The generation of T-cell memory to protect against tuberculosis

Claudio Counoupas¹,² & James A Triccas¹,³,⁴

¹ Discipline of Infectious Diseases and Immunology, Faculty of Medicine and Health, The University of Sydney, Camperdown, NSW, Australia
² Tuberculosis Research Program, Centenary Institute, Sydney, NSW, Australia
³ Charles Perkins Centre, The University of Sydney, Camperdown, NSW, Australia
⁴ Marie Bashir Institute for Infectious Diseases and Biosecurity, The University of Sydney, Sydney, NSW, Australia

Keywords
Clinical trials, correlates of protection, tuberculosis, vaccine

Abstract
Tuberculosis (TB) kills more individuals each year than any other single pathogen and a more effective vaccine is critical for the global control of the disease. Although there has been recent progress in the clinical testing of candidates, no new vaccine has been licensed for use and correlates of protective immunity in humans have not been defined. Prior Mycobacterium tuberculosis infection does not appear to confer long-term protective immunity in humans; thus mimicking the natural immune response to infection may not be a suitable approach to develop improved TB vaccines. Data from animal testing are used to progress vaccines through the "vaccine pipeline", but studies in animals have not been able to predict efficacy in humans. Furthermore, although the generation of conventional CD4+ T-cell responses are considered necessary to control infection with M. tuberculosis, these do not necessarily correlate with protection induced by candidate vaccines and other immune components may play a role, including donor unrestricted T cells, tissue-resident memory T cells and anti-M. tuberculosis antibodies. This review will summarize the current understanding of the protective immune responses following M. tuberculosis infection or vaccination, with a particular focus on vaccines that have recently entered clinical trials.

INTRODUCTION
Tuberculosis has been responsible for more deaths than any other infectious disease in history.¹ Despite the availability of a vaccine (Mycobacterium bovis Bacille Calmette-Guérin or BCG) and effective antibiotics, globally there are an estimated 1.7 million deaths and 10.4 million new TB cases each year.² The limited efficacy of the BCG vaccine, emergence of multidrug-resistant strains of Mycobacterium tuberculosis and HIV coinfection all contribute to the inability of current programs to adequately control TB. The development of a more effective and easily administrable vaccine is necessary for optimal TB control and progress toward ending the global TB epidemic.

There are a number of reasons why an effective new TB vaccine has yet to be developed. Unlike common childhood infections, where pre-exposure vaccination is highly effective, the high proportion of the population latently infected with M. tuberculosis (estimated to be 1.7 billion individuals³) suggests post-exposure vaccines would be necessary for optimal control; modeling of new vaccine efficacy supports this.⁴ The choice of antigens and delivery systems is also critical for TB vaccine design. There is no consensus on the most immunodominant antigens of M. tuberculosis and antigen expression differs between active and latent/chronic infection.⁵ Furthermore, vaccine delivery should be safe for use in immunocompromised individuals, a potential limitation of live vaccines, while adjuvants for use with subunit vaccines should not induce deleterious inflammation and ideally be capable of mucosal delivery, to target the lung as the primary site of infection. Finally, the immunological mechanisms responsible for protection against TB are not completely defined. While essentially all the current vaccines used in infant/childhood
vaccination programs induce protective antibody responses, such responses are not considered essential for protection against *M. tuberculosis* and the stimulation of long-term memory CD4+ T-cell responses is the goal of anti-TB vaccination strategies. However, the precise phenotype of protective CD4+ T cells is unknown, hindering attempts to rationally design vaccines targeting optimal protective immunity. In this review, we will dissect the adaptive memory response to *M. tuberculosis* infection and discuss recent developments in the progression and immunological characterization of TB vaccine candidates.

**M. TUBERCULOSIS INFECTION AND THE GENERATION OF PROTECTIVE IMMUNITY**

A large body of data now exists on T-cell behavior following pathogen exposure. Most of this information, such as the kinetics of T-cell expansion/contraction and the development of T-cell memory stems from the studies of CD8+ T-cell responses against acute viral infection or fast-growing bacteria such as *Listeria monocytogenes*. This is because of the ease of using such model organisms, as opposed to slow-growing pathogens such as *M. tuberculosis*, and the availability of tetramers and transgenic T-cell receptor mice to examine antigen-specific CD8+ T-cell immunity. However, a number of studies have identified the critical role for CD4+ T cells in immunity against *M. tuberculosis*, aided by the recent availability of reagents and models for precise definition of T-cell immunity to *M. tuberculosis* infection. Delayed priming of *M. tuberculosis*-specific T cells in the lungs of infected mice has been reported and this presumably contributes to the chronic nature of *M. tuberculosis* infection. Antigen load is the key determinant in the priming of CD8+ T cells after encounter with mycobacteria and more recent data demonstrate that the abundance and duration of antigen expression defines the function of CD4+ T cells in mice and humans. This further highlights the complexity of TB vaccine design, as the selection of stage-specific antigens would be required to provide protection during active and/or chronic phases of infection.

**DOES THE IMMUNE RESPONSE “REMEMBER” M. TUBERCULOSIS? IMPLICATIONS FOR TB VACCINE DEVELOPMENT**

The rationale for the development of all vaccines currently in use is the “mimicking” of natural immunity induced by the pathogen. This is because of the fact that exposure to common childhood infections, such as measles and chickenpox, results in long-term protection against re-infection. However, recurrent TB is common; for example, studies in Uzbekistan revealed that a third of TB patients successfully treated were subsequently re-diagnosed with recurrent TB after an approximate 2-year follow up. In some studies, re-infection was identified as the major cause of recurrence, as opposed to reactivation of latent infection.

So how do these findings impact on vaccine development? These studies indicate that primary infection may negatively impact on protective immunity, possibly due to the chronic nature of *M. tuberculosis* infection. Evidence from the literature supports a model where high level, persisting antigen, such as that occurring during *M. tuberculosis* infection, may compromise the effective generation of CD4+ T-cell memory responses. For example, persistent viral infection in humans results in CD4+ T cells that display a phenotype and function resembling “effector” type cells, rather than memory cells that were generated in response to short-lived protein antigens. These findings parallel those with CD8+ T cells, in which chronic viral infection appears to favor the generation of effector CD8+ T cells, which fail to acquire the key properties of memory cells. Indeed, more recent analysis of antigen-specific responses revealed that chronic *M. tuberculosis* infection led to functional exhaustion of CD4+ T cells in mice and humans. Conversely, limited antigen expression correlates with poor T-cell expansion and poorly persistent live vaccines display limited protection against *M. tuberculosis* infection in mice. Thus, a model could be put forward where the immunizing dose and persistence of protective antigens would dictate the quality of the resultant T-cell response (Figure 1) and new vaccine design should be instructed by these parameters. This is further supported by the recent observation that low antigen dose provides optimal post-exposure protection with the H56 fusion protein vaccine candidate in mice, with high antigen dose altering the functional avidity of vaccine-specific CD4+ T cells. Dose escalation studies in humans of H56-containing vaccines demonstrated that low dose of vaccine antigen induced durable antigen-specific CD4 T cells, irrespective of *M. tuberculosis* infection status.
IFN-γ deficiency in humans leads to disseminated mycobacterial infection.\textsuperscript{20,21} However, numerous studies in mice and humans have revealed that IFN-γ is not a reliable correlate of protection and indeed high levels of IFN-γ may be a more reliable marker of immunopathology and bacterial load (reviewed in Reference 22). CD4\textsuperscript{+} T cells expressing multiple cytokines (e.g. IFN-γ, TNF and/or IL-2), were found to strongly correlate with protection in a murine model of *Leishmania major*\textsuperscript{23} and similar findings have been described in mice for *M. tuberculosis* infection.\textsuperscript{24,25} However, despite CD4\textsuperscript{+}IFN-γ/TNF/IL-2\textsuperscript{+} T cells being induced after boosting of BCG vaccines with the MVA85A vaccine in humans, no improved protection over BCG was observed.\textsuperscript{26} Thus, other properties of CD4\textsuperscript{+} T-cell responses may be required for protective responses. Many studies have correlated with the level of antigen-specific central memory T cells (T\textsubscript{CM}) and effector memory T cells with the ability to contain chronic infections, including TB. In humans infected with *M. tuberculosis* CD4\textsuperscript{+} T cells appear to be more of an effector memory T cells as opposed to T\textsubscript{CM} phenotype, suggesting chronic infection impairs the generation of protective T cells.\textsuperscript{27,28} Lindenstrøm et al. demonstrated that BCG protection wanes in chronically infected mice in parallel with the loss of T\textsubscript{CM} of a defined phenotype (CD4\textsuperscript{+}IL-2\textsuperscript{+}KLRG1\textsuperscript{-}) which could be counteracted by boosting BCG with the H1/IC31 vaccine.\textsuperscript{25} Importantly, a similar T\textsubscript{CM} subset were identified in humans vaccinated with H1/IC31 and it would be of interest to examine if these cells correlate with protection in efficacy trials.\textsuperscript{29} Finally, enhanced protection in mice conferred by vaccination with a recombinant BCG strain (VMP1002) correlated with high numbers of T\textsubscript{CM} and adoptive transfer of these cells confirmed their critical role in protection.\textsuperscript{30} Thus, vaccines with the capacity to preferentially induce long-lasting T\textsubscript{CM} subsets may provide optimal protection against *M. tuberculosis* infection in humans.

IL-17–secreting CD4\textsuperscript{+} T cells (Th17 cells) play a critical role in immunity against a number of bacterial and fungal pathogens (reviewed in Reference 31). Th17 cells have been reported to either play a protective role\textsuperscript{32,33} or confer pathological effects\textsuperscript{34} during mycobacterial infection. In particular, it has been reported that Th17 cells are important in enhancing the recruitment of neutrophils and Th1 CD4\textsuperscript{+} T cells by the secretion of various chemokines, which correlates with the control of *M. tuberculosis* infection.\textsuperscript{32} Similar to that observed with IFN-γ, the production of IL-17 alone does not seem to be sufficient to mediate protection\textsuperscript{32} but Th17 responses do correlate with improved protection by the VPM1002 vaccine in mice.\textsuperscript{35} Thus, vaccine-induced production of IL-17 may contribute to protection by inducing the recruitment of neutrophils and circulating CD4\textsuperscript{+} T cells to the site of infection, as well as inducing early maturation of the granuloma.\textsuperscript{36}

A role for CD8\textsuperscript{+} T cells?

While CD8\textsuperscript{+} T cells are critical for the control of viral and some bacterial infections, their role in immunity
against mycobacteria is less clear. T-cell adoptive transfer studies and studies in gene-deficient mice support a role for CD8\(^+\) T cells in containment of \(M\. \textit{tuberculosis}\) infection, particularly during the chronic stage (reviewed in Reference 37). In mice, BCG is a poor stimulator of CD8\(^+\) T-cell responses compared with \(M\. \textit{tuberculosis}\) and this is the rationale for the development of vaccines targeting CD8\(^+\) T-cell expansion.\(^{38}\) However, viral and subunit vaccines that lead to strong CD8\(^+\) T-cell responses in mice did not improve protection against \(M\. \textit{tuberculosis}\) infection.\(^{39,40}\) Both these studies, however, used the immunodominant \(M\. \textit{tuberculosis}\) antigen, TB10.4, and TB10.4-specific CD8\(^+\) T cells appear unable to recognize \(M\. \textit{tuberculosis}\)-infected macrophages,\(^{41}\) which suggests that \(M\. \textit{tuberculosis}\) may subvert the CD8\(^+\) T-cell responses as a virulence strategy. Despite this, a number of TB vaccines in clinical trials are designed to elicit a strong CD8\(^+\) T-cell response (Table 1). The VPM1002 vaccine induces a defined subset of CD8\(^+\) T cells expressing IL-17 in humans;\(^{42}\) however, the potential pathological role of these cells in inflammation indicates that their expansion may need to be tightly regulated.\(^{43}\) A recombinant human cytomegalovirus vaccine that stimulates CD8\(^+\) T cells results in protective immunity against \(M\. \textit{tuberculosis}\) infection in nonhuman primates, and it would be of interest to observe if a similar finding is observed in human trials.\(^{44}\)

**NEW CORRELATES OF VACCINE-INDUCED PROTECTION AGAINST TB**

More recent analysis of pathogen immunity has identified T-cell populations other than TCM or effector memory T cells that may play a role in vaccine efficacy, in particular tissue-resident memory T cells.\(^{45,46}\) Resident memory T cells reside in the mucosal tissues such as the lung and do not recirculate through the blood or the lymphatics. Lymphocytes resident in the lung are sufficient for BCG-induced protection of mice against \(M\. \textit{tuberculosis}\) infection, as blocking egress of cells from the secondary lymphoid organs did not alter the protective effect of the vaccine.\(^{46}\) Mucosal delivery of BCG in multiple animal models has shown an improvement in protective efficacy compared with parenteral delivery\(^{47,48}\) although intriguingly earlier studies showed no difference between vaccination routes.\(^{49}\) The reason for this difference is unclear but may relate to use of antibiotics to clear bacterial load after immunization in the Palendira study, as it was subsequently identified that vaccine persistence and BCG antigen load impacts T-cell immunity.\(^{10,17}\) Investigating other BCG delivery routes may be an important consideration, as intravenous (i.v.) delivery of BCG in nonhuman primates improves protection against aerosol \(M\. \textit{tuberculosis}\) challenge and i.v. administration of attenuated \textit{Plasmodium falciparum} sporozoites promotes sustained protection against malaria challenge in humans.\(^{50}\)

---

**Table 1. Immune response induced by selected TB vaccine candidates in clinical trials**

| Vaccine type                  | Vaccine name | Clinical trial stage\(^{a}\) | Human immune response elicited\(^{b}\) | Vaccine efficacy\(^{c}\) | References |
|------------------------------|-------------|------------------------------|----------------------------------------|--------------------------|------------|
| Whole-cell mycobacterial strains | MTBVAC (attenuated \(M\. \textit{tuberculosis}\)) | Phase 2a | m.f. CD4\(^+\) and CD8\(^+\) T cells | N.D. | 65 |
|                              | VPM1002 (Recombinant BCG) | Phase 3 | m.f. CD4\(^+\) and CD8\(^+\) T cells; IL-17\(^+\) CD8\(^+\) T cells | N.D. | 42 |
|                              | DAR-901 (killed \(M\. \textit{obuense}\)) | Phase 2b | IFN-\(\gamma\) production by CD4\(^+\) T cells; Anti-LAM antibodies | N.D. | 66 |
|                              | BCG Revaccination | Phase 2b | m.f. CD4\(^+\) T cells | Sustained QFT conversion 45.4\% \((P = 0.03)\) | 63 |
| Recombinant viral vectors     | MVA85A     | Phase 2b | m.f. CD4\(^+\) T cells; no CD8\(^+\) T cells | TB disease 17.3\% protection (N.S.) | 26 |
|                              | Ad5Ag85A   | Phase 1 | m.f. CD4\(^+\) and CD8\(^+\) T cells | QFT conversion -3.8\% (N.S.) | 67 |
| Protein in adjuvant           | H4:IC31    | Phase 2b | m.f. CD4\(^+\) T cells; no/low CD8\(^+\) | Sustained QFT conversion 30.5\% \((P = 0.16)\) | 29, 63 |
|                              | H56:IC31   | Phase 2b | m.f. CD4\(^+\) T cells; no/low CD8\(^+\); H56-specific IgG | N.D. | 19, 68 |
|                              | M72A501\(_E\) | Phase 2b | m.f. CD4\(^+\) Th1; M72-specific IgG | TB disease 54% protection \((P = 0.04)\) | 64 |
|                              | ID93/GLA-SE | Phase 2a | m.f. CD4\(^+\) Th1; no/low CD8\(^+\); ID93-specific IgG | N.D. | 69 |

\(^{a}\)Clinical trial phase taken from associated references and/or www.clinicaltrials.gov.  
\(^{b}\)m.f., multifunctional (T cells capable of simultaneously producing multiple cytokines).  
\(^{c}\)N.D., not determined; QFT, QuantiFERON-TB Gold in-tube assay (as a marker of \(M\. \textit{tuberculosis}\) infection); N.S., not significant.
Pulmonary delivery of fusion proteins with *Bacillus subtilis* spores\(^5^1\) or recombinant Influenza A expressing *M. tuberculosis* antigens also induced lung-resident memory T cells, which could confer protection in the absence of circulating T\(_{\text{CM}}\).\(^5^2\) More recently, a new subset termed stem cell memory T cells have been identified, that represent the earliest and longest lasting developmental stage of memory T cells and have a greater self-renewing capacity and proliferative potential compared with T\(_{\text{CM}}\) and effector memory T cells.\(^5^3\) In *M. tuberculosis*-infected individuals and BCG vaccines, antigen-specific CD4\(^+\) stem cell memory T cells are detected and display effector expression including Th1 cytokine release and expression of cytotoxic molecules.\(^5^4\) Thus, a major challenge in TB vaccine development is to determine the relative importance of each of these memory T cells and define if effective vaccines should induce all or some of these subsets for maximal protection.

In addition to the “conventional” T cells described above, that recognize peptide antigens presented in the context of classical MHC class I or MHC class II molecules, unconventional T cells with invariant or semi-variant TCRs may also play a role in anti-TB immunity. Donor unrestricted T cells are a diverse set of T-cell groups including Natural Killer T cells, CD1-restricted T cells and mucosa-associated invariant T cells, the latter recognizing the evolutionarily conserved major histocompatibility complex-like molecule MR1. The antigens recognized by these cells encompass a wide range of natural occurring molecules such as lipids and metabolites, many of which are derived from microbes including *M. tuberculosis*. Humans CD1-restricted T cells recognizing *M. tuberculosis* cell wall products have been identified,\(^5^5\) while mucosa-associated invariant T cells have been shown to detect intracellular infection with *M. tuberculosis*.\(^5^6\) Donor unrestricted T cells can produce effector cytokines, display cytolytic activity and are thought to represent an early line of defence against *M. tuberculosis*.\(^5^7\) Therefore, targeting unconventional T-cell responses in humans may represent a feasible strategy for the early control of mycobacterial infection, particularly with the use of live vaccines; indeed, mucosa-associated invariant T cells recognize macrophages infected with BCG\(^5^8\) and it would be of particular interest to determine whether other live vaccines in human trials (e.g. VPM1002 and MTBVAC, see Table 1) activate Donor unrestricted T cells.

A role for antibodies in TB vaccines?

The possible role of antibodies in the control of TB is covered elsewhere in this special feature. In terms of vaccine development, most focus has been on targeting T-cell responses and only a small number of vaccines have been developed to specifically target humoral responses, or examine antibody generation after vaccination. A conjugate vaccine candidate composed of Ag85B fused with Arabinomannan, a mycobacterial target of the humoral response, could improve survival of *M. tuberculosis*-infected mice and guinea pigs.\(^5^9\) Vaccination of mice with the ID93/GLA-SE vaccine, currently in clinical trials, revealed vaccine-induced memory T-cell responses are impaired in B cell-deficient mice.\(^6^0\) Antibody responses have been examined in humans after protein/adjuvant vaccines; however, a correlation with protective immunity is yet to be examined (Table 1). Post hoc analysis of the MV85A vaccine trial demonstrated that the production of IgG antibodies specific for the Ag85A was associated with reduced TB disease risk,\(^6^1\) and as such the potential targeting of humoral immunity to improve anti-TB immunity warrants further exploration.

**CONCLUSION**

Despite many years of concerted research and development, a new TB vaccine to replace the existing BCG has yet to emerge. This is partly because of the fact that defined correlates of protection have not been identified. Identifying such correlates would allow the early demonstration of new vaccine efficacy, thus permitting prioritization of candidate TB vaccines for human efficacy testing and minimize the time and cost associated with late-stage efficacy trials. The importance of “conventional” T-cell subsets (CD4\(^+\) T cells and CD8\(^+\) T cells) in response to infection with *M. tuberculosis* is well established, and while particular cells subsets, such as multifunctional Th1 CD4\(^+\) T cells, appear essential for protection, their presence is not a reliable correlate of vaccine-mediated protection against TB disease.\(^6^2\) While animal models have been instrumental in the selection of vaccine candidates for clinical progression, clinical trial endpoints will be required for defining correlates of protective immunity. The recent demonstration of the protective effects of BCG revaccination against *M. tuberculosis* infection in adolescents\(^6^3\) and the M72/AS01\(_E\) protein/adjuvant against pulmonary TB in adults with latent TB infection\(^6^4\) provides opportunities to define the parameters that correlate with protection against TB and instruct the development of improved vaccines. Such analysis would need to include the multitude of other immune parameters associated with *M. tuberculosis* infection, including tissue resident and/or stem cell memory T cells, Th17 cells, B cells or unconventional T cells.\(^5^7\) The exact contribution of this spectrum of immune responses to protective immunity in humans and their role as correlates of protection are to
be resolved, highlighting the complexity of vaccine development against pathogens highly evolved to combat immune detection and clearance.

**ACKNOWLEDGMENTS**

We acknowledge the support by a National Health and Medical Research Council (NHMRC) Project Grant Scheme (APP1043519), the NHMRC Centre of Research Excellence in Tuberculosis Control (APP1043225) and the European H2020 grant TBVAC2020 15 643381.

**CONFLICT OF INTEREST**

None declared.

**REFERENCES**

1. Paulson T. Epidemiology: a mortal foe. *Nature* 2013; **502**: S2–S3.
2. World Health Organization. Global Tuberculosis Report 2018. Geneva: World Health Organization; 2018. Licence: CC BY-NC-SA 3.0 IGO.
3. Houben RM, Dodd PJ. The global burden of latent tuberculosis infection: a re-estimation using mathematical modelling. *PLoS Med* 2016; **13**: e1002152.
4. Young D, Dye C. The development and impact of tuberculosis vaccines. *Cell* 2006; **124**: 683–687.
5. Meier NR, Jacobsen M, Ottenhoff THM, Ritz N. A systematic review on Novel *Mycobacterium tuberculosis* antigens and their discriminatory potential for the diagnosis of latent and active tuberculosis. *Front Immunol* 2018; **9**: 2476.
6. Kaufmann SH, Weiner J, von Reyn CF. Novel approaches to tuberculosis vaccine development. *Int J Infect Dis* 2017; **56**: 263–267.
7. Badovinac VP, Porter BB, Harty JT. Programmed contraction of CD8+ T cells after infection. *Nat Immunol* 2002; **3**: 619–626.
8. Wherry EJ, Teichgraber V, Becker TC, et al. Lineage relationship and protective immunity of memory CD8 T cell subsets. *Nat Immunol* 2003; **4**: 225–234.
9. Wolf AJ, Desvignes L, Linas B, et al. Initiation of the adaptive immune response to *Mycobacterium tuberculosis* depends on antigen production in the local lymph node, not the lungs. *J Exp Med* 2008; **205**: 105–115.
10. Ryan AA, Nambiar JK, Wozniak TM, et al. Antigen load governs the differential priming of CD8 T cells in response to the bacille Calmette-Guérin vaccine or *Mycobacterium tuberculosis* infection. *J Immunol* 2009; **182**: 7172–7177.
11. Moguche AO, Musovosri M, Penn-Nicholson A, et al. Antigen availability shapes t cell differentiation and function during tuberculosis. *Cell Host Microbe* 2017; **21**: 695–706.e5.
12. Cox H, Kebede Y, Allamuratova S, et al. Tuberculosis recurrence and mortality after successful treatment: impact of drug resistance. *PLoS Med* 2006; **3**: e384.
13. van Rie A, Warren R, Richardson M, et al. Exogenous reinfection as a cause of recurrent tuberculosis after curative treatment. *N Engl J Med* 1999; **341**: 1174–1179.
14. Han S, Asoyan A, Rabenstein H, Nakano N, Obst R. Role of antigen persistence and dose for CD4+ T-cell exhaustion and recovery. *Proc Natl Acad Sci USA* 2010; **107**: 20453–20458.
15. Wherry EJ, Barber DL, Kaech SM, Blattman JN, Ahmed R. Antigen-independent memory CD8 T cells do not develop during chronic viral infection. *Proc Natl Acad Sci USA* 2004; **101**: 16004–16009.
16. Bold TD, Banai N, Wolf AJ, Ernst JD. Suboptimal activation of antigen-specific CD4+ effector cells enables persistence of *M. tuberculosis* in vivo. *PLoS Pathog* 2011; **7**: e1002063.
17. Pinto R, Saunders BM, Camacho LR, Britton WJ, Gicquel B, Triccas JA. *Mycobacterium tuberculosis* defective in phthiocerol dimycocerosate translocation provides greater protective immunity against tuberculosis than the existing bacille Calmette-Guérin vaccine. *J Infect Dis* 2004; **189**: 105–112.
18. Billeskov R, Lindenstrom T, Woodworth J, et al. High antigen dose is detrimental to post-exposure vaccine protection against tuberculosis. *Front Immunol* 2017; **8**: 1973.
19. Suliman S, Luabeya AKK, Geldenhuys H, et al. Dose optimization of H56:IC31 vaccine for tuberculosis-endemic populations. A double-blind, placebo-controlled, dose-selection trial. *Am J Respir Crit Care Med* 2019; **199**: 220–231.
20. Cooper AM, Dalton DK, Stewart TA, Griffin JP, Russell DG, Orme IM. Disseminated tuberculosis in interferon gamma gene-disrupted mice. *J Exp Med* 1993; **178**: 2243–2247.
21. Seneviratne SL, Doffinger R, Macfarlane J, et al. Disseminated *Mycobacterium tuberculosis* infection by *Leishmania major* infection due to interferon gamma gene deficiency. Response to replacement therapy. *Thorax* 2007; **62**: 97–99.
22. Bhatt K, Verma S, Ellner JJ, Salgame P. Quest for safety and efficacy of MVA85A, a new tuberculosis vaccine, in infants previously vaccinated with BCG: a randomised, placebo-controlled phase 2b trial. *Lancet* 2013; **381**: 1021–1028.
27. Wang X, Cao Z, Jiang J, et al. Association of mycobacterial antigen-specific CD4+ memory T cell subsets with outcome of pulmonary tuberculosis. J Infect 2010; 60: 133–139.

28. Arrigucci R, Lakehal K, Vir P, et al. Active tuberculosis is characterized by highly differentiated effector memory TH1 cells. Front Immunol 2018; 9: 2127.

29. Mears H, Geldenhuyis HD, Kagina BM, et al. H1:iC31 vaccination is safe and induces long-lived TNF-α-IL-2+CD4 T cell responses in M. tuberculosis infected and uninfected adolescents: a randomized trial. Vaccine 2017; 35: 132–141.

30. Vogelzang A, Perdomo C, Zedler U, et al. Central memory CD4+ T cells are responsible for the recombinant Bacillus Calmette-Guérin ΔAureChly vaccine’s superior protection against tuberculosis. J Infect Dis 2014; 210: 1928–1937.

31. Curtis MM, Way SS. Interleukin-17 in host defence against bacterial, mycobacterial and fungal pathogens. Immunology 2009; 126: 177–185.

32. Khader SA, Bell GK, Pearl JE, et al. IL-23 and IL-17 in the establishment of protective pulmonary CD4+ T cell responses after vaccination and during Mycobacterium tuberculosis challenge. Nat Immunol 2007; 8: 369–377.

33. Wozniak TM, Saunders BM, Ryan AA, Britton WJ. Mycobacterium bovis BCG-specific Th17 cells confer partial protection against Mycobacterium tuberculosis infection in the absence of gamma interferon. Infect Immun 2010; 78: 4187–4194.

34. Cruz A, Fraga AG, Fountain JJ, et al. Pathological role of interleukin 17 in mice subjected to repeated BCG vaccination after infection with Mycobacterium tuberculosis. J Exp Med 2010; 207: 1609–1616.

35. Desel C, Dorhoi A, Bandermann S, Grode L, Eisele B, Kaufmann SH. Recombinant BCG ΔAureC hly+ induces superior protection over parental BCG by stimulating a balanced combination of type 1 and type 17 cytokine responses. J Infect Dis 2011; 204: 1573–1584.

36. Okamoto Yoshida Y, Umemura M, Yahagi A, et al. Essential role of IL-17A in the formation of a mycobacterial infection-induced granuloma in the lung. J Immunol 2010; 184: 4414–4422.

37. Lin PL, Flynn JL. CD8 T and Mycobacterium tuberculosis infection. Semin Immunopathol 2015; 37: 239–249.

38. Grode L, Seiler P, Baumann S, et al. Increased vaccine efficacy against tuberculosis of recombinant Mycobacterium bovis bacille Calmette-Guérin mutants that secrete listeriolysin. J Clin Invest 2005; 115: 2472–2479.

39. Florido M, Pillay R, Gillis CM, et al. Epitope-specific CD4+, but not CD8+, T-cell responses induced by recombinant influenza A viruses protect against Mycobacterium tuberculosis infection. Eur J Immunol 2015; 45: 780–793.

40. Lindenstrom T, Aagaard C, Christensen D, Agger EM, Andersen P. High-frequency vaccine-induced CD8+ T cell specific for an epitope naturally processed during infection with Mycobacterium tuberculosis do not confer protection. Eur J Immunol 2014; 44: 1699–1709.

41. Yang JD, Mott D, Sutivisesak R, et al. Mycobacterium tuberculosis-specific CD4+ and CD8+ T cells differ in their capacity to recognize infected macrophages. PLoS Pathog 2018; 14: e1007060.

42. Loxton AG, Knaul JK, Grode L, et al. Safety and immunogenicity of the recombinant Mycobacterium bovis BCG vaccine VPN1002 in HIV-unexposed newborn infants in South Africa. Clin Vaccine Immunol 2017; 24: e00439-16.

43. Srenathan U, Steel K, Taams LS. IL-17+ CD8+ T cells: differentiation, phenotype and role in inflammatory disease. Immunol Lett 2016; 178: 20–26.

44. Hansen SG, Zak DE, Xu G, et al. Prevention of tuberculosis in rhesus macaques by a cytomegalovirus-based vaccine. Nat Med 2018; 24: 130–143.

45. Muruganandah V, Sathkumara HD, Navarro S, Kupz A. A systematic review: the role of resident memory T cells in infectious diseases and their relevance for vaccine development. Front Immunol 2018; 9: 1574.

46. Connor LM, Harvie MC, Rich FJ, et al. A key role for lung-resident memory lymphocytes in protective immune responses after BCG vaccination. Eur J Immunol 2010; 40: 2482–2492.

47. Perdomo C, Zedler U, Kuhl AA, et al. Mucosal BCG vaccination induces protective lung-resident memory T cell populations against tuberculosis. MBio 2016; 7: e01686-16.

48. Verreck FAW, Tchilian EZ, Vervenne RAW, et al. Variable BCG efficacy in rhesus populations: pulmonary BCG provides protection where standard intra-dermal vaccination fails. Tuberculosis 2017; 104: 46–57.

49. Palendira U, Bean AG, Feng CG, Britton WJ. Lymphocyte recruitment and protective efficacy against pulmonary mycobacterial infection are independent of the route of prior Mycobacterium bovis BCG immunization. Infect Immun 2002; 70: 1410–1416.

50. Lyke KE, Ishizuka AS, Berry AA, et al. Attenuated PSpZV Vaccine induces strain-transcending T cells and durable protection against heterologous controlled human malaria infection. Proc Natl Acad Sci USA 2017; 114: 2711–2716.

51. Copland A, Diogo GR, Hart P, et al. Mucosal delivery of fusion proteins with Bacillus subtilis spores enhances protection against tuberculosis by Bacillus Calmette-Guérin. Front Immunol 2018; 9: 346.

52. Florido M, Muflihah H, Lin LCW, et al. Pulmonary immunization with a recombinant influenza A virus vaccine induces lung-resident CD4+ memory T cells that are associated with protection against tuberculosis. Mucosal Immunol 2018; 11: 1743–1752.

53. Gattinoni L, Speiser DE, Lichterfeld M, Bonini C. T memory stem cells in health and disease. Nat Med 2017; 23: 18–27.

54. Mpande CAM, Dintwe OB, Musvosvi M, et al. Functional, antigen-specific stem cell memory (TSCM) CD4+ T cells are induced by human Mycobacterium tuberculosis infection. Front Immunol 2018; 9: 324.

55. Van Rhijn I, Moody DB. CD1 and mycobacterial lipids activate human T cells. Immunol Rev 2015; 264: 138–153.
56. Gold MC, Napier RJ, Lewinsohn DM. MR1-restricted mucosal associated invariant T (MAIT) cells in the immune response to *Mycobacterium tuberculosis*. *Immunol Rev* 2015; 264: 154–166.

57. Huang S. Targeting innate-like T cells in tuberculosis. *Front Immunol* 2016; 7: 594.

58. Chua WJ, Truscott SM, Eickhoff CS, Blazevic A, Hoft DF, Hansen TH. Polyclonal mucosa-associated invariant T cells have unique innate functions in bacterial infection. *Infect Immun* 2012; 80: 3256–3267.

59. Hamasur B, Haile M, Pawlowski A, et al. *Mycobacterium tuberculosis* arabinomannan-protein conjugates protect against tuberculosis. *Vaccine* 2003; 21: 4081–4093.

60. Dubois Cauwelaert N, Baldwin SL, Orr MT, et al. Antigen presentation by B cells guides programming of memory CD4+ T-cell responses to a TLR4-agonist containing vaccine in mice. *Eur J Immunol* 2016; 46: 2719–2729.

61. Fletcher HA, Snowden MA, Landry B, et al. T-cell activation is an immune correlate of risk in BCG vaccinated infants. *Nat Commun* 2016; 7: 11290.

62. Kagina BM, Abel B, Scriba TJ, et al. Specific T cell frequency and cytokine expression profile do not correlate with protection against tuberculosis after Bacillus Calmette-Guérin vaccination of newborns. *Am J Respir Crit Care Med* 2010; 182: 1073–1079.

63. Nemes E, Geldenhuys H, Rozot V, et al. Prevention of *M. tuberculosis* infection with H4:IC31 vaccine or BCG revaccination. *N Engl J Med* 2018; 379: 138–149.

64. Van Der Meeren O, Hatherill M, Nduba V, et al. Phase 2b controlled trial of M72/AS01E vaccine to prevent Tuberculosis. *N Engl J Med* 2018; 379: 1621–1634.

65. Spertini F, Audran R, Chakour R, et al. Safety of human immunisation with a live-attenuated *Mycobacterium tuberculosis* vaccine: a randomised, double-blind, controlled phase I trial. *Lancet Respir Med* 2015; 3: 953–962.

66. von Reyn CF, Lahey T, Arbeit RD, et al. Safety and immunogenicity of an inactivated whole cell tuberculosis vaccine booster in adults primed with BCG: a randomized, controlled trial of DAR-901. *PLoS One* 2017; 12: e0175215.

67. Smaill F, Jeyanathan M, Smieja M, et al. A human type 5 adenovirus-based tuberculosis vaccine induces robust T cell responses in humans despite preexisting anti-adenovirus immunity. *Sci Transl Med* 2013; 5: 205ra134.

68. Luabeya AK, Kagina BM, Tameris MD, et al. First-in-human trial of the post-exposure tuberculosis vaccine H56:IC31 in *Mycobacterium tuberculosis* infected and non-infected healthy adults. *Vaccine* 2015; 33: 4130–4140.

69. Penn-Nicholson A, Tameris M, Smit E, et al. Safety and immunogenicity of the novel tuberculosis vaccine ID93 + GLA-SE in BCG-vaccinated healthy adults in South Africa: a randomised, double-blind, placebo-controlled phase 1 trial. *Lancet Respir Med* 2018; 6: 287–298.