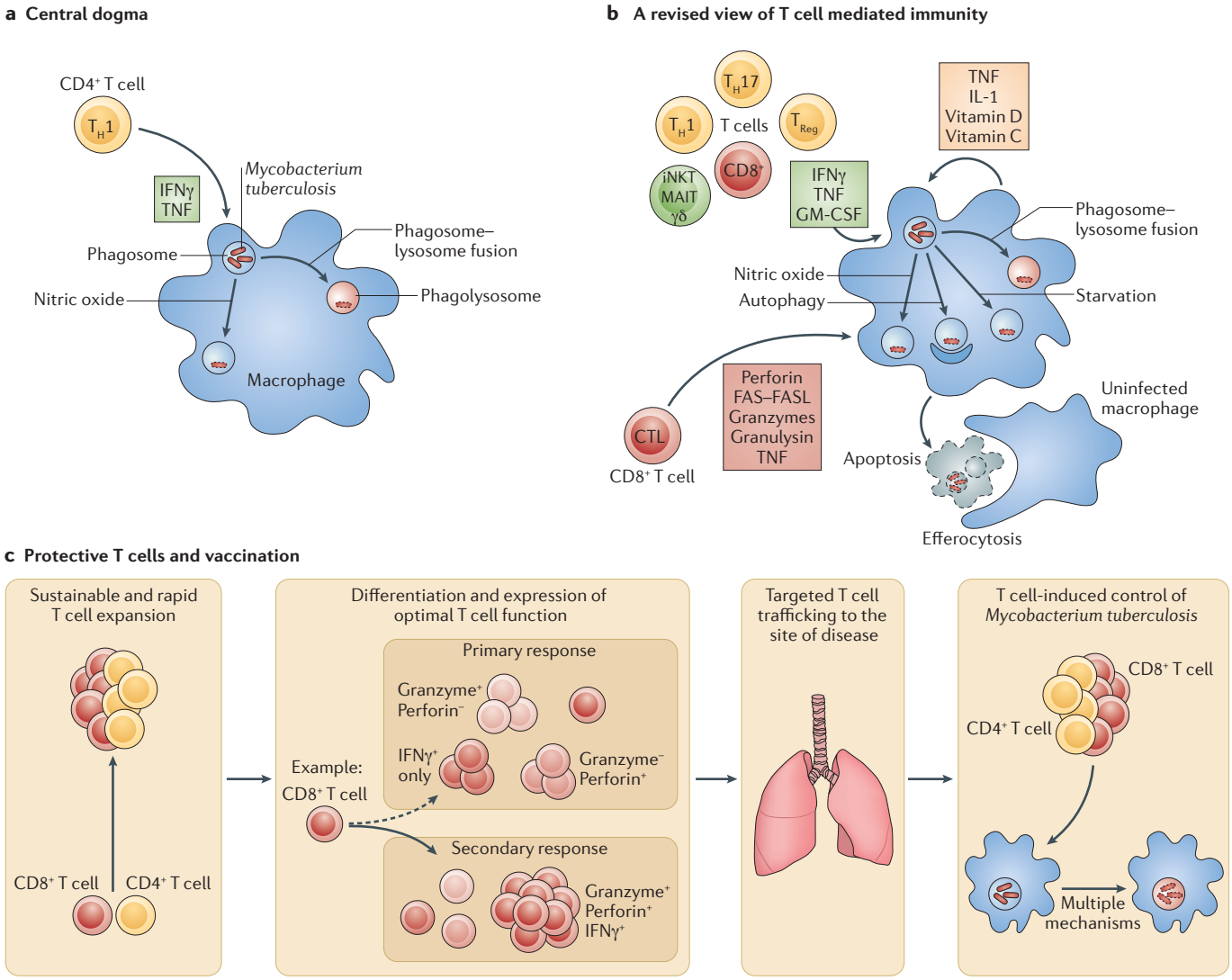


Figure 2 | **Paradigms of protective immunity to TB. a** | The 'central dogma' of protective immunity to tuberculosis (TB) is that $CD4^+$ T cells produce interferon-γ (IFNγ) (T helper 1 (T_H1) cells), which synergizes with tumour necrosis factor (TNF; produced by the T cell or the macrophage), and together these activate macrophage antimicrobial activity that is capable of restricting the growth of *Mycobacterium tuberculosis*. Two pathways activated by IFNγ that are capable of killing *M. tuberculosis* are nitric oxide production and phagosome–lysosome fusion, which acidifies the bacterial phagosome. **b** | 'A revised view of T cell-mediated immunity' incorporates additional T cell subsets ($CD4^+$ T cells, $CD8^+$ T cells and unconventional T cells: γδ T cells, mucosal-associated invariant T (MAIT) cells and CD1-restricted T cells) and includes additional mechanisms by which T cells mediate killing of *M. tuberculosis*. These include additional cytokines (for example, granulocyte–macrophage colony-stimulating factor (GM-CSF)) and cytolysis of infected macrophages. The cytolytic mechanisms vary and can include cytotoxic granules, which can deliver antimicrobial peptides, such as granulysin, but can also deliver granzymes, which can trigger apoptotic cell death. Cytotoxic T lymphocyte (CTL) activity mediated by FAS ligand (FASL)–FAS or TNF can also lead to apoptosis. Apoptosis can have a beneficial effect on the outcome of infection, as infected apoptotic cells can be engulfed by bystander macrophages, which are capable of destroying the apoptotic cells, including any intracellular bacteria. Finally, several components of the innate response, including interleukin-1 (IL-1) and vitamins, can synergize with cytokines that are produced by T cells. **c** | 'Protective T cells and vaccination' focuses on the desired features of protective T cell responses. Rational vaccine design should aim to elicit protective T cells by optimizing their action on infected cells in several ways. Vaccine-elicited memory T cells must rapidly expand and generate secondary effector T cells that undergo sustained proliferation following activation. Whereas the functions of primary effector T cells are heterogeneously expressed, vaccination can lead to more homogenous expression of effector functions during the recall response. Such T cells, which are often identified as multifunctional T cells, may have a greater protective potential. Primed effector and memory T cells should efficiently traffic to sites of infection, but the kinetics of the response must be balanced with respect to T cell subsets and limit the potential for T cell exhaustion, excessive inflammatory pathology or an ineffective response that hinders T cell–target contact.

Similar complexity is observed in patients with MSMD: more than 300 cases of MSMD have been described, but *M. tuberculosis* infection was present in only four cases; the rest were infections with BCG or NTM species[24]. Although such bias might reflect the relative exposure to BCG or NTM compared with *M. tuberculosis*, IFNγ-activated pathways might be more important for immunity against NTM than against *M. tuberculosis*[24,30]. Although these rare cases of TB and immunodeficiency are instructive,

most people who develop active TB have no obvious defects in their T cell compartment and generate *M. tuberculosis*-specific IFNγ responses. Thus, whereas HIV and MSMD patient data establish that T cells and IFNγ are required for immunity against *M. tuberculosis*, T cells that produce IFNγ do not seem to be sufficient to prevent active disease.

The shortcomings of the 'central dogma' also apply to disease progression and vaccine-induced protection in otherwise healthy people, as more T cells that secrete IFNγ, or greater IFNγ levels, do not correlate with protection[31]. In fact, patients whose T cells produce greater amounts of IFNγ are more likely to progress to active disease than patients with weaker responses[32], which supports the idea that IFNγ levels correlate better with bacterial burden than with disease control. Such a correlation between increased *M. tuberculosis* bacterial burden and increased T cell IFNγ production has been observed in humans, non-human primates (NHPs) and mice[32–35].

Similar conclusions can be drawn from vaccination studies[36]. BCG vaccination can elicit protective T cells in experimental animals, but IFNγ production by these T cells has not been predictive of vaccine-induced protection[36,37]. The only predictor of protection in mice that are vaccinated with BCG is an increased number of antigen-specific $CD8^+$ T cells[36]. In some human studies, increased IFNγ production by T cells has been observed after BCG vaccination or adult re-vaccination, but protection was not evaluated[38–41]. One study in South African infants who were vaccinated with BCG addressed the relationship between vaccine-induced protection, T cell frequency and cytokine profile but found no correlation between the number of BCG-elicited T cells that produce IFNγ or multiple cytokines (such as IFNγ, IL-2 and tumour necrosis factor (TNF)) and the development of culture-positive TB[42].

These data raise the question: if $CD4^+$ T cells and IFNγ are important, why doesn't IFNγ production by $CD4^+$ T cells correlate with protection? The idea that, because $CD4^+$ T cells produce IFNγ, their IFNγ production must be important is an assumption that has little supporting data. Several studies have shown that $CD4^+$ T cells protect mice against *M. tuberculosis* independently of IFNγ[43–47]. Transgenic $CD4^+$ T cells, which are specific for the *M. tuberculosis* antigen ESAT6 (6 kDa early secretory antigenic target) retain their ability to protect mice against *M. tuberculosis* even when they are unable to produce IFNγ or TNF[44]. Similarly, the ability of IFNγ-null memory T cells to mediate protection is only slightly diminished compared with wild-type memory T cells[45,48]. These studies show that, although $CD4^+$ T cells and IFNγ are important for *M. tuberculosis* control, T cell functions other than IFNγ production can mediate protection. Furthermore, it is not known how much IFNγ is needed, which cells are required to produce it and whether more is better[36]. Also, the inflammatory microenvironment in which IFNγ is produced might be important, as the balance between IFNγ and different cytokines, such as IL-10 and other T helper 2 (T_H2) cell cytokines, is likely to influence disease outcome[49]. Therefore, it is crucial to identify factors that are required for resistance and that correlate with susceptibility in individuals with intact immune systems, as opposed to components of the immune response that are necessary for protection but that do not predict clinical outcome or disease state.

The idea that IFNγ is necessary but not sufficient for bacterial control following mycobacterial infection is supported by multiple studies in mice. For example, several knockout mice (such as TNF-, granulocyte–macrophage colony-stimulating factor (GM-CSF)-, IL-1- and IL-6-null mice) die rapidly following *M. tuberculosis* infection, similarly to IFNγ-null animals[50–53]. As these mice produce IFNγ, their failure to control *M. tuberculosis* indicates that additional pathways besides IFNγ production are essential for immunity. Although the data from knockout mice and MSMD families is irrefutable, more mechanistic insights into the protective pathways that lead to *M. tuberculosis* control are needed. It is also important to remember that, although IFNγ inhibits *M. tuberculosis* replication in mouse macrophages[54], it is not sufficient to control *M. tuberculosis* growth in human macrophages[55,56]. Similarly, nitric oxide (NO) production by murine macrophages can kill *M. tuberculosis*, but its production by human alveolar macrophages and its role in controlling *M. tuberculosis* in these cells is controversial[57,58]. These observations reinforce the idea that we must look beyond the $CD4^+$ T cell–IFNγ central dogma (FIG. 2a) to identify other immunological functions that protect against *M. tuberculosis*.

Reassessing protective immunity

In recent years, many studies have looked past the central dogma and have shown that different pathways are involved in protective immunity during TB. These studies reveal characteristics of protective T cells that should be incorporated in the design of new vaccines against *M. tuberculosis* (FIG. 2b).

Other mediators that activate macrophages. The cytokines TNF, GM-CSF and IL-1β and vitamins C and D are all implicated as mediators that activate macrophages to control the growth of *M. tuberculosis*. Mice that lack TNF are highly susceptible to *M. tuberculosis* infection, and TNF production by T cells has been shown to be important for resistance against *M. tuberculosis*[50,59]. TNF synergizes with IFNγ in stimulating NO production by macrophages, maintaining granuloma structure and limiting immunopathology, possibly by modulating IL-10 levels, by inhibition of T_H2 cell responses and by limiting neutrophil infiltration[60–62]. The widespread use of TNF blockers to treat patients with autoimmune diseases for which TNF is a pathogenic factor, such as rheumatoid arthritis, has resulted in many cases of reactivated latent TB. This establishes TNF as an important mediator of resistance to *M. tuberculosis* in people[63].

Mice lacking GM-CSF are highly susceptible to *M. tuberculosis*, and GM-CSF treatment of mouse and human macrophages restricts the intracellular growth of *M. tuberculosis* and *Mycobacterium avium*[52,64,65,66]. GM-CSF is produced by a range of cells, including leukocytes, epithelial cells and fibroblasts, and loss of this cytokine leads to abnormalities in surfactant recycling and the development of a lung disease that resembles human pulmonary alveolar proteinosis[67]. Overexpressing GM-CSF in epithelial cells reverses these lung abnormalities, but susceptibility to *M. tuberculosis* remains, which suggests that GM-CSF production by other cells — perhaps T cells — contributes to protection in mice. This idea is supported by the observation that iNKT cell production of GM-CSF contributes to host resistance against tuberculosis[66]. In addition, the presence of GM-CSF-specific autoantibodies that block GM-CSF function has been linked to both cryptococcal meningitis and pulmonary TB in otherwise healthy people, which indicates that GM-CSF has an important role in host defences against infection in people[68].

Mice that lack IL-1β — a pro-inflammatory cytokine that is produced by macrophages — or its receptor are highly susceptible to *M. tuberculosis* infection, and IL-1β directly inhibits intracellular growth of *M. tuberculosis*[47,51,69–71]. Although mice that lack IL-1β die prematurely from infection, IL-1β can also be detrimental by recruiting pathogenic T_H17 cells and neutrophils to the lungs,

which results in tissue inflammation[46,72,73]. IL-1β also activates human macrophages to control bacterial replication[69,71,74].

Stimulation with either IFNγ or a ligand that triggers Toll-like receptor (TLR2) and TLR1 induces the nuclear vitamin D receptor (VDR) and enzymes that catalyse the conversion of vitamin D to its bioactive form[22,75]. Signalling through VDR elicits the production of the human cathelicidin LL-37, which is an antimicrobial peptide that directly kills *M. tuberculosis*[76]. Beyond its role in cathelicidin production, vitamin D is involved in autophagy, phagosome–lysosome fusion and IL-1β production[22,77,78]. Dissecting the role of vitamin D has been challenging. Multiple studies show decreased levels of bioactive vitamin D in patients with TB, but whether this is a cause or an effect of TB is unknown, and whether Vitamin D supplementation benefits treatment is still uncertain[79].

Vitamin C might be important for immunity against *M. tuberculosis,* as vitamin C affects *M. tuberculosis* survival and growth[80]. Given the established association between malnutrition and susceptibility to TB, it is important to determine whether specific nutritional deficiencies contribute to susceptibility to *M. tuberculosis*.

Killing of infected macrophages. In addition to cytokine production, T cells — particularly $CD8^+$ T cells — kill cells that they recognize as 'foreign'. $CD8^+$ T cells that have the capacity to kill target cells are called cytotoxic T lymphocytes (CTLs). *M. tuberculosis* elicits $CD8^+$ T cell responses in people and in animal models, and these $CD8^+$ T cells function as CTLs *in vivo*[81–84]. Three different molecular pathways mediate CTL activity: exocytosis of cytotoxic granules containing proteins that cause the lysis and apoptosis of target cells, such as perforin, granulysin and granzymes; FAS (also known as CD95) or FAS ligand (FASL; also known as CD95L), which are cell surface proteins that mediate death signalling; and TNF[84]. The increased susceptibility of $Fas^{-/-}$, $FasL^{-/-}$ and $perforin^{-/-}$ mice to *M. tuberculosis* corroborate the importance of these pathways for immunity[85,86]. Importantly, perforin is required for CTL-mediated protection[84]. Human $CD8^+$ T cells also require perforin to restrict *M. tuberculosis* growth, and granulysin is an important granule constituent[87]. Other than perforin, the crucial effector molecules for mouse $CD8^+$ T cells are unknown[87].

How killing of infected macrophages by $CD8^+$ T cells impairs *M. tuberculosis* survival is an active area of investigation. All three killing pathways induce target cell apoptosis, which is associated with reduced bacterial viability[88]. The engulfment of apoptotic, infected cells by uninfected macrophages — which is a process known as efferocytosis — leads to the rapid association of the bacteria trapped in the phagocytosed apoptotic cell (known as the 'efferosome') with lysosomes and the killing of *M. tuberculosis*[89].

T cells orchestrate granuloma formation. In addition to detecting infected macrophages, T cells have a key role in the formation of granulomas. T cell-derived cytokines (such as TNF) and chemokines (such as CC-chemokine ligand 3 (CCL3)) recruit inflammatory macrophages, neutrophils and B cells to the granuloma[90]. IFNγ and TNF maintain granuloma architecture in mice and people[17,63,91–93]. The importance of $CD4^+$ T cells in shaping the granuloma microenvironment is inferred from HIV-positive subjects who form dysfunctional granulomas that fail to contain *M. tuberculosis*[94] and from studies in guinea pigs and rabbits[95,96]. Recent imaging studies in people and NHPs indicate that granulomas function autonomously and are more dynamic than previously appreciated[8,97,98]. Granulomas change over time independently of each other with respect to size and metabolic activity: some shrink, whereas others expand. Although $CD4^+$ T cells promote granuloma formation early after *M. tuberculosis* infection, they also contribute to transmission by promoting granuloma necrosis accompanied by erosion into airways during later disease stages[99].

Balancing pro- and anti-inflammatory signals. In many chronic infections, including TB, immune-mediated tissue injury is more detrimental than the pathogen itself. Therefore, there are mechanisms to counter-regulate pro-inflammatory immune cells and prevent the harmful effects of excessive inflammation; however, these effects might also dampen protective immunity.

$Foxp3^+$ ($CD4^+CD25^+$) regulatory T cells (T_{Regs}) suppress inflammation and limit immune responses by producing immunosuppressive cytokines, such as IL-10 and transforming growth factor β (TGFβ), and by directly interacting with other cells via inhibitory cell surface molecules[100]. T_{Regs} are generated following *M. tuberculosis* infection in humans, NHPs and mice[101–104]. In mice, T_{Reg} elimination can improve protective immunity, as shown by the survival of fewer bacteria; however, whether this occurs at the risk of greater tissue injury has not been addressed[104–106].

Chronic antigen stimulation and exposure to inflammatory cytokines leads to a state of T cell exhaustion that is manifested by a progressive loss of T cell function over time, which has been best documented during chronic viral infection. There is great interest in the mediators of exhaustion, as blocking them might 're-invigorate' T cell immunity and promote pathogen clearance during chronic infection. One such mediator, programmed cell death 1 (PD-1), is a cell surface receptor that is expressed by antigen-activated T cells. Interaction of PD-1 with its ligands transduces a signal that inhibits T cell proliferation and cytokine production[107]. Disruption of the PD-1–ligand interaction, by the use of neutralizing antibodies or knockout of PD-1 in mice, increases the number and function of *M. tuberculosis*-specific T cells in the lungs of infected mice[108–110]. However, in the absence of PD-1 signalling, dysregulation of $CD4^+$ T cells leads to increased bacterial burden, the destruction of lung tissue and the death of infected mice[108,110]. These data suggest that T cell exhaustion might be a beneficial regulatory mechanism that prevents overt immunopathology.

Neutrophils have an early protective role against *M. tuberculosis* in the lungs by producing IL-1β, TNF, defensins, cathelicidins, lipocalin, NADPH oxidase and superoxides[76,111–113]. Neutrophils also participate in T cell priming, including cross-presentation of class I-restricted antigens, which is a process that is important for the stimulation of $CD8^+$ T cells by intracellular pathogens[114]. However, when the short-lived neutrophils die, the pro-inflammatory contents of their granules can be released; thus, an excess of neutrophils can promote tissue damage.

Although IFNγ is a pro-inflammatory cytokine, it also limits inflammation, at least in part by the direct and indirect inhibition of neutrophils. IFNγ can have anti-proliferative effects on T cells and modulate their function, including inhibiting $CD4^+$ T cell production of IL-17, which is a cytokine that drives neutrophilic inflammation[115]. In addition, IFNγ acts directly on neutrophils to inhibit their accumulation in the lung[46,72]. In fact, we view neutrophil infiltrates in the lung as a sign of failed T_H1 cell immunity, which leads to accelerated tissue destruction during chronic *M. tuberculosis* infection[46]. Similarly, NO restrains inflammation by inhibiting IL-1β production by macrophages. Whereas NO production by mouse macrophages mediates the antimicrobial activity of IFNγ, NO also inhibits

Box 2 | **A role for non-conventional T cells in immunity to tuberculosis**

iNKT cells

Invariant natural killer T (iNKT) cells are a T cell subset that recognize lipid and glycolipid antigens. Subjects with active tuberculosis (TB) have reduced iNKT cell numbers in the peripheral blood compared with latently infected or healthy individuals[145–147]. Treatment of infected mice with α-galactosylceramide (αGalCer), which is a potent activator of iNKT cells, improves disease outcome and synergizes with antibiotics[130,158]. αGalCer also stimulates human iNKT cells to lyse *Mycobacterium tuberculosis*-infected macrophages and kill intracellular bacteria *in vitro*[160]. Mouse iNKT cells cultured with *M. tuberculosis*-infected primary macrophages restrict bacterial growth, and the adoptive transfer of iNKT cells limits bacterial growth *in vivo*[155]. Activated iNKT cells also have adjuvant-like properties and conjugation of αGalCer to bacille Calmette–Guérin (BCG) augments its efficacy as a vaccine[161]. Why iNKT cells are dispensable in the intact mouse but exert a major protective role once activated needs investigation. Also, the use of αGalCer in human tuberculosis (TB) still has not been explored.

Group I CD1-restricted T cells

CD1-restricted T cells that recognize the mycobacterial lipid glucose monomycolate or C32-phosphomycoketide can be detected in the peripheral blood of patients with *M. tuberculosis*[148–150]. The effector functions of these T cells, and whether they can be elicited by vaccination, are still not fully understood.

γδ T cells

Human γδ T cells recognize small organic phosphate antigens and alkylamines and expand in response to *M. tuberculosis*-infected cells *in vitro*. Exciting data indicate that they generate a recall response following BCG vaccination and *M. tuberculosis* challenge in non-human primates (NHPs)[120,153,162,163]. Although γδ T cells are not required for bacterial control in mice, they are the main source of interleukin-17 (IL-17) in the lung during *M. tuberculosis* infection[13,164]. Activation of γδ T cells by IL-2 and phosphoantigen treatment results in reduced bacterial burdens and attenuated lesions in the lungs of NHPs that are infected with *M. tuberculosis*[159].

MAIT cells

Mucosal-associated invariant T (MAIT) cells, which are found in human lung and peripheral blood, recognize *M. tuberculosis*-infected cells[151,156]. MAIT cells can have antimicrobial activity against bacteria and yeast, but their role during *M. tuberculosis* infection still requires investigation[152].

T_H17 cells

IL-17 has an early role in the recruitment of antigen-specific interferon-γ (IFNγ)-secreting T helper 1 (T_H1) cells, particularly after BCG vaccination[163,165,166], as well as in early granuloma formation. However, persistence of T_H17 cells can be detrimental. IL-17 promotes neutrophil recruitment and inflammation and, if not ultimately suppressed by IFNγ, can exacerbate tissue damage[46,73,115,164].

Cell type	iNKT cell	Group I CD1-restricted T cell	MAIT cell	γδ T cell	T_H17 cell
Antigen presenting molecule	CD1d	CD1a, CD1b, CD1c	MR1	Several including butyrophilin[141]	MHC II
Antigen type	Lipids	Lipids	Riboflavin metabolites	Phosphoantigens	Peptides
Required for mouse *M. tuberculosis* immunity?	No[14,86,142,143]	Not found in mice	Unknown	No[13]	No[144]
Detected in *M. tuberculosis*-infected people?	Yes[145–147]	Yes[148–150]	Yes[151,152]	Yes[153]	Yes[154]
Respond to *M. tuberculosis*-infected macrophages?	Yes [66, 155]	Yes[148]	Yes[156]	Yes[153]	Yes[157]
Stimulation leads to improved antimycobacterial function?	Yes[130,158] (αGalCer)	Unknown	Unknown	Yes[159] (IL-2 and phosphoantigen)	Unknown

MHC II, major histocompatibility complex II; MR1, major histocompatibility complex class I-related gene protein.

NLRP3 inflammasome assembly, which curtails the production of IL-1β[116].

Collectively, these data support the idea that T cells are uniquely positioned to influence the balance of pro- and anti-inflammatory signals. These results strengthen the idea that the function of $CD4^+$ T cells and IFNγ is broader than activating macrophages and is necessary for optimal immunity during *M. tuberculosis* infection. Thus, IFNγ functions as a key negative regulator of innate immunity, including neutrophils and IL-1β, both of which might be beneficial early in *M. tuberculosis* infection but have detrimental effects if they persist into the chronic phase of infection. The anti-inflammatory role of T cells might prevent over-exuberant protective responses that cause harmful immunopathology and tissue damage during chronic infection[46,116]. Measuring surrogates of pro- or anti-inflammatory signals — for example, by expression profiling[117] or by measuring the monocyte/lymphocyte ratio[118] in peripheral blood — could help to identify individuals who are at risk of developing active TB.

Other cells participate in the immune response to M. tuberculosis. Although it is generally accepted that conventional $CD4^+$ and $CD8^+$ T cells mediate protection against *M. tuberculosis*, many other cell types participate in the immune response (see BOX 2 for the contribution of non-conventional T cells). The TB mouse model is $CD4^+$ T cell-centric and it is difficult to prove a role even for conventional $CD8^+$ T cells. Other T cell subsets are not present or are qualitatively different in the mouse compared to humans. Similarly, the contribution of B cells and antibody-mediated immunity needs further clarification[119]. Thus, these different cell types need to be investigated in other models. Both $CD8^+$ T cells and non-conventional T cells seem to have a quantitatively greater role in immunity to *M. tuberculosis* in NHPs than in other animal models[81,120]. Understanding the roles of these different cell types during *M. tuberculosis* infection might provide opportunities to discover new protective effector functions and to develop methods to augment their function as part of new vaccination or treatment strategies.

Lessons for developing T cell vaccines

Is natural immunity against TB sufficient? Many pathogens do not elicit protective immunity, including common pathogens that cause urinary tract infections (such as enteric Gram-positive and Gram-negative bacteria), pathogens that cause sexually transmitted infections such as *Chlamydia trachomatis*, *Neisseria gonorrhea* and *Treponema pallidum* and group A streptococci that cause pharyngitis. Other pathogens, such as poxviruses, induce long-term

protection — an observation that is the basis of vaccination. It is still unclear why some pathogens, but not others, induce protective immunity against reinfection. Which is the case for TB? If only around 10% of infected people develop active disease during their lifetime, one must concede that natural immunity works well, even if it doesn't lead to sterilization. What about the 10% who develop symptomatic disease? Although progression to symptomatic disease can sometimes be attributed to acquired immunodeficiency (caused by AIDS, TNF blockade, corticosteroids, autoantibodies etc.), in many cases, immunocompetent individuals also develop active TB, which indicates failure of their immune systems to control the infection. Why does the immune system fail to enforce latency and enable active disease to emerge in immunocompetent individuals?

People who have previously been treated for TB are at higher risk of developing additional episodes of disease[121–125]. Can this be attributed to relapse after inadequate treatment? Or do these individuals have a defect in immunity that might explain why they developed disease in the first place? If these are the patients who we are trying to protect with vaccination, we need to understand why they are susceptible to TB. This is important, as vaccines that aim to augment typical immune responses might fail to protect people with immune defects. Such people might not respond normally to vaccines or they might be resistant to their effects, suggesting that natural immunity in these individuals is defective.

As an example, after aerosol *M. tuberculosis* infection, C3HeB/FeJ mice develop necrotic granulomatous lesions and die rapidly. However, these mice have robust T cell responses[126,127]. The genetic basis for their susceptibility has been mapped to several loci, and the dominant locus, *Ipr1*, is preferentially expressed by macrophages and alters their death modality[128]. Macrophages that express the resistant allele of *Ipr1* are more prone to apoptosis following intracellular infection, whereas macrophages that express the susceptible allele undergo necrosis, which is associated with higher bacterial loads and more tissue destruction. This is an important insight as some people might develop active TB because their macrophages are unable to control intracellular *M. tuberculosis* growth, rather than because they have dysfunctional T cells. Similarly, an increase in type I and type II interferon-inducible genes is found in the peripheral blood of individuals with active pulmonary TB[117]. Surprisingly, this gene signature is mostly accounted for by changes in neutrophil gene expression. These data support the theory that, just as for macrophages, alterations in neutrophil functions can have an effect on disease susceptibility and progression. Thus, even vaccines that elicit strong T cell responses might not be effective at protecting such people from TB because their macrophages, neutrophils or other cell types cannot respond appropriately to the T cell signals. Without understanding why people are susceptible to disease, we cannot predict how to protect them.

Finally, instead of mimicking natural immunity, vaccine-induced protection against TB might require 'uncommon' or 'unnatural' immunity, as recently discussed by the Gates Foundation (grand challenges TB vaccine accelerator; see further information). For example, such unnatural protective immunity is induced after tetanus toxoid vaccination, but is not observed after natural infection with *Clostridium tetani*[129]. Examples of unnatural immunity to *M. tuberculosis* are iNKT cells (BOX 2). These cells are dispensable for protection against primary infection in immunocompetent mice, but their activation can prolong the survival of inbred strains of susceptible mice[130]. It might be possible to induce such unnatural or uncommon immunity — for example, by engineering BCG to express the bacterial toxins listeriolysin or perfringolysin, which alter the route of antigen presentation and lead to more efficient stimulation of $CD8^+$ T cells[131–133]. Incorporating into vaccine design such strategies that stimulate a broader immune response than current vaccines might have a greater effect on the induction of protective immunity than incorporating strategies that only stimulate a stronger response to a single antigen.

Quantity versus quality. The goal of vaccination is to elicit a population of long-lived memory T cells that, after *M. tuberculosis* challenge, will rapidly proliferate, acquire optimal effector functions, traffic to the lungs, recognize *M. tuberculosis*-infected cells, control bacterial replication and lead to sterilization (FIG. 2c). We assume that successful vaccination will elicit $CD4^+$ and $CD8^+$ T cells, which are specific for one or more mycobacterial antigen or antigens and whose functions will include IFNγ production (that is, a T_H1 cell response). However, T_H1 cell responses are unable to sterilize the host during active disease, and as we cannot define protective immunity, there is no way to measure successful immunization other than empirically quantifying changes in pathogen burden after challenge or, in people, after natural exposure — an approach that is slow and cumbersome.

For infections that can be prevented by humoral immunity, antibody titres correlate with protective immunity. For T cell-based vaccines, we are not sure whether the number of elicited T cells will correlate with protection or whether a change in one of the many functions that T cells perform will be a more useful measure. For example, re-exposure to antigen *in vivo* induces $CD8^+$ T cells to more frequently and persistently co-express effector molecules (such as perforin, granzyme A, granzyme B, FASL and IFNγ) and to more efficiently kill than $CD8^+$ T cells that are stimulated by the antigen the first time[134]. This suggests that an important function of T cell vaccination is to induce and coordinate the expression of effector molecules. Similarly, several different types of intermediate to long-term antigen-specific T cells (central, effector and tissue-resident memory cells) persist after infection or vaccination, with the potential to rapidly respond to infectious challenge[135]; however, it is unclear which population (or populations) are most effective in preventing TB.

Collectively, the data summarized in this Opinion article suggest that vaccines that elicit large numbers of T cells with the capacity to only produce IFNγ might not be optimal for protecting against TB. We should be looking for changes in T cell function, rather than numbers, as key factors that will lead to a substantial breakthrough in vaccine design. Furthermore, as several studies have shown different pathways to be involved in protective immunity against TB (such as those mediated by IL-1β, GM-CSF, vitamin C, vitamin D and cytolysis), vaccine design should aim at arming T cells with the capacity to modulate such pathways in cells that are infected with *M. tuberculosis* (FIG. 2c). Finally, we must avoid the trap of thinking that there is one type of T cell that will mediate protection alone. The host response to *M. tuberculosis* elicits many different types of T cells and, even if all of them do not kill *M. tuberculosis*, it is likely that they all have a role in orchestrating a successful immune response.

Conclusion

An important obstacle to vaccine development is our incomplete understanding of what constitutes protective immunity against *M. tuberculosis*. It is difficult to define the goals of vaccination without first knowing what the immune system is capable of. Although a vaccine that prevents infection is

everyone's first choice, the consensus seems to be that a vaccine that enforces latency and prevents transmission is a more realistic goal. However, would we feel differently if we understood why some people do not become infected despite repeated exposure? Or why some granulomas function autonomously, with some apparently able to control and eradicate *M. tuberculosis* and others not[98]?

This lack of knowledge supports our main suggestion for vaccine design: that characterization of additional immune mediators and cell types, even ones that seem to have minor roles during natural infection, is an essential first step. An effective vaccine might need to engage multiple immune mechanisms that are activated during a typical infection and might need to skew the host response in ways that are not seen during natural infection.

TB is a chronic disease, and *M. tuberculosis* evades detection by antibodies by occupying an intracellular niche. Thus, a vaccine that generates $CD4^+$ and $CD8^+$ central memory T cells with high proliferative potential, as well as a cohort of potent $CD8^+$ effector memory and resident memory T cells that are poised to rapidly kill infected cells in the lungs can be expected to be an ideal T cell vaccine candidate for disease prevention. Alternative approaches that — for example — stimulate unconventional T cell subsets and B cell and antibody responses alongside conventional T cells should be further investigated. However, we think that continuing to develop T cell vaccines aimed at boosting childhood BCG vaccination by solely varying the antigen will probably continue to fail. It is not enough to target specific antigens without a better understanding of how vaccines modulate T cell subsets and their functions and trafficking. IFNγ production by $CD4^+$ T cells is essential in certain situations but it will probably not be sufficient as a protective response after vaccination. We think that the premise that IFNγ production by $CD4^+$ T cells is required for protection during infection has never been conclusively shown, and the assumed role of IFNγ in vaccine-induced immunity is made on the basis of an over-interpretation of published data. In addition, we think that it is important to make vaccines that elicit multiple T cell subsets that express diverse protective functions. For example, we predict that a vaccine that elicits $CD4^+$ T cells that produce GM-CSF and IFNγ, and $CD8^+$ T cells that function as cytolytic effectors, in addition to producing IFNγ, would be more protective than vaccines that elicit only IFNγ. Finally, we must broaden the ways in which vaccine candidates are evaluated and the biomarkers that are used to measure their effects. Without defined correlates of protection, this will be challenging. Ongoing efforts to expand the ways in which vaccine candidates are evaluated and to embrace the diversity and heterogeneity of T cells need to be supported.

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Competing interests statement
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