

Christopher L. Karp
Christopher B. Wilson
Lynda M. Stuart

Tuberculosis vaccines: barriers and prospects on the quest for a transformative tool

Authors' addresses

Christopher L. Karp¹, Christopher B. Wilson¹, Lynda M. Stuart^{1,2}

¹Discovery and Translational Sciences, Global Health, The Bill & Melinda Gates Foundation, Seattle, WA, USA.

²Benaroya Research Institute at Virginia Mason, Seattle, WA, USA.

Correspondence to:

Christopher L. Karp

The Bill & Melinda Gates Foundation

P.O. Box 23350

Seattle, WA 98102, USA

Tel.: +1 206 709 3100

e-mail: chris.karp@gatesfoundation.org

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Summary: The road to a more efficacious vaccine that could be a truly transformative tool for decreasing tuberculosis morbidity and mortality, along with *Mycobacterium tuberculosis* transmission, is quite daunting. Despite this, there are reasons for optimism. Abetted by better conceptual clarity, clear acknowledgment of the degree of our current immunobiological ignorance, the availability of powerful new tools for dissecting the immunopathogenesis of human tuberculosis, the generation of more creative diversity in tuberculosis vaccine concepts, the development of better fit-for-purpose animal models, and the potential of more pragmatic approaches to the clinical testing of vaccine candidates, the field has promise for delivering novel tools for dealing with this worldwide scourge of poverty.

Keywords: *Mycobacterium tuberculosis*, vaccine, immunopathogenesis

Introduction

Thoroughly conscious ignorance... is the prelude to every real advance in knowledge. James Clerk Maxwell (1)

For tuberculosis (TB) to be removed from the top rank of global health problems in any reasonable time frame, transformative tools and strategies will need to be developed. A vaccine that effectively prevented pulmonary TB holds promise for being just such a tool—decreasing both transmission and disease burden in this infection in which the two are closely linked (2). Recognition of this promise and the concerted emergence of the philanthropic and political will to take on this highly prevalent disease of poverty have led to a global effort to develop new, efficacious TB vaccines. There has been a growing realization, however, that the development path is not at all clear. Interrelated problems retarding progress include (i) fundamental immunobiological ignorance of host-pathogen interactions in *M. tuberculosis* infection; (ii) the lack of a correlate of vaccine-induced protection in the absence of a vaccine with demonstrable efficacy, together with the realization that the presumptive mechanistic correlate that has driven TB vaccine discovery to date may well be inadequate or

incorrect; (iii) the generation of relatively few vaccine concepts despite a plethora of vaccine candidates—with the dominant concept appearing to run counter to the available evolutionary evidence; and (iv) an apparent lack of fit-for-purpose animal models. Given these issues, there is currently no way to de-risk vaccine candidates before the performance of large, very costly efficacy trials, nor clear methods for data-driven prioritization of candidates for entry into such trials. This untenable situation is something of a *reductio* of fundamental problems underlying the global health vaccine R&D enterprise in general. We examine these issues and discuss possible paths forward.

Barriers to successful TB vaccine discovery and development

Immunobiological ignorance

While much has been learned about the intricate dance between host and pathogen in TB in recent years, it remains unclear what sorts of vaccine-inducible immune responses are likely to provide protection against disease. Answers have classically been sought in the natural response to *M. tuberculosis* infection, as 90% of immunocompetent individuals with *M. tuberculosis* infection never suffer from TB disease. Moreover, prior *M. tuberculosis* infection may substantially reduce the risk of progression to disease after reinfection in some populations (3). Given this, one might be tempted to consider infection with *M. tuberculosis* to be a reasonably efficacious vaccine, albeit one with an unacceptably high rate of severe side effects (e.g. TB disease). In any case, mimicking and improving on the immunological mechanisms responsible for maintaining clinical latency (and providing concomitant immunity against disease due to re-exposure) in the bulk of those with natural infection would appear to be a rational goal for vaccine development—albeit a high bar to attain.

Much of what is understood about protection from disease in natural human infection with *M. tuberculosis* comes from outlier situations (genetic, autoimmune, infectious, iatrogenic) in which there is demonstrable default on the part of the host immune system. The critical role of the IL-12/IFN- γ axis in the control of mycobacterial disease in general has been underlined by the syndromes of Mendelian susceptibility to mycobacterial disease due to mutations in the IL-12 and IFN- γ receptors (4–6), as well as by the sequelae of developing high-titered autoantibodies to IFN- γ (7). These findings are concordant, of course, with the hyper-susceptibility to TB exhibited by mice that are unable

to generate Th1 responses (8–10). The fact that TB is the most common fatal opportunistic infection in HIV/AIDS worldwide (11) further underscores the likely critical role for CD4⁺ T-cell responses in successful control of *M. tuberculosis* infection, as does the fact that impairment of *M. tuberculosis* antigen-specific Th1 responses occurs early after HIV infection, concomitant with a dramatically elevated risk of developing TB, and prior to demonstrable peripheral CD4⁺ T-cell depletion (12). Finally, the increased risk of reactivation disease observed in patients on anti-TNF and anti-IL-6 therapy (13, 14), as well as the susceptibility to TB exhibited by mice lacking TNF, GM-CSF, and IL-1 β (15–17), has underscored the important role of these proinflammatory cytokines in controlling *M. tuberculosis*.

Th1 and proinflammatory responses [likely interrelated: Th1 cells are a relevant source of TNF (18)] may provide an ‘envelope’ of protective responses that is necessary to prevent disease in natural infection. However, it is not at all clear that such responses would be necessary and sufficient for an efficacious TB vaccine to induce. Firstly, although aiming to drive Th1 responses to subunit or whole-cell vaccines (either alone or as a boost to BCG) has represented the dominant paradigm in modern TB vaccinology (19), Th1 responses have failed as correlate of protection to date. BCG drives robust Th1 responses, yet the vigor of BCG-driven Th1 responses does not correlate with protective efficacy (19–22). Similarly, the ability to drive Th1 responses, which represent a standard gating criterion for essentially all modern TB vaccines, has not predicted success either preclinically or in the one human efficacy trial in which the protective efficacy of a contemporary TB vaccine was assessed (19, 23). Secondly, the patterns of tuberculous disease associated with the above situations of host Th1 and proinflammatory default actually represent a somewhat different spectrum of disease than classical adult pulmonary TB, with a high incidence of disseminated disease as opposed to localized destructive inflammatory lesions in the lungs. The former is the spectrum of disease prevented by BCG vaccination of infants. Basal Th1 and proinflammatory competence is thus clearly necessary for prevention of disseminated disease. But, such responses appear necessary but not sufficient for prevention of pulmonary TB and are likely to be elicited in the immunocompetent host by BCG vaccination, non-tuberculous mycobacterial infection, or *M. tuberculosis* infection itself. Finally, the localized destructive lesions of pulmonary TB appear to be the result of overly vigorous inflammatory responses. Longitudinal studies of high-risk individuals (close contacts of individuals with TB) have

revealed that active TB is frequently heralded by robust increases in *M. tuberculosis*-specific Th1 responses (24–26). Furthermore, disease can be ameliorated by adjunctive inhibition of TNF during anti-microbial therapy in animal models (27, 28). Thus, neither overly weak nor overly strong inflammatory responses are likely to lead to a successful interaction with *M. tuberculosis*, and the stable clinical latency exhibited by 90% of those infected likely requires this fine balance. This makes considerable theoretical sense when viewed through the lenses of the damage response (29) or disease tolerance (30) models of host–pathogen interactions. In sum, it remains unclear whether driving Th1 and pro-inflammatory responses, either *de novo* or as a boost for BCG, represents a compelling strategy for TB vaccine development (31).

Moving away from a focus on Th1 responses, devising vaccines that can elicit the pattern of immune responses that lead to successful immune responses in natural infection, i.e. mimicking the mechanisms and benefits of clinical latency, in the absence of the risks associated with infection remains an attractive target. However, it may be a very difficult target to hit. Latency, defined operationally by evidence of long-lasting T-cell reactivity with *M. tuberculosis* antigens [via tuberculin skin tests (TST) or IFN- γ release assays (IGRA)] in the absence of clinical symptoms, appears to represent a broad spectrum of responses, from sterilizing immunity to subclinical active disease. This spectrum of latent disease is itself part of a broader, highly dynamic spectrum of responses to infection that includes active and fulminant disease (32). The dynamic nature of this deserves underscoring. Histological and functional imaging analysis of infected humans and macaques has revealed that individual granulomas can pursue independent trajectories in the same lung (33–35). The co-existence of control, loss of control, and tissue destruction at the same time but in different foci of infection in the same individual suggests critical importance for local, microenvironmental (structural? stochastic?) factors in the outcome of infection. If local expression of the same general pattern of immunological responsiveness can lead to diametrically opposing outcomes in different places in the same lung, there are obvious implications both for being able to define ‘the target’ immunologically and for devising strategies to hit it.

Stepping back a bit from these complexities, an unbiased systems biology approach revealed the presence of a type I IFN transcriptional signature in blood during active pulmonary TB (36). This signature was subsequently validated in several different populations (37–41). Importantly, the

signature waxes with disease activity and wanes with treatment (42). Notably, longitudinal studies have shown that such signatures can arise months prior to clinical progression to active disease (personal communication, W. Hanekom). The strong, reproducible association of this type I IFN activity with active disease raises the question of the immunobiology that underlies this association. Biological plausibility is high (43–46). Increased type I IFN activity may well underlie the early susceptibility to TB observed with HIV infection, which is associated with robust, sustained type I IFN production (47, 48). It may even contribute to the spring peak in incidence of active TB observed in temperate zones (49) as an amplification effect on the dynamic flux of granuloma activity exerted by respiratory virus-induced type I IFNs. However, it remains to be determined whether there is a causal link between type I IFNs and disease or whether it is indirect and epiphenomenal. Studies that establish if type I IFNs are sufficient to induce reactivation in animal models of latency (34) or if blockade of type I IFN activity blocks progression of latently infected individuals at high risk of progression to disease (e.g. those with a robust correlate of risk signature) may help establish causality. If the type I IFN signature of high risk of progression to disease were validated, it should allow for better-stratified clinical studies of TB drugs (e.g. for secondary prophylaxis) and vaccines. However, without understanding the biology of this type I IFN signature, it remains unclear how to harness these findings in the service of vaccine discovery.

Thus, if the goal of vaccination is to increase the proportion of infected individuals that achieve and maintain clinical latency to greater than 90%, we currently do not know what sorts of immune responses, elicited either *de novo* or as a boost to BCG, are sufficient to achieve this goal. The considerations sketched above do however suggest the sorts of immunobiological knowledge that would be useful to obtain if we are going to rationally develop vaccination approaches that mimic and improve on the protection seen with natural infection.

Vaccine concepts and candidates

There is no shortage of vaccine candidates in the global pipeline, with at least 13 candidates in clinical studies, including subunit vaccines (adjuvanted proteins, virally vectored proteins), live whole-cell vaccines [recombinant BCG (rBCG), attenuated *M. tuberculosis*, inactivated mycobacteria], and mycobacterial lysates (50). This large number of candidates represents relatively few concepts. The dominant concept, explicit in the case of subunit vaccines and generally

implicit in the case of whole-cell vaccines, is to drive immunodominant CD4⁺ T-cell responses (with or without immunodominant CD8⁺ T-cell responses) to prevent the transition from latency to disease (or to prevent primary progression). This is generally envisaged as a boost for neonatal BCG vaccination and thought of in the context of driving specific patterns of CD4⁺ T-cell responses (Th1 and/or Th17), as noted above. There are what may be biologically important distinctions among the vaccine candidates, including the combination of antigens expressed in diverse phases of the interaction of *M. tuberculosis* with the host in the case of subunit vaccines. The central focus on driving immunodominant T-cell responses and preventing the progression from infection to disease remains.

Recent data have cast some doubt on the wisdom of targeting antigens containing immunodominant T-cell epitopes. *M. tuberculosis* and *H. sapiens* appear to have been doing their intricate dance for at least 70 000 years (51). This is more than enough time for the host immune system to have left traces of immune pressure in the mycobacterial genome, especially given the high mortality caused by tuberculosis after the Neolithic demographic transition. Seminal work by Ernst and Gagneaux leveraged the power of whole genome sequencing to interrogate the evolutionary pressures exerted by the immune system on the TB genome. Surprisingly, the known T-cell epitopes, both CD4⁺ and CD8⁺, are hyperconserved in *M. tuberculosis*; indeed, the sequences encoding them appear to represent the most conserved regions of the *M. tuberculosis* genome, harboring the lowest ratio of non-synonymous to synonymous changes (52–54). This evidence of strong purifying selection suggests that stable human T-cell recognition of *M. tuberculosis* is beneficial, overall, to the mycobacterium. While these data may seem a bit counterintuitive at first, cogent biological hypotheses suggest themselves. On one hand, inflammatory tissue destruction in the lung is central for mycobacterial transmission from one human host to another. As noted above, T cells are almost surely critical to this process, something underscored by the observation that those with HIV co-infection tend not to present with cavitory lesions and are thought to be less infectious than immunocompetent patients with pulmonary TB (55). Thus, T-cell epitope conservation may well be key for facilitating mycobacterial spread. On the other hand, without the above-described envelope of T cell-dependent protective responses, there is failure of mycobacterial containment, with disseminated disease and (in the absence of treatment) death in the short term. Causing dissemination and high mortality would likely be the Scylla to the Charybdis

of failing to induce holes in the lung from the evolutionary point of view of *M. tuberculosis*. T-cell epitope conservation may also be important to avoid this fate as well. Whatever the evolutionary reasons, the fact that *M. tuberculosis* appears to have evolved to ensure such T-cell recognition suggests caution for using antigens with immunodominant epitopes as vaccine candidates.

If a better evolutionary understanding of *M. tuberculosis* has cast some doubt on the wisdom of engineering vaccines to drive immunodominant α/β T-cell responses, what about the other part of the dominant paradigm: having as the goal of vaccination, preventing the transition from latency to disease (or primary progression)? Preventing lung disease is clearly a critical goal, as it would prevent morbidity and mortality as well as transmission. Increasing the fraction of infected individuals who never get disease to greater than 90% is one way of doing this. Might prevention of infection or—to be biologically clearer—prevention of sustained infection be another way to the same goal? There is evidence suggesting that this occurs in natural infection: abortive infection, as defined by evidence of robust sensitization of T-cell responses by TSTs and/or IGRAs followed by the reversion of such tests, is recognized in humans studied longitudinally (56) and has been reported in guinea pigs infected in natural transmission models (57). There is also cross-sectional evidence suggesting that BCG vaccination has efficacy in preventing sustained infection, in both children and adults (58–63). [Whether *M. tuberculosis* infection itself raises the bar to subsequent superinfection, as opposed to disease (3), remains unclear for technical reasons. Superinfection is not detectable by assaying the T-cell response].

Prevention of sustained infection may thus be a rational target for novel vaccination strategies. The characteristic delay observed in the onset of the development and pulmonary trafficking of adaptive immune effector responses after pulmonary inhalation of *M. tuberculosis* presents a challenge. In natural infection of humans, adaptive immune responses (as measured by TSTs) only become detectable more than 40 days after infection (64, 65). Similarly, in standard aerosol infection models in mice, the development of antigen-specific CD4⁺ T-cell responses takes 9–14 days (66–68), it taking a further 20 days or so for the antigen-specific T-cell response to stop mycobacterial growth in the lung (69). In the interim, *M. tuberculosis* growth is progressive. With vaccination of mice, the accumulation of effector CD4⁺ T cells in the lungs occurs more quickly, but it still takes some 15 days (69–71). In standard mouse models, sterile immunity is not seen. As noted above, however, the development

of (apparently) sterile immunity appears to occur at some rate in natural infection. How this rate might be increased by vaccination remains a matter for conjecture and experiment at present. Strategies for accelerating the expression and pulmonary localization of memory α/β T-cell effector responses, exploiting 'non-standard' T-cell populations with semi-invariant T-cell receptors that localize to mucosal sites, and/or exploiting antibody responses all come to mind. They are all understudied. In part, this is due to a poverty of fit-for-purpose animal models.

Animal models

A plethora of animal models have been used in TB pathogenesis and vaccinology research, including infection of the airways of mice, guinea pigs, rabbits, non-human primates, and cattle with *M. tuberculosis*. None appear to be terrific mimics of the broad spectrum of human outcomes of exposure to *M. tuberculosis*. Why this is so remains unclear. Various facets of the lack of mimicry of the human spectrum may be due to fundamental species differences in immune cells and pathways (e.g. mice lack CD1a-c restricted T cells and $V\gamma_9/V\delta_2$ T cells), mode and dose of infection [e.g. guinea pigs were 'known' to be hyper-susceptible to TB and poor mimics of human pathology, but natural transmission studies revealed their ability to develop apparently sterile protection and to exhibit a range of disease pathology including well-defined granulomas, caseous necrosis and cavitary disease (57)], *M. tuberculosis* strain employed (with standard use of lab-adapted strains), and/or the use of non-physiological, stress-inducing housing conditions (e.g. mice are routinely cold-stressed) (72). Of course, models are just that – models. The important issue is how they are employed.

While animal models are used in the current paradigm as gating criteria for vaccine development, there is no way of knowing if these models are predictive. As is the case with correlates of protection, this will not become clear until efficacy is shown in a human efficacy trial. There are, however, reasons to question the current use of animal models. Firstly, as noted, the models in standard use are not good mimics of the human spectrum of infection and disease. Secondly, while the standard models are models of primary progression with dissemination, vaccine-induced prevention of the progression from infection to disease is generally not seen [except in low-dose infection of cynomolgus monkeys which can lead to latency (34, 73)]. The standard readout is mycobacterial load, not disease prevention; and the gating criterion of success in the current paradigm (typically 0.5

log lower mycobacterial load than that achieved with BCG vaccination) is arbitrary, absent evidence that it predicts outcomes in humans. Thirdly, while preventing primary progression is important, a major goal of vaccination has also been prevention of the transition from latent infection to active disease. There currently is no biologically relevant model in which to test this. While latent infection can be seen (e.g. in cynomolgus monkeys, as noted above), we lack a physiologically relevant way to drive the transition from latency to active disease. Blockade of TNF or IFN- γ , or administration of high dose steroids, can certainly reverse latency. But this is at the likely cost of blocking the very effector mechanisms that are critical for either basal or vaccine-induced protection. Fourthly, while the development of sustained memory is critical, the current standard practice is to challenge a mere 6–8 weeks after vaccination. Fifthly (and in the same vein), while most of the vaccines in the current pipeline are aimed at adolescent or adult boosting of infant BCG vaccination, the current practice involves rapid, sequential vaccination of models with BCG and the intended boost, the latter occurring while the response to BCG is still developing.

Preclinical testing of vaccines aimed at protection against sustained infection is even more difficult with current animal models. The history of HIV vaccinology is instructive here. Until 10 years ago, the standard practice was to immunize non-human primates with a vaccine candidate, and then challenge them with a large intravenous bolus of simian immunodeficiency virus (SIV). Protection against infection is very difficult to observe with such an approach. It is also quite non-physiological: 60–80% of HIV mucosal transmission events involve a single transmitted-founder virus (74). The field subsequently moved to repetitive ultra-low dose mucosal challenge, which allowed more physiological modeling of protection from infection. It has similarly been known since the studies of Riley (75, 76), who modeled natural transmission of *M. tuberculosis* by venting the air from a TB ward through guinea pig cages, that such transmission likely involves a single organism in an airborne droplet nucleus. The standard models of airway infection used in TB vaccinology involve challenge with non-physiologically high doses that aim at 100% infection. Such models are unlikely to be useful for testing vaccine approaches to the prevention of sustained infection. This is even more problematic in the case of testing the hypothesis that antibodies against the surface capsular polysaccharides, lipids, and/or proteins might alter the initial interaction between

M. tuberculosis organisms in droplet nuclei and alveolar macrophages in such a way as to raise the bar to successful infection. As *M. tuberculosis* grows *in vitro* as a clumped, sticky mass, the standard practice is to vortex the mycobacteria in detergent to obtain single organisms – stripping away the natural surface of the infecting mycobacteria, a surface that may well uniquely contain antigens to which protective antibodies could bind.

Clinical development

In the absence of animal models known or likely to be predictive, or a validated correlate of protection, there is neither a rational way to up- or down-select particular candidates for clinical development, nor is there a reason to think that there is a high prior probability of success for any particular candidate (remembering that most vaccine candidates fail). Further, as the standard test of efficacy is prevention of disease, and only 10% of immunocompetent hosts with sustained *M. tuberculosis* infection develop disease, trial sizes are daunting. In the current paradigm, there is thus no way to de-risk vaccine candidates before the performance of large, very costly efficacy trials. This is clearly an unsustainable situation, something that has become clear to the global health community at large in the aftermath of the recent phase 2b demonstration of a lack of efficacy for the MVA85A vaccine in infants (23).

Potential paths forward

The barriers to TB vaccine research and development outlined above are considerable. However, contemplation of these barriers suggests potential paths forward. These are discussed under headings that mirror those above.

Immunobiological ignorance

It is close to axiomatic that real progress in TB vaccine research and development will depend on a deeper understanding of molecular and cellular immunopathogenesis—of the varied ways, successful and unsuccessful, that humans interact with *M. tuberculosis*. For this, the field should be poised to take advantage of recent developments in the fields of genomics and immunology, driven by enabling technologies such as next generation sequencing and high dimensional immune profiling that have great potential for deep interrogation of the human immune response. Promising avenues forward include the careful study of outlier populations, the exploitation of correlates of risk for disease to focus immunological investigation, and (perhaps) the devel-

opment of human challenge models. A deep understanding of the immunobiology of human infection should provide a foundation on which to develop the next generation of vaccines, vaccines that could exploit common or uncommon immune responses observed in natural infection. Such study, together with insights from molecular and cellular mycobacteriology, may also suggest rationales and methods for driving ‘unnatural’ protective responses. (In the latter regard, it should be noted that, although it is commonly bemoaned that the ‘hard vaccines’, i.e. HIV, TB, and malaria, will have to do better in terms of inducing protective responses than natural infection, some of our first highly successful vaccines, tetanus and diphtheria toxoid vaccines, do just this.)

Outlier populations

Cohorts of individuals with an apparent high degree of resistance to infection with *M. tuberculosis* have been reported: highly exposed, persistently TST (or IGRA) negative (77, 78). The likely existence of this uncommon phenotype suggests the possibility of uncommon immunity that might confer sterilizing protection against sustained infection and suggests utility for the close study of such individuals. The fact that the identification of such outliers hinges on the demonstration of a lack of sustained reactivity of conventional T cells with mycobacterial proteins highlights a major limitation of our diagnostic tools. Such highly exposed, persistently TST-negative individuals may have developed an effective, conventional T-cell response to infection, with test reversion upon the achievement of what may be sterile protection. That is, T-cell reactivity as a measure of infection may have been transient. It is also possible, however, that infection led to an uncommon, protective adaptive immune response that is not assayable by TSTs, e.g. is due to unusually effective antibody responses or the activation of an ‘unconventional’ T-cell class having non-peptide specificities (*vide infra*). In either case, study of such individuals might illuminate uncommon mechanisms of immunity that confer protection from sustained infection.

Such studies have not been harnessed for vaccine discovery, however. They have primarily focused on genetic association analysis, which, in turn, has led to the identification of loci that appear to modify the risk of infection (77, 78). However, the pathways implicated to date are integral to the innate immune system and identify variants that are likely to result, e.g. in alveolar macrophages that are hyper-responsive to *M. tuberculosis* or mycobacterial ligands, exhibiting altered phagocytosis or increased cytokine production (77,

78). Although such findings increase our understanding of pathogenesis and may inform host-directed therapies (79), they have been relatively unhelpful in informing vaccine strategies. For this, it will be necessary to define whether such resistance to infection is, in some cases, the result of an uncommon adaptive immune response. For cohort studies of stably resistant individuals in high-exposure settings (e.g. close household contacts, miners) to inform vaccine approaches, there must be a focus on identifying those components of the adaptive immune system that confer protection and that can be both endowed with memory and targeted through vaccination. As an example, the identification of antibody specificities associated with resistance having opsonophagocytic killing capacity for *M. tuberculosis*, or the identification of novel T-cell specificities associated with resistance, would provide new targets for vaccine development. For this, cohorts of outliers (and controls) should undergo deep immune profiling applying novel next generation sequencing technologies for TCR and BCR (antibody) sequencing, single cell analysis, and multi-parameter flow cytometry. Both peripheral blood and lung compartments should be profiled, the latter with appropriate attention being paid to lung-localized T-cell populations with semi-invariant TCRs. Of course, it remains possible that such elite control of infection is the result of innate immune responses that are not amenable to a vaccine approach.

Exploiting correlates of risk

The signatures of high risk for progression to tuberculous lung disease discussed above have obvious utility for stratifying trial design and, by allowing the selection of individuals with a high risk of progression to disease for vaccine or drug interventions, could significantly decrease study size. In addition, such signatures could be used to stratify immunological analyses to facilitate cross-sectional studies of individuals with latent infection of similar duration, with and without high risk for progression to disease. High dimensional, lung-directed, comparative immunophenotyping of such groups of individuals might identify key differences in the adaptive immune cell populations or specificities that are associated with progression to disease and, by extrapolation, the types of responses that might prevent the transition to active disease. It is noted that such studies would be complex, especially as they are likely to be impacted by HLA heterogeneity. Another risk of this approach has been noted above: as different foci of infection in the same lung can undergo diametrically opposing trajectories (control, quies-

cence; loss of control, disease), it may be that tissue biopsy of different foci would be necessary, which would be difficult to justify in humans. Analysis of such would obviously be aided if the signatures of risk were locally derived and specific to foci undergoing a loss of control. Similar to the step-wise approach that was used in the systems biological analysis of correlates of risk research, noted above, the hypotheses generated in such cross-sectional studies would need to be validated in longitudinal studies of individuals transitioning from signature-negative to signature-positive status. Such foundational studies might define the nature of beneficial, vaccine-targetable responses in the lungs of those individuals who sustained control of infection and, by comparison to those who did not, generate new hypotheses for vaccine strategies aiming to prevent progression from latent to active disease.

The importance of moving from correlates of risk to understanding the underlying biology deserves underscoring. The type I IFN signature that forms an important part of the signature of risk is a case in point. Understanding the biology would have fundamental implications for being able to harness these findings for vaccine discovery, host-directed therapies, and model development.

Human challenge models

The above approaches involve cross-sectional and/or prospective natural history studies. Another approach of increasing importance in vaccine research and development is the use of controlled human infection models. In such studies, healthy individuals undergo controlled challenge either with a wildtype pathogen that can be detected early and is rapidly responsive to therapy [e.g. controlled human malaria infection, or challenge with enterotoxigenic *E. coli* (80, 81)] or with an attenuated pathogen strain that elicits an attenuated disease course [e.g. dengue virus challenge (82)]. Such studies offer a remarkable opportunity for deep analysis in controlled settings, have proved invaluable for understanding pathogenicity and immunopathogenesis, and have dramatically facilitated vaccine development. Malaria vaccine development is the poster child for the latter use, controlled human malaria infection models having been essential to the development of RTS as well as for irradiated sporozoite vaccine approaches (80, 83). Challenge trials are increasingly being used to generate new vaccine concepts and to discover and/or validate new vaccine antigens.

An important question for the TB field is whether a truly informative, safe human challenge model could be

developed. The safety hurdles are obvious, as is the current lack of any sensitive measure of mycobacterial load or persistence. One strategy that has already been explored is the use of a surrogate challenge strain such as BCG inoculated intradermally (84). However, to be truly informative, lung challenge will likely be necessary. Intrabronchial challenge with BCG could likely be performed safely (85), but BCG would obviously not be the most informative mycobacterium to use. When attenuated strains are employed in other human infectious challenges, they are often vaccine candidates that proved too 'hot' for clinical development, but for which safety data are available from phase I trials. In this regard, attenuated recombinant *M. tuberculosis* strains developed as vaccine candidates might prove useful as challenge strains. Complementary approaches might involve engineering the development of auxotrophic strains that would not replicate *in vivo* along with the inclusion of reporters to monitor mycobacterial burden. The development of techniques for the robust, inducible transcriptional and posttranscriptional extinguishing of gene expression in *M. tuberculosis* (86) suggests a path toward the development of a challenge strain in which limited *in vivo* replication could be allowed to occur under highly controlled circumstances. Using such genetic switch techniques, a strain could be engineered in which the transcriptional repression (and gene product degradation) of two or more unlinked genes whose expression is essential for mycobacterial viability was placed under the tight (negative) control of an ingested regulator. Halting the ingestion of the regulator after a defined period would effect cure. Pulmonary challenge with such strains could provide a very useful provocation tool for eliciting informative immune responses in the sorts of cross-sectional and prospective immunophenotyping studies discussed above. Pulmonary challenge could also allow acute, local responses to *M. tuberculosis* to be profiled. The benefits of such a challenge model would, perforce, be derived primarily from insight into the immune responses that occur early after exposure. As such, they would likely have utility in informing and testing vaccine concepts that aim to interdict infection (such as vaccines driving antibodies).

TB is primarily a chronic infection, however. Most aspects of immunopathogenesis will not be recapitulated in acute challenges with highly attenuated mycobacteria. The responses important for protection from progression to disease are unlikely to be analyzable in such a model. As such, a human challenge model for TB would likely be of limited use in informing vaccines that aim to control primary or secondary progression, which is the dominant current

approach in the field. Thus, instead of being a broadly enabling tool, it is likely that human challenge models would have fairly restricted use. As the regulatory path to a controlled infection model in TB would not be trivial, the field should carefully consider whether there would be sufficient benefit or application of such a model to warrant the extensive effort required to establish it.

Vaccine concepts and candidates

There is a clear need for increasing the diversity of TB vaccine concepts along at least two axes: (i) increasing the immunological 'space' being sampled, and (ii) adding an additional proximal goal of vaccination: the prevention of sustained infection as well as prevention of progression. Some relevant considerations for this approach are sketched out here, starting with whole-cell approaches.

Whole-cell vaccines and lysates

The intrinsic multivalency of whole-cell vaccines, cutting broadly across the activatable 'immune space', has considerable theoretical appeal. Diverse approaches have been taken, including attempting to wring more benefit from existing BCG(s), the generation of rBCGs, defined molecular attenuation of *M. tuberculosis*, and the use of inactivated mycobacteria or mycobacterial lysates.

BCG has been the most widely used vaccine in the world (87, 88). Efficacy against disseminated disease (miliary disease, meningitis) in infants is 60–80% (89). As noted above, this benefit is likely provided by *M. tuberculosis* infection itself in older immunocompetent individuals (though not in standard animal models), efficiently activating immune responses that protect against uncontrolled dissemination. The efficacy of BCG in preventing pulmonary disease is much less impressive, ranging from negative to 80%, with the lowest efficacy being seen in endemic areas with high transmission and rates of disease (88, 90, 91). In some settings and populations, impressive, highly durable immunity against pulmonary TB has been seen. In Native Americans, retrospective analysis suggests persistence of protection against pulmonary TB (efficacy = 52%; 95% CI: 27–69%) for 50–60 years (92). Overall, however, the duration of protection afforded by BCG appears to average around 10 years (93). While apparently not a factor in the setting of the impressive efficacy and duration observed in studies among Native Americans (92), the fact that BCG actually represents several genetically diverse vaccines (94) with likely differences in efficacy (95) may help explain some of

the observed variability in protection against disease and sustained infection afforded by BCG vaccination. However, exposure to non-tuberculous mycobacteria (NTM) is hypothesized to play a major role (87). It is important to be clear about this effect. The most plausible underlying mechanism is that exposure to NTM provides benefits similar to BCG vaccination and hence masks the ability to observe vaccination-induced protection (87). While immune responses driven by NTM might also theoretically inhibit BCG replication, and hence lower antigenicity, the relevance of such 'blocking' remains unclear, given the rapid clearance of BCG in naive hosts (96). It also remains possible that subsequent exposure to NTM can compromise (or augment) the BCG response, a phenomenon that can be generated in reductive mouse models (97). Whether variable force of infection also plays an important role in the variable efficacy of BCG in protecting against sustained infection or lung disease remains unclear. Another interesting question that remains unanswered is what percentage of the lung disease prevention observed with BCG vaccination is the actually the result of preventing sustained infection (58–63). Overall, it is certainly clear that BCG, as currently administered, is not a sufficient tool to control disease and transmission in those areas of the world with the highest transmission, despite nearly universal use in such areas. It may be possible to build on the available benefits of BCG, either through revaccination (88), direct administration to the airways (98), or supplementing the antigenicity (part of the problem may be that BCG lacks critical *M. tuberculosis* antigens) and/or the immunogenicity of BCG, a fundamental rationale behind the development of rBCGs (99).

BCG appears to have important positive effects that are broader than just protection from infection and disease due *M. tuberculosis*. Observational studies from several parts of the world have provided evidence that BCG vaccination has positive effects on all-cause mortality in early life, especially in areas with high infectious infant mortality, that is greater than its contributions to protection against TB alone, although robust data from randomized controlled trials remain scarce. The original observations derive from Calmette and Guerin themselves (100, 101); more recently, the epidemiological data have been reviewed by SAGE (102). While the mechanisms underlying these apparent heterologous beneficial effects of BCG remain unclear, the findings are certainly biologically plausible. BCG infection of mice significantly increases resistance to subsequent infection with *Listeria* and poxvirus (103, 104). Vaccination of human neonates with BCG has been shown to alter the quality and

amplitude of immune responses to heterologous vaccines (105). The immunostimulatory properties of BCG infection are routinely harnessed for bladder cancer therapy (106). More generally, there are numerous mechanisms by which BCG vaccination might affect concurrent and subsequent infectious challenges, including innate immune priming and memory, induction of the acute phase response with secretion of antimicrobial effector molecules by the liver, upregulation of myeloid cell production, maturation and function, induction of cross-reactive T cells and antibodies, and altering the kinetics of immune maturation (107–111). There are obvious potential implications of these beneficial heterologous effects, including the possibility that the different BCGs employed around the world vary in their induction of such effects. For the present discussion, however, concern focuses on the intended use of whole-cell vaccines currently in development, including rBCG and attenuated *M. tuberculosis*. While the use of such vaccines as boosts for neonatal BCG is not concerning, these have generally been viewed as BCG replacements (99). It will clearly be necessary to test such 'replacements' against BCG in well-designed studies that measure all-cause infant mortality as a primary endpoint prior to effecting replacement of BCG with such vaccines.

Diverse approaches have been taken to recombinant augmentation of BCG: the overexpression of immunodominant *M. tuberculosis* T-cell antigens that are already present in BCG; the introduction of immunodominant *M. tuberculosis* T-cell antigens that are missing in BCG; the reintroduction of genes deleted during BCG attenuation; and increasing BCG exposure to the cytosol to upregulate CD8⁺ T-cell responses (99, 112). The rBCG Δ ureC:hlly (VPM1002) vaccine, currently in phase II trials, took the latter approach—expressing listeriolysin (which effects membrane perforation at acid pH) in the setting of urease C deletion (which prevents BCG from neutralizing phagosomal pH) (113). All of these rBCG approaches aim at increasing the response to immunodominant T-cell antigens, something that should give some pause in the face of the evolutionary biology concerns discussed above. Despite this, it remains possible that in the context of the broader immune activating characteristics of BCG, increased efficacy without the generation of locally destructive inflammatory responses may be seen with these approaches.

Attenuation of *M. tuberculosis* by targeted genetic deletion represents another approach to the development of a live, whole-cell vaccine. The attenuated *M. tuberculosis* candidate farthest along the development path is MTBVAC, a clinical

isolate with deletions in *phoP* (which encodes a transcription factor that plays an important role in regulating the virulence of *M. tuberculosis*) and *fadD26* (which encodes an enzyme critical to the synthesis of some complex lipid virulence factors) (114). The obvious promise of this approach is the generation of something approaching the same protection that is delivered by natural *M. tuberculosis* infection without the attendant risk of progression to disease. However, the protection afforded by natural *M. tuberculosis* infection is likely dependent upon persistent infection. Such concomitant immunity is evident in various parasitic infections, both protozoal and helminthic. In animal models, concomitant immunity appears to depend on the presence, at the time and site of exposure, of short-lived effector T cells whose stable presence (renewed by the activation of naive T cells and/or the re-stimulation of memory T cells) is maintained by persistent infection (115). In addition, the protection afforded by natural *M. tuberculosis* infection might depend on the expression of antigens, such as latency-associated antigens, that are only expressed during sustained infection. Neither attenuated *M. tuberculosis* vaccines nor rBCG vaccines (which appear to be functionally more attenuated than BCG) lead to persistent infection, a potential problem if either of these hypotheses is correct.

Evolutionary considerations have suggested that *M. tuberculosis* was likely considerably less virulent, with longer latency, prior to the crowding associated with the Neolithic transition (54). Such considerations suggest the possibility that if such genomes could be recovered, pre-Neolithic *M. tuberculosis* strains might point the way to combining the attenuation (and hence safety) of recombinantly attenuated *M. tuberculosis* vaccines with the antigenic persistence of a quasi-commensal *M. tuberculosis*. Whether genomic recovery of such strains is possible with current techniques for analysis of paleo-DNA remains unclear.

Inactivated, whole-cell vaccines are also in the clinic. Heat killed *M. vaccae* (the vole bacillus) has shown some efficacy as a boost for BCG in prevention of TB in HIV⁺ patients (116), is undergoing a phase I trial as a boost for BCG in adolescents and adults in HIV⁺ and HIV⁻ patients in trials sponsored by Aeras, and is in a phase III trial for the prevention of pulmonary TB in TST-positive individuals in China (50). RUTI, composed of fragmented *M. tuberculosis* formulated liposomally, has shown safety and tolerability in a phase 2a trial aimed at the indication of preventing TB in latently infected individuals pretreated with 1 month of isoniazid (117). These approaches would appear to have the benefit of the capacity for broad immunological activation,

but they lack the hypothetical benefits of antigenic persistence, the expression of infection stage-specific antigens, and the 'natural' presentation of immunogenic structures that occurs with infection.

There are some final points to be made on whole-cell vaccines as a class. Firstly, whatever their efficacy, whole-cell vaccines have promise as probes (and challenges) of the immune system to enable better understanding of the immunopathogenesis of TB. Secondly, the HIV/TB syndemic is a daunting target for vaccine development, given that the immunosuppression associated with HIV infection will likely attenuate vaccine immunogenicity and effectiveness. That said, several rBCG and attenuated *M. tuberculosis* candidates appear safer than BCG in immunocompromised mice and may provide greater safety than BCG in HIV-infected infants. Thirdly, while a clear potential benefit of all whole-cell vaccine approaches is the breadth of their immune activating capabilities, it needs pointing out that the current 'gold standard' of immunogenicity of both subunit and whole-cell vaccines is the magnitude of the classical CD4⁺ and CD8⁺ T-cell responses induced (with a focus on Th1 responses). Rational harnessing of the broader immune activation afforded by these vaccines will demand quantification of such responses that, in turn, will demand bringing high dimensional immunological analysis to bear. Finally, the breadth of the immune activation induced by whole-cell vaccines might actually represent their Achilles heel. We will need to do better with a vaccine than the natural immunity to TB commonly observed after infection with *M. tuberculosis*. The immune response to whole-cell vaccines, like that to natural infection, likely involves a complex mix of responses that are beneficial to the host and beneficial to *M. tuberculosis*—the end result of extensive coevolution and coadaptation. Reductive approaches, and the testing of reductive subunit vaccine concepts, may well be needed to tease apart the specifically beneficial components of common successful immunological responses to infection, as well as to define the potential for the induction of uncommon or unnatural protective immunity to TB. Hence, there is a clear role for rational research on subunit vaccines.

Subunit vaccines: CD4⁺ and CD8⁺ T cells

The immunological concept that has dominated TB vaccinology to date is that of driving immunodominant CD4⁺ and/or CD8⁺ T-cell responses. However, as reviewed above, evolutionary data question the wisdom of such approaches. It of course remains possible that there are unrecognized parts

of the genome under T-cell immune pressure (our knowledge of T-cell epitopes, especially CD8⁺ epitopes, is less than encyclopedic at this juncture) that might provide good antigen candidates. It is also possible that there are proteins that are not immunogenic in natural infection that due to a particular expression pattern in the human host, if rendered immunogenic, might provide effective, 'unnatural' vaccine-induced protective immunity. However, the practical difficulties involved in selecting and testing such gene products as vaccine candidates appear prohibitive with standard technologies and models. An alternative strategy for unnatural immunity that circumvents intrahost variability might be to develop vaccines that induce T-cell responses that are restricted not by conventional class I and II MHC, but rather by unconventional HLA molecules such as HLA-E (118). Exploitation of HLA-E could have multiple advantages, as in addition to being relatively invariant when compared to conventional MHC molecules (118), HLA-E is known to present *M. tuberculosis*-derived antigens (119, 120) and its expression is not decreased during HIV infection (121).

Attempts to improve vaccine design for subunit approaches aimed at driving classical T-cell responses might be facilitated by alternative approaches to antigen choice. It may, for example, be useful to combine genomic approaches with unbiased identification of the TB antigens that are actually presented during infection. The standard technique for this is somewhat indirect and relies on prediction algorithms and overlapping peptides to identify those antigens that stimulate *M. tuberculosis*-specific T-cell clones (122–124). However, these peptides may not actually be presented on infected cells, e.g. because the proteins from which they are derived are only transiently expressed (125). For these reasons, it might be advantageous to combine standard epitope discovery approaches with orthogonal, unbiased techniques for peptide discovery such as peptide elution and mass spectrometry. Although technically challenging and complicated by the possibility that there may be differential presentation of epitopes during latent and active disease, this type of approach is now feasible and worthy of further exploration (126).

Finally, it may be that altering the localization or kinetics of expression of immunodominant T-cell responses could overcome the apparent evolutionary constraints and lead to earlier containment. That is, one potential failing of current vaccine strategies may not be their antigen specificity, but rather their failure to position the relevant cells at the portals of infection. It is therefore possible that current approaches could be more effective if we were able to be more

deliberate in inducing local lung immune responses through vaccination. (Such could, of course, also lead to more effective tissue destruction and disease promotion.) Over the past 5 years, the field of immunology has come to better understand both the cellular and molecular mechanisms that are important in establishing such local, tissue-specific defenses. In addition to effector memory T cells that circulate through tissue and lymphoid organs, there are tissue-resident memory T cells (Trm) (127). These cells are established when effector T cells enter sites of inflammation and then differentiate into memory T cells *in situ* and are subsequently constrained to the tissue. Trms are long-lived and well poised for early microbial interdiction, either directly or through recruiting additional effector T cells in an antigen-dependent manner. Because of these properties, it is conceivable that strategies that deliberately aim to establish Trm in the lung might increase vaccine efficiency (128). One such possibility would be via the use of the recently developed cytomegalovirus (CMV) vaccine vector used in SIV/HIV vaccine research that induces large numbers of class II- and HLA-E-restricted CD8⁺ T cells in tissues throughout the body (129, L. Picker, personal communication). Current data from mouse models suggest that this end might also be achieved by drawing the cells into the tissue during their expansion in the effector phase using an inflammatory or chemokine stimulus, or by modifying the route of vaccine administration (130, 131). Pertinent to this approach, aerosol administration of BCG and other TB vaccines are being considered, with one potential benefit being improved induction of lung-resident Trm cells (98). As an alternative, vaccines that induce unconventional T cells with semi-invariant T-cell receptors (TCRs), such as $\gamma\delta$ T cells and mucosa-associated innate T cells (MAITs), might be useful as they have a natural propensity for populating mucosal sites (*vide infra*).

Subunit vaccines: B cells and antibodies

The intracellular nature of *M. tuberculosis* replication in macrophages led early TB vaccinologists to dismiss the potential role of antibodies in protection, and this view subsequently became entrenched in the field. However, this assumption may be incorrect, especially when one considers that antibodies are known to protect against viral infection and modify disease progression, despite the obligate intracellular lifecycle of viruses. Furthermore, essentially all of our current, effective vaccines to both viral and bacterial pathogens work via inducing antibody responses (BCG and varicella zoster vaccination in the elderly being possible exceptions).

The possibility of humoral immunity being effective against TB thus deserves revisiting. Several groups have shown that serum or antibody transfer can modify the course of infection – specifically, dissemination from the lung in standard mouse models (132, 133) – although the data are conflicting (132). However, whether antibody responses can protect against sustained infection has not been testable, for the reasons (high infective dose; the use of surface-stripped *M. tuberculosis* as inocula) noted above. There are reasons to hypothesize that antibodies might provide a path to vaccine-inducible sterilizing protection from infection as they have the potential for altering the initial interaction between *M. tuberculosis*—in a droplet nucleus inhaled into an alveolus—with alveolar macrophages. Blocking this interaction, facilitating entry into a microbicidal compartment in alveolar macrophages, and driving alveolar macrophage microbicidal effector functions via FcR engagement are all possibilities. Of course, it remains possible that such antibodies might just more efficiently target *M. tuberculosis* to alveolar macrophages in the absence of facilitating killing.

The development and use of natural transmission models, in which infection occurs by sustained exposure to another infected vertebrate (e.g. human to guinea pig, non-human primate to guinea pig) will be necessary for a direct test of the hypothesis that antibodies against the normal surface constituents of this encapsulated organism (134–136) can protect against infection. A rational approach would be the generation and adoptive transfer of antibodies (alloantibodies, not xenoantibodies due to the prolonged course of infectious exposure necessary in natural transmission models) to surface glycans, lipids, and proteins of *M. tuberculosis*. Only a subset of antibodies directed against surface structures of *M. tuberculosis* is likely to have the requisite specificities and effector functions. Opsonophagocytosis assays employing alveolar macrophages may be helpful in their identification. Antibody isotype is another important consideration, both in preclinical proof of concept studies as well in potential human vaccines. The dominant isotype in the upper airway is IgA, whereas below the level of the bronchioles, including the alveolus, IgG predominates. Which isotype(s) are needed to protect the respiratory tract from *M. tuberculosis* infection would need to be defined.

Subunit vaccines: T cells with semi-invariant TCRs

'Unconventional' T cells with invariant or semi-invariant TCRs are a diverse group, including $\gamma\delta$ T cells, group 1 CD1- (CD1a-c) restricted $\alpha\beta$ T cells, group 2 CD1- (CD1d)

restricted iNK T cells, and MR1-restricted mucosal associated innate T cells (MAITs). The specific features of each are discussed below, along with our current understanding (or ignorance) of the degree to which these various T-cell classes have the requisite memory, effector, and localization properties to provide for vaccine-inducible protection against TB. The antigens for these cells are not conventional peptides but diverse lipids and metabolites, including those found in or induced by microbes. Most of these T-cell subsets have non-polymorphic restricting elements that present their cognate antigens. Over the past 10 years, all have been shown to have potential reactivity with *M. tuberculosis*-derived or -induced products. Although initially thought to be very restricted in the antigens that they recognize, recent analysis of their TCR sequences has in many cases revealed CDR3 diversity, consistent with broader antigenic specificity. Consistent with this, an increasing number of ligands have been found for these unconventional T cells, including many from mycobacteria. However, whole categories of these T cells are absent from mice; hence, their role in TB immunity may well have been overlooked. Furthermore, in humans, many of these T-cell subsets exist at a low frequency in the blood but at much higher frequencies in tissues, especially during inflammation. Definition of the function of these unconventional T cells thus demands direct interrogation of relevant model species or of human tissues. Despite being difficult to study for these practical reasons, their specificity for *M. tuberculosis*-derived ligands, their pulmonary localization, and the non-polymorphic nature of their restricting elements make these invariant T cells attractive targets that may be exploitable in the next generation of TB vaccines.

$\gamma\delta$ T cells are infrequent in blood but enriched in mucosal sites (137). During infection, $\gamma\delta$ T cells expand rapidly and can come to outnumber other lymphocytes in inflamed mucosal sites (138, 139). $\gamma\delta$ T cells rearrange their γ and δ chains, resulting in junctional diversity, and adopt a memory phenotype (140–142). Although $\gamma\delta$ T cells exist in mice, the $V\gamma_9/V\delta_2$ cells that have been implicated as important effector cells in TB in humans and primates (143) are unique to these species. [Murine $\gamma\delta$ T cells appear to play an immunoregulatory role in standard mouse models of TB (144).] The identity of the molecule that presents ligands to the $V\gamma_9/V\delta_2$ TCR remains unclear, although recent reports suggest that the butyrophilin, BTN3A1, may be the restricting element (145). $V\gamma_9/V\delta_2$ T cells are thought to respond to non-peptidic phospho-antigens such as HMB-PP (E)-4-hydroxy-3-methyl-but-2-enyl pyrophosphate), a microbial

compound that is an intermediate of the isopentenyl pyrophosphate (IPP) biosynthetic pathway (139). Importantly, HMB-PP is a metabolite common to most pathogenic bacteria including *M. tuberculosis* (139). Mycobacteria induce expansion of $V\gamma_9/V\delta_2$ T cells with a more restricted TCR use than that induced by synthetic $V\gamma_9/V\delta_2$ TCR agonists such as Phosphostim[®], suggesting that there are pathogen-specific, mycobacterial derived product(s) that stimulate $V\gamma_9/V\delta_2$ T cells (146). In addition to recognizing such products, numerous lines of evidence suggest that $V\gamma_9/V\delta_2$ T cells participate in the immune response to *M. tuberculosis* (147, 148). Human $V\gamma_9/V\delta_2$ T cells that have been expanded with lysates of *M. tuberculosis* inhibit intracellular replication of mycobacteria (149). $V\gamma_9/V\delta_2$ T cells convert from making proinflammatory cytokines during early TB disease to an inactivated or immunomodulatory profile in the lung during severe late disease (150, 151). In macaque studies, $V\gamma_9/V\delta_2$ T cell expansion with a non-specific stimulus (Phosphostim[®] and IL-2) has been shown to enhance resistance to TB (152).

MAIT cells are a subset of $\alpha\beta$ T cells that localize to mucosal sites. Their TCRs are semi-invariant, comprised of an α chain, $V\alpha_{7.2}-J\alpha_{33}$, paired with various β chains in humans (153). These T cells can recognize riboflavin derivatives generated by bacteria and presented by MR1, a non-polymorphic and highly conserved MHC-related molecule (154). MAITs are expanded by the microbiome (155), suggesting memory properties. Ligand recognition induces expression of a variety of chemokines and cytokines including IFN- γ , TNF, and IL-17A (156, 157). MAITs are able to lyse infected cells and promote killing of intracellular bacteria, implicating them in cell-mediated defense (158). Recent work has shown that there is TCR heterogeneity in MAITs, suggesting clonal specificities for different microbial stimuli (159, 160). Furthermore, differing MAIT clones also appear to have different functional phenotypes (160), an observation that suggests the possibility that they may be educated by prior exposure to their cognate ligand (156). During infection, MAITs localize to the airways (161). Airway epithelial cell infection with *M. tuberculosis* can stimulate their *in vitro* activation (162). What is not yet clear is what role (effectors? immunoregulators?) MAITs play in TB. The recent identification of the nature of the ligands that stimulate MAITs should facilitate identification of the products from TB that can induce the activation of TB-specific MAIT clones *in vivo*, and help elucidate their functional relevance.

CD1-restricted T cells that respond to a variety of lipid and glycolipid antigens (163), including some derived from

M. tuberculosis (164, 165), represent another interesting population of T cells. The group 1 CD1 family, comprised of CD1a, CD1b, and CD1c, presents lipid antigens to $\alpha\beta$ T cells. These cells are not found in mice but are present in guinea pigs, which therefore serve as a tractable model in which they can be studied (166, 167). The protective role of CD1-restricted T cells was demonstrated over 10 years ago using the guinea pig model, at which time the possibility of a lipid-based vaccine was suggested (168). In these studies, immunization with mycobacterial lipids encapsulated into liposomes and delivered with adjuvant showed protection comparable to that induced by BCG. In humans, lipoproteins are major drivers of the polyclonality of the T-cell response in TB, and many of these cells are CD1-restricted (169). The use of CD1b-tetramers has enabled the identification and further characterization of these *M. tuberculosis*-specific T cells (170). Interesting recent data using tetramer technology and next generation sequencing has revealed that there is expansion of CD1b-restricted $\alpha\beta$ T cells with a high-affinity, germline-encoded, mycolyl lipid-reactive (GEM) TCR (α -chain: V TRAV1-2, J TRAJ9) in unrelated patients with TB (171, 172). Like MAITs, GEMs expand in a pathogen-specific manner, and secrete inflammatory cytokines supporting their potential role in host defense (171). More generally, the CD1b repertoire appears to be composed of a mix of cells with high-affinity, conserved TCRs (such as GEMs) and cells with low-affinity, diverse TCRs (171, 173), something that may well be true of the broader group 1 CD1 family. So, how are GEMs and other CD1-restricted T cells with semi-conserved TCRs selected, and can we manipulate their function? These TCRs do not appear to self-reactive and hence are unlikely to have been selected on self-antigen. Intriguingly, GEM T cells are found in the naive repertoire of individuals that have not been infected by *M. tuberculosis* or BCG, and it is possible that lipids derived from environmental mycobacteria or components of our microbiota may have driven selection of these cells (171). Potentially useful features of GEMs are that their TCRs express a common CDR3 motif, show relatively little interindividual variation and—similar to public MHC-restricted TCRs—appear to arise by convergent events in genetically diverse individuals' CDR3s (171). Manipulating public T-cell responses is an attractive strategy in vaccination, as it might allow one to target genetically diverse individuals through a predictable antigen-specific TCR, and to drive relatively homogenous responses.

NKT cells represent a final group of CD1-restricted cells, CD1d-restricted in this case. These cells have an $\alpha\beta$ TCR and

share features with both NK cells and conventional T cells (174). Type 1 NKT cells express an invariant TCR, $V_{\alpha 24}\text{-}J_{\alpha 18}\text{:}V_{\beta 11}$ TCR (and hence are referred to as invariant or iNKT), whereas type 2 NKT cells are more diverse. All NKT cells recognize lipid antigens. iNKT cells recognize glycolipids from a variety of microbes including *M. tuberculosis* and can produce numerous cytokines including IFN- γ , IL-4, and GM-CSF (175, 176). Notably, GM-CSF produced by iNKT cells during infection controls *M. tuberculosis* growth in macrophages (177). In mouse models, therapeutic activation of iNKT cells with α -galactosylceramide has therapeutic efficacy against *M. tuberculosis* infection that is additive with isoniazid, suggesting that activation of iNKT cells might be useful as a host-directed therapy (178). But could NKT cells be vaccine-inducible? Until recently this seemed unlikely, as there was little evidence for a memory iNKT population. However, recent work suggests that immunization of mice with NKT ligand-bearing APC can induce effector memory iNKT cells that last for several months in the lung (179). Furthermore, TCR analysis has suggested clonal expansion (179), raising the intriguing possibility that it might be feasible to target these cells in an antigen-specific manner through vaccination. Whether a defined mycobacterial antigen could be identified and used to expand these cells in a *M. tuberculosis*-specific manner remains unclear.

In summary, harnessing these unconventional T cells may represent an alternative path to the development of a TB vaccine. It may, for example, be possible to focus the broad immune response induced by BCG priming by boosting with antigens that are specific for these unconventional T cells to expand only that aspect of the immune response that is beneficial to the host. However, it should be noted that, to date, no vaccine has been developed with the specific intention of acting via such cells. It remains to be demonstrated whether the deliberate induction of any of these unconventional T-cell responses, either alone or in the context of a vaccine with broader activation properties, can induce durable protective immunity to *M. tuberculosis*. If durable, vaccine-induced protective immunity cannot be induced through these unconventional T cells, their activation may nonetheless be beneficial in host-directed therapy, as an adjunct to anti-mycobacterial drugs.

Fit-for-purpose animal models

While a strong case can be made for harnessing recent technical advances in the interrogation of the human immune response to help liberate the TB pathogenesis and vaccinolo-

gy fields from the historical constraints of extant animal models, clear awareness of the limitations of the animal models in current use also suggests ways for generating potentially transformative, fit-for-purpose models.

Nardell's (57) revisiting of the Riley model of transmission from humans to guinea pigs has underscored the fact that natural transmission can dramatically alter the spectrum of infection and disease, in this case to a closer mimic of that exhibited by humans. Whether this relates to infectious dose (likely a single organism) or to the biological state of the infecting inoculum remains unclear. As discussed above, such natural transmission models will be essential for testing the hypothesis that antibodies against surface structures of *M. tuberculosis* can raise the bar to infection. To control the input variables, a model in which the donor is an experimentally infected non-human primate, as opposed to a naturally infected human, would be desirable. For adequate N, throughput and respiratory sampling capacity, guinea pigs are probably the optimal downstream species. Being able to mimic and replace the vertebrate middleman with *in vitro*-cultured mycobacteria delivered in a physiological way to the respiratory tree would simplify the model even more.

More physiological challenge models are also likely to be critical for testing other vaccine strategies aimed at preventing sustained infection. The hard-won insights of the SIV/HIV vaccine field suggest the likely utility of pursuing ultra-low dose repetitive challenge, with challenge number required for sustained infection as the primary readout. The exigencies of mycobacterial growth will demand the generation of tagged strains to allow for such analysis.

The standard animal models of today are largely models of primary progression with dissemination. The natural challenge model experience holds out the possibility that ultra-low dose challenge will also be associated with a low degree of primary progression and increased establishment of latency. To directly test vaccines aimed at preventing the transition from latency to active pulmonary disease, the establishment of tractable models of latency will be a necessary first step. Low-dose infection of cynomolgus monkeys is especially promising in this regard (34). There may also be a role for rabbit models (180). What will also be needed is a physiologically relevant way to drive the transition from latency to active disease. As noted above, testing the effects of type I IFNs and other mediators associated with the signature of risk for progression to disease in humans may be useful. More generally, intensive study of human TB should allow the iterative development of animal models designed

for testing new approaches to preventive (and therapeutic) intervention.

Other important considerations for improving the usefulness of animal models in TB vaccine research include attention to the use of clinical strains that cause disease in high transmission settings, assuring an adequate time between vaccination and challenge to assure that memory responses are in fact being analyzed, attention to the timing of BCG vaccination for vaccines aiming at boosting of such responses long after infancy, and standardization of improved models to allow optimal head-to-head testing of vaccine concepts and candidates.

Clinical development: efficacy trials

Our inability to de-risk vaccine candidates before the performance of large, very expensive efficacy trials in the current paradigm for the clinical development of TB vaccines is clearly not sustainable. Creative trial design will be essential for moving forward. One attractive solution would be to dramatically decrease trial size by: (i) focusing enrollment on those at highest risk of the outcome event, and (ii) increasing the false discovery rate, in essence looking not for efficacy but for plausibility of efficacy: for a reason to select a vaccine with a low prior probability of technical and regulatory success for a follow-up, better-powered efficacy trial (181, 182).

Performing prevention of sustained infection trials in very high transmission settings, such as in adolescents in the Western Cape of South Africa (183), is one way to focus enrollment on those at highest risk. There are obvious complexities to trials testing the prevention of sustained infection. For one, endpoints based on sustained evidence of robust T-cell reactivity with *M. tuberculosis* antigens (via TST, IGRA, or novel IGRAs containing *M. tuberculosis* antigens that are not present in the vaccine candidates being tested) are not as firm as disease endpoints. For such studies as well as drug treatment studies, the development of sensitive ways of detecting and quantifying mycobacterial load in humans would obviously be a major advance. Secondly, it is at least theoretically possible that a vaccine might be highly efficacious in preventing infection, but just in those individuals (90%) who would never develop disease after infection anyway. There would thus be a need for prevention of disease trials in follow-up. Finally, it should be acknowledged that such trials could well leave something on the table; that is, a vaccine with high efficacy in preventing disease might not show comparable efficacy in preventing infection.

Given the high rates of recurrent disease after successful treatment seen in high transmission settings (184), another way to enrich for endpoints could be by performing prevention of recurrence trials in such settings. As with prevention of infection trials, there are risks and complexities, including the facts that recurrence is a mix of reinfection and relapse, and that those who end up developing TB are at very high risk for recurrent disease. Preventing recurrence is likely, for both host-centric and environmental reasons, to be an especially high bar for a vaccine to pass.

This latter point brings up a more general consideration. The efficacy of secondary prophylaxis with isoniazid, while apparent in low transmission settings, has been difficult to demonstrate clearly in areas with a very high force of infection (185). Similarly, as noted above, the efficacy of BCG in protection against pulmonary TB may well be compromised in such settings. If these interventions had been first tested under such conditions, their clear value in other settings might not have become apparent.

Despite these concerns, plausibility of efficacy trials, especially prevention of infection studies, provide a pragmatic way for moving towards achieving proof of concept in humans in a vaccine field in which the current paradigm for clinical development is not consistent with available human or financial resources.

Conclusions

The road to a more efficacious vaccine that could be a truly transformative tool for the control of TB is clearly quite difficult. Given that, if a highly sensitive, highly specific, sputum-independent point of care diagnostic along with a 3–4 week course of curative therapy were in hand, a strong case might be made to step back from TB vaccine research and development for now. But, of course, neither exist. Despite the difficulties involved, we actually see this as a hopeful time. Abetted by better conceptual clarity, clear acknowledgment of the degree of our immunobiological ignorance, the availability of powerful new tools for dissecting the immunopathogenesis of human TB, the attendant promise of generating more creative diversity in TB vaccine concepts that test a broader immunological space, the development of better fit-for-purpose animal models, and the potential of more pragmatic approaches to the clinical testing of vaccine candidates, the field has promise for delivering a transformative tool for dealing with this worldwide scourge of poverty.

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