The vaccine discovery paradigm in tuberculosis (TB) has been to mimic the natural immune response to infection. With an emphasis on interferon (IFN)-γ as the main protective cytokine, researchers have selected dominant antigens and administered them in delivery systems to promote strong T helper (Th)1 responses. However, the Bacillus Calmette–Guérin (BCG) vaccine is a strong inducer of Th1 cells, yet has limited protection in adults, and further boosting by the Modified-Vaccinia-Ankara (MVA)85A vaccine failed to enhance efficacy in a clinical trial. We review the current understanding of host–pathogen interactions in TB infection and propose that rather than boosting Th1 responses, we should focus on understanding protective immune responses that are lacking or insufficiently promoted by BCG that can intervene at critical stages of the TB life cycle.

Understanding the cellular immune response to Mycobacterium tuberculosis (MTB)

MTB has coevolved with humans for >70,000 years, predating human emergence from Africa [1]. This has resulted in the world’s most successful bacterial pathogen, equipped to establish itself within the human host for decades as a latent infection without overt disease. Today >2 billion people worldwide are infected with MTB and each year >8.6 million people develop acute pulmonary tuberculosis (TB) and 1.3 million people die from the disease [2]. TB therefore remains second only to HIV as the infectious disease responsible for the most human deaths [2]. With increasing number of cases involving multidrug-resistant (MDR), extensively drug resistant (XDR), and total drug-resistant (TDR) TB, an effective vaccine is greatly needed.

Overall, any novel vaccination strategy must answer two main questions: (i) what antigens to target? and (ii) what kind of immune response to induce? In the classic vaccinology approach, these questions are answered simultaneously by mimicry of the natural immune response to infection using an attenuated pathogen with a broad antigen profile based on the assumption that the natural immune response to infection is sufficient for protection against a future infection. Although this has resulted in the development of many highly successful vaccines against acute infections and such medical triumphs as the eradication of smallpox, it is becoming increasingly clear that this classical approach to vaccination against TB is insufficient to control the global epidemic. In this context it is important to bear in mind that with the exception of herpes zoster in which cellular immunity correlates with protection, all licensed vaccines mediate protection through antibodies [3]. The current TB vaccine, BCG – an attenuated strain of Mycobacterium bovis – reduces TB incidence in children. However, neither BCG nor prior MTB infection promotes an immune response sufficient to prevent reactivation or reinfection with MTB in adult life, highlighting the challenges of developing a vaccine against an infection that requires cellular immunity to contain an established infection. Adaptive cellular immunity can control TB disease, but only rarely completely eradicate infection [4], giving rise to reactivation as well as reinfection with new strains later in life (Box 1). Thus, MTB can establish a long-term latent TB infection (LTBI), wherein a successful immune response is characterized as one that prevents overt disease where bacterial growth and immunopathology leads to lung tissue destruction, cavity formation, and wasting disease. Towards control of primary, reactivation, and reinfection TB, a vaccine able to promote long-term containment (if not clearance) of infection and prevent the development of contagious lung disease would have a major impact on transmission and global health [5].

In the past 10 years there has been substantial progress in the TB vaccine field, with more than a dozen novel vaccines in clinical trials [6,7]. Recently, one of these new vaccines, MVA85A, became the first TB vaccine since BCG itself to complete an efficacy trial [8]. In a study powered to detect a 60% reduction in incidence of TB cases in an endemic area, 2795 BCG-vaccinated infants were boosted with this MVA expressing the MTB antigen 85A or a placebo control and thereafter followed for 3 years. During its preclinical development program, this vaccine was demonstrated to boost BCG-primed immune responses, promoting powerful Th1 responses measured by IFN-γ EliSpots. However, the outcome of the trial was very disappointing with no detectable improvement of protection against TB [8,9].

In light of these results, here we re-examine our current understanding of cellular immune response to MTB. In particular, we focus on protective immune responses that are lacking or insufficiently promoted by BCG.

MTB infection and immunity

MTB infection begins with inhalation of an infectious bacterium and engulfment by antigen presenting cells (APCs) such as alveolar macrophages and dendritic cells.
Box 1. The life cycle of TB infection/disease and its impact on vaccination strategies

TB can be broadly characterized as primary disease (following a recent exposure) or LTBI, which is clinically defined as an individual demonstrating pre-existing cellular memory against MTB antigens (e.g., via a positive tuberculin skin test) but without clinical disease symptoms. The relative contribution of MTB reactivation caused by the original strain and/or reinfection with a new strain of MTB in this latent TB group of individuals is not well defined. Classical observations of healed TB lesions without culturable bacteria from autopsies suggest that MTB clearance can occur [76]. However, the lifelong maintenance of cellular responses to TB antigens, reports of disease with strains having an identical genotype to isolates from the same individual 30 years earlier [77], and the isolation of MTB DNA from traffic accident victims with no previous history of TB disease [78] suggest that sterile immunity is rare and that infection can continue lifelong in most cases. In particular, reactivation of LTBI seems to be the main mechanism responsible for TB disease in the adult population in low-endemic regions [79]. In some high-incidence areas, high rates of reinfection have been reported, especially in HIV coinfected individuals [80]. Recent genome sequence analyses of MTB samples from HIV-negative TB patients with recurrent TB in mid/high-incidence regions found that reinfection accounted for ~5% of cases in a South Indian cohort and ~10% of cases in a retrospective study within Thailand, Malaysia, and South Africa [81,82]. Preventive chemotherapy (6–9 months of isoniazid treatment) is frequently offered as treatment for LTBI in low-incidence areas to prevent reactivation, however in a recent large population intervention study in South African miners, such treatment was demonstrated to have minimal impact on the subsequent incidence rates [83]. This important study highlights the limitations of the natural immune response against TB infection in protecting susceptible individuals from subsequent reinfection. From a vaccination point of view, we do not know if post-exposure vaccination to protect against reactivation would differ from vaccination to protect against reinfection and this important point should be addressed in future vaccine research.

Box 2. Host–pathogen interaction in various stages of TB infection

Cellular dynamics in different stages of TB infection: Compared to most infections, the induction of adaptive immunity against MTB is delayed in infected individuals [84], and similarly, in animal models the first response is detected around 2 weeks after infection. This delayed adaptive response seems to be actively induced by MTB through a deferred macrophage recruitment and antigen dissemination to the lung draining lymph node [10]. The result is that pulmonary TB infection in the initial stage remains almost unnoticed by the immune system. Once initiated, a CD4+Th1/Th17 response dominates the early response to infection [10,11]. Th17 cells promote the recruitment of other cell types including neutrophils and CXC56+ Tfh CD4 T cells [65]. CD8 T cell responses increase later during infection as a consequence of the increasing bacterial burden [85,86]. Regulatory T (Treg) cells delay the initial early adaptive response, but also control immune-mediated pathogenesis to prevent disease in later stages of infection [87]. Ultimately, the infection plateaus and an equilibrium between host immune response and pathogen replication is established. In 5% of infected individuals, this stage progresses to active pulmonary disease whereas the remaining 95% contain the infection as LTBI, where bacteria survive in a low- or non-replicating stage inside granulomas. Different stages of a granuloma (from small focused granulomas where bacterial growth is contained to active granulomas with active mycobacterial growth) often coexist [21,73,88]. This provides an ongoing challenge for the immune system and has profound influence on the dynamic development of immune responses and the maintenance of immunological memory located in the lung alveoli. Thereafter, APCs carry MTB to the regional lymph nodes where an immune response is initiated [10]. This results in the expansion of MTB-specific T cells that subsequently home to the infected site in the lung and form structured granulomas where T cells are localized adjacent to and surrounding the infected APCs (Box 2). Cellular immunity dominates the immune response, and CD4 T cell Th1 immunity at the site of infection is critical to MTB control [10,11]. In addition, Th17 cells can accelerate the initial response and promote the recruitment of other immune cells to the site of infection [12]. The Th1 cytokines IFN-γ and tumor necrosis factor (TNF)-α are absolutely required for control of bacterial growth in both animal models and in humans. IFN-γ and TNF-α-deficient mice are unable to control MTB infection [13,14], and people with IFN-γ receptor defects are highly susceptible to mycobacterial infections [15]. These cytokines promote the control of bacterial growth in the activated macrophages by a combination of expression of reactive oxygen and nitrogen intermediates, supported by lysosomal enzyme attack as well as autophagy [6,10,16]. IFN-γ also promotes expression of antimicrobial peptides (cathelicidin and defensin-β2) delivered to MTB phagosomes via vitamin-D-dependent pathways in human phagocytes [17]. In response, MTB has evolved a set of strategies including arrest of phagosome maturation and lysosomal movements, modulation of cell death pathways, prevention of antigen presentation, and release of anti-inflammatory factors that allow it to evade its own elimination and establish latent infection [16,18]. The actual state of the bacteria in LTBI is not completely understood, but most data from in vivo transcription studies of bacteria isolated from infection studies in animal models have demonstrated that the bacteria transform into a slow or nonreplicative state (so-called dormant MTB) that express...
The dormant MTB does not cause disease, but resists elimination and represents a source of continuous antigen exposure that influences the maintenance of immunological memory to TB (Box 3). Later, MTB can undergo resuscitation into a highly replicative and metabolically active stage characteristic of active TB disease.

MTB infection is therefore characterized by complex host–pathogen interactions where specific immune responses required to control infection as well as the bacterial counter-response to these measures have been identified. In light of these insights we can re-evaluate the strengths and weaknesses of our current vaccine against MTB, and how we can improve upon it.

Improving the BCG vaccine

*M. bovis* BCG is the only vaccine currently available against TB and has been so for >80 years. BCG is one of the most widely administered vaccines worldwide and has been part of the World Health Organization Expanded Program on Immunization (EPI) for childhood vaccination since the early 1970s. BCG vaccination prevents disseminated disease in children, but despite inducing a strong Th1 response, the efficacy of BCG is highly variable (ranging from 0 to 80%) [23], and the vaccine inadequately prevents adult pulmonary TB in high-TB-endemic regions where the presence of abundant atypical mycobacteria in the environment interfere with vaccine activity [24]. It is clear that an improved vaccine is needed to reduce the global TB burden and currently various strategies are being pursued in clinical trials (Box 4). Any novel booster vaccine for the adult population needs to be designed with the epidemiology of high-endemic regions such as Sub-Saharan Africa in mind. In these regions the incidence of LTBI increases during childhood to reach 60–70% in some of the most afflicted populations at the age of >25 years [25]. The result is that a vaccine strategy that targets the adolescent or adult population in high-endemic regions, in most cases, will be administered post-exposure to individuals with LTBI. A vaccine that targets LTBI should therefore be capable of promoting an efficient adaptive immune response in the complex immune modulatory environment of an ongoing MTB infection and target MTB that have adapted to this environment. Some of the most recently developed vaccines have therefore been evaluated and optimized in post-exposure animal models and target LTBI by incorporating latency-expressed MTB antigens [19] (Box 4).

One of the largest obstacles in improving upon BCG is that we still do not completely understand which critical aspects of BCG-elicted immunity are most important for protection against MTB, and which are missing for providing long-lasting protection against TB disease. The immune response to BCG is clearly Th1 skewed, and results in a pool of MTB-specific CD4+ T cells that provide an early response to MTB infection and is associated with some level of protection measured as a reduction in bacterial load and increased survival of infected animals [26–29]. However, several studies have reported that the strength of the IFN-γ response following BCG vaccination does not correlate with protection [30–32]. Moreover, BCG vaccination does not result in sterile eradication or efficient long-term containment of the infection as clearly demonstrated by the spread of the global TB epidemic amongst BCG-vaccinated individuals. Instead of boosting more of the same Th1 response that BCG promotes, we discuss supplementing BCG with antigens or immune responses that have been demonstrated to be involved in protection against TB but are insufficiently induced by BCG (Figure 1).

**Box 3. Immunological memory and TB protection**

CD4 T cells can be subdivided based upon their anatomical location, expression of various cell surface markers, and cytokine secretion [97]. TEm cells have low expression of CD62L and chemokine CC receptor (CCR7) required for trafficking into secondary lymphoid organs. They are found in peripheral tissues and produce effector cytokines such as IFN-γ and TNF-α. In response to continuous antigen from persistent TB infection they are gradually converted to terminal TEM cells, which have lost their ability to proliferate and express the killer cell lectin-like receptor G1 (KLRG1) [46,98]. Recent data furthermore suggest that differentiated T cells that express the KLRG1 marker lack the ability to migrate into the lung parenchyma and are trapped in the lung vasculature and therefore functionally impaired and unable to protect against bacteria multiplying in the lung tissue [99]. TCM cells are opposite to TEM cells based on most of these fundamental parameters. They are characterized by a high expression of CD62L and CCR7 (which direct them through lymph nodes) and they produce abundant IL-2 [97]. The TCM cell subset is of particular importance for the maintenance of immunological memory after vaccination due to its continuous proliferation, and was demonstrated by adoptive transfer studies in the TB mouse model to mediate a highly efficient protection in the lung against an aerosol challenge with MTB [100,101].

Data from TB-infected individuals supports the general observations from animal models. TCM cells expressing IL-2 are associated with LTBI and treated disease, whereas T cells in patients with active TB are predominately TEM cells that express IFN-γ/TNF-α [102–104]. This suggests that CD4 T cell functional capacity during infection is driven toward terminally differentiated T cells by bacterial load and continuous antigen exposure.

**Box 4. Vaccine strategies against TB**

TB vaccines can be administered at different stages of infection to control disease.

- **Pre-exposure vaccines** are administered prior to infection with MTB. The current vaccine, BCG, is given as a pre-exposure vaccine during the first weeks of life. Two types of pre-exposure vaccines are currently being evaluated in clinical trials.
  - Viable mycobacteria are designed to replace BCG as prime vaccines. There are two different vaccines in clinical trials; the recombinant BCG *ΔureC hly*+ (VPM1002) and an attenuated mycobacteria with two gene deletions (MTBVAC) [6,7].
  - Subunit vaccines comprise MTB protein antigens expressed in viral vectors or delivered in adjuvant and are designed as BCG boosters. There are currently several different subunit vaccines in clinical trials based on viral delivery (MVA85A and Crucell Ad35) and protein in adjuvant (H4/IC31 and M72F/AS01E) [6,7].

- **Post-exposure vaccines** target adolescents and adults with LTBI. The most recent subunit vaccine candidates have been tailored for this strategy by integrating latency antigens of MTB with the goal of enhancing immune pressure and control of infection to prevent reactivation of TB in the more than two billion people latently infected [19]. A few of these novel vaccines (H56/IC31 and ID93/GLA-SE) have recently initiated clinical trials [6,7].

**Supplementing the antigenic profile of BCG**

**Missing virulence factors**

BCG is an attenuated strain originally derived from *M. bovis* through >13 years of continuous culture. In this
process BCG lost several large genomic regions encoding potentially relevant antigens. In fact, a recent comparison of 13 BCG and five MTB substrains revealed that 124–188 (>25%) of the 483 experimentally verified human T cell epitopes within MTB are missing from BCG [33]. The major attenuating event in the development of BCG was the loss of the region of difference 1 (RD1) that encodes essential components of the type VII secretion system ESAT-6 secretion system-1 (ESX-1), and thus at least nine potential antigens present in virulent MTB [34]. In fact, the ESX-1-secreted proteins early secreted antigen-6 kDa (ESAT-6) and culture filtrate protein-10 kDa (CFP10) are recognized by nearly all TB patients and are the basis for highly sensitive and specific novel TB-specific diagnostics [35]. In a recent comprehensive screening of the complete TB genome for antigens recognized by TB-infected individuals, the two major antigenic islands were centered on ESAT-6/CFP10 and Rv3615/3616 [36], which may relate to the high expression of these antigens throughout the MTB lifecycle (Box 2). Rv3615/3616 are not part of the RD1, but are required for a functional ESX-1 secretion apparatus that facilitates their own secretion, and are in this way functionally removed in BCG [37]. The fact that several of the premier antigen targets recognized during TB infection are missing from BCG suggests that at least part of the failure of BCG from a vaccine perspective lies in its inability to induce immune responses to these proteins. Indeed, insertion of the MTB RD1 segment into BCG results in reduced bacterial load after MTB challenge, however, the other side of this coin is reduced safety with increased virulence in immunocompromised mice [38]. Along the same lines, vaccination of BCG-primed animals including non-human primates with ESAT-6-containing vaccines promotes a strong protective immune response and improves protection [26,28]. Interestingly, in a recent study, post-exposure vaccination with ESAT-6-containing vaccines was found to have a unique ability to prevent reactivation of TB in a large screening of different prophylactically protective vaccines in a mouse model of latency in which BCG itself lacked efficacy [39]. Thus, the spectrum of immune responses promoted by BCG is incomplete and lacks key antigens that play a major role in both the virulence of MTB and as targets of effective protective immunity in the host.
Missing latency/resuscitation antigens
As MTB adapts to the immune altered host environment and transitions into nonreplicating persistence it upregulates a transcriptional program distinct from the genes expressed during early logarithmic growth [19] (Box 2). These latency genes are upregulated during in vitro conditions such as nutrient starvation and hypoxia, and encode many antigens that are recognized by human T cells isolated from individuals with LTBI [22,40,41]. Notably, due to its attenuation, BCG does not enter into dormancy after vaccination and thus although many of the latency genes are present in the BCG genome the vaccine seems to have a greatly impaired ability to promote responses to the corresponding antigens [42].

Some of the antigens encoded by these genes have been demonstrated to have protective vaccine activity and the potential to target MTB in the mouse model of chronic/latent infection, either on their own or combined with early antigens as part of multistage vaccines [29,43]. Recent attempts to express some of these latency antigens (e.g., nutrient starvation antigen Rv2659 and hypoxia-induced antigen Rv1733) into recombinant BCG have also been encouraging with improved control of late stages of MTB infection [44]. Supplementation of BCG-promoted responses by recombinant engineering or by boosting with multistage subunit vaccines that encode key targets expressed during latency may therefore represent a promising avenue towards increased long-term containment of persistent infection.

Overall, attenuation of M. bovis to the vaccine BCG required the loss of virulence factors for safety, but in return removed expression of key protective immune targets. By identifying these factors it is now possible to develop optimal subunit vaccines to supplement BCG or directly engineer improved live vaccines that complement these antigens without compromising safety.

Supplementing the immune profile of BCG

Immunological memory
BCG-induced Th1 immunity has been shown to decline with time after vaccination and is generally thought to last no more than 10–15 years, which should be compared to the lifelong protection found with some vaccines (e.g., polio or measles) that mediate their effect through antibodies. Although this limitation of BCG may simply reflect the normal turnover of a vaccine-promoted CD4+ T cell response, there is increasing evidence to suggest that BCG may suffer from an insufficient ability to promote immunological memory (see Box 3 for an introduction to immunological memory and TB). In a direct comparison of the longevity and composition of T cell responses promoted by BCG and the subunit vaccine H1/CAF01 (Ag85B genetically fused to ESAT-6 administered in a liposome adjuvant) in mice, it was demonstrated that the subunit vaccine very efficiently maintained a population of long-lived central memory T (T_CMS) cells for up to 2 years post-vaccination, whereas this population was only transiently expressed after BCG vaccination, which instead promoted a response dominated by IFN-γ/TNF-α+ effector memory T (T_EMF) cells [45]. This pattern was also seen in chronically MTB-infected mice, where animals boosted with the subunit vaccine maintained a robust T_CMS cell population in the infected organs in contrast to BCG-vaccinated animals that were characterized by terminally differentiated effector T (T_EMF) cells and failure to control bacterial replication [46]. In agreement with this observation, preventing T_CMS cells from exiting the lymph nodes had no influence on the protection by BCG, indicating that the majority of the T cell response promoted by BCG is tissue-resident T_CMS and T_EMF cells [47]. A lack of long-lived T_CMS cells may therefore represent one of the major shortcomings of BCG [46,48], and in a recent study of BCG vaccination in humans, it was observed that although the vaccine-promoted T cell responses had phenotypic markers that resemble T_CMS cells, their cytokine profiles (mostly IFN-γ producing) and their lack of proliferation were functionally characteristic of T_EMF cells [49].

Important for the discussion of vaccine-promoted memory, the induction of T_CMS cells is dependent upon both delivery [50] and vaccine dose [51]. Mice vaccinated with the MTB subunit vaccine, H28, delivered in either the MVA vector or the liposomal adjuvant CAF01, showed a clear difference in their CD4 T cell phenotype, where the viral-vectorized response was characterized by reduced interleukin (IL)-2-producing T_CMS cells compared to the adjuvanted subunit vaccine [50]. The quality of immune memory is also influenced by vaccine dose, and a low antigen dose in cationic liposomes (which forms an antigen depot at the injection site) was found to correlate with increased T_CMS cell induction and better protection in animal models [51]. These observations were recently mirrored in a human clinical trial where TB-specific memory responses were maintained at constant levels for >2.5 years after the administration of the H1 subunit vaccine in the depot-forming adjuvant IC31 [52].

CD8 T cells
Compared to MTB, BCG primes a reduced CD8 T cell response that is associated with its general avirulence, as well as the specific loss of the ESX-1 secretion apparatus (described above) that confers ability to escape the phagosome and promote class I MHC-restricted antigen presentation [53,54]. Depletion and gene knockout studies in mice have suggested a preferential role for CD8 T cells in the control of chronic/latent MTB infection, [55,56], whereas prophylactic protection by CD8 T cells is a field with contrasting evidence from the literature [57–59]. In humans, anti-TNF antibodies can deplete CD8 T cell mediated anti-MTB activity from peripheral blood mononuclear cells, which has been proposed as a mechanism for the increased reactivation of latent TB in patients receiving anti-TNF immunotherapy [60].

There have been various attempts to increase CD8 T cell responses after BCG vaccination. Recombinant BCG has been engineered to facilitate escape from the phagosome to increase class I MHC antigen presentation [61], and virus- and DNA-based subunit vaccines aimed at boosting BCG-induced CD8 T cells have also been developed [58]. However, CD4 T cell responses are also boosted by these constructs, and the improved protection provided in preclinical studies has not yet been shown to be dependent upon enhancement of CD8 T cells [27,55]. Although there is
a lack of direct evidence for a distinct role for boosting or potentiating the relatively modest CD8 T cell component promoted by BCG, further studies are needed in larger animals and humans where CD8 T cells may play a more pronounced role.

**Th17 cells**

Several studies have shown that adoptive transfer of MTB-specific Th17 cells derived from mice deficient for IFN-γ or the Th1-promoting transcription factor T-bet can reduce bacterial load after MTB challenge [62,63]. The main role of Th17 cells in restricting MTB infection seems to be a chemokine-mediated recruitment of Th1 and chemokine CXC receptor (CXCR)5+ T follicular helper (Tfh) cells to the site of infection in the lung [64]. BCG promotes Th17 T cells that are involved in the protection against MTB challenge [65], and increasing IL-17 responses after BCG vaccination (by blocking IL-10) improved protection against MTB in mice [66]. In a recent study comparing wild-type BCG with the vaccine candidate ΔureC hly+ BCG (a recombinant BCG that is devoid of urease C and expresses membrane-perforating listeriolysin), a significantly enhanced MTB specific IL-17 response was noted following vaccination [27]. This was associated with early recruitment of MTB-specific T cells to the lungs of MTB challenged mice and a significantly reduced bacterial load. Taken together, the data indicate that improving upon BCG-promoted Th17 memory response could have a beneficial influence on protection against TB. Adding an additional Th17 response on top of BCG can be achieved, for example, by subunit vaccines, as recently demonstrated with a TB vaccine based on the CAF01 liposome adjuvant that signals through the C-type lectin receptor, Mincle, on APCs, and promotes long-lived MTB-specific Th17 memory responses [67].

**Protective antibodies**

When considering how to supplement immune responses to BCG vaccination, we also need to briefly discuss antibodies. The contribution of humoral immunity to protection against MTB is a field with conflicting data that has recently been discussed in excellent review articles [68,69]. There is no doubt that cell-mediated immunity (CMI) plays the major role for protection against many intracellular pathogens, but there are clear examples such as *Chlamydia trachomatis* and *Cryptococcus neoformans*, where pre-existing antibodies against surface-associated antigens play an important role in controlling the initial stage of infection [70]. In the context of TB, an antibody response in the alveolar space could opsonize the bacteria and result in increased intracellular killing in macrophages or neutralize essential MTB virulence factors involved in required processes such as nutrient uptake (reviewed in [68]). The main antibody targets should obviously be looked for among surface-presented carbohydrates and proteins and importantly for the current discussion, epitope differences between BCG and MTB have been reported [71,72]. A subunit booster vaccine that incorporates these structures from MTB could therefore, in theory, reduce (or prevent) the initial bacterial implantation and the bacterial load that the CMI response would subsequently have to combat. Although protective antibodies clearly are the most speculative among the possibilities suggested, it is still an interesting avenue that has so far not been rigorously pursued in TB vaccine discovery.

Overall, the identification of these protective cellular (and potentially humoral) responses that are insufficiently primed by BCG, and whose enhancement improves protection in animal models, gives direction for the specific nature of the immune responses which should be focused on and evaluated as we look toward novel vaccine strategies against TB.

**Concluding remarks**

The central requirement for IFN-γ in natural control of MTB is well established, and enhancing the magnitude of the Th1 response has been the central paradigm to the improvement of BCG-induced immunity. In this regard, IFN-γ release by MTB-specific T cells has been the standard comparator for ‘vaccine take’ and relative immunogenicity in most clinical trials of TB vaccines to date, and the guiding principle behind the development of the MVA85A vaccine.

As discussed above, there are several possible approaches that supplement BCG immune responses or provide antigens lacking in BCG and which represent a fundamentally different strategy than boosting Th1 responses primed by BCG. The different immune effector responses that we have discussed target either preferentially the early stage of MTB infection (Th17/antibodies) or the late persistent stage of infection (T(h)17/antibodies) – both stages of the MTB lifecycle where improvements of BCG are needed if we are to break the global cycle of transmission (Figure 2). Overall, the key to vaccination is memory. This may be true in two distinct ways in the case of TB. Long-lasting immune memory after prophylactic vaccination is the key to an accelerated anamnestic response, and for TB this may be particularly important to enable the host to counteract the delay of adaptive immunity ingeniously orchestrated by MTB (Box 2). Furthermore, the unique ability of MTB to establish lifelong persistent LTBI is a cardinal challenge for immune memory. LTBI represents a mixture of lesions in various states of activity [21,73]. These infectious foci are under constant immune control, consistent with reactivation of clinical disease in HIV* individuals and those receiving anti-TNF therapy [74]. Without a substantial TCM cell pool, constant flaring of lesions may too quickly deplete the memory buffer and result in a lack of immune control. For a TB vaccine it is therefore critically important to maintain a sufficient population of self-renewing memory cells both to contain persistent infection and to provide the accelerated response needed to prevent new (re)infections in areas of high transmission.

Several outstanding questions remain in TB vaccine development including the following. What characterizes an efficient immune response that contains MTB infection? Can we develop vaccines that specifically target the early stages of TB infection and/or the late persistent stage of infection? Can vaccine immune responses prevent early implantation or lead to sterilizing immunity? Although we still cannot say exactly what an optimally protective
immune response against TB looks like, given the prevalence of reactivation and reinfection TB, we can at least say that it is not simply more of what MTB promotes, as is clearly demonstrated by failure of the natural immune response to TB infection to protect against reinfection (Box 1). This idea is further supported by the evidence of hyper-conservation of human T cell epitopes in the TB genome, suggesting that T cell responses induced by MTB are, in fact, evolutionarily advantageous to the bacteria [75]. After all, TB is an immune ‘Goldilocks’ problem: a strong immune response is required to control infection, but is also integral in driving disease pathology and dissemination. The trick to vaccination may be to not let the bacteria steer the immune response too much – a steering that may eventually end up in depleted functional memory and inflammation.

Just now there are several vaccine candidates undergoing clinical trials that represent vaccines with different immune profiles and modes of action (live virus/live mycobacteria/adjuvanted subunits) (Box 4). It will be important to go beyond the prevailing focus on various readouts for IFN-γ when these trials are monitored, and assess a wide range of immune parameters in standardized assays that are harmonized between the different laboratories involved. This will allow signatures to be identified and correlated with clinical success or failure in future proof-of-concept phase IIb clinical trials. In the meantime, we should maintain momentum in innovative vaccine discovery to ensure a diverse preclinical pipeline representing different immune profiles and antigen combinations that promote responses beyond (or at least in addition to) IFN-γ and the Ag85 antigens. A future vaccine strategy that blocks transmission by preventing reactivation of contagious TB or the establishment of new infections would represent game-changing improvements compared to BCG.

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