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A broader evaluation of vaccine-induced T cell immunity against tuberculosis

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Although Bacillus Calmette-Guérin (BCG) vaccine, the only licensed vaccine against tuberculosis (TB), is the most widely used vaccine worldwide, TB is the second leading global killer from a single infectious agent responsible for over one million deaths annually. With the increasing threat of the emergence of drug-resistant TB, there is intense research toward better and more efficacious vaccines against TB. Indeed, TB vaccine research has blossomed in recent years: demonstration of sterilizing immunity against *Mycobacterium tuberculosis (Mtb)* challenge in non-human primates, the potential benefit of BCG revaccination in humans, and a phase IIb vaccine with ~50% efficacy against developing active disease. Consequently, several vaccines are set to begin phase 3 trials in 2024, and new candidates have entered phase 1 including mRNA-based TB vaccines. However, despite the enthusiasm, there are no known correlates of protection against TB, the antigens that induce protective immunity are incompletely defined, and the overreliance on Th1 cytokine production as an "absolute" measure of protection is increasingly debatable. In this perspective, I highlight the recent milestones in TB Vaccine research and the remaining challenges and propose suggestions for future considerations.

KEYWORDS

vaccine-induced, T cells, responses, tuberculosis, evaluation

Introduction

The gradual yet promising decline in global tuberculosis (TB) mortality over the past two decades has been negated by the healthcare system disruptions caused by the COVID-19 pandemic. Fortunately, the trajectory of TB-related deaths is trending downward to levels similar of the pre-COVID-19 era, with over 1.3 million deaths recorded in 2022 (1). While there have been significant improvements in shortening the treatment duration for TB, including multi-drug resistant TB (2–6), effective TB treatment is expensive, and the increasing prevalence of drug-resistant TB (including extensively drug-resistant TB) remains a cause for concern (1, 7). Consequently, the essential role of an effective vaccine against TB and the urgency to develop such vaccines to contribute to the global efforts to eliminate TB cannot be overstated. Since exposure to *Mtb* results in different infection outcomes, vaccine candidates for TB can have several indications—prevention of infection, prevention of disease, prevention of recurrence, or therapeutic (vaccine administered during the time of TB therapy to enhance immune responses targeting viable bacteria despite the presence of drugs). While emerging modeling studies suggest that the number of people with immunological evidence of *Mtb* exposure is likely overestimated (8, 9), people with latent TB (10–12) remain a large reservoir for the current global TB burden and it is believed that a post-exposure vaccine that prevents the development of TB disease

will be essential in eliminating TB (13). Vaccination against TB has relied solely on BCG vaccine, the only licensed vaccine for over 100 years, usually administered at birth. BCG vaccine is effective in infants reducing the incidence of disseminated and severe TB, but it has limited efficacy against active disease in older children, adolescents, and adults (14–16). BCG vaccine also protects against other diseases through mechanisms that have only recently come to greater light (17–20). Mathematical modeling studies show that even partially efficacious new TB vaccines will significantly impact health (21, 22). In this perspective, I will discuss the recent milestones in TB Vaccine research and the remaining challenges and offer suggestions for future considerations.

Recent advances in TB vaccine research

Since BCG is the "gold standard" vaccine against TB, the efficacy of new vaccine candidates in animal experimental studies is always benchmarked on the protection conferred by BCG vaccine or whether the subunit vaccines can augment the efficacy of BCG vaccine (23–29). Different categories of TB vaccines are in the development pipeline including live whole cell, inactivated whole cell or lysates, protein subunit, viral vector, and recently mRNA vaccines—extensively reviewed in Zhuang et al. (30), Zhou and Zhang (31), and Lai et al. (32). Here, I highlight how the knowledge gathered and discussed in published reviews can be used to comprehensively define correlates of protection against TB. Several vaccination approaches have been employed to improve the efficacy of candidate TB vaccines including different routes of administration (28, 33–41), prime-boost approaches with or without BCG as part of the vaccine schedule (42–45), as well as BCG revaccination in humans (46–50). Additionally, alternative formulations of vaccines in the preclinical stage have generated encouraging results (23, 24, 29, 51, 52) indicating that the efficacy of some previously studied vaccine candidate *Mtb* antigens can be improved upon. Significant reports using these strategies have demonstrated that it is possible to achieve sterilizing immunity against *Mtb* infection through vaccination in non-human primates—a model that more closely resembles TB disease in humans. Using the RhCMV/TB vaccine, Hansen et al. demonstrated that effective immune responses could intercept *Mtb* infection in its earlier stages, rendering complete vaccine-mediated immune control of highly pathogenic *Mtb* (28). In that study, rhesus cytomegalovirus vectors encoding *Mtb* antigen inserts (acute phase proteins 85A, 85B, and ESAT-6; latency proteins Rv1733, Rv3407, and Rv2626; and resuscitation proteins Rpf A, Rpf C, and Rpf D), RhCMV/TB—elicited and maintained highly effector-differentiated, circulating, and tissue-resident *Mtb*-specific $CD4^+$ and $CD8^+$ memory T cells that reduced pulmonary and extrapulmonary infection and disease by 68% compared with unvaccinated controls. Importantly, 14 out of 34 RhCMV/TB vaccinated animals showed no TB disease by computed tomography (CT) scans or at necropsy (unvaccinated controls and intradermal BCG vaccinated animals had higher necropsy scores); 10 of the vaccinated animals with no TB disease had negative *Mtb*-culture across all tissues sampled. Using different routes of BCG administration (intradermal, aerosol, and intravenous) in non-human primates, Darrah et al. demonstrated that a high-dose intravenous BCG vaccination achieved sterilizing immunity against *Mtb* challenge (40). Compared to unvaccinated animals, there was no difference in thoracic, lung, and lymph node colony forming units (CFU) in animals that received low-dose intradermal, high-dose intradermal, aerosol, and aerosol plus intradermal BCG vaccine. On the other hand, there was a significant reduction in thoracic, lung, and thoracic lymph node CFU in animals that received high-dose intravenous BCG vaccine. Most significantly, six out of 10 animals that were vaccinated intravenously had no detectable thoracic and lung CFU and only one out of 10 had thoracic lymph node CFU. In follow-up intravenous BCG dose-ranging experiments, the same group demonstrated graded immune responses and 50% protection (including sterilizing immunity) in macaques (53). Further analysis of protected vs. non-protected animals discovered that intravenous BCG-mediated protection correlated with *Mtb*-specific CD4 Th1/Th17 and NK cells in the airways. The intravenous BCG has also demonstrated efficacy against *Mtb* challenge in simian immunodeficiency virus -infected macaques, including sterilizing immunity in 9 out of 12 animals (41).

Other studies have demonstrated the superiority of mucosal BCG vaccination in mouse and non-human primate models compared to the standard intradermal route (54–58). Dijkman et al. showed that mucosal BCG vaccination prevented infection in vaccinated animals repeatedly challenged (for eight consecutive weeks) with a limiting dose of *Mtb* (1 CFU at each challenge time) on the same lung lobe, through a mechanism that involved polyfunctional Th17 cells, interleukin 10, and immunoglobulin A as the correlates of local protective immunity (59). This study demonstrated that establishing immunity at the site of infection using BCG is capable of limiting the establishment of infection and averting disease, especially with a low dose that closely resembles natural human infection (60–62).

After several disappointing results of clinical TB vaccine trials, two recent studies invigorated the enthusiasm and hope for more efficacious TB vaccines. The first was a phase 2b subunit vaccine, $M72/AS01_E$ (consisting of two *Mtb* proteins PPE18 and PepA) that was administered as a prevention of disease in three countries Kenya, South Africa, and Zambia. The participants in this study were HIV-negative adults with evidence of prior *Mtb* exposure but with no clinical symptoms at the time of enrollment into the study and randomized to receive two doses of the vaccine or placebo 30 days apart and then followed for 3 years with the primary endpoint being microbiologically confirmed active, pulmonary TB with no evidence of HIV infection (63). The initial analysis done at approximately 2.3 years of follow-up showed the vaccine provided 54.0% protection against active pulmonary TB disease with no safety concerns (63). The final vaccine efficacy analysis at month 36 showed 49.7 % protection against active pulmonary TB disease in vaccine recipients compared to placebo, M72-specific antibodies and polyfunctional $CD4^+$ T cells (IFNγ, TNF, or IL2 producing) increased after the first dose and were maintained throughout the follow-up period (64). The significance of this study was the demonstration for the first time that a subunit vaccine can protect *Mtb*-infected individuals from progression to active TB disease.

The safety and immunogenicity of the M72 vaccine have also been evaluated in people living with HIV (PLHIV) in the MESA-TB study (Clinical Trial: NCT04556981) and found to be well-tolerated with no safety signals and found to be immunogenic in virally suppressed, ART-treated PLHIV (*Linda Han, Union World Conference on Lung Health, November 2023*). Buoyed by the phase 2b results, and after delays occasioned by the recent pandemic, the M72/AS01$_E$ vaccine launched phase 3 clinical trials in March 2024 in South Africa with other centers nearing rollout as well.

The second clinical trial evaluated BCG revaccination aimed at preventing *Mtb* infection among high-risk adolescents (HIV uninfected and QuantiFERON TB test negative) (46). This randomized, partially blinded trial aimed to assess the protective effect of BCG revaccination as well as to evaluate a new recombinant protein vaccine candidate H4 (containing *Mtb* antigens Ag85B and Tb10.4) formulated with the adjuvant IC31. Since the vaccines were evaluated on interferon-gamma release assay negative (IGRA^{neg}) participants, the primary endpoint was conversion from a negative to a positive IGRA, as an indicator of *Mtb* infection compared to placebo control, that is, a prevention of infection vaccine. Although none of the vaccine arms demonstrated significant protection against IGRA conversion compared to placebo, BCG revaccination showed 45.4% efficacy in preventing sustained IGRA conversion, which was interpreted as preventing latent TB infection. H4:IC31 had only 30.5% efficacy. Studies to validate the efficacy of BCG revaccination in a larger cohort are ongoing (Clinical Trial: NCT04152161) in South Africa. However, results of human BCG revaccination studies in different regions have been conflicting (49, 65, 66) partly because the endpoint in these studies is different: immunity against *Mtb* infection is not the same as immunity against TB disease. Differences in the force of infection (higher force of infection in South Africa than in Brazil), unmatched ages of study participants and prior *Mtb* sensitization could also account for the discrepancy in the results. The significance of BCG revaccination is self-explanatory: if BCG revaccination can improve the protective efficacy of this century-old vaccine in humans, we would have an inexpensive, readily deployable tool to help improve TB control globally (21, 48, 67).

Overall, TB vaccine research is in the ascendency: there are several vaccines in active phase 2b/3 clinical trials reviewed in Zhuang et al. (30), Zhou and Zhang (31), and Lai et al. (32), alternative vaccination strategies are beginning to identify immune signatures associated with vaccine-mediated protection (25, 53, 55, 59, 68), and new technologies like mRNA vaccines are being incorporated in the preclinical studies. However, the low number of TB vaccine candidates in preclinical development is a constant reminder that the TB research community needs to double the efforts to identify new targets that can create chinks in the armor of *Mtb*.

Challenges for TB vaccine research

Antigen selection

The complete sequencing of *Mtb* genome (69) and advances in computational biology (70, 71) have vastly increased our understanding of the complexity of *Mtb* as a pathogen from centuries of coevolution with humans. Many studies have been conducted to elucidate the identities of *Mtb* proteins and identify their role in *Mtb* survival. Relevant to vaccination, protein subunit vaccines elicit immune responses to specific antigens from the target pathogen, and since they are not viable, subunit vaccines are generally safe and can be given to immunocompromised individuals without the risk of infection. Since subunit TB vaccines target certain proteins from *Mtb*, the breadth of the immune response is narrower than for the whole organism vaccines, making the choice of antigenic target critical and an arduous task considering over 4,000 potential immunogenic proteins in the *Mtb* genome (69). An earlier *Mtb* vaccine antigen discovery study investigated 94 *Mtb* genes selected based on well-defined criteria and evaluated for IFNγ recall responses in previously *Mtb*-exposed healthy individuals demonstrated that distinct *Mtb* antigens varied in their ability to confer protection against Mtb challenge in mice (72). A recent longitudinal human cohort study in which *Mtb*-exposed individuals were followed for several years during which some participants developed TB disease (progressors) while others did not (controllers) identified antigenic peptides targeted by T cell receptor similarity groups associated with control or progression, demonstrating that distinct *Mtb* antigens are associated with infection outcomes (73). In this study, epitopes from *Mtb* antigens PE13 and CFP10 were associated with control while epitopes from EspA were associated with progression. Other TCR specificity groups were significantly enriched in progressors with incomplete protein level resolution. A study is underway to develop an mRNA vaccine containing these TCR Informed TB Antigen, TITAN, constructs of CFP10, PE13, Wbb11, and PPE18 (74). Considering the essential role of human CD4 T cell responses in controlling *Mtb* infection (75–78), it is unprecedented that the immunodominant *Mtb* antigens contain the most hyperconserved T cell epitopes, with rare exceptions (79, 80). We recently discovered that *Mtb* antigens showing evidence of diversifying evolutionary selection (80) induce predominantly Th17 responses in healthy people with a history of *Mtb* exposure (IGRA$^+$) while the conserved immunodominant *Mtb* antigens induce Th1 responses (81). *Mtb* antigen availability can determine the quality of T cell responses (82); antigens expressed at high levels during the chronic phase of infection drive terminal T cell differentiation while antigens more abundant in the acute phase generate less differentiated T cells. Therefore, the selection of antigens to include as potential vaccine candidates remains a bottleneck in TB vaccine discovery (83).

Live attenuated vaccines for example MTBVAC and VPM1002, reviewed in Nieuwenhuizen et al. (84) and Martín et al. (85), provide an opportunity to overcome the challenge of distinct antigen selection as vaccine candidates because of the shared similarity in the sequence of organisms in the *Mtb* complex (MTBC) family. However, two significant considerations are necessary to overcome for future live attenuated vaccines, the safety of the vaccines and whether the attenuated bacteria still elicit the desired immune responses that could be protective.

Correlates of protection

Mtb exposure results in a spectrum of infection outcomes. While symptomatic active TB is the ultimate clinical definition of the disease, with improvements in case finding and a battery

of diagnostic approaches, it is increasingly recognized that there are more people with TB disease, but asymptomatic, than initially estimated (12). Defining the correlates of protection in humans is challenging because of the spectrum of infection outcomes. While evidence indicates that Th1-polarized immune responses can limit *Mtb* growth (86, 87), vaccination studies have shown that vaccine immunogenicity is not the same as a correlate of protection (88, 89). The identification of correlates of vaccine-induced protection has been hindered by the complexity of immune responses involved in the immunity to TB that includes cells of both innate and adaptive immune system (90–92). Th1 responses have been the main component in attempts to identify correlates of protection against TB, but emerging data support that Th1 cytokines alone, though essential, are insufficient for effective control of TB. IL17-producing T cells, less differentiated T cells, specific antibody isotypes, NK cells, and γδT cells have been shown to correlate with vaccine-induced protection (23–26, 53, 59, 68, 93, 94). Some immune markers that correlate with vaccine-induced protection are associated with TB clinical states, suggesting that some mediate control in natural infection (95–97). A summary of studies that identify vaccine-induced correlates of protection is shown in Table 1.

Adjuvants are critical components of TB subunit vaccines (105–107), additional summary in Table 1, and are crucial in optimizing antigen presentation and modulating the vaccine-specific immune response. Vaccine formulations that favor Th1 responses, including induction of polyfunctional Th1 cytokines—IFNγ, TNF, and IL2 have had limited success (88). Excessive Th1 polarization favors the generation of terminally differentiated T cells that do not effectively migrate into the lung parenchyma during *Mtb* infection (94, 108, 109). T cells with Th1 and Th17 properties, referred to as Th1* or Th1Th17, were associated with granuloma that restricted *Mtb* growth (110) or with asymptomatic *Mtb* infection (111) in non-human primates, suggesting the role of Th1* cells in TB control. Further evidence from animal vaccination studies indicates that IL17 and Th17 responses in combination with Th1 responses are necessary for protective immunity to TB (25, 40, 53, 59). Studies in mice show that adjuvants that induce Th17 responses confer superior protection against *Mtb* challenge (23, 24, 100, 101, 112) and emerging evidence indicates that CAF®10b adjuvant can drive memory antibody, Th1 and Th17 vaccine-specific responses across species (113). Therefore, studies that identify correlates of vaccine-induced protection should consider the contribution of adjuvants in the formulation and ideally include an adjuvant-only group for comparisons in preclinical stages.

Since TB is a disease of the tissues, primarily the lung, immune responses at the site of infection would more likely identify correlates of protection when *Mtb* exposure does not result in active disease or correlates of risk when there is active disease. Indeed, intravenous BCG-induced airway Th1/Th17 and NK cells were recently shown to associate with protection in non-human primates (53). For ethical and practical reasons, our knowledge of immunity to TB in humans has relied mainly on studies of peripheral blood. Efforts to standardize and validate TB human infection studies to accelerate TB vaccine development are ongoing (114) with a recent report demonstrating that aerosol BCG-controlled human infection model was sufficiently well tolerated (115). Evidence from animal experimental models indicates that vaccination strategies that favor the establishment of lung tissue-resident memory (TRM) T cells often confer superior protection than vaccinations that do not generate TRM populations in the lung (116). The route of vaccination appears to play an important role in engendering TRM T cells with mucosal vaccinations more adept at generating lung TRM cells (56, 58, 59, 102, 104). However, intravenous BCG administration can also generate TRMs (40). Studies of resected human lung tissue showed that *Mtb*-responsive T cells are enriched in TB-diseased lung tissue compared to matched peripheral blood and express markers consistent with a TRM phenotype (117). Unlike in animal model studies where the time of infection with *Mtb* is known and controlled, human lung resection was done on chronic advanced TB disease patients, and the protective potential of lung TRM cells observed in this study may have been lost in this disease setting.

In summary, it is evident that no single measure of T cell immunity is the ideal correlate of protection against TB disease and thus a systems immunology approach will be vital in advancing knowledge in this area. In the context of subunit vaccines, the critical contribution of adjuvants in orchestrating immune cell interactions should be carefully evaluated.

Other challenges

Drawing parallels from the global response to the COVID-19 pandemic, it was clear that with the right political will and available resources, it is possible to develop vaccines faster and rapidly deploy them to save lives. TB vaccine R&D is acutely underfunded (118) despite the devastation caused by TB, highlighting the lukewarm commitment by governments to tackle the disease. On the research front, the outcome of clinical trials takes several years to determine, and since the rate of progression is generally low in the community, many participants are required. In turn, this causes a delay in policy-making decisions while adding to the overall cost of TB vaccine R&D. There are efforts to use mathematical modeling to design studies to reduce the vaccine trial durations (119, 120). It is worth considering beforehand how new TB vaccines would be integrated with other control programs like treatment and diagnostics to minimize delays in distribution and address the concern of vaccine hesitancy.

Measurement of vaccine efficacy

T cell cytokine profile is a constant consideration in evaluating vaccine-induced cellular immunity because of the central role of T cells in the control of *Mtb* infection (75–78, 121, 122). In this category, IFNγ production has been the most dominant cytokine due to the high susceptibility to TB in animals lacking IFNγ (86, 123, 124) and humans with deficiencies in IFNγ signaling (125, 126). However, IFNγ response alone is not sufficient for the control of TB (127). Several lines of evidence have shown the existence of IFNγ-independent mechanisms of T cell-mediated control of TB in animals (128, 129) and some contacts of active TB cases do not make IFNγ responses to *Mtb* antigen stimulation

TABLE 1 Correlates of vaccine-induced T cell responses in experimental animal models.

Vaccine description	Host	Route of vaccination	Immune signature	Measure of efficacy	References
ESAT6 with LT-IIb adjuvant	Mouse	Mucosal	IL17-dependent formation of iBALT structures through induction of CXCL13	Lower lung bacterial burden compared to unvaccinated or sham vaccinated controls	(98)
Ag85B-hPIV2 vector	Mouse	Mucosal	iBALT formation with induction of Th17, and $CD11b^{+}CD11c^{+}$ cells		(99)
Subunit vaccines i – H83/CAF01: (MPT70, Rv3020c, Rv3019c and ESAT6) ii – H89/CAF01: (MPT70, Rv3020c, Rv3019c and Rv1198)	Mouse	Subcutaneous	FDS as a measure of T cell differentiation state, higher FDS means more terminally differentiated T cell repertoire. H83 vaccine rescued CD4 T cells from terminal differentiation conferring long-term protection compared to H89 vaccine. H83 vaccine specific cells had low expression of KLRG1 than saline control group.	Lower lung bacterial burden compared to Saline or BCG vaccinated controls	(94)
Subunit vaccines i-MPT70/CFA01 ii- ESAT6/CAF01	Mouse	Subcutaneous	Lower FDS for MPT70 vaccinated than ESAT-vaccinated or saline controls. Lower proportion of cytokine expressing $KLRG1^{+}$ $CD4^{+}$ T cells in MPT70 vaccinated compared to ESAT6 vaccinated mice.	Reduced lung bacterial burden compared to Saline controls	(100)
Subunit vaccine H107/CAF01: (PPE68, ESAT6*, EspI, EspC, EspA, MPT64, MPT70, MPT83)	Mouse	Subcutaneous, intravenous, or intradermal	Low FDS for H107 specific T cells. Induction of Th17 cells ($IL17^{+}$; $ROR\gamma T^{+}$ $CD4^{+}$ T cells)	Reduced lung bacterial burden compared to BCG vaccinated and Unvaccinated controls	(26)
Subunit vaccine H107e/CAF01: (PPE68, ESAT6*, $EspI^{\#}$, EspC, EspA, MPT64, MPT70, MPT83)	Mouse	Subcutaneous	Boosted BCG specific long-lived Th17 ($IL17^{+}$; $ROR\gamma T^{+}$ $CD4^{+}$) cells	Lower lung bacterial burden compared to BCG vaccinated and Unvaccinated controls	(25)
Subunit vaccine 5Ag/RR-CDG or 5Ag-ML-RR-cGAMP: (5Ag = Ag85B, ESAT6, Rv1733c, Rv2626c, and RpfD)	Mouse	Mucosal	Type I IFN independent mediated protection that includes induction of lung parenchyma $CXCR3^{+}$ $KLRG1^{-}$ T cells and Th1 ($IFN\gamma^{+}$) and Th17 ($IL17^{+}$) responses.	Lower lung bacterial burden compared to PBS controls or 5Ag construct without RR-CDG adjuvant.	(101)
Subunit vaccine 5Ag/ML-RR-cGAMP (CDN) or 5Ag/MLPA	Mouse	Mucosal	Induction of Th1, Th17 and Th1* cells. IL17 and IFNγ dependent protection. Induction of expression of Tnfsf8 (CD153).	Reduced bacterial burden in the lung lobe compared with unvaccinated or MPLA adjuvanted vaccine	(24)
Subunit NE-TB vaccine (nanoemusion adjuvant with Ag85B and ESAT6)	Mouse	Mucosal	Induction of IL-17^{+} T-cell responses in the lungs and spleen	Reduced lung bacterial burden lower % inflammation per lung lobe Decreased chemokine (CXCL9 and CXCL2) induction. Improved B cell lymphoid follicle formation.	(23)
rIAV expressing Mtb Ag85B	Mouse	Mucosal	Induction of lung $CD4^{+}$ TRM ($CD69^{+}CD11a^{+}$ $CD44^{hi}CD62L^{lo}$) independent of circulating memory T cells. Polyfunctional Th1 cytokines (IFNγ/TNF/IL2 or IFNγ/TNF) resident in the lung parenchyma.	Lower lung bacterial burden compared to unimmunized controls	(102)
Recombinant vaccine (SeV85AB)	Mouse	Mucosal	Induction of lung $CD8^{+}$ TRM ($CD103^{+}CXCR3^{+}KLRG1^{-}$). Induction of Ag85AB-specific polyfunctional $CD8^{+}$ T cells ($IL2^{+}TNF^{+}$ and $IFN\gamma^{+}TNF^{+}$).	Lower lung bacterial burden. % reduction in inflammation per lung lobe Enhanced BCG responses in BCG prime-SeV85AB boost. Enhanced Ag85AB-specific cytotoxic $CD8^{+}$ T cell responses.	(45)

(Continued)

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TABLE 1 (Continued)

Vaccine description	Host	Route of vaccination	Immune signature	Measure of efficacy	References
Fusion protein CysVac2 ajuvanted with Advax (CysVac2/Advax)	Mouse	Mucosal	Induction of lung-resident ($CD4^+CD44^{hi}CD62L^{low}CD69^+$ IV^-), antigen specific memory Th17 cells ($IL17^+ROR\gamma T^+$). Serum CysVac2-specific IgG1, IgG2 and IgA. Formation of iBALT structures in the lung.	Reduced bacterial burden in the lung and spleen	(103)
BCG	Mouse	Mucosal	Superior induction of *Mtb*-antigen specific $CD4^+IFN\gamma^+$ T cells in the lung and spleen. Induction of $CD4^+$ TRM defined as $CXCR3^+PD\text{-}1^+KLRG1^-i.v^-$ or $CD44^{hi}$ $CD62L^{lo}$ $CD103^+$ $CD69^+$ T cells. Increased expression of transcripts typical of tissue residency *Itgae* (CD103), *Itgal* (VLA-1) and regulatory T cells (*Foxp3* and *Il10*). Increased frequency and durable *Mtb* antigen-specific CD4+ ($IFN\gamma^+$, TNF^+ or $IL2^+$) T cells in the lung parenchyma and BAL. Enhanced proliferative capacity of lung parenchymal $CD4^+$ T cells. Increased frequency of effector memory ($CD44^{hi}$ $CD62L^{lo}$ $CD69^{lo}$) T cells in the lung.	Superior reduction of lung bacterial burden compared with subcutaneous vaccination and unvaccinated controls.	(56, 58, 104)
Viral vector vaccine (RhCMV/TB) encoding Mtb proteins Ag85A, Ag85B, ESAT-6, Rv1733, Rv3407, Rv2626, Rpf A, Rpf C and Rpf D.	Rhesus Macaques	Subcutaneous	Highly effector differentiated circulating and tissue resident memory Mtb-specific $CD4^+$ and $CD8^+$ memory T cells.	Superior reduction of bacterial burden and necropsy disease score compared with unvaccinated controls or intradermal BCG vaccination.	(28)
BCG	Rhesus Macaques	Mucosal	Local (lung) $IL17A^+CD4^+$ T cells. Production of PPD-specific IL10. Induction of IgA in the BAL.	Reduction of TB disease - Limited dissemination of disease - Reduced lung involvement (FDG uptake) - Reduced bacterial burden in the lungs and lung draining LN	(59)
BCG	Rhesus Macaques	Intravenous	TB-specific CD4 Th1/Th17 ($TNF^+IFN\gamma^+$ and TNF^+IL17^+) and NK cells in the airway. Innate cell transcription signature (type1 interferon and RIG-I-like receptor signaling pathways) at day 2 correlated with protective airway CD4 T cells (Th1/Th17) at week 8. IgM titers in the plasma and lungs.	Reduced lung inflammation (total FDG activity). Lower number of lung granuloma (serial PET-CT scans). Fewer lung bacterial burden in IV-vaccinated animals compared to other routes of vaccination.	(40, 41, 53, 68, 93)
Mtb mutant in SigH (MtbΔ*sigH)*	Rhesus Macaques	Mucosal	Recruitment of iBALT and lung $CD4^+$ and $CD8^+$ T cells expressing activation and proliferation markers. Strong central memory $CD4^+$ and $CD8^+$ T cell responses in the lung. Significant increase in polyfunctional Th1 cells ($IFN\gamma^+TNF^+IL2^+$).	Reduction in bacterial burden in the lung and bronchial LN. Significantly diminished clinical manifestations (body temperature, body weight, serum CRP, thoracic radiograph scores). Reduced granulomatous pathology. Lower % lung involvement. Higher % survival compared to unvaccinated and BCG vaccinated controls.	(37)

*Fusion protein contains four copies of ESAT6; #EspI without a proline-rich fraction (Δ75-294) for increased expression of H107e compared to the original construct H107. FDS, Functional Differentiation Score; LT-IIb, type II heat-labile enterotoxin; hPIV2, Human parainfluenza type 2 virus; CAF01, cationic adjuvant formulation 1; RR-CDG, R,R stereochemical configuration of cyclic diguanylate; ML-RR-cGAMP, mixed linkage-R,R stereochemical configuration cyclic guanosine monophosphate adenosine-monophosphate; CDN, cyclic dinucleotides; MPLA, monophosphoryl lipid A; NE, nanoemulsion based adjuvant; FDG, F-fluorodeoxyglucose; PET-CT, positron emission tomography–computed tomography; rIAV, recombinant influenza A viruses; SeV85AB, Sendai Virus expressing Mtb Ag85A and Ag8B; CysVac2, fusion protein of Mtb antigens Ag85B and CysD; CRP, C-reactive protein; BAL, Broncho-alveolar lavage; LN, lymph node.

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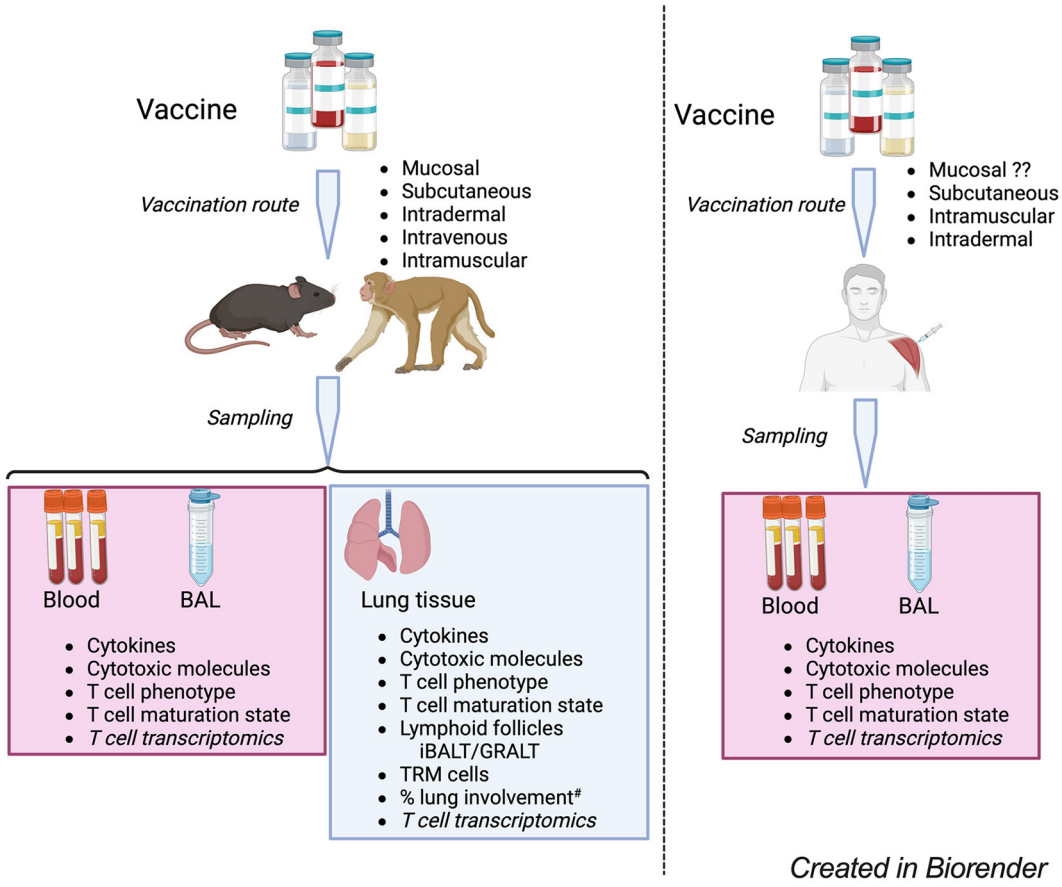

FIGURE 1
A model for the evaluation of TB vaccine-induce T cell responses in experimental models and humans. Candidate TB vaccines are delivered via various routes and samples blood, BAL – bronchoalveolar lavage – fluid and lung tissue (for experimental models) collected for analysis of vaccine-specific T cell responses. The T cell phenotype analysis considers markers of T cell lineages such as Th1, Th17, regulatory T cells as well as expression of chemokine receptors, inhibitor/exhaustion receptors. T cell maturation state analysis to consider the T cell differentiation and proliferative capacity; T cell transcriptomics to consider vaccine induced changes in the expression of T cell transcripts associated with different functions to identify novel pathways involved in vaccine response. Experimental models, analysis of % lung involvement# should consider the role of T cells in tissue repair and integrity, looking at T cell expression of makers like amphiregulin that can mitigate lung tissue damage. #Lung involvement can be measured by PET-CT/FCG uptake, together with microscopy approaches to define the cellular composition of the involved tissue.

(130, 131). Therefore, the cytokine repertoire of vaccine-induced T cell immunity should consider T cell functions beyond IFNγ production guided by existing literature (81, 132–135).

Beyond the cytokines, CD4 T cells that express CD153 offer superior protection from *Mtb* infection across species (136–138), and expression of CD153 on *Mtb*-antigen-specific T cells is inversely associated with bacterial load and disease severity in humans (139). Studies in mice demonstrate that vaccines that favor the generation of less differentiated T cells offer superior protection (26, 94, 100). Studies that incorporate the measurement of exhaustion markers could shed more light on the phenotype of protective vaccine-induced T cells.

Studies of *Mtb*-infected lungs provide further details of cellular organization in the lung tissue that may restrict growth and limit the dissemination of the bacteria. Lymphoid follicles [variously termed inducible bronchus-associated lymphoid tissue (iBALT) or granuloma-associated lymphoid tissue (GRALT)] are protective in mice and macaques (140–145) and have been described in resected *Mtb* infected human lung tissue (146). Vaccines can induce the formation of tertiary lymphoid structures (37, 98, 99), establish TRM populations in the lung (45, 103, 116), and provide greater *Mtb* control. It is thus evident that no single T cell feature is sufficient to define protective immunity against TB and the correlate of protection will be a T cell signature of different phenotypes and functions. Figure 1 illustrates some features to consider in defining vaccine-induced T cell responses against TB.

Evaluating vaccine efficacy is often biased toward the host responses but consideration of how vaccines restrict *Mtb* growth is also important. Enumerating CFU is the standard practice after vaccination (26, 53, 59) in animal model studies with few studies reporting the total bacterial counts as measured by bacterial chromosome equivalents (CEQ) (147–149). Similar approaches are limited in clinical settings, not least because of the number of participants involved. Intensive research on the utility of mycobacterial growth inhibition assay (MGIA)—to measure vaccine efficacy is an area of active research [extensively reviewed in Painter et al. (150)]. Finally, it is worth considering the role of vaccine induced T cell responses in mitigating lung tissue repair

and integrity (151, 152) since that will have significant impact on TB transmission in humans. A host and bacteria-pronged approach to identify vaccine-induced control of *Mtb* infection will advance efforts toward defining correlates of protection against TB.

Conclusion

The results of the Phase 2b trial of the subunit vaccine, M72/AS01E, and the number of Phase 3 clinical trials show that a new TB vaccine, with cautious optimism, is within reach. However, with very few candidates in Phase 1 and 2a stages of development, the TB vaccine research community cannot afford to take their eyes off the ball. It is important to maximize and effectively use available specimens from both experimental and clinical trials for a multifactorial approach using advances in systems immunology to improve chances of identifying correlates of vaccine-induced protection. These efforts will require the integration of innate, cellular, and humoral arms of the immune system, and resource mobilization through consortia dedicated to this cause, like the Gates Foundation-led efforts to identify correlates of M72 vaccine and BCG-revaccination studies. Human TB studies of people living with HIV, anti-TNF treatment and anti-PD1 therapy for cancer treatment have shown that TB is an immunological disease and a deeper understanding of immunity to *Mtb* infection is extremely important to evaluate and characterize new immunological correlate of protection in individuals with different immune backgrounds. To this end, new TB subunit vaccines in the clinical development stage should consider vulnerable populations, people living with HIV, people with diabetes and individuals taking biologic drugs to treat inflammatory diseases such as rheumatoid arthritis and psoriasis in the early stages for evaluation of the candidates across the heterogeneity of the population and minimize the logistical and financial costs of testing the vaccines in these populations at later time.

Data availability statement

All relevant data is contained within the article: The original contributions discussed in the perspective are included in the article/supplementary material cited, further inquiries can be directed to the corresponding author/s.

Ethics statement

Ethical approval was not required for the studies involving humans because the manuscript does not include original data. The studies were conducted in accordance with the local legislation and institutional requirements. The participants provided their written informed consent to participate in this study. Ethical approval was not required for the study involving animals in accordance with the local legislation and institutional requirements because there is no original data presented in this manuscript.

Author contributions

PO: Conceptualization, Funding acquisition, Writing – original draft, Writing – review & editing, Data curation.

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Conflict of interest

The author declares that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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