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Can what have we learnt about BCG vaccination in the last 20 years help us to design a better tuberculosis vaccine?

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ABSTRACT

The BCG vaccine will, in 2021, have been in use for 100 years. Much remains to be understood, including the reasons for its variable efficacy against pulmonary tuberculosis in adults. This review will discuss what has been learnt about the BCG vaccine in the last two decades, and whether this new information can be exploited to improve its efficacy, by enhancing its ability to induce either antigen-specific and/or non-specific effects. Many factors affect both the immunogenicity of BCG and its protective efficacy, highlighting the challenges of working with a live vaccine in man, but new insights may enable us to exploit better what BCG can do.

1. The BCG vaccine is capable of inducing protection against tuberculosis in some groups and settings

It is often stated that the BCG vaccine does not provide protection against tuberculosis. This is not true. The systematic review published by Mangtani et al. [1] included randomised controlled trials investigating whether BCG vaccination induced protection against tuberculosis. BCG vaccination is protective in some age groups and in some settings – in neonates against pulmonary and disseminated forms of tuberculosis, and at latitudes of 40° and above it gives better protection against pulmonary disease than in vaccinees living closer to the equator. In school age children protection was stronger if vaccination was restricted to those who were skin test negative to PPD using the Mantoux skin test [1]. These observations support the generally held view that exposure to other mycobacteria can reduce the protection induced with BCG vaccination through either masking the protection that BCG induces, or by blocking multiplication of the live BCG thereby preventing it from inducing protection [2], a consensus that has strengthened in the last 20 years. Overall, it is not correct to say that the BCG vaccine is unable to protect – as it can protect infants and young children against disseminated forms of TB, and adults against pulmonary TB in some circumstances [1]. Most of the world's children receive BCG vaccination with two-thirds of those countries giving BCG vaccination estimated to have >90% vaccine coverage [3]. BCG vaccination is recommended by the WHO to be given shortly after birth, however, when vaccine coverage is usually assessed at 1 year of age, in some settings many infants have been vaccinated later than the WHO recommends [4,5]. It is also clear that this wide vaccine coverage has been insufficient to control the spread of tuberculosis. Given that in 2019, there were 10 million individuals diagnosed with tuberculosis (TB) and 1.4 million deaths [6], we need an improved TB vaccine or vaccination regimen [7].

2. BCG-induced protective immunity can be long-lived – but this may depend on the type of immunity being measured

Another comment often made about the BCG vaccine is that it fails to induce long-lasting immunity, generally assumed to be mediated by classical T-cell memory. Again, this is not correct, as despite concerns about the induction of long-term immunological memory by BCG in mice, in certain settings, BCG can induce very long-lived protection in man – for as long as 50–60 years in Alaskan natives and American Indians [8]. Although many studies have not included longer term follow-up, the metanalysis by Abubakar et al. [9] identified one trial and four observational studies where protection lasted for 15 years or more. A recent retrospective population-based cohort analysis of BCG vaccination studies in Norway also found 58% protection against pulmonary tuberculosis 10–19 years after vaccination; however, this effect was diminished at later time-points [10].

Such longevity might be possible for antigen-specific immune responses as a result of antigen-specific T-cell memory but so far there is no evidence that non-specific protection as discussed below can last so long. The effects of non-specific trained immu-

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to wane by 12 months after BCG vaccination [11], although longer effects might result from the epigenetic reprogramming of cells in the bone marrow [12]. Observational studies have suggested that longer-term non-specific effects can persist in individuals for more than a few years after vaccination; one Danish case-cohort study found that BCG vaccination was associated with protection against natural deaths (but not against accidental deaths, murders or suicides) for decades [13].

3. BCG vaccines are variable in composition

There is evidence that different strains of the BCG vaccine can induce varying degrees of T-cell immunity to mycobacterial or heterologous antigens. Infant BCG vaccination studies in Uganda, Nigeria, South Africa and Australia suggested that BCG Denmark may induce higher proportions than BCG Bulgaria or BCG Russia of single or multiple cytokine producing CD4+ T-cells responding to PPD, BCG or heterologous antigens and higher cytokine production by these cells [14–16]. In Australia, vaccination with BCG Japan outperformed both BCG Russia and BCG Denmark in terms of Th1 cytokine, IL-10, MCP-1 or MIP-1β production in response to mycobacterial antigens [16]. In Brazil, not only was the extent of cytokine production by healthy adult peripheral blood and umbilical cord mononuclear cells different in response to BCG Moreau, BCG Denmark and BCG Pasteur but also the rates of apoptosis: BCG Moreau induced the strongest cytokine production and the greatest degree of apoptosis [17]. Collectively, these studies suggest that different strains of BCG can induce differing classical immune responses.

There are some subtleties here though: any potential strain-specific antimycobacterial or heterologous effects of BCG might be susceptible to confounding, such as delivery route (discussed below) or the number of viable bacilli in the vaccine. *Mycobacterium bovis* BCG can be tricky to grow, even for experienced vaccine producers, and the proportion of live and dead bacilli can vary in different vaccine batches. This makes it hard to compare different BCG strains directly. Even if grown and prepared in exactly the same way, which not all BCG vaccines are, the rate of growth can also vary. A study by Biering-Sørensen et al. [18] showed that slower growing batches resulted in greater vaccination site scarring and increased cytokine production in response to mycobacterial or heterologous stimuli. In another study, different strains of BCG were found to differ in proportions of viable bacilli and to induce divergent cytokine profiles in whole blood from newborns and adults [19]. Interestingly, the number of viable BCG bacilli in this study correlated with levels of GM-CSF, PDGF-AB/BB, IL-1β, TNFα and IFNγ, cytokines known to have roles in antimycobacterial and trained immunity [19], suggesting that the viability of BCG might affect the degree of innate training. Indeed, gamma-irradiation of BCG decreased its ability to induce trained immunity and related cytokine production *in vitro,* although it did not abolish training completely [20].

Potential influences of BCG strains on heterologous downstream effects may be even more difficult to capture. While in normal birth weight infants from Guinea-Bissau BCG Denmark was associated with higher rates of scar formation compared to BCG Russia, there was no significant difference in rates of health consultations between infant groups vaccinated with these two BCG strains [21]. In another study in Guinea-Bissau, no significant differences in morbidity or mortality by 6 weeks of age were observed in newborns given BCG Russia, BCG Denmark and BCG Japan [22]. This suggests that if different strains of BCG affect the non-specific effects induced by BCG, the impact is likely to be limited, although more studies are needed.

In summary, whatever BCG does, live BCG usually does it better than dead bacilli – and this includes not only protection but induction of non-specific trained immunity. Despite these differences in immunogenicity and in composition, the different strains of BCG were not found to be associated with protection against TB in the Mangtani systematic review [1].

4. What has been learnt about the immunogenicity of BCG vaccination?

If the BCG vaccine is given to either adolescents or infants in the UK, strong T-cell responses to cross-reactive mycobacterial antigens such as PPD are induced. The last two decades have largely been the era of cytokines for measurement of immunogenicity, with a focus on the measurement of IFNγ secretion.

Comparisons of different geographic settings in a series of trials in adolescents and young adults showed that whereas BCG vaccination induced protection against pulmonary tuberculosis in the UK, in Malawi it failed to induce any protection against tuberculosis (although it did induce some protection against leprosy) [23]. PPD stimulation of diluted whole blood samples from UK adolescents showed minimal IFNγ production prior to BCG vaccination, and a marked increase that was greatest at 3 but that remained strong at 12 months following vaccination [24]. In contrast, in Malawi most adolescents and young adults were pre-sensitised to PPD before BCG vaccination and did not show significant increases in response following BCG vaccination. The ability to make a strong IFNγ response in such assays is associated with changes in DNA methylation [25]. When South African infants progressing to a diagnosis of TB were stratified into groups of high, medium or low IFNγ responders using an ELISPOT assay in which PBMC were stimulated with BCG, the high responders showed the slowest rate of progression to TB [26]. BCG vaccination of UK infants can induce polyfunctional T-cells making IFNγ, TNFα and IL-2 [27], a cell type also attracting much interest as a possible correlate of protection, but in a cohort of South African infants there was no association of these responses with progression to TB disease [28]. T-cell responses are needed though, as shown by how susceptible those with HIV infection are to *M. tuberculosis* infection, or the rapidly progressive infections seen in SCID mice, as well as the increased mycobacterial growth in mice lacking the ability to produce or respond to IFNγ; in mice and in man there are similar examples of genetic mutations in the IFNγ-IL-12 axis resulting in susceptibility to mycobacterial disease [29]. IFNγ provides valuable information about immunogenicity and may play a role in protection but measuring it alone has not delivered a confirmed correlate of protection. A number of other immunological components, such as various cell types, antibodies and cytokines have been proposed to be associated with protection against tuberculosis (Table 1), but confirmed correlates of protection are still needed.

5. Can measuring mycobacterial growth inhibition directly provide a better estimate of protection?

Mycobacterial growth inhibition (MGI) assays have recently been exploited to investigate the association between immunity and the ability to restrict mycobacterial growth following BCG vaccination. They can also provide a system in which the contributions of various cells and cytokines can be dissected.

UK infants showed a marked induction of MGI following BCG vaccination [27]. In healthy adults, historical BCG vaccination was associated with improved mycobacterial growth inhibition *ex vivo* on its own or in the presence of isoniazid or rifampicin [30]. Interestingly, this study detected a possible association between NK cell frequency and inhibition of mycobacterial growth,

Table 1
Immunological components associated with protection against TB.

Immunological component	Association with protection against TB	Study	References
	Cellular components		
BCG-responsive high-IFNγ producing PBMCs	Lower risk of progression to TB disease	BCG-vaccinated infants	[26]
CD4+IFNγ+TNFα+IL-2+ T-cells	Enhanced inhibition of BCG growth in MGIT	BCG-vaccinated infants	[27]
Th17 cells			
CD4+ central memory T-cells	Enhanced inhibition of BCG growth in MGIT	*M. tuberculosis* exposed uninfected individuals	[34]
CD4+IFNγ+TNFα+ T-cells	Control of *M. tuberculosis* induced lung pathology at study week 6	BCG-vaccinated NHPs	[53]
CD4+ T-cells:	Reduction of thoracic *M. tuberculosis* burden	BCG-vaccinated NHPs	[54]
CD154+IFNγ+IL-2+TNFα+			
CD154+IL-2+TNFα+			
CD8+ T-cells:			
IFNγ+TNFα+IL-2+			
IFNγ+TNFα+			
Peak CD4+ and CD8+ T-cell counts			
CD4+PD-1+KLRG1- T-cells	Reduction of *M. tuberculosis* burden in lungs and spleen	BCG-vaccinated mice	[57]
Epigenetically reprogrammed monocytes	Reduction of *M. tuberculosis* burden in lungs, spleen and bone marrow	BCG-vaccinated and non-vaccinated murine parabiont and adoptive bone marrow transplant models	[12]
NK cells	Enhanced inhibition of BCG growth in MGIT	Historically BCG-vaccinated adults	[30,31]
B-cells, CXCL10+ CD14$_{dim}$ monocytes	Enhanced inhibition of BCG growth in MGIT	*M. tuberculosis* exposed uninfected individuals	[34]
Neutrophils	Reduction of *M. tuberculosis* burden in the lungs	BCG-vaccinated mice	[83]
	Soluble components		
IFNγ	Control of mycobacterial infection	Human and mice gene deficiencies	[29]
CXCL9, CXCL10	Enhanced inhibition of BCG growth in MGIT	*M. tuberculosis* exposed uninfected individuals	[34]
IL-1β, TNFα, IL-6	Elevated		
IL-10	Reduction of pulmonary and extrathoracic *M. tuberculosis* burden	BCG-vaccinated NHPs	[59]
αPPD-IgA			
αAg85A-IgG	Lower risk of progression to TB disease	BCG-vaccinated infants	[26]
αAM-IgG, αAM-, αLAM-IgM	Enhanced survival of mice infected with *M. tuberculosis*	*M. tuberculosis* infection in mice	[103]
	Improved clearance of LAM from the circulation and spleen	Exogenous LAM challenge in mice	
α19-kDa-IgG	Negative correlation between DTH responses to PPD and α19-kDa-IgG levels	Factory workers unexposed to TB	[104]
αAM-, αLAM-, αHBHA-, α16-kDa-α-crystalin-, and αMPB83-IgG, anti-mycobacterial IgG, IgA	Reduced susceptibility to TB or progression to disease	Murine or NHP *M. tuberculosis* infection, passive serum or polyclonal IgG transfer, B-cell deficiency models, functional assays	[105]

Abbreviations:
AM – arabinomannan
DTH – delayed-type hypersensitivity
HBHA – heparin binding hemagglutinin
MPB83 – mycobacterial cell surface lipoprotein
LAM – lipoarabinomannan
NHP – non-human primate

NB: *The list of studies or reviews presented in this table is not comprehensive.*

with a tendency for higher proportions of NK cells in BCG-vaccinated individuals. Further analyses showed that while overall and cytotoxic NK cell frequencies were associated with *ex vivo* inhibition of mycobacterial growth in BCG-naïve individuals, cytokine-producing NK cell responses correlated with control of mycobacterial growth in BCG-vaccinated individuals [31]. BCG vaccination has been shown to enhance NK cell activation and cytokine production in response to mycobacterial or heterologous stimuli in infants and adults, an effect that lasted 3 to 4 months [32,33]. BCG also protected SCID mice from lethal *Candida* infection with a partial role demonstrated for NK cells [33].

Activated monocytes or macrophages are considered protective against TB and their efficiency in containing mycobacterial infections has also been explored in mycobacterial growth inhibition assays. A study by Joosten et al. found that enhanced secretion of CXCL9 and CXCL10 by non-classical monocytes was associated with greater mycobacterial growth inhibition in individuals who were exposed to TB but not infected, compared to TB patients or healthy controls, although central memory T-cell responses and B-cell frequencies were also associated with control of mycobacterial growth [34]. PBMCs from those exposed to TB also showed some features consistent with innate immune training, e.g. elevation of innate immune cytokines IL-1β, TNFα or IL-6 in response to BCG stimulation and higher CXCL10 production in response to heterologous stimuli, although monocyte TNFα was not associated with improved mycobacterial growth inhibition.

6. Other explanations for variable responses to BCG

The complexity of measuring such vaccine-induced immune responses in the "real world" is very considerable. Immune status

is affected by our environment, health, nutrition, microbiome, age, and more [35]. Marked differences in the IFNγ and broader cytokine responses following *in vitro* stimulation of diluted whole blood with PPD were observed between Malawian and UK infants who were BCG vaccinated 3 or 12 months previously [36,37]. A study of cytokine responses in diluted whole blood cultures stimulated with PPD for 6 days in Ugandan infants given BCG at birth showed the development of immune responses that peaked 4–10 weeks post vaccination, but with considerable individual variation, and some infants failed to make a detectable cytokine response [38]. The literature on how delaying BCG vaccination affects the immunogenicity of BCG vaccination has not shown any consistent improvement with delayed vaccination [39]. The genetics of the vaccinees, other vaccines they are given, nutrition, seasonality and more, will influence these responses. However, certain additional factors have received more attention in the last decade.

7. Maternal influences on the response to vaccination in their infants

Newborn infants receiving BCG vaccination should be immunologically naïve, and any confounding effects of environmental or non-tuberculous mycobacteria should not be present. However, young infants may have an immature immune system that has been influenced by their mothers' immune or infection status [40,41]. For example, latent TB infection (LTBI) in a woman might influence how her baby responds to BCG vaccination. In Uganda, cytokine responses in BCG-vaccinated infants showed no association with the mothers' LTBI status [38]. This is perhaps surprising, as mycobacterial antigens might have crossed the placenta and induced either sensitisation or tolerance in the infant. Other common infections, such as malaria or other parasitic infections in the mothers during pregnancy can have broad immunomodulatory effects on the immune system of the newborns/infants. Although helminth infections in the mother had limited effects on the response to BCG in Uganda [42], viral infections such as CMV alter CD8 T-cells and rate of progression to TB in infants [26,43].

The BCG vaccination status of the mother may also have some effects on Th2 cytokine responses in BCG vaccinated infants to *M. tuberculosis* culture filtrate proteins or cord blood IL-10, IFNγ or immune cell growth factor responses to innate stimuli [42,44]. A possible beneficial association of previous maternal immunisation with BCG and lower rates of parent-reported infections was found in infants at 0–3 months of age in the Danish BCG study [45], and maternal BCG scar was also associated with lower infant mortality risk in Guinea-Bissau [46]. Whether this reflects an as yet unknown biological mechanism or was associated with confounding healthcare practices within a family is not clear.

8. Would BCG be more protective if given by another route?

Although when first used in 1921 the BCG vaccine was given orally, it is now given intradermally. One area of recent and active research is whether BCG (and other novel TB vaccines) might be more protective if given by routes other than the standard intradermal route. Intradermal vaccination is tricky and well-trained staff are needed to give intradermal vaccines such as BCG. Some countries have therefore used a multipuncture device to deliver BCG; this has the added benefit of reducing scarring which in some cultures is regarded by parents/guardians as of major importance. For example, in Japan and South Korea BCG Japan has been delivered percutaneously with a multi-puncture device, but skin test responses and IFNγ responses to PPD in Korean children aged 4–7 years given BCG Pasteur intradermally or BCG Tokyo by multi-puncture device were comparable [47]. A larger trial of BCG given percutaneously or intradermally in South Africa found that there was no difference in the protective efficacy of BCG given by these routes [48,49]. A strength of this South African study was that the same BCG strain (Japan 172) was given by both routes. Presence of a BCG scar is often used as a proxy for BCG vaccination history, although scars can disappear over time and not all of those vaccinated develop a scar. Both the presence and the size of BCG scar in children that have received BCG vaccination has been associated with improved survival indicating non-specific protection [50,51].

Studies in animal models have shown that BCG can be more protective if given intravenously rather than by other routes. Early studies in which BCG was given to non-human primates (NHPs) were published as long ago as 1970 [52] but there has been a recent revival of interest in giving BCG by this route. Vaccinating Rhesus macaques by the intravenous route induced better protection than giving BCG intradermally, or intradermally with boosting by intratracheal administration [53]. A larger study in Rhesus macaques used positron emission tomography–computed tomography (PET–CT) imaging with ^{18}F-fluorodeoxyglucose, confirming the improved protection given by intravenous BCG, and showed that 9/10 animals given BCG intravenously failed to show any lung lesions [54]. In another study, giving BCG to mice intravenously was shown to alter the differentiation of haematopoietic stem cells, promoting myelopoiesis and enhancing the activation status of bone marrow-derived macrophages [12]. In addition, compared to subcutaneous immunization, BCG delivered intravenously could be detected in the bone marrow for 7 months after BCG vaccination, suggesting prolonged interaction with the immune system. However, giving BCG intravenously in man is not likely to be practical and could induce adverse events including disseminated disease in immunosuppressed individuals.

Alternative delivery routes delivering BCG directly into the mucosa or lungs may also be worth exploring [55]. Compared to intravenous immunization, aerosol vaccination with BCG gave less bacterial dissemination and reduced bacterial counts in the lungs [56]. In mice, intranasal BCG induced better protection in the lungs than the standard intradermal vaccination, with induction of antigen-specific tissue-resident T-cells expressing a PD-1$^+$ KLRG1$^-$ -cell-surface phenotype [57]. BCG can also induce protection in mice when given by the sublingual route [58]. In NHPs, mucosal delivery was associated with improved Th17 cell and IL-10 responses, slower IGRA conversion, lower pathology in the lungs and better control of *M. tuberculosis* growth in the lungs or lymphoid tissues compared to the intradermal route [59]. We still need a better understanding of how to maximise beneficial immune responses in the lungs while avoiding excessive immune activation.

9. Do different routes of administration also affect induction of innate training?

BCG vaccination can also induce non-specific protection against unrelated pathogens [60,61] and reduce all-cause mortality in infants [62–66]. Importantly, this vaccine can induce a phenomenon known as "trained immunity", resulting in epigenetic or metabolic reprogramming of the innate immune cells and enhanced surface marker expression or cytokine responses upon secondary stimulation [33,67,68], suggesting that this mechanism can contribute to the non-specific effects of BCG and protection against infectious diseases [69]. Adults vaccinated with BCG and then given yellow fever vaccine were shown to have reduced viraemia compared to BCG-naïve controls and this effect was associated with enhanced IL-1β production [70]. Infant BCG vaccination studies in Guinea-Bissau and the UK, as well as Australia demonstrated that production of cytokines associated with innate immunity was

enhanced in BCG vaccinated infants compared to unvaccinated controls upon secondary stimulation with heterologous stimuli [32,71–73]. This phenomenon is not restricted to BCG alone as another live mycobacterial vaccine – MTBVAC has been shown to enhance innate cytokine responses to LPS in human monocytes and in mice to improve resistance to an otherwise lethal infection with *S. pneumoniae* [74].

BCG revaccination was able to increase reversion of interferon-gamma release assay (IGRA) positivity in South African adolescents who had been BCG vaccinated at birth [75], which has led to renewed interest in giving a repeat BCG vaccination. In children, two randomised trials have provided some evidence that a repeat BCG vaccination may reduce all-cause mortality [76]; for example, in infants in Guinea Bissau who had received their diptheria, pertussis and tetanus (DPT) booster before their BCG revaccination at 19 months, there was some evidence of a reduction in mortality between 19 and 60 months [77]. In the South African H4/BCG trial, it was observed that the BCG revaccinated group had a lower rate of upper respiratory tract infections than in either the H4:IC31 group or the placebo group [75]. In an Indonesian study in which BCG was given monthly for 3 months to elderly individuals, the prevalence of acute respiratory infections was reduced [78]. These studies indicate that revaccination or boosting as well as primary vaccination with BCG may be able to induce or enhance innate memory with beneficial effects on survival; similar effects have also been observed with other live attenuated vaccines such as smallpox or oral polio vaccine [76].

The route of BCG administration can affect mycobacteria-specific immune responses and efficacy of the BCG vaccine. However, can different routes of BCG delivery affect the extent of innate immune training? So far, most studies of BCG-dependent innate immune training in humans have used intradermal BCG vaccination. However, recent exposure to tuberculosis has also been associated with innate immune training, suggesting that aerosol or mucosal interaction with mycobacteria can imprint innate immune responses [34]. Immunising calves with aerosolised BCG was associated with induction of trained immunity in PBMCs, although cytokine production by alveolar macrophages was not affected [79]. In humans, alveolar macrophages expressed lower levels of activation markers CD11b and HLA-DR after intradermal immunisation with BCG, although this study did not examine BCG-dependent changes in alveolar macrophage cytokine responses [80]. It is possible that induction of trained immunity in the lungs might be regulated or contributed to by adaptive immune cells, as adenovirus-dependent priming of alveolar macrophages in mice was found to be dependent on IFNγ produced by CD8+ T-cells in a model of *S. pneumoniae* infection [81].

Is this different if the BCG vaccine is delivered by other routes? In mice, intravenous delivery of BCG induced stronger haematopoietic cell expansion and differentiation compared to subcutaneously injected vaccine and was capable of priming bone marrow derived macrophages (BMDMs), enhancing their ability to control *M. tuberculosis* growth *in vitro* [12]. Intradermal BCG vaccination of humans also polarised haematopoietic stem cell differentiation into myeloid cells [82], suggesting that some BCG associated changes in the innate immune system can occur irrespective of the delivery route.

In another study, mice were vaccinated with BCG subcutaneously and their ability to control growth of *M. tuberculosis* in the lungs was compared with other routes of immunisation: intravenous, intranasal, aerosol or intramuscular [83]. A protective effect of similar extent on mycobacterial growth in the lungs was found for most immunisation routes despite varying colony forming units of BCG, except for low-dose aerosolised BCG which did not induce protection. In subcutaneously vaccinated mice, the protection against *M. tuberculosis* growth in the lung was independent of T-cell responses, suggesting that BCG mediated protection via innate immune cells [83]. Interestingly, depletion of neutrophils in this model was associated with diminished protection by BCG [83], supporting findings in humans, where intradermal BCG was associated with a neutrophil transcriptional signature and elevated neutrophil counts in BCG-vaccinated infants [82].

There also seem to be differences in how BCG, delivered via the skin, affects the innate immune cells. In humans, intradermal BCG vaccination induced a trained phenotype in monocytes in NOD2 dependent manner, enhancing accessibility of proinflammatory genes for transcription and cytokine production upon secondary stimulation with mycobacterial or heterologous antigens [67,82], with similar changes happening in the NK cells [33] and NK cell cytokine responses associated with inhibition of mycobacterial growth years in these historically vaccinated individuals [31]. However, control of *M. tuberculosis* growth in lungs of subcutaneously BCG-vaccinated mice was not mediated by NOD2 dependent pathways, monocytes or NK cells [83]. Further investigation would be required to clarify whether these differences reflect the influence of route of vaccine delivery or differences in human and murine trained innate responses, as differences in regulation of trained immunity by long non-coding RNAs in human and murine models have been reported previously [84].

10. How can what we have learnt about BCG accelerate the development of new TB vaccines?

There is a pipeline of candidate TB vaccines in development, of varying types. Some are recombinant BCG vaccines, designed to be safer in infants who are HIV infected, or to induce improved protection by inclusion of additional antigens from *M. tuberculosis*. Some are other live mycobacterial vaccines, including *M. tuberculosis* itself with mutations that reduce its virulence, or environmental non-tuberculous mycobacteria. It is likely that any issues that affect growth of BCG bacilli in a BCG vaccine, will similarly affect the growth of another live mycobacterial vaccine. Other vaccine candidates include subunit or recombinant proteins in adjuvant, which would be given as a booster vaccine following BCG vaccination, that would depend on BCG vaccination having induced an effective primary immune response. Similarly, the vaccines that consist of viral vectors that deliver one or more antigens, are usually intended to boost a pre-existing immune response rather than induce a primary immune response. The TB vaccine portfolio is therefore very dependent on what BCG vaccination does or does not do. It may also be beneficial if primary vaccination (for example with a live mycobacterial vaccine) can induce non-specific innate training.

One surprising result from a recent vaccine trial of the subunit H4 vaccine to prevent infection rather than disease, was that repeat BCG given as the control arm, was more effective at inducing reversion to IGRA-negative status than the subunit vaccine (although neither vaccine provided significant protection against IGRA conversion, taken as indication of *M. tuberculosis* infection) [75]. This was surprising because in a number of earlier studies performed in different settings such as Malawi [23] or Brazil [85], there was no improvement seen with a repeat BCG vaccination although with data from a longer-term follow-up of the BCG-REVAC trial there was some evidence that repeat BCG could be protective in an area of Brazil with low prevalence of non-tuberculous mycobacteria [86].

11. Could inducing greater innate training improve the protection given by BCG or these new vaccines?

Trained immunity has been associated with protection against heterologous infections and has been implicated in the non-

specific effects of BCG. However, improving innate immunity may also be able to enhance the protective efficacy of new TB vaccines [87,88]. BCG could be detected in the bone marrow 7 months after BCG vaccination and where it could reprogram haematopoietic stem cells (HPSCs) inducing their differentiation into epigenetically primed myeloid cells capable of reducing growth of *M. tuberculosis* [12]. In another mouse immunisation model, BCG vaccination induced protection against *M. tuberculosis* in a neutrophil-dependent manner [83]. Of interest, BCG vaccination of human adults induced transcriptional signatures consistent with myeloid cell differentiation or neutrophil responses, epigenetically imprinting both the HPSCs and CD14+ cells [82]. Not only BCG, but the recombinant *M. tuberculosis* vaccine, MTBVAC, has also been shown to induce trained immunity *in vitro*, resulting in elevated production of IL-1β, TNFα or IL-6 upon secondary stimulation [74]. BCG-dependent enhancement of these cytokines could be exploited, as the cytokines could act as adjuvants to induce improved Th1 or Th17 responses that are considered protective against TB. Mycobacterial component-based vaccines, such as RUTI have also been shown to improve inhibition of mycobacterial growth *ex vivo* in association with phenotypic changes in monocytes from vaccinated mice [89]. Metabolites, such as fumarate, or the fungal component β-glucan, can also induce innate immune training *in vivo* [90–92]. BCG and β-glucan can induce features of trained immunity in cells from both neonates and adults [93], suggesting that microbial components, and metabolites might be exploited in combination with BCG or other anti-TB vaccines to enhance innate immunity and possibly protective T-cell responses not only in adults, but also in neonates, the main target group for immunisation against TB.

It may also be necessary to optimise vaccine regimens to maximise innate training. Just as for adaptive T-cell (and antibody) responses, BCG-dependent innate training (or that induced by other live vaccines such as MTBVAC [74] may be susceptible to external factors, resulting in variability. In healthy adults, circadian rhythms have been shown to modulate both heterologous and mycobacteria-specific cytokine production, with individuals administered BCG vaccine in the morning showing higher differences from baseline at 2 weeks or 3 months post-immunisation than individuals vaccinated in the evening [94]. What is learnt from BCG may help in the design of better vaccination strategies for both tuberculosis and other diseases [95].

It is also possible that trained innate or heterologous effects of BCG might be sex-specific, enhancing some immune responses more in males than in females or vice versa [96,97]. It should be noted that such effects are subtle and often result in trends rather than large-scale effects on all-cause morbidity or mortality [98]. While neither the systematic review by the WHO SAGE committee in 2014, nor its update in 2016 found sex-differential effects on all-cause mortality in BCG-vaccinated infants [63,64], some recent studies showed that beneficial effects of BCG on all-cause mortality can be observed at different time points since vaccination in males and females [65].

Finally, it is not fully clear for how long the effects of trained immunity last. Some studies in healthy adults showed that enhancement of cytokine production is transient and unlikely to last beyond a few months after vaccination [11], although as noted above some longer term protection may be induced [13]. However, while variability and lack of longevity of trained immunity might limit prime-boost strategies, vaccines, compounds and metabolites inducing this phenomenon could still be exploited as adjuvants. This rationale underpins new trials of BCG vaccination as an interim protective measure against COVID-19, for example in front-line healthcare workers [99,100].

12. Conclusions

The BCG vaccine has been used for almost one hundred years but we still have a lot to understand about it [101]. BCG vaccination can induce long-lasting protection against tuberculosis, and induces T-cell responses, but there is considerable variability in individual responses to vaccination between and within different settings, which may result in both BCG itself and other factors affecting responses to vaccination [102]. Measuring growth inhibition of BCG or *M. tuberculosis* itself may be more informative but we still lack proven correlates of protection. New routes of administration are being investigated such as giving BCG intravenously or by aerosol. BCG revaccination is also attracting interest. We need to understand better what BCG does and does not do, in order to develop more effective vaccination regimes to protect against tuberculosis, using either BCG, a modified BCG vaccine, or a new TB vaccine. We also need to investigate whether increasing innate training might enhance the efficacy of BCG vaccination. Finally, when developing new vaccines, we need to avoid the loss of any beneficial non-specific protective effects that BCG vaccination provides to infants.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

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References

[1] Mangtani P, Abubakar I, Ariti C, Beynon R, Pimpin L, Fine PEM, et al. Protection by BCG vaccine against tuberculosis: a systematic review of randomized controlled trials. Clin Infect Dis 2013;58:470–80. https://doi.org/10.1093/cid/cit790.

[2] Andersen P, Doherty TM. The success and failure of BCG — implications for a novel tuberculosis vaccine. Nat Rev Microbiol 2005;3:656–62. https://doi.org/10.1038/nrmicro1211.

[3] World Health Organization. WHO-UNICEF estimates of BCG coverage 2020. https://apps.who.int/immunization_monitoring/globalsummary/timeseries/tswucoveragebcg.html (accessed August 11, 2020).

[4] Thysen SM, Byberg S, Pedersen M, Rodrigues A, Ravn H, Martins C, et al. BCG coverage and barriers to BCG vaccination in Guinea-Bissau: an observational study. BMC Public Health 2014;14:1037. https://doi.org/10.1186/1471-2458-14-1037.

[5] Kagoné M, Yé M, Nébié E, Sie A, Schoeps A, Becher H, et al. Vaccination coverage and factors associated with adherence to the vaccination schedule in young children of a rural area in Burkina Faso. Glob Health Action 2017;10:1399749. https://doi.org/10.1080/16549716.2017.1399749.

[6] World Health Organization. Tuberculosis 2020. https://www.who.int/news-room/fact-sheets/detail/tuberculosis (accessed August 11, 2020).

[7] Brazier B, McShane H. Towards new TB vaccines. Semin Immunopathol 2020;42:315–31. https://doi.org/10.1007/s00281-020-00794-0.
[8] Aronson NE, Santosham M, Comstock GW, Howard RS, Moulton LH, Rhoades ER, et al. Long-term efficacy of BCG vaccine in american indians and alaska natives: a 60-year follow-up study. JAMA 2004;291:2086–91. https://doi.org/10.1001/jama.291.17.2086.
[9] Abubakar I, Pimpin L, Ariti C, Beynon R, Mangtani P, Sterne J, et al. Systematic review and meta-analysis of the current evidence on the duration of protection by bacillus Calmette-Guérin vaccination against tuberculosis. Health Technol Assess (Rockv) 2013;17. https://doi.org/10.3310/hta17370.
[10] Nguipdop-Djomo P, Heldal E, Rodrigues LC, Abubakar I, Mangtani P. Duration of BCG protection against tuberculosis and change in effectiveness with time since vaccination in Norway: a retrospective population-based cohort study. Lancet Infect Dis 2016;16:219–26. https://doi.org/10.1016/S1473-3099(15)00400-4.
[11] Kleinnijenhuis J, Quintin J, Preijers F, Benn CS, Joosten LAB, Jacobs C, et al. Long-lasting effects of BCG vaccination on both heterologous Th1/Th17 responses and innate trained immunity. J Innate Immun 2014;6:152–8. https://doi.org/10.1159/000355628.
[12] Kaufmann E, Sanz J, Dunn JL, Khan N, Mendonça LE, Pacis A, et al. BCG educates hematopoietic stem cells to generate protective innate immunity against tuberculosis. Cell 2018;172:176–90. https://doi.org/10.1016/j.cell.2017.12.031.
[13] Rieckmann A, Villumsen M, Sørup S, Haugaard LK, Ravn H, Roth A, et al. Vaccinations against smallpox and tuberculosis are associated with better long-term survival: a Danish case-cohort study 1971–2010. Int J Epidemiol 2017;46:695–705. https://doi.org/10.1093/ije/dyw120.
[14] Anderson EJ, Webb EL, Mawa PA, Kizza M, Lyadda N, Nampijja M, et al. The influence of BCG vaccine strain on mycobacteria-specific and non-specific immune responses in a prospective cohort of infants in Uganda. Vaccine 2012;30:2083–9. https://doi.org/10.1016/j.vaccine.2012.01.053.
[15] Kiravu A, Osawe S, Happel A-U, Nundalall T, Wendoh J, Beer S, et al. Bacille Calmette-Guérin vaccine strain modulates the ontogeny of both mycobacterial-specific and heterologous T cell immunity to vaccination in infants. Front Immunol 2019;10:2307. https://doi.org/10.3389/fimmu.2019.02307.
[16] Ritz N, Dutta B, Donath S, Casalaz D, Connell TG, Tebruegge M, et al. The influence of Bacille Calmette-Guérin vaccine strain on the immune response against tuberculosis. Am J Respir Crit Care Med 2012;185:213–22. https://doi.org/10.1164/rccm.201104-0714OC.
[17] Ponte C, Hacker M, Moraes M, Castello-Branco L, Silva F, Antas P. The patterns of in vitro cell-death and inflammatory cytokines induced by distinct BCG vaccine strains are differentially induced in human mononuclear cells. Hum Vaccin Immunother 2018;14:28–35. https://doi.org/10.1080/21645515.2017.1382788.
[18] Biering-Sørensen S, Jensen KJ, Aamand SH, Blok B, Andersen A, Monteiro I, et al. Variation of growth in the production of the BCG vaccine and the association with the immune response. An observational study within a randomised trial. Vaccine 2015;33:2056–65. https://doi.org/10.1016/j.vaccine.2015.02.056.
[19] Angelidou A, Conti M-G, Diray-Arce J, Benn CS, Shann F, Netea MG, et al. Licensed Bacille Calmette-Guérin (BCG) formulations differ markedly in bacterial viability, RNA content and innate immune activation. Vaccine 2020;38:2229–40. https://doi.org/10.1016/j.vaccine.2019.11.060.
[20] Arts RJW, Blok BA, Aaby P, Joosten LAB, Jong D, Meer JWM, et al. Long-term in vitro and in vivo effects of γ-irradiated BCG on innate and adaptive immunity. J Leukoc Biol 2015;98:995–1001. https://doi.org/10.1189/jlb.4MA0215-059R.
[21] Frankel H, Byberg S, Bjerregaard-Andersen M, Martins CL, Aaby P, Benn CS, et al. Different effects of BCG strains – a natural experiment evaluating the impact of the Danish and the Russian BCG strains on morbidity and scar formation in Guinea-Bissau. Vaccine 2016;34:4586–93. https://doi.org/10.1016/j.vaccine.2016.07.022.
[22] Schaltz-Buchholzer F, Bjerregaard-Andersen M, Øland CB, Golding C, Stjernholm EB, Monteiro I, et al. Early Vaccination With Bacille Calmette-Guérin-Denmark or BCG-Japan Versus BCG-Russia to Healthy Newborns in Guinea-Bissau: A Randomized Controlled Trial. Clin Infect Dis 2019. https://doi.org/10.1093/cid/ciz1080.
[23] Ponnighaus JM, Msosa E, Gruer PJK, Liomba NG, Fine PEM, Sterne JAC, et al. Efficacy of BCG vaccine against leprosy and tuberculosis in northern Malawi. Lancet 1992;339:636–9. https://doi.org/10.1016/0140-6736(92)90794-4.
[24] Black GF, Weir RE, Floyd S, Bliss L, Warndorff DK, Crampin AC, et al. BCG-induced increase in interferon-gamma response to mycobacterial antigens and efficacy of BCG vaccination in Malawi and the UK: two randomised controlled studies. Lancet 2002;359:1393–401. https://doi.org/10.1016/S0140-6736(02)08353-8.
[25] Hasso-Agopsowicz M, Scriba TJ, Hanekom WA, Dockrell HM, Smith SG. Differential DNA methylation of potassium channel KCa3.1 and immune signalling pathways is associated with infant immune responses following BCG vaccination. Sci Rep 2018;8:13086. https://doi.org/10.1038/s41598-018-31537-9.
[26] Fletcher HA, Snowden MA, Landry B, Rida W, Satti I, Harris SA, et al. T-cell activation is an immune correlate of risk in BCG vaccinated infants. Nat Commun 2016;7:11290.
[27] Smith SG, Zelmer A, Blitz R, Fletcher HA, Dockrell HM. Polyfunctional CD4 T-cells correlate with in vitro mycobacterial growth inhibition following Mycobacterium bovis BCG-vaccination of infants. Vaccine 2016;34:5298–305. https://doi.org/10.1016/j.vaccine.2016.09.002.
[28] Kagina BMN, Abel B, Scriba TJ, Hughes EJ, Keyser A, Soares A, 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;182:1073–9. https://doi.org/10.1164/rccm.201003-0334OC.
[29] Kumararatne DS. Tuberculosis and immunodeficiency—of mice and men. Clin Exp Immunol 1997;107:11–4. https://doi.org/10.1046/j.1365-2249.1997.d01-910.x.
[30] Prabowo SA, Zelmer A, Stockdale L, Ojha U, Smith SG, Seifert K, et al. Historical BCG vaccination combined with drug treatment enhances inhibition of mycobacterial growth ex vivo in human peripheral blood cells. Sci Rep 2019;9:4842. https://doi.org/10.1038/s41598-019-41008-4.
[31] Prabowo SA, Smith SG, Seifert K, Fletcher HA. Impact of individual-level factors on Ex vivo mycobacterial growth inhibition: Associations of immune cell phenotype, cytomegalovirus-specific response and sex with immunity following BCG vaccination in humans. Tuberculosis 2019;119:. https://doi.org/10.1016/j.tube.2019.101876101876.
[32] Smith SG, Kleinnijenhuis J, Netea MG, Dockrell HM. Whole Blood Profiling of Bacillus Calmette–Guérin-Induced Trained Innate Immunity in Infants Identifies Epidermal Growth Factor, IL-6, Platelet-Derived Growth Factor-AB/BB, and Natural Killer Cell Activation. Front Immunol 2017;8:644. https://doi.org/10.3389/fimmu.2017.00644.
[33] Kleinnijenhuis J, Quintin J, Preijers F, Joosten LAB, Jacobs C, Xavier RJ, et al. BCG-induced trained immunity in NK cells: Role for non-specific protection to infection. Clin Immunol 2014;155:213–9. https://doi.org/10.1016/j.clim.2014.10.005.
[34] Joosten SA, van Meijgaarden KE, Arend SM, Prins C, Oftung F, Korsvold GE, et al. Mycobacterial growth inhibition is associated with trained innate immunity. J Clin Invest 2018;128:1837–51. https://doi.org/10.1172/JCI97508.
[35] Grassly NC, Kang G, Kampmann B. Biological challenges to effective vaccines in the developing world. Philos Trans R Soc B Biol Sci 2015;370:20140138. https://doi.org/10.1098/rstb.2014.0138.
[36] Lalor MK, Ben-Smith A, Gorak-Stolinska P, Weir RE, Floyd S, Blitz R, et al. Population Differences in Immune Responses to Bacille Calmette-Guérin Vaccination in Infancy. J Infect Dis 2009;199:795–800. https://doi.org/10.1086/597069.
[37] Lalor MK, Floyd S, Gorak-Stolinska P, Ben-Smith A, Weir RE, Smith SG, et al. BCG Vaccination Induces Different Cytokine Profiles Following Infant BCG Vaccination in the UK and Malawi. J Infect Dis 2011;204:1075–85. https://doi.org/10.1093/infdis/jir515.
[38] Lubyayi L, Mawa PA, Nabakooza G, Nakibuule M, Tushabe JV, Serubanja J, et al. Maternal Latent Mycobacterium tuberculosis Does Not Affect the Infant Immune Response Following BCG at Birth: An Observational Longitudinal Study in Uganda. Front Immunol 2020;11:929. https://doi.org/10.3389/fimmu.2020.00929.
[39] Dockrell HM, Smith SG. What Have We Learnt about BCG Vaccination in the Last 20 Years?. Front Immunol 2017;8:1134. https://doi.org/10.3389/fimmu.2017.01134.
[40] Dauby N, Goetghebuer T, Kollmann TR, Levy J, Marchant A. Uninfected but not unaffected: chronic maternal infections during pregnancy, fetal immunity, and susceptibility to postnatal infections. Lancet Infect Dis 2012;12:330–40. https://doi.org/10.1016/S1473-3099(11)70341-3.
[41] Marchant A, Sadarangani M, Garand M, Dauby N, Verhasselt V, Pereira L, et al. Maternal immunisation: collaborating with mother nature. Lancet Infect Dis 2017;17:e197–208. https://doi.org/10.1016/S1473-3099(17)30229-3.
[42] Elliott AM, Mawa PA, Webb EL, Nampijja M, Lyadda N, Bukusuba J, et al. Effects of maternal and infant co-infections, and of maternal immunisation, on the infant response to BCG and tetanus immunisation. Vaccine 2010;29:247–55. https://doi.org/10.1016/j.vaccine.2010.10.047.
[43] Müller J, Tanner R, Matsumiya M, Snowden MA, Landry B, Satti I, et al. Cytomegalovirus infection is a risk factor for tuberculosis disease in infants. JCI Insight 2019;4:. https://doi.org/10.1172/jci.insight.130090e130090.
[44] Mawa PA, Webb EL, Filali-Mouhim A, Nkurunungi G, Sekaly R-P, Lule SA, et al. Maternal BCG scar is associated with increased infant proinflammatory immune responses. Vaccine 2017;35:273–82. https://doi.org/10.1016/j.vaccine.2016.11.079.
[45] Kjærgaard J, Birk NM, Nissen TN, Thøstesen LM, Pihl GT, Benn CS, et al. Nonspecific effect of BCG vaccination at birth on early childhood infections: a randomized, clinical multicenter trial. Pediatr Res 2016;80:681–5. https://doi.org/10.1038/pr.2016.142.
[46] Berendsen MLT, Øland CB, Bles P, Jensen AKG, Kofoed P-E, Whittle H, et al. Maternal Priming: Bacillus Calmette-Guérin (BCG) Vaccine Scarring in Mothers Enhances the Survival of Their Child With a BCG Vaccine Scar. J Pediatric Infect Dis Soc 2020;9:166–72. https://doi.org/10.1093/jpids/piy142.
[47] Lee H, Cho SN, Kim HJ, Anh YM, Choi JE, Kim CH, et al. Evaluation of cell-mediated immune responses to two BCG vaccination regimes in young children in South Korea. Vaccine 2011;29:6564–71. https://doi.org/10.1016/j.vaccine.2011.07.003.
[48] Davids V, Hanekom WA, Mansoor N, Gamieldien H, Sebastian JG, Hawkridge A, et al. The Effect of Bacille Calmette-Guérin Vaccine Strain and Route of Administration on Induced Immune Responses in Vaccinated Infants. J Infect Dis 2006;193:531–6. https://doi.org/10.1086/499825.
[49] Hawkridge A, Hatherill M, Little F, Goetz MA, Barker L, Mahomed H, et al. Efficacy of percutaneous versus intradermal BCG in the prevention of

tuberculosis in South African infants: randomised trial. BMJ 2008;337:. https://doi.org/10.1136/bmj.a2052a2052.

[50] Schaltz-Buchholzer F, Berendsen M, Roth A, Jensen KJ, Bjerregaard-Andersen M, Kjær Sørensen M, et al. BCG skin reactions by 2 months of age are associated with better survival in infancy: a prospective observational study from Guinea-Bissau. BMJ Glob Heal 2020;5:. https://doi.org/10.1136/bmjgh-2020-002993e002993.

[51] Storgaard L, Rodrigues A, Martins C, Nielsen BU, Ravn H, Benn CS, et al. Development of BCG Scar and Subsequent Morbidity and Mortality in Rural Guinea-Bissau. Clin Infect Dis 2015;61:950–9. https://doi.org/10.1093/cid/civ452.

[52] Barclay WR, Anacker RL, Brehmer W, Leif W, Ribi E. Aerosol-Induced Tuberculosis in Subhuman Primates and the Course of the Disease After Intravenous BCG Vaccination. Infect Immun 1970;2:574 LP – 582. https://doi.org/10.1128/IAI.2.5.574-582.1970.

[53] Sharpe S, White A, Sarfas C, Sibley L, Gleeson F, McIntyre A, et al. Alternative BCG delivery strategies improve protection against Mycobacterium tuberculosis in non-human primates: Protection associated with mycobacterial antigen-specific CD4 effector memory T-cell populations. Tuberculosis 2016;101:174–90. https://doi.org/10.1016/j.tube.2016.09.004.

[54] Darrah PA, Zeppa JJ, Maiello P, Hackney JA, Wadsworth MH, Hughes TK, et al. Prevention of tuberculosis in macaques after intravenous BCG immunization. Nature 2020;577:95–102. https://doi.org/10.1038/s41586-019-1817-8.

[55] Stylianou E, Paul MJ, Reljic R, McShane H. Mucosal delivery of tuberculosis vaccines: a review of current approaches and challenges. Expert Rev Vaccines 2019;18:1271–84. https://doi.org/10.1080/14760584.2019.1692657.

[56] Nuermberger EL, Yoshimatsu T, Tyagi S, Bishai WR, Grosset JH. Paucibacillary Tuberculosis in Mice after Prior Aerosol Immunization with Mycobacterium bovis BCG. Infect Immun 2004;72:1065–71. https://doi.org/10.1128/IAI.72.2.1065-1071.2004.

[57] Bull NC, Stylianou E, Kaveh DA, Pinpathomrat N, Pasricha J, Harrington-Kandt R, et al. Enhanced protection conferred by mucosal BCG vaccination associates with presence of antigen-specific lung tissue-resident PD-1+ KLRG1− CD4+ T cells. Mucosal Immunol 2019;12:555–64. https://doi.org/10.1038/s41385-018-0109-1.

[58] Eickhoff CS, Blazevic A, Killoran EA, Morris MS, Hoft DF. Induction of mycobacterial protective immunity by sublingual BCG vaccination. Vaccine 2019;37:5364–70. https://doi.org/10.1016/j.vaccine.2019.07.034.

[59] Dijkman K, Sombroek CC, Vervenne RAW, Hofman SO, Boot C, Remarque EJ, et al. Prevention of tuberculosis infection and disease by local BCG in repeatedly exposed rhesus macaques. Nat Med 2019;25:255–62. https://doi.org/10.1038/s41591-018-0319-9.

[60] de Castro MJ, Pardo-Seco J, Martinón-Torres F. Nonspecific (Heterologous) Protection of Neonatal BCG Vaccination Against Hospitalization Due to Respiratory Infection and Sepsis. Clin Infect Dis 2015;60:1611–9. https://doi.org/10.1093/cid/civ144.

[61] Hollm-Delgado M-G, Stuart EA, Black RE. Acute Lower Respiratory Infection Among Bacille Calmette-Guérin (BCG)–Vaccinated Children e73 LP-e81. Pediatrics 2014;133. https://doi.org/10.1542/peds.2013-2218.

[62] Aaby P, Roth A, Ravn H, Napirna BM, Rodrigues A, Lisse IM, et al. Randomized trial of BCG vaccination at birth to low-birth-weight children: beneficial nonspecific effects in the neonatal period?. J Infect Dis 2011;204:245–52. https://doi.org/10.1093/infdis/jir240.

[63] Higgins J, Soares-Weiser K, Reingold A. Systematic review of the non-specific effects of BCG, DTP and measles containing vaccines 2014:1–34. https://www.who.int/immunization/sage/meetings/2014/april/3_NSE_Epidemiology_review_Report_to_SAGE_14_Mar_FINAL.pdf (accessed August 27, 2020).

[64] Higgins JPT, Soares-Weiser K, López-López JA, Kakourou A, Chaplin K, Christensen H, et al. Association of BCG, DTP, and measles containing vaccines with childhood mortality: systematic review. BMJ 2016;355. https://doi.org/10.1136/bmj.i5170.

[65] Biering-Sørensen S, Jensen KJ, Monterio I, Ravn H, Aaby P, Benn CS. Rapid protective effects of early BCG on neonatal mortality among low birth weight boys: observations from randomized trials. J Infect Dis 2018;217:759–66. https://doi.org/10.1093/infdis/jix612.

[66] Prentice S, Webb EL, Akello F, Kiwudhu F, Akurut H, Elliott A. BCG-induced non-specific protection against heterologous infectious disease in Ugandan neonates: an investigator-blind randomised controlled trial. Lancet Infect Dis n.d.

[67] Kleinnijenhuis J, Quintin J, Preijers F, Joosten LAB, Ifrim DC, Saeed S, et al. Bacille Calmette-Guérin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. Proc Natl Acad Sci 2012;109:17537 LP – 17542. https://doi.org/10.1073/pnas.1202870109.

[68] Arts RJW, Carvalho A, La Rocca C, Palma C, Rodrigues F, Silvestre R, et al. Immunometabolic pathways in BCG-induced trained immunity. Cell Rep 2016;17:2562–71. https://doi.org/10.1016/j.celrep.2016.11.011.

[69] Netea MG, Joosten LAB, Latz E, Mills KHG, Natoli G, Stunnenberg HG, et al. Trained immunity: A program of innate immune memory in health and disease. Science (80-) 2016;352:aaf1098. https://doi.org/10.1126/science.aaf1098.

[70] Arts RJW, Moorlag SJCFM, Novakovic B, Li Y, Wang S-Y, Oosting M, et al. BCG vaccination protects against experimental viral infection in humans through the induction of cytokines associated with trained immunity. Cell Host Microbe 2018;23(89–100):. https://doi.org/10.1016/j.chom.2017.12.010e5.

[71] Freyne B, Donath S, Germano S, Gardiner K, Casalaz D, Robins-Browne RM, et al. Neonatal BCG vaccination influences cytokine responses to toll-like receptor ligands and heterologous antigens. J Infect Dis 2018;217:1798–808. https://doi.org/10.1093/infdis/jiy069.

[72] Jensen KJ, Larsen N, Biering-Sørensen S, Andersen A, Eriksen HB, Monteiro I, et al. Heterologous immunological effects of early BCG vaccination in low-birth-weight infants in Guinea-Bissau: a randomized-controlled trial. J Infect Dis 2015;211:956–67. https://doi.org/10.1093/infdis/jiu508.

[73] Freyne B, Messina NL, Donath S, Germano S, Bonnici R, Gardiner K, et al. Neonatal BCG vaccination reduces interferon-γ responsiveness to heterologous pathogens in infants from a randomized controlled trial. J Infect Dis 2020;221:1999–2009. https://doi.org/10.1093/infdis/jiaa030.

[74] Tarancón R, Domínguez-Andrés J, Uranga S, Ferreira AV, Groh LA, Domenech M, et al. New live attenuated tuberculosis vaccine MTBVAC induces trained immunity and confers protection against experimental lethal pneumonia. PLOS Pathog 2020;16:. https://doi.org/10.1371/journal.ppat.1008404e1008404.

[75] Nemes E, Geldenhuys H, Rozot V, Rutkowski KT, Ratangee F, Bilek N, et al. Prevention of M. tuberculosis Infection with H4:IC31 Vaccine or BCG Revaccination 138-149. N Engl J Med 2018;379. https://doi.org/10.1056/NEJMoa1714021.

[76] Benn CS, Fisker AB, Whittle HC, Aaby P. Revaccination with live attenuated vaccines confer additional beneficial nonspecific effects on overall survival: a review. EBioMedicine 2016;10:312–7. https://doi.org/10.1016/j.ebiom.2016.07.016.

[77] Roth AE, Stabell Benn C, Ravn H, Rodrigues A, Lisse IM, Yazdanbakhsh M, et al. Effect of revaccination with BCG in early childhood on mortality: randomised trial in Guinea-Bissau. BMJ 2010;340:. https://doi.org/10.1136/bmj.c671c671.

[78] Wardhana Datau EA, Sultana A, Mandang VVV, Jim E. The efficacy of Bacillus Calmette-Guerin vaccinations for the prevention of acute upper respiratory tract infection in the elderly. Acta Med Indones 2011;43:185–90.

[79] Guerra-Maupome M, Vang DX, McGill JL. Aerosol vaccination with Bacille Calmette-Guerin induces a trained innate immune phenotype in calves. PLoS ONE 2019;14:. https://doi.org/10.1371/journal.pone.0212751e0212751.

[80] Koeken VACM, van der Pasch ES, Leijte GP, Mourits VP, de Bree LCJ, Moorlag SJCFM, et al. The effect of BCG vaccination on alveolar macrophages obtained from induced sputum from healthy volunteers. Cytokine 2020;133:155135. https://doi.org/10.1016/j.cyto.2020.155135.

[81] Yao Y, Jeyanathan M, Haddadi S, Barra NG, Vaseghi-Shanjani M, Damjanovic D, et al. Induction of autonomous memory alveolar macrophages requires T cell help and is critical to trained immunity. Cell 2018;175:1634–50. https://doi.org/10.1016/j.cell.2018.09.042.

[82] Cirovic B, de Bree LCJ, Groh L, Blok BA, Chan J, van der Velden WJFM, et al. BCG vaccination in humans elicits trained immunity via the hematopoietic progenitor compartment. Cell Host Microbe 2020;28:322–34. https://doi.org/10.1016/j.chom.2020.05.014.

[83] Bickett TE, McLean J, Creissen E, Izzo L, Hagan C, Izzo AJ, et al. Characterizing the BCG induced macrophage and neutrophil mechanisms for defense against mycobacterium tuberculosis. Front Immunol 2020;11:1202. https://doi.org/10.3389/fimmu.2020.01202.

[84] Fanucchi S, Fok ET, Dalla E, Shibayama Y, Börner K, Chang EY, et al. Immune genes are primed for robust transcription by proximal long noncoding RNAs located in nuclear compartments. Nat Genet 2019;51:138–50. https://doi.org/10.1038/s41588-018-0298-2.

[85] Rodrigues LC, Pereira SM, Cunha SS, Genser B, Ichihara MY, de Brito SC, et al. Effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: the BCG-REVAC cluster-randomised trial. Lancet 2005;366:1290–5. https://doi.org/10.1016/S0140-6736(05)67145-0.

[86] Barreto ML, Pereira SM, Pilger D, Cruz AA, Cunha SS, Sant'Anna C, et al. Evidence of an effect of BCG revaccination on incidence of tuberculosis in school-aged children in Brazil: second report of the BCG-REVAC cluster-randomised trial. Vaccine 2011;29:4875–7. https://doi.org/10.1016/j.vaccine.2011.05.023.

[87] Khader SA, Divangahi M, Hanekom W, Hill PC, Maeurer M, Makar KW, et al. Targeting innate immunity for tuberculosis vaccination. J Clin Invest 2020;129:3482–91. https://doi.org/10.1172/JCI128877.

[88] Koeken VACM, Verrall AJ, Netea MG, Hill PC, van Crevel R. Trained innate immunity and resistance to Mycobacterium tuberculosis infection. Clin Microbiol Infect 2019;25:1468–72. https://doi.org/10.1016/j.cmi.2019.02.015.

[89] Prabowo SA, Painter H, Zelmer A, Smith SG, Seifert K, Amat M, et al. RUTI vaccination enhances inhibition of mycobacterial growth ex vivo and induces a shift of monocyte phenotype in mice. Front Immunol 2019;10:894.

[90] Arts RJW, Novakovic B, ter Horst R, Carvalho A, Bekkering S, Lachmandas E, et al. Glutaminolysis and fumarate accumulation integrate immunometabolic and epigenetic programs in trained immunity. Cell Metab 2016;24:807–19. https://doi.org/10.1016/j.cmet.2016.10.008.

[91] Moorlag SJCFM, Khan N, Novakovic B, Kaufmann E, Jansen T, van Crevel R, et al. Beta-Glucan induces protective trained immunity against mycobacterium tuberculosis infection: a key role for IL-1. Cell Rep 2020;31. https://doi.org/10.1016/j.celrep.2020.107634.

[92] Pérez-Hernández CA, Kern CC, Butkeviciute E, McCarthy E, Dockrell HM, Moreno-Altamirano MMB, et al. Mitochondrial signature in human monocytes and resistance to infection in *C. elegans* during fumarate-induced innate immune training. Front Immunol 2020;11:1715. https://doi.org/10.3389/fimmu.2020.01715.

[93] Namakula R, de Bree LCJ, A. Tvedt TH, Netea MG, Cose S, Hanevik K. Monocytes from neonates and adults have a similar capacity to adapt their cytokine production after previous exposure to BCG and β-glucan. PLoS One 2020;15:e0229287. https://doi.org/10.1371/journal.pone.0229287.
[94] de Bree LCJ, Mourits VP, Koeken VACM, Moorlag SJCFM, Janssen R, Folkman L, et al. Circadian rhythm influences induction of trained immunity by BCG vaccination. J Clin Invest 2020. https://doi.org/10.1172/JCI133934.
[95] Angelidou A, Diray-Arce J, Conti MG, Smolen KK, van Haren SD, Dowling DJ, et al. BCG as a case study for precision vaccine development: lessons from vaccine heterogeneity, trained immunity, and immune ontogeny. Front Microbiol 2020;11:332. https://doi.org/10.3389/fmicb.2020.00332.
[96] Koeken VACM, de Bree LCJ, Mourits VP, Moorlag SJCFM, Walk J, Cirovic B, et al. BCG vaccination in humans inhibits systemic inflammation in a sex-dependent manner. J Clin Invest 2020. https://doi.org/10.1172/JCI133935.
[97] Darboe F, Adetifa JU, Reynolds J, Hossin S, Plebanski M, Netea MG, et al. Minimal sex-differential modulation of reactivity to pathogens and toll-like receptor ligands following infant bacillus calmette-guérin russia vaccination. Front Immunol 2017;8:1092. https://doi.org/10.3389/fimmu.2017.01092.
[98] de Bree LCJ, Koeken VACM, Joosten LAB, Aaby P, Benn CS, van Crevel R, et al. Non-specific effects of vaccines: current evidence and potential implications. Semin Immunol 2018;39:35–43. https://doi.org/10.1016/j.smim.2018.06.002.
[99] Netea MG, Giamarellos-Bourboulis EJ, Domínguez-Andrés J, Curtis N, van Crevel R, van de Veerdonk FL, et al. Trained immunity: a tool for reducing susceptibility to and the severity of SARS-CoV-2 infection. Cell 2020;181:969–77. https://doi.org/10.1016/j.cell.2020.04.042.
[100] Curtis N, Sparrow A, Ghebreyesus TA, Netea MG. Considering BCG vaccination to reduce the impact of COVID-19. Lancet 2020;395:1545–6. https://doi.org/10.1016/S0140-6736(20)31025-4.
[101] Scriba TJ, Mizrahi V. Renewing the fight against TB with an old vaccine. Cell 2020;180:829–31. https://doi.org/10.1016/j.cell.2020.02.024.
[102] Zimmermann P, Curtis N. Factors that influence the immune response to vaccination. Clin Microbiol Rev 2019;32:e00084–e118. https://doi.org/10.1128/CMR.00084-18.
[103] Correia-Neves M, Sundling C, Cooper A, Källenius G. Lipoarabinomannan in active and passive protection against tuberculosis. Front Immunol 2019;10:1968.
[104] Bothamley GH, Beck JS, Potts RC, Grange JM, Kardjito T, Ivanyi J. Specificity of antibodies and tuberculin response after occupational exposure to tuberculosis. J Infect Dis 1992;166:182–6. https://doi.org/10.1093/infdis/166.1.182.
[105] Achkar JM, Chan J, Casadevall A. B cells and antibodies in the defense against Mycobacterium tuberculosis infection. Immunol Rev 2015;264:167–81. https://doi.org/10.1111/imr.12276.