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A century of BCG: Impact on tuberculosis control and beyond

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Abstract

BCG turns 100 this year and while it might not be the perfect vaccine, it has certainly contributed significantly towards eradication and prevention of spread of tuberculosis (TB). The search for newer and better vaccines for TB is an ongoing endeavor and latest results from trials of candidate TB vaccines such as M72AS01 look promising. However, recent encouraging data from BCG revaccination trials in adults combined with studies on mucosal and intravenous routes of BCG vaccination in non-human primate models have renewed interest in BCG for TB prevention. In addition, several well-demonstrated non-specific effects of BCG, for example, prevention of viral and respiratory infections, give BCG an added advantage. Also, BCG vaccination is currently being widely tested in human clinical trials to determine whether it protects against SARS-CoV-2 infection and/or death with detailed analyses and outcomes from several ongoing trials across the world awaited. Through this review, we attempt to bring together information on various aspects of the BCG-induced immune response, its efficacy in TB control, comparison with other candidate TB vaccines and strategies to improve its efficiency including revaccination and alternate routes of administration. Finally, we discuss the future relevance of BCG use especially in light of its several heterologous benefits.

KEYWORDS
adaptive, BCG, innate, revaccination, TB, trained immunity, vaccination

1 | INTRODUCTION

Mycobacterium bovis Bacille Calmette-Guerin (BCG) is the sole vaccine currently in use for the control of tuberculosis, a disease that has claimed about a billion lives in the past 200 years, more in fact than malaria, smallpox, influenza, HIV/AIDS, cholera, and plague.[1] As of 2021, BCG has been in use for 100 years. It was developed at the Pasteur Institute, France, by physician Albert Calmette and veterinarian Camille Guèrin by passaging virulent *Mycobacterium bovis (M bovis)* 230 times from 1908 to 1921 until an attenuated

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TABLE 1 Factors that influence BCG Efficacy

Variables	Study cohort	Country	Study findings/trial outcomes	Reference
BCG Strains	Infants	Hong Kong (RCT)	Low dose of the more virulent strain, BCG-Pasteur provided a significantly greater (40%) protection against childhood forms of TB than a less virulent strain, BCG-Glaxo.	248
		Mexico	BCG Denmark and BCG Brazil (Moreau)-immunized newborns increased expression of cytokines related to adaptive immunity (IFN-γ, IL-12, and IL-27), while BCG Japan induced cytokines associated with acute inflammatory responses (IL-1α/β, IL-6, and IL-24).	249
		Australia (RCT)	BCG Denmark and BCG Japan administered at birth induced higher proportions of *Mtb*-specific polyfunctional IFN-γ^{+}TNF-α^{+}IL-$2^{+}$$CD4^{+}$ T cells than BCG Russia.	152
		Uganda	BCG-induced scarring was associated with higher IFN-γ responses to heterologous stimuli at 1 year after birth, strain efficacy: BCG Denmark>BCG Bulgaria>BCG Russia)	12
		Kazakhstan	Infants vaccinated at birth with 3 vaccine strains were monitored for clinical and culture-positive TB for 3 years and TB meningitis for 21 months. Efficacy against TB was in the order, BCG Japan>BCG-Serbia>BCG-Russia, while all were 70% effective against meningitis.	151
		Nigeria & South Africa	BCG-Denmark mounted significantly higher frequencies of CD4+ T cells, durable polyfunctional cytokine response to BCG and heterologous vaccine antigens (Tetanus and Pertussis) than BCG-Bulgaria and BCG-Russia strains.	13
		Guinea-Bissau (RCT)	BCG strains did not affect morbidity; however, BCG-Denmark and BCG-Japan were more immunogenic than BCG-Russia.	14
NTM	School children and adolescents	India, Malawi and UK (RCT)	The frequency of responders and the magnitude of IFN-γ induced in response to PPDs of NTM species are far greater in Malawian and Indian populations, where the efficacy of BCG against pulmonary TB is 0%, compared to the UK, where the efficacy of BCG is 80%.	157,158
Geographic Location (latitude)	Infants and children	NA	BCG provides greater protection at higher latitudes compared to that at equator. Lower BCG efficacy may be due to high prevalence of NTM at the equator.	250,251
Time postvaccination	1- to 19-year-olds	American Indians and Alaska Natives (USA) RCT	BCG vaccination of TST-negative infants and children from low TB-endemic regions were followed up to 60 years. Efficacy persisted for 50 years, although it waned over time (80 to 50%) indicating that a single dose of the vaccine induces durable protection.	144
	12- to 50-year-olds	Norway	BCG-vaccinated TST-negative children and adults followed up to 44 years displayed a vaccine efficacy of 67% against pulmonary TB up to 9 years, 63% (10-19 years), 50% (20-29 years), and 40% (30-40 years), indicating BCG offers long-lasting protection that wanes with time.	145

(Continues)

TABLE 1 (Continued)

Variables	Study cohort	Country	Study findings/trial outcomes	Reference
	Infants and children {<1 yr, ≥1 yr <2 yr, ≥2 yr <5 yr, ≥5 yr}	South Africa	Infants mount comprehensive T cell responses. Reduced expansion and proliferation of BCG-specific effector $CD4^+$ T cells was associated with increased regulatory T cells in older children. Recall responses to BCG wane with age and may contribute to susceptibility to TB.	146
Age at Immunization	Infant/ Children/Adults	UK MRC	BCG-vaccinated TST-negative 14- to 15-year-olds were followed up to 20 years and demonstrated a vaccine efficacy of 84% during the first five years, and gradually decreased, averaging 77% over the whole period.	18
	Infants and Adults	Chingleput South India (RCT)	Two BCG vaccines (French and Danish) at high and low doses were simultaneously tested in infants and adults. Vaccine recipients re-evaluated 15 years after BCG vaccination. Vaccine efficacy in children was found to be 17%, while vaccination of adolescents or adults offered no protection.	19
	Infants (0 and 10 weeks of age)	South Africa (RCT)	Infants who received delayed BCG vaccination (10 weeks of age) demonstrated higher frequencies of BCG-specific $CD4^+$ T cells, particularly polyfunctional IFN-γ^+TNF-α^+IL-2^+ T cells at 1 year of age.	147
	Infants (0 and 4 1/2 months old)	Gambia (RCT)	Vaccination at birth induced a broad Th1/Th2/Th17/Treg anti-*Mtb* response but postponing by 4 1/2 months reduced the Th1/Th17 response	148
	Infants (0 and 2 months old)	Australia (RCT)	Comparable proportions of *Mtb*-specific cytokine-producing $CD4^+$ and $CD8^+$ T cells and polyfunctional IFN-γ^+TNF-α^+IL-2^+ $CD4^+$ T cells at birth and 2 months of age. Delay of BCG immunization does not confer any immunological advantage in cellular immunity.	252
	Infants (≤1year) and school-age children (10- to 15-year-olds)	UK	BCG-vaccinated UK-born infants (≤1year) from Black and Asian Minority Ethnic (BAME) communities and school-age children (10- to 15-year-olds) from native white population were followed for up to 20 years, vaccine efficacy ~50% and gradually waned after 10 years and 20 years in infants and school-age children, respectively.	253
Helminth infection	Infants (10-14 months old)	Kenya	Prenatal sensitization to Schistosomiasis persisted into childhood and reduced PPD-specific IFN-γ responses induced by BCG vaccination at birth but led to a biased Th2 response accompanied by enhanced IL-4 and IL-5 production.	254
	18-to-24-year-old college students	Ethiopia (RCT)	Deworming TST-negative helminth-infected subjects with albendazole prior to BCG vaccination increased T cell proliferation as well as PPD-specific IFN-γ production and enhanced BCG efficacy.	255
	Adults	Ethiopia (RCT)	Helminth-infected individuals from the placebo group had lower frequencies of PPD-responsive IFN-γ and IL-12 but higher frequencies of TGF-β producing cells compared to those who received anti-helminth therapy prior to BCG vaccination.	256
Socioeconomic status (SES)	6-9-year-old children	Dominican Republic	Malnourished children from rural areas in Santo Domingo were less likely to have a BCG scar than were urban children with adequate nutritional status.	257

(Continues)

TABLE 1 (Continued)

Variables	Study cohort	Country	Study findings/trial outcomes	Reference
	Infants	Indonesia	Both SES and nutritional status shape the response toward BCG vaccination at 10 months of age. Infants born to low SES families have smaller BCG scar size due to IgE levels compared to infants born in high SES families. Infants born with better nutritional status were found to have bigger BCG scar size.	258
Diet/nutritional Status	Infants	UK	Vitamin D deficiency is linked to TB susceptibility. BCG-vaccinated infants have higher concentrations of vitamin D in the serum. Vitamin D may play an immuno-regulatory role following BCG vaccination and contribute to its heterologous effects.	259
	Infants	China (RCT)	Vitamin A and vitamin D supplementation for 3 months enhanced PPD-specific responses in BCG-vaccinated infants and may be beneficial in TB-endemic countries or in cases of vitamin deficiency.	260

form, namely *M bovis* bacille Calmette Guèrin or *M bovis* BCG, incapable of causing disease in healthy humans, monkeys, guinea pigs, horses, and other animals, was obtained.[2] BCG was first used as a vaccine for TB control in humans in 1921.[3] Since then, it has been one of the most widely used vaccines in the world and is part of the immunization programs of many countries. The use of BCG as a TB vaccine has steadily declined in the developed world owing to the dramatic reduction in TB cases and spread in these parts. However, regions with a very high TB burden, for example, India, Africa, and South America still administer BCG vaccine after birth for TB prevention.[4]

M bovis a virulent mycobacterium species responsible for bovine tuberculosis is the parent for all available BCG vaccine strains. The genome sequences of *M bovis* and *M tuberculosis* are 99.95% similar.[5] However, there are significant genomic differences between BCG and *M bovis* and *M tuberculosis*. Using subtractive genomic hybridization, it was found that the 9.5kb RD-1 region is absent from all attenuated BCG strains but conserved in all laboratory and clinical isolates of *M bovis* and *M tuberculosis* capable of causing disease.[6] The RD-1 region encompasses 9 proteins: Rv3871, PE35, PPE68, ESAT-6, CFP-10, Rv3876, Rv3877, Rv3878, and Rv3879c.[7] Reintroduction of RD-1 into BCG results in changes in colony morphology and protein expression patterns such that they resemble that of virulent mycobacteria, for example, *M tb* and *M bovis*[6,8]; RD-1 knock-in of BCG grows faster and causes more immunopathology and granuloma formation compared to control in immune-compromised SCID mice.[8] These experimental evidences indicate that the loss of RD-1 from BCG contributes toward its attenuation. Apart from the absence of RD-1, propagation of BCG around the world for vaccination over the years has resulted in several additional genomic changes like SNPs, insertions, and deletions; about 129 open reading frames (ORFs) are absent from various BCG strains currently in use for vaccination.[9] Based on these changes, BCG can be classified into early (Russia, Japan, Moreau, Birkhaug, and Sweden) and late (Prague, Glaxo, Danish, Tice, Phipps, and Pasteur) strains.[2] BCG strains used for vaccination vary across different regions of the world; BCG Russia is used in Russia and former USSR countries, BCG Denmark in several European countries, BCG Pasteur in Canada and Australia and several countries immunize with more than one BCG strain, for example, India which uses both BCG Russia and Denmark.[10,11] There is evidence to suggest that anti-TB protection might vary with the strain of BCG used for vaccination (Table 1).[12-14] In Uganda, mycobacterial immune responses between infants vaccinated with BCG-Russia, Bulgaria, and Denmark were compared and it was found that vaccination with BCG-Denmark induced a higher percentage of scarring and anti-mycobacterial immune responses.[12] Similar results were reported in another clinical trial which compared BCG-Russia and Denmark.[13] Here too, BCG-Denmark induced a greater magnitude of mycobacteria-specific $CD4^{+}$T cell responses and polyfunctionality compared to BCG-Russia.[13] Finally, a very large clinical trial in infants in Guinea-Bissau that compared BCG-Denmark, Russia, and Japan found that BCG-Denmark and Japan were more immunogenic than BCG-Russia.[14] Results from these clinical trials suggest that the BCG strain used for immunization may impact vaccination outcomes (Table 1).

BCG is the most commonly administered vaccine in the world and has been used for TB prevention for 100 years. A meta-analysis of 1264 studies on BCG vaccination showed that on an average BCG vaccination provides 50% protection from different forms of TB across ages.[15] BCG protection against disseminated, meningeal, and pulmonary TB is greatest when administered at birth or school age.[16] Also, using a mathematical model, it has been estimated that 90% global BCG vaccination coverage prevents 117 132 TB deaths per birth cohort up to the age of 15.[17] In adults, BCG-induced protection against TB in different populations can range from very high (incidence rate ratio 0.22) to very low (incidence rate ratio 1.05).[18-20] However, the consensus from data that has emerged from numerous trials is that BCG efficacy is variable and appears to be influenced by numerous factors such as age at vaccination, latitude, exposure to non-tuberculous mycobacteria, BCG strains and route of administration (Table 1).[21]

BCG is cheap, widely available and the sole WHO recommended vaccine for TB. Importantly, it is very safe across all age groups and

most populations/communities except in HIV positive and other immune-compromised individuals. While concerns about its variable/limited efficacy in preventing the occurrence of TB in humans beyond childhood remain, promising results from recent studies primarily using primate models of TB have boosted confidence that BCG intrinsically has the potential to provide protection from adult TB too. This review aims to (a) provide a detailed analysis of BCG-induced immune responses implicated in protection from TB; (b) discuss the role of BCG in TB control including recent evidence that BCG has the potential to protect against TB; (c) discuss strategies that can be employed to improve BCG efficacy; (d) discuss the mechanisms that underpin the non-specific protective effects of BCG including modulation of trained immunity; and finally (e) discuss the relevance of future use of BCG including how it may be exploited in the context of new and emergent pathogens, like SARS-CoV-2.

2 | BCG-INDUCED IMMUNE RESPONSES

The immune correlates of protection for TB are still not clearly outlined and neither are the protective anti-TB immune responses induced by BCG vaccination. While it is possible to study anti-*Mtb* responses post-BCG vaccination in controlled animal challenge experiments, such a strategy cannot be followed for human studies. Animal experiments on BCG vaccination followed by TB challenge have yielded important information on BCG-induced immune responses; however, longitudinal studies in humans to study BCG immune responses have not been very successful in identifying a definitive correlate of protection perhaps due to the fact that BCG vaccine efficacy is influenced by many factors and is variable across the world (summarized in Table 1). Therefore, identification of a correlate of anti-TB protection induced by BCG is still an area of active investigation. Nevertheless, BCG-induced immune responses have now been studied for a century, and we currently have significant data on its wide-reaching impact on innate as well as adaptive aspects of anti-mycobacterial immunity (brief diagrammatic representation in Figure 1).[22] This is discussed in the sections below.

2.1 | Innate immune responses

BCG vaccine is administered intradermally, and hence, the immune response to it is initiated by resident epidermal neutrophils, macrophages, and dendritic cells (DCs) (Figure 1). This is in contrast to the initiation of the Mtb immune response which happens in the pulmonary tract and alveolar macrophages are the key players. The first interaction between BCG and the host immune system takes place via PAMPs on BCG and PRRs on neutrophils, macrophages, and DCs. The PAMPs present in BCG are primarily cell wall components like peptidoglycans, arabinogalactans, and mycolic acids.[23] There are various mycobacterial proteins that can also serve as PAMPs (mostly TLR ligands), for example, members of the PE/PPE family[24] and heat-shock proteins.[25] The PRRs that recognize and bind to mycobacterial PAMPs are CD11b, CD18, $F_c\gamma$RII, and $F_c\gamma$RIII on neutrophils[26]; complement receptor 3 (CR3),[27] TLR2/4/9,[28] mannose receptor (MR),[29] macrophage inducible Ca^{2+}-dependent lectin (MINCLE) receptor on macrophages[30]; the cytosolic nucleotide-binding oligomerization domain (NOD)-like receptors, for example, NOD2 on monocytes[31] and; DC-SIGN, CD11b, CD11c, and CD205 on dendritic cells[32-35]; and Dectin-1 expressed on macrophages, DCs, and neutrophils.[36,37] There is redundancy in PRRs that recognize BCG PAMPs such that absence of one does not majorly influence the generation of an overall immune response.[38,39]

Culture studies from skin biopsies of injection/immunization spots have revealed that live BCG numbers peak at 2 weeks and last till 4 weeks after vaccination suggesting that this is perhaps the timeline for the initial innate immune response at the site of immunization.[40] Also, the cells that accumulate at the BCG blister site comprise primarily of $CD15^+$ neutrophils with a small percentage of $CD14^+$ monocytes and a miniscule fraction of $CD3^+$ T cells.[40] The neutrophil response is not only a component of the BCG-induced innate immune response but also vital for the generation of the adaptive T cell response.[41] However, in vitro experiments that have studied the BCG immune response in whole blood cultures have found that the BCG-induced innate response comprises of signatures not just derived from neutrophils, but from monocytes, $CD56^+$NK cells, NKT cells γδ T cells, and MAIT cells as well.[42] The major innate functional responses are generation of ROS/RNI primarily by neutrophils and secretion of chemokines and cytokines such as IL-6, TNF-α, MIP-1α, MIP-1β, IL-8, and IL-1α mainly by monocytes, neutrophils and to a lesser extent by $CD56^+$NK cells, NKT cells γδ T cells, and MAIT cells.[42] Innate mediators are released very early on (1-3 hours post-BCG stimulation). Cytokines that are released later on (16-30 hours post-BCG stimulation) are adaptive cytokines like IL-2, IFN-γ, and IL-17.[42]

Macrophages are excellent phagocytes that are key antigen-presenting cells and also serve as specialized niches for mycobacterial survival and replication.[43] There is ample experimental evidence which shows that BCG-induced macrophage activation postvaccination has significant impact on anti-mycobacterial immune responses; both adaptive and innate.[44] Postinternalization mycobacteria including BCG reside in phagosomes. Mycobacteria through the course of evolution have acquired several mechanisms to prevent phagosome-lysosome fusion and supress other macrophage effector functions like generation of ROS/RNI, autophagy, secretion of effector cytokines like TNF-α, IL-12, and IL-6, thus enabling their survival, replication, and dissemination in the host.[24,45] However, BCG is a potent activator of macrophage/monocytes responses. Studies in mice have shown that mycobacterial killing by macrophages can be observed as early as 7 days post-BCG vaccination suggesting that this effector function of macrophages is independent of the initiation of adaptive immune responses.[46] Also, depletion of macrophages compromises clearance of mycobacteria (H37Ra) post-BCG vaccination in mice.[47] BCG-vaccinated guinea pigs when challenged with H37Rv or Erdman show enhanced phagosome-lysosome fusion, significantly enhanced mycobacterial clearance and increased secretion

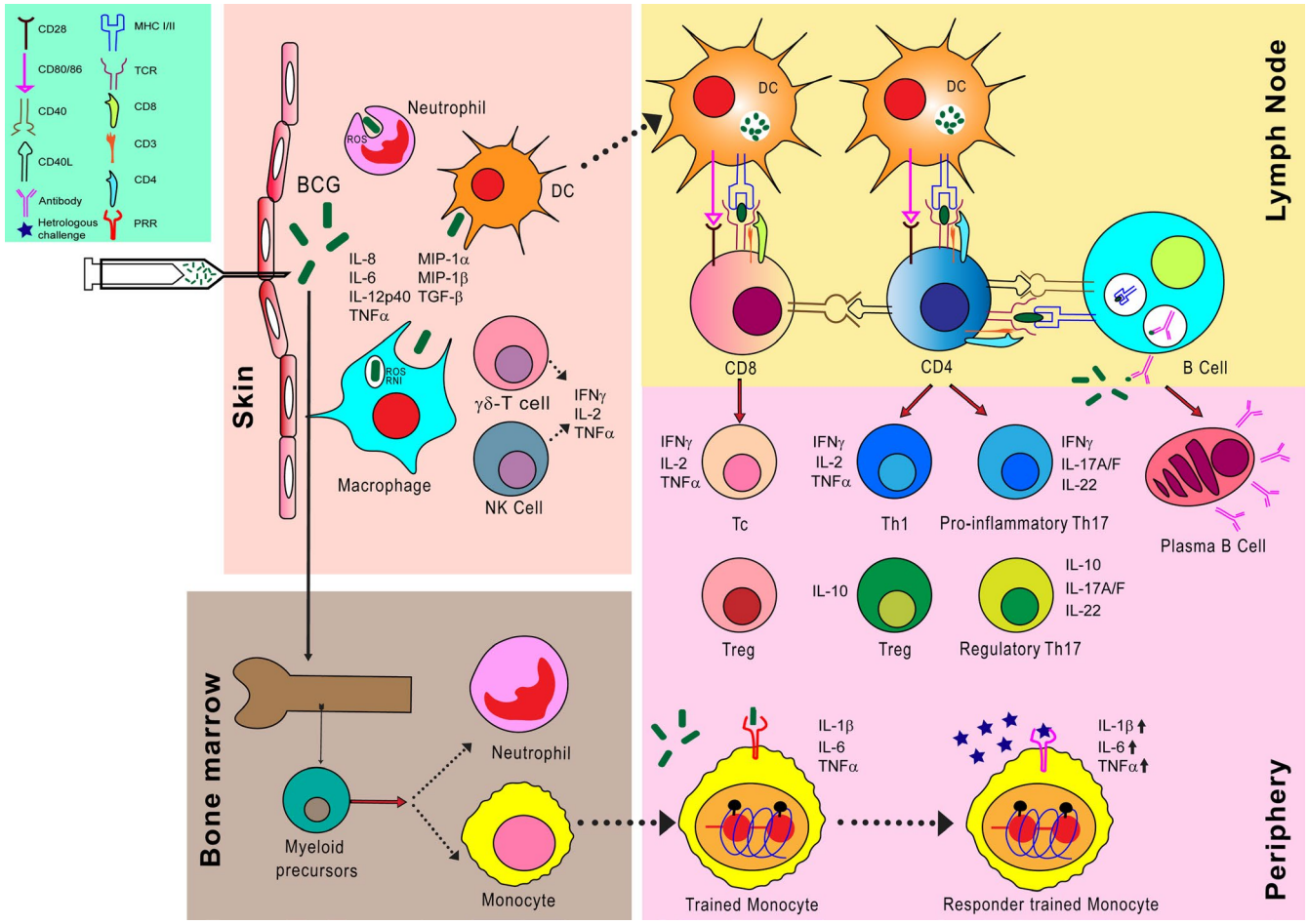

FIGURE 1 A diagrammatic overview of innate, adaptive, and trained immune responses generated post intradermal BCG vaccination. BCG immunization leads to sequential immune responses that unfold at the site of injection, in the lymph node, periphery, and even the bone marrow (depicted as differently colored regions in the figure). BCG when administered intradermally is recognized by several PRRs like TLRs, NOD-2, CD11b, $F_c\gamma$RII, $F_c\gamma$RIII, and mannose receptor present on neutrophils, monocytes, macrophages, and DCs which are the first responders that help in initiation of the immune response. The cytokine and chemokines that are secreted by activated macrophages, neutrophils and dendritic cells are IL-6, IL-8, TNF-α, IL-12p40, MIP-1α, MIP-1β, TGF-β, etc. In addition, neutrophils produce ROS and macrophages produce ROS and RNI postphagocytosis. Other innate cells that can be measured in the periphery 8-12 weeks post-BCG vaccination are IL-2, IFN-γ, TNF-α producing NK and γδ T cells. From the site of vaccination, BCG antigens are trafficked to the lymph node primarily by DCs. In the lymph node, DCs present processed BCG antigens to specific $CD4^+$ and $CD8^+$T cells to give rise to effector adaptive responses. The major $CD4^+$ effector populations that expand in response to BCG and can be measured in the periphery 8-12 weeks postvaccination are IFN-γ^+IL-2^+TNF-α^+ Th1 and IFN-γ^+IL-$17A^+$ Th17 cells along with $CD4^+$ Treg and IL-10^+IL-17^+ regulatory Th17 cells. Similarly, Tc or cytotoxic $CD8^+$ T cells and $CD8^+$ Treg cells are the $CD8^+$ effector T cells that expand and can be measured in the periphery 8-12 weeks postvaccination. In addition to T cells, B cells are also activated and differentiate into antibody-producing plasma B cells. Apart from the conventional innate and adaptive immune responses, BCG is also involved in generation of "trained immunity." BCG can cause epigenetic changes in monocytes primarily in the promoter regions of cytokine genes such as IL-1β, IL-6, and TNF-α. These epigenetic signatures persist long after primary exposure to BCG such that when there is an insult or challenge from an agent totally unrelated to BCG, for example, LPS, C. albicans, and yellow fever virus, these trained monocytes are able to mount a more efficient immune response. Apart from this BCG can induce transcriptional changes in bone marrow myeloid precursor cells which boosts myelopoiesis, that is, increased generation of monocytes and neutrophils which can form an army of trained cells including monocytes in the periphery capable of responding to heterologous challenges such as *C albicans*, yellow fever virus, LPS from Gram-ve bacteria, and other respiratory viruses

of TNF-α, IFN-γ, TGF-β, and IL-12p40.[48] In vitro infection of human monocyte-derived macrophages with BCG leads to secretion of IL-6, IL-12, TNF-α, and IL-10; cytokines that influence macrophage function as well as Th differentiation, from both infected as well as bystander cells.[49] BCG-induced secretion of cytokines IL-6, TNF-α, MIP-1α, MIP-1β, IL-8, and IL-1α by monocytes is regulated by their own expression-autocrine regulation in a feed-forward fashion; blocking with IL-RA, anti-TNF-R and anti-IL-6R dampens expression of IL-6, TNF-α, MIP-1α, MIP-1β, IL-8, and IL-1α.[42] Also, their secretion is regulated by ROS, NF-κB, and JAK1/2 signaling.[42]

Neutrophils comprise 60% of all blood cells and form the first line of defense by being the first cells to traffic to the site of infection.

The majority of cells present at the site of BCG immunization up to 4 weeks are neutrophils.[40] Intranasal infection with BCG and *Mtb* in mice leads to rapid recruitment of neutrophils to the lungs.[50] Studies in a mouse model of aerosol BCG infection showed that the early phagocytic response in the lungs is dominated by $Gr1^{int/hi}$ granulocytes/neutrophils.[51] Stimulation of purified blood neutrophils from healthy donors with BCG for 1.5-3.5 hours leads to enhanced mRNA expression of IL-1α, IL-1β, IL-8, MIP-1α, MIP-1β, GRO-α, TGF-β, MCP-1, IL-2Rγ, IL-10Rα, and IL-6R.[52] Whole blood cultures stimulated with BCG for 16 hours lead to enhanced expression of IL-8, MIP-1α, and MIP-1β in neutrophils as determined by flow cytometry.[42] The enhanced expression of these mediators is dependent on ROS and NF-κB-mediated signaling.[42] Neutrophils can internalize BCG and other mycobacteria and can eliminate them either by ROS-dependent mechanisms or non-oxidative mechanisms which involve phagolysosome fusion and release of anti-microbial peptides.[53] Apart from this, neutrophils can traffic internalized BCG to draining lymph nodes where the immune response can be further amplified by DCs and T cells. In fact, both mouse and human DCs co-cultured with BCG infected neutrophils stimulate IFN-γ responses in T cells.[41] However, neutrophil activation in response to BCG and other mycobacteria can be beneficial as well as harmful. Two strains of mice namely C3Heb/FeJ and C3H/HeOuJ mice exhibited varying BCG-induced protection upon *Mtb* challenge. Enhanced and prolonged BCG protection in the former was due to reduced frequencies of $CD11b^{+}Gr1^{+}$ neutrophils in the lungs compared to the latter upon aerosol *Mtb* challenge.[54] Also, it is now well documented that TB disease severity in humans is associated with a neutrophil-derived Type-I IFN molecular signature.[55]

Dendritic cells serve as a bridge between the innate and adaptive arms of the immune system. Post-BCG vaccination, epidermal DCs serve as carriers of processed antigens to the draining lymph nodes where they present them to T cells (Figure 1).[26] Antigen presentation and priming of T cells by DCs postexposure to BCG has been found to be dependent on IL-1R, MyD88 pathway,[56] and BATF-3.[57] BCG exposure promotes DC maturation into potent antigen presentation cells as indicated by enhanced expression of cell surface MHC-II, CD40, CD44, CD54, CD80, and CD86, markers of DC activation and molecules involved in antigen presentation.[58] However, studies have also shown that BCG stimulation of DC leads to secretion of IL-10 and IL-4 which might skew Th differentiation toward Th2 phenotype and diminish BCG efficacy.[59,60]

Apart from monocytes, macrophages, neutrophils, and DCs, other innate immune cells that are part of the BCG-induced innate response are NK cells[52,61]; innate lymphoid cells or ILCs[62] and innate-like T cell subset known as mucosal-associated invariant T or MAIT cells.[63] However, their role in protection remains to be confirmed.

2.2 | Adaptive immune responses

Our in-depth understanding on what constitutes protective immunity to TB is limited. Nevertheless, studies evaluating the immunogenicity of BCG and other TB vaccines have shed light on the probable correlates of protection against TB. Notably, the evidence for protective T cell responses induced upon BCG immunization was demonstrated by $CD4^{+}$ and $CD8^{+}$ T cells that were adoptively transferred from BCG-vaccinated mice into mice lacking both T and B cells (*rag1*$^{-/-}$), against aerosol BCG challenge. In this model, $CD4^{+}$ T cells were implicated as the main effector cells that reduced bacterial burden in the lung and spleen, while the cytotoxic $CD8^{+}$ T cells prevented extrapulmonary dissemination.[64] Although the effector role of $CD8^{+}$ T cells has not been fully characterized in the context of BCG vaccination, $CD8^{+}$ T cells are able to reduce bacterial burden in the lung of BCG-vaccinated CD4 knock-out mice challenged with *Mtb* at later stages of disease.[65] Moreover, depletion of $CD8^{+}$ T cells in the BCG-vaccinated rhesus macaques compromised BCG vaccine-induced immunity against tuberculosis.[66]

Several mechanistic studies have provided valuable insight into the requirements for BCG-induced protection against TB that was originally thought to be executed primarily by Th1 cells mostly through the production of IFN-γ.[67,68] Indeed, increased susceptibility to *Mtb* infection in IFN-γ knock-out mice[69] and in patients who have deleterious mutations in genes that encode major proteins in the type-1 cytokine (IL-12/IL23-IFN-γ) axis[70] underpin the importance of classical Th1 effectors in TB immunity. Consequently, IFN-γ secreted by $CD4^{+}$ Th1 cells had been considered as the benchmark against which protective immunity of BCG or other candidate TB vaccines were evaluated (Figure 1).[71]

Protective efficacy of BCG vaccination against TB has been validated in various animal models. Notably, many studies have provided extensive evidence that immunization of C57BL/6 or BALB/c mice with BCG or recombinant BCG (rBCG) vaccines induced a dominant Th1-type immune response, characterized by elevated expression of Th1 signature cytokines IFN-γ, IL-2, and TNF-α.[72-75] On the contrary, other studies indicated the prevalence of a mixed Th1/Th2-type response associated with reduced IFN-γ and increased IL-10 levels in mice immunized with rBCG expressing Ag85B or heat-killed BCG.[76,77] The pivotal role of Th1 immunity and IFN-γ in BCG-induced protection was best illustrated in infants wherein BCG administered at birth induced a Th1-biased response like vaccinated adults that persisted for 1 year and indicated the development of immunological memory after vaccination, whereas a Th2-type immune response was dominant in unvaccinated infants.[68,78] Additionally, IFN-γ-secreting BCG-specific T cells were associated with reduced risk of TB disease over the next three years of life.[79]

However, this long-established paradigm has been repeatedly debated over the last few decades by some animal and human studies that contradict the existing mechanisms of BCG-induced immune protection in their quest for better correlates of protection. These reports undermine the notion that a single immune marker can exclusively predict protection provided by BCG vaccination and have conclusively shown a lack of correlation between IFN-γ produced by $CD4^{+}$ T cells and BCG-mediated protection against TB.[71,80-82] Bone

marrow–derived macrophages from IFN-γR knock-out mice when cultured with BCG-primed $CD4^+$ T cells substantially inhibited intracellular *Mtb* growth in vitro via a NO-dependent mechanism. Furthermore, BCG-vaccinated IFN-γ-deficient mice demonstrated significant protection against a subsequent virulent *Mtb* challenge that was significantly lost upon depletion of $CD4^+$ T cells,[81] consistent with $CD4^+$ T cell deficient mice that succumb to tuberculosis despite a transient reduction in IFN-γ levels.[83] Similarly, a study in BCG-vaccinated humans that investigated relationships between immune-mediated *Mtb* growth inhibition and potential surrogate markers of protective TB immunity concluded that mycobacterial growth inhibition does not correlate with IFN-γ production.[80] Therefore, these reports reinforced that $CD4^+$ T cell–mediated IFN-γ-independent mechanisms are associated with BCG-induced immune protection against *Mtb* infection.

Polyfunctional $CD4^+$ T cells, which predominantly express different combinations of effector cytokines (IFN-γ, TNF-α, and IL-2), display greater association with protective T cell immune responses in infectious diseases than IFN-γ-secreting monofunctional T cells.[84] Vaccine-induced protection against *Mtb* infection in mice strongly correlated with the magnitude and quality of polyfunctional $CD4^+$ T cells.[85] However, BCG-vaccinated infants followed up to 2 years to identify those who developed culture-positive TB demonstrated enhanced Th1 responses and polyfunctional cytokine profile but none of these correlated with protection against TB.[86] Yet another study that involved BCG-vaccinated adults who were administered with a booster dose of MVA85A (modified vaccinia virus Ankara expressing antigen 85A) revealed that the frequency of polyfunctional T cells was significantly higher compared to BCG vaccination alone, leading to the hypothesis that the heterologous vaccine might augment the efficacy of BCG.[87] However, the results obtained from the phase 2b trial in infants given this prime-boost strategy indicated that MVA85A booster immunization failed to enhance protection despite the expansion of polyfunctional T cells.[88] These studies substantiate that polyfunctional T cells though essential for mycobacterial immunity do not always correlate with protective immunity, consistent with reports that detected increased multifunctional *Mtb*-specific $CD4^+$ T cells in active TB compared to latent TB subjects.[89]

2.3 | Th17 cells: Key players emerging in BCG-mediated protective immunity to TB

Several studies have highlighted the contribution of IL-17- and IL-22-producing $CD4^+$ T cell subsets toward protective anti-mycobacterial immunity,[90,91] while others have demonstrated reduced frequencies of Th17 cells and low serum IL-17 levels in TB patients compared to LTBI subjects to be associated with high mortality in TB patients.[92-94] Consistent with these reports, recent studies from our laboratory have provided further evidence in this aspect, whereby $IL\text{-}10^+$ regulatory Th17 cells enriched in latent TB subjects were skewed toward $IFN\text{-}\gamma^+$ pro-inflammatory Th17 cells in active TB and HIV-infected patients; however, a balanced Th17 response was restored following anti-tubercular and anti-retroviral therapy, respectively.[95,96] Additionally, a correlation between the inhibition of Th17 responses and progression from infection to active TB was revealed by transcriptional and clinical analyses of healthy South African adolescents.[97] Subsequently, Th17 cells have steadily emerged as key players in vaccine-induced protection against TB,[98,99] even in the absence of IFN-γ.[100] In an *Mtb* challenge model, adoptive transfer of in vitro primed ESAT6-specific Th17 cells into naive mice, conferred similar level of protection as achieved by vaccination. Importantly, IL-23 is critical, while IL-12 and IL-21 are not required for the quick generation and activation of protective Th17 recall responses. On the contrary, IFN-γ produced by adoptively transferred Th17 cells is detrimental to long-lasting protective recall immunity against *Mtb* challenge.[101] BCG-stimulated Th17 responses in the lung are diverse and are either linked to increased protection against *Mtb* infection or tissue damage and lung pathology. Therefore, IL-17A is important in generating an effective $CD4^+$ T cell immune response that leads to restrained inflammation and *Mtb* containment in the lung.[102] On the other hand, IL-17A-dependent increased influx of neutrophils into the lung due to repeated BCG immunization leads to exacerbated pathology owing to excessive inflammation and uncontrolled *Mtb* growth.[103]

Importantly, Th17 cells are considered indispensable for enhanced BCG-induced protection against *Mtb* challenge in tuberculosis-susceptible mice.[104,105] In further support of Th17 cells as critical mediators of immunity to BCG, it has been shown that accelerated Th1 memory responses in the lung of BCG-vaccinated mice are dependent on IL-17A and IL-23 derived from Ag-specific memory Th17 cells, and that these lung-resident memory Th17 cells quickly respond to *Mtb* infection.[102] Furthermore, sterile granulomas in the lungs of *Mtb*-infected cynomolgus macaques had moderately higher frequencies of T cells that produced IL-10 in combination with any of Th1 (IL-2 and TNF-α) and/or Th17 (IL-17A) cytokines than non-sterile granulomas, were linked to containment of *Mtb*.[106]

Additionally, Th17 cells correlated with improved immunogenicity in preclinical models of novel TB vaccines. For instance, VPM1002, the recombinant BCG vaccine candidate in which the urease C gene is replaced with the listeriolysin O encoding gene from Listeria monocytogenes (BCGΔureC:hly), elicited a profound increase in Th17 cells and offered superior protection compared with parental BCG in mice.[107] Besides being safe, well-tolerated, and immunogenic in newborn infants with additional increase in $CD8^+$ T cells producing IL-17.[108] Additionally, rBCG-CMX (composed of immune-dominant epitopes from Ag85C, MPT51, and HspX) immunized mice presented higher amounts of Th1, Th17, and polyfunctional-specific T cells that may be responsible for the reduction in the inflammatory lung lesions induced by *Mtb* challenge in BALB/c mice and the reduction in the bacterial load.[109] Hence, antigen-specific IL-17 and $IFN\text{-}\gamma$ or IL-10 co-producing $CD4^+$ T cells are regarded as one of the major determinants for protective efficacy of TB vaccines, the effects of which are considerably abrogated either by depletion or neutralizing antibodies to IL-17.

Importantly, intravenous and high-dose intradermal BCG administration was associated with protection in non-human primates challenged with virulent *Mtb*, since these immunization regimens particularly induced PPD-responsive $CD4^+$ T cells expressing the

Th1/Th17 phenotype that constituted almost 10% of total $CD4^+$ Th1 response in BAL compared to low-dose intradermal or aerosol BCG delivery.[110] Other NHP studies have also shown this cell subset to be associated with protection against *Mtb*.[111,112] Notably, PBMC-mediated mycobacterial growth inhibition in vitro correlated significantly with the frequencies of polyfunctional and IL-17^+ $CD4^+$ T cells at 4 months post-BCG vaccination of infants.[113] Consistent with studies in NHP models, we have recently demonstrated that BCG revaccination of young adolescents induced Ag85A- and BCG-specific IL-10 expressing regulatory Th17 cells (Figure 1).[114] Thus, antigen-specific Th1/Th17 cells may be considered as an attractive candidate for an immune correlate of protection against TB.

2.4 | Effect of BCG on immune-regulatory mechanisms

The impact of BCG on immune-regulatory or inhibitory mechanisms has been most well studied in context of influence of BCG vaccination on regulatory T cell (Treg) cell frequency and function. Optimal Treg cell function is crucial for maintaining a balanced immune response. The role of Tregs in active TB disease is dichotomous as experimental evidence from both animal models and human studies over the years indicates.[115] During the acute stages of *Mtb* infection, Treg frequencies increase in lungs and hamper development of protective T cell responses.[116] However, in the chronic phase of infection presence of Tregs can improve disease outcome by controlling over-exuberant immune responses[117,118] but the expansion of activated HLA-DR^+CD4^+ T cells refractory to Treg mediated suppression can serve as a deterrent to this effect.[119] Similar to active *Mtb* infection BCG inoculation has been shown to increase Treg frequencies in animal models as well as human studies (Figure 1).[120-123] This increase in Treg frequencies postadministration of BCG is believed to reduce its vaccination efficacy.[124,125] Absence of Tregs in BCG-vaccinated mice leads to higher cytotoxic T cell and Th1 responses[125] and marginally but significantly reduced bacterial burden postchallenge with *Mtb*.[124] However, another study showed that depletion of Tregs had no effect on BCG vaccination efficacy.[126] Rather, the ability of BCG to expand IL-13 producing $CXCR3^+CD4^+$ Treg was found to reduce mortality by diminishing damage causing inflammation in *Mtb*-infected type-2-diabetes mice.[123] On the other hand, Treg responses in humans postvaccination are heterogenous.[121] Individuals with high scarring/skin inflammation postvaccination exhibit an elevated pro-inflammatory response (high frequency of $CD4^+$IFN-γ^+IL-2^+TNF-α^+) compared to individuals who had low scarring/localized skin inflammation but a more pronounced expansion in BCG-specific $CD8^+$ Treg cells[121] suggesting that perhaps Treg cells indeed counter-regulate BCG-induced inflammation and may be detrimental to generation of vaccine-induced protective responses. This ability of BCG to increase Treg frequencies and dampen inflammation can be possibly exploited in its use as therapy in autoimmune disorders such as multiple sclerosis and type-1 diabetes (Table 2).[127,128] Patients with long-term type-1 diabetes when given 2 doses of BCG and monitored over a period of 8 years were found to have reduced near-normal levels of hemoglobin A1c (marker of disease) after 3 years and also elevated expression of regulatory markers FoxP3 and CTLA-4[128] suggesting that the mechanism behind BCG-mediated reduction in type-1 diabetes disease severity is BCG-induced immune-regulatory processes. The normal levels of hemoglobin A1c were maintained for the next 5 years suggesting that such effects of BCG vaccination are reasonably long-lasting.[128] Similar beneficial effects of BCG were observed in patients with multiple sclerosis or MS.[127] Multiple sclerosis patients who were given BCG had fewer lesions 6 months postvaccination compared to unvaccinated patients as monitored by brain MRI.[127] BCG-induced expansion of IL-10^+CD4^+ Treg cells in experimental autoimmune encephalomyelitis or EAE mice and the subsequent dampening of disease[129] could also be a possible mechanism behind suppression of MS lesions post-BCG vaccination.[127] BCG can influence immune-metabolism. It shifts the balance in cellular metabolism towards glycolysis as energy source rather than oxidative phosphorylation.[130] This shift known as the Warburg effect highly favors expansion and function of Treg cells which prefer glycolysis to obtain energy.[131] BCG-induced Treg expansion by modulation of immune-metabolism might be useful for tackling several inflammatory disorders.

Apart from Tregs, BCG can also modulate levels of regulatory cytokine IL-10.[60,111,125,132] BCG has been shown to increase IL-10 *in vitro*[60] as well as in vaccinated animals.[111,125] The absence of IL-10 in mice increases Th1, Th17, and cytotoxic T cell responses post-BCG vaccination and reduced bacterial burden after *Mtb* challenge[125,132] suggesting that IL-10 might block generation of BCG-induced protective anti-mycobacterial responses. However, this might not be true of higher animals like macaques where BCG vaccination through the mucosal route enhanced mycobacteria-specific IL-10 production in lungs post-*Mtb* challenge and this correlated with protection. In humans, BCG vaccination induces expansion of IL-10^+Th17 regulatory cells[114] which are a marker of controlled rather than active *Mtb* infection.[95] Expansion in Treg frequencies and IL-10 production post-BCG vaccination might cause dampening of protective pro-inflammatory responses. At the same time, they might also be signatures of a balanced immune response which is protective in chronic TB disease.

3 | BCG VACCINATION AND TB CONTROL

3.1 | How successful has BCG vaccination at birth been in TB eradication?

Although the incidence of active TB disease has declined considerably since its discovery 100 years ago, BCG is still in use in TB-endemic countries.[133] However, several low-burden countries have withdrawn their universal immunization policy in childhood due to the changing epidemiology of TB but continued with restricted BCG vaccination for high-risk populations.[4,134] Other deterrents for its usage include probable interference with tuberculin skin test (TST) reactivity and its highly variable efficacy (ranging from 0%-80%) and duration of

TABLE 2 Heterologous benefits of BCG beyond TB protection

Infection/disease condition	Effect of BCG	Mechanism of action	Reference
Neonatal Sepsis	BCG vaccination in infants protects against neonatal sepsis.	BCG vaccination triggers granulopoiesis, that is, increase in neutrophils thereby inducing protection.	261
Respiratory Infections	BCG vaccination in infants reduces incidence of acute lower respiratory tract infections caused by respiratory syncytial virus.		206
	BCG revaccination reduces incidence of upper-respiratory tract infection in adults.		163
	BCG vaccination reduces incidence upper-respiratory tract infections and pneumonia in elderly.		207,208
Leprosy	BCG vaccination and revaccination offers considerable protection against leprosy. BCG is the only vaccine available for leprosy prevention.		199-201
NTM infections	BCG vaccination provides 47% protection against Buruli ulcer caused by *M ulcerans*. BCG-vaccinated children have 96% protection against NTM infections.		202,203,262
Yellow Fever Virus (YFV)	BCG-vaccinated individuals have reduced viremia when challenged with an attenuated strain of YFV.	BCG induces "trained immunity" the mechanism for which is primarily IL-1β guided epigenetic reprogramming of monocytes. Such trained monocytes respond better to a heterologous challenge like YFV.	211
SARS-CoV-2 infection/ COVID-19	Countries with mandatory BCG vaccination have reduced spread and deaths per million from COVID-19 compared to countries without mandatory BCG vaccination.	BCG-induced "trained immunity" is being speculated as the mechanism behind possible protection against COVID-19.	241,242
	Individuals from Netherlands vaccinated with BCG anytime in the past 5 years prior to the COVID-19 outbreak reported less incidence of sickness, shorter duration of sickness and significantly reduced fatigue due to COVID-19 compared to unvaccinated controls.		244
Melanoma	BCG vaccination in infancy/childhood reduces the risk development of melanoma.		234
	Injection of BCG into nodules induced regression in 90% lesions.	BCG when injected into skin lesions promotes local inflammatory responses which include secretion of CXCL9, CXCL10 and CXCL10 by primed innate immune cells leading to recruitment of $CXCR3^{+}$Th1 cells; secretion of IL-32 by monocytes leading to increased cross-presentation; epigenetic reprogramming of primed monocytes leading to trained immunity.	263,264
Non-muscle invasive bladder cancer	BCG therapy reduces tumor progression	BCG activates tumor infiltrating T cells, thus improving anti-tumor immunity; reduces tolerance in the tumor environment; increases Ag presentation.	265-269

(Continues)

TABLE 2 (Continued)

Infection/disease condition	Effect of BCG	Mechanism of action	Reference
Type-1 diabetes	Patients given BCG have reduced Hb1Ac implying reduced disease severity.	BCG induces expansion of Treg cells by increasing glycolysis which favors Treg expansion and function. Increase in Tregs ameliorates severity of autoimmune disease.	127,128,130,131
Multiple Sclerosis	Patients who were given BCG had fewer lesions 6 months postvaccination.		

protection (20-60 years) against pulmonary TB (PTB).[135] Furthermore, BCG's adverse side effects like disseminated BCG infection in severely immune-compromised children, BCG-associated lymphadenitis, and osteomyelitis raised major concerns about its safety.[136,137] Additionally, a small proportion (0.4%) of household contacts of newly diagnosed leprosy patients developed paucibacillary leprosy within 12 weeks post-BCG vaccination.[138] The mechanisms of this observation remain to be elucidated but imply that BCG vaccine may in some individuals be detrimental in leprosy, possibly due to cross-reactive immune responses.[138] This raises questions on the use of BCG in populations that are highly exposed to mycobacteria. While this is an important consideration, our recent data on BCG revaccination of young healthy adults living in a TB-endemic area with a positive IGRA test, implying *Mtb* infection, did not show an increased incidence of TB in these revaccinated subjects over the 2 years that the study was conducted.[114]

Notably, BCG has been extremely effective at preventing life-threatening tuberculous meningitis and extrapulmonary disseminated TB in infants and young children.[139,140] A meta-analysis and systematic review of case-control studies conducted predominantly in Brazil (Latin America) and India (Asia) from 1980 to 1996 to investigate the protective efficacy of BCG, revealed an overall 73% decline in the incidence of TB meningitis, with greater protection in Latin American (87%) compared to Asian countries (69%), while the incidence of miliary TB was reduced by 77%.[139] Similarly, multiple randomized controlled trials performed in the United States, UK, Canada, Puerto Rico, and India demonstrated that BCG vaccination leads to ~90% reduction in disease severity and progression in infants or environmental non-tuberculous mycobacteria (NTM)-unexposed TST-negative school-going children, with enhanced protection achieved against meningeal and miliary tuberculosis compared to pulmonary tuberculosis.[16]

3.2 | Limitations, Variable efficacy, and Failure of BCG in preventing Adult TB

BCG is undeniably the most reliable vaccine for prevention of TB, particularly disseminated TB in children; however, its protective efficacy against pulmonary TB in adults is highly variable.[21,26,141-143] Table 2 summarizes the list of studies and trials conducted all over the world to substantiate the various hypotheses that have been proposed to explain the divergent outcomes of protection against TB induced upon BCG vaccination. Notably, the benefits of BCG vaccination seem to wane with age; thus, effectiveness against *Mtb* infection and disease is maximum during childhood and gradually declines over time.[144-146] Further, delayed immunization of TST-negative infants by a few weeks or months or in school-age children has demonstrated greater protection; however, this phenomenon is best observed in low TB-endemic countries where protective TB immunity has lasted until 50 years.[147,148] Moreover, genetic variability amid BCG strains that has risen due to culture protocols and multiple passages can potentially manipulate the immunogenicity of BCG.[149] Meta-analysis and systematic review of data from case studies and randomized clinical trials that were conducted in diverse populations but lacked direct comparison of different BCG strains could not account for the variable efficacy of BCG.[16,150] However, subsequent trials and studies that were longitudinally monitored up to several years following BCG vaccination provided extensive evidence for the variability in the efficiency against TB among BCG strains.[151] Additionally, in vitro studies demonstrated that BCG-mediated immune responses in humans are strain-dependent.[12,152]

Another potential cause for the variable efficacy conferred by BCG against pulmonary disease is non-tuberculous mycobacteria (NTM) interference.[153-155] Interestingly, high prevalence of NTMs in the tropical regions may be related to the reduced BCG efficacy observed in TB-endemic areas.[135,156] Pre-existing baseline immunity induced by NTMs mask the effects of BCG; hence, further increment of the anti-TB immune response is not detected upon BCG vaccination. Similarly, NTMs might block the replication of BCG due to cross-protective immunity, thereby limiting BCG-mediated protection against TB.[157,158]

Therefore, a plethora of factors responsible for the variable efficacy of BCG include host factors (genetic diversity, age, sex, and comorbidities), factors related to the vaccine (strain, dose, and delivery route) and the *Mtb* (virulence of circulating strains). Furthermore, environment factors (geographic location/latitude, climate), external factors (pre-existing immunity due to environmental/NTM, helminth/parasitic infections, and drugs/antibiotics), nutritional status (malnutrition, diet, micronutrients), and socioeconomic/psychosocial determinants (poor ventilation, hygiene, stress, and low income) may influence the effectiveness of BCG vaccine.

3.3 | How does efficacy of BCG compare with other TB vaccine candidates?

3.3.1 | VPM1002

VPM1002, a recombinant BCG vaccine, was genetically developed to improve the efficacy of BCG and widen the protective T cell immune response.[159,160] Consequently, its safety and immunogenicity

in adults and neonates have been proven by encouraging results from phase I and phase IIa clinical trials.[108,161] Interestingly, the less virulent VPM1002 has been demonstrated to be more efficacious than canonical BCG since it leads to improved stimulation of $CD4^+$ and $CD8^+$ T cells mostly of central memory phenotype as well as enhanced activation of both Th1, follicular T helper cells (Tfh) and Th17 cells.[107,162] Currently additional clinical trials have been initiated whose results are awaited. These include (a) a phase III clinical trial in HIV exposed and unexposed neonates as a replacement for BCG with prevention of infection (POI) as clinical end point, (b) a trial named priMe that aims to investigate the efficacy of VPM1002 and BCG in neonates from Sub-Saharan Africa, and (c) an ongoing phase III clinical trial in India with VPM1002 to assess the prevention of recurrence (POR) in cured TB patients who have the propensity to relapse or get reinfected within 1 year after completion of drug treatment (NCT 03152903).[162]

3.3.2 | H4:IC31, H56:IC31 vaccines and BCG

Recently, Aeras C-040-404, a phase 2 POI trial of H4:IC31 and BCG regimens, conducted among healthy HIV-uninfected adolescents BCG-vaccinated at birth from TB-endemic regions of South Africa, revealed that BCG revaccination significantly reduced the rate of sustained QuantiFERON-TB Gold In-tube (QFT-GIT) conversion, a secondary end point thought to be a marker of sustained *Mtb* infection, with an efficacy of 45.4% ($P = .03$), while the efficacy of H4:IC31 vaccine was shown to be 30.5% ($P = .16$).[163] Simultaneously, another randomized phase 1b trial (HVTN 602/Aeras A-042) was conducted for investigating the safety and immunogenicity of H4:IC31, H56:IC31 vaccines and BCG revaccination in QFT-GIT-negative, HIV-uninfected, healthy adolescents (aged 12-17 years) in Cape Town. All vaccines were safe and well-tolerated, with no reports of severe adverse events. H4:IC31 and H56:IC31 elicited $CD4^+$ T cells recognizing vaccine-matched antigens and H4- and H56-specific IgG binding antibodies. The highest vaccine-induced $CD4^+$ T cell response rates were for those recognizing Ag85B in the H4:IC31 and H56:IC31 vaccinated groups, while BCG revaccination elicited robust, polyfunctional BCG-specific $CD4^+$ T cells.[164]

4 | POSSIBLE STRATEGIES TO IMPROVE BCG EFFICACY

Even though BCG is the only vaccine available for TB control, but unfortunately it is not entirely efficacious due to several reasons discussed above and outlined in Table 1. Therefore, it is worthwhile to consider possible strategies to improve its efficacy as discussed below.

4.1 | I. Altered route of administration

Protection conferred by the routine intradermal injection of live attenuated BCG is variable; hence, better and alternative immunization strategies are required to improve TB prevention. Multiple studies across different species (mice and non-human primates) have demonstrated that BCG vaccination by delivery to the lung mucosa is more effective against aerosol *Mtb* challenge than parenterally delivered BCG which may be due to the direct effect on the local environment in the lung.

4.1.1 | Murine model

Mucosal BCG vaccination via nasal route confers superior protection in BALB/c mice compared to subcutaneous vaccination against pulmonary TB.[165] Interestingly, intranasal BCG administration significantly enhanced protective splenic immune responses in *Mtb*-infected mice that persisted 10 months after vaccination and was characterized by increased frequencies of $CD4^+$ and $CD8^+$ T cells and elevated levels of IFN-γ, IL-9, IL-11, and IL-21.[166] Importantly, mucosal (intratracheal and intranasal) BCG vaccination generated T effector memory and resident memory T cells in the lung compared to subcutaneous vaccination. Adoptive mucosal transfer of these airway-resident memory T cells expressing a PD-1^+ KLRG1^- cell surface phenotype into naive mice contributed to host defense against pulmonary TB.[167,168] Pulmonary (intratracheal) vaccination of mice with a higher dose of BCG (10^7 CFU) provided better protection against airway challenge with a virulent strain of *Mycobacterium tuberculosis* compared to that of conventional subcutaneous vaccination.[105,169] Therefore, murine studies strongly suggest that BCG delivered directly to the respiratory mucosa may prove to be a more effective route of vaccination.

4.1.2 | Non-human primates (NHP) model

NHP are regarded as the most clinically relevant models for evaluating vaccine efficacy as well as identifying correlates of protection against human TB.[170] Moreover, BCG's variable efficacy against *Mtb* infection and TB disease in NHP closely resemble that of humans.[171] Five decades ago, initial studies in rhesus macaques reported that BCG delivered by intradermal (ID), subcutaneous (SC), or intramuscular (IM) routes were not as effective in thwarting *Mtb* infection as intravenous (IV) route.[172] Recent studies further corroborated these findings and revealed potential mechanisms that might be responsible for the remarkable protection induced by mucosal or intravenous (IV) BCG administration versus failure of intradermal immunization against aerosol *Mtb* infection of highly susceptible rhesus macaques.[110,111,173-175] Intradermal BCG vaccination provides complete protection against extrapulmonary dissemination of TB, however, is incapable of preventing pulmonary TB, associated with increased bacterial burden in the lungs and granuloma severity.[176] On the contrary, localized immune responses induced by direct delivery of BCG into the lung mucosa deferred infection in some of the macaques challenged repeatedly with a low dose of *Mtb*, while entirely prevented *Mtb* infection and TB disease development in others.[111,174] Mucosal BCG vaccination of macaques led to

the enrichment of PPD-specific polyfunctional $CD4^+$ Th1/Th17 cells that expressed IL-17 in combination with canonical Th1 cytokines (IFN-γ, TNF-α, and/or IL-2) and $CD8^+IFN\text{-}\gamma^+TNF\text{-}\alpha^+$ T cells preferentially in the bronchoalveolar lavage (BAL) compared to unvaccinated and intradermally vaccinated groups. Additionally, IL-10, granzyme B, GM-CSF, and IgA antibodies were also enhanced in the lung of mucosally vaccinated NHP and correlated with improved protection compared to parenteral immunization.[111]

Latest reports indicated that macaques challenged with 10-100 CFU of a highly pathogenic *Mtb* Erdman strain 5-6 months postintravenous BCG vaccination exhibited rapid clearance of *Mtb*, reduced *Mtb* growth and fewer granulomas, decreased lung pathology and improved survival compared to unvaccinated and intradermally and/or aerosol vaccinated animals.[110,173] The probable mechanisms for unprecedented protection elicited by IV BCG vaccination may be linked to increased frequencies of PPD-specific $CD4^+$ and $CD8^+$ T cells in the BAL, blood, spleen, bone marrow, peripheral lymph nodes and lung parenchyma, high proportion of tissue-resident memory (T_{RM}), effector memory (T_{EM}) and transitional memory (T_{TM}) T cells in the lung and high level of IgA antibodies in BAL and plasma.[110]

Similarly, studies investigating the immunogenicity and efficacy of aerosol versus parenteral BCG vaccination against ultra-low-dose inhaled *Mtb* infection revealed that disease progression and extrapulmonary dissemination were appreciably inhibited, while bacterial burden was significantly reduced in extra-thoracic sites (spleen, liver, and kidneys) in response to aerosol in comparison with intradermal BCG delivery.[177,178] White et al probed the mechanisms behind this enhanced protection mediated by vibrating mesh nebulizer (VMN)-delivered aerosol BCG and showed an induction of Th1 and Th17 cytokine responses, as well as $CD4^+$ T cell responses in BAL fluid cells producing distinct cytokines. Additionally, they found a significant increase in frequencies of peripheral central memory (T_{CM}) T cells and in PPD-specific IFN-γ-producing cells 10-13 weeks following aerosol vaccination.[177] More recently, the induction and continued presence of circulating *Mtb*-specific T_{CM} and transitional effector memory $CD8^+$ T cells post-BCG vaccination and *Mtb* challenge suggests their contribution toward disease control observed in aerosol BCG-vaccinated macaques.[178]

4.1.3 | Human studies

Orally delivered BCG sub-strain Moreau Rio de Janeiro was well-tolerated in healthy adults from Brazil and UK and significantly boosted PPD- and Ag85-specific IFN-γ responses to previous childhood intradermal BCG immunization that lasted for 3 months postvaccination.[179] Similarly, a small-scale trial that investigated the safety and immunogenicity of oral and/or intradermal administration of Danish strain of BCG in healthy individuals reported no deleterious effects.[180] Interestingly, using systems immunology approaches, it was suggested that a combination of intradermal and oral BCG vaccination regimens may lead to enhanced protection against TB due to the synergistic induction of systemic (blood) and mucosal (BAL) immune responses, respectively.[180] Aerosolized BCG was first administered in the late 1960s to children and young adults and did not report serious adverse side effects.[181] Currently, phase I clinical trials are investigating the safety of aerosol delivery of BCG in BCG-naive healthy adults (NCT02709278 and NCT03912207).[182]

Therefore, improving protective efficacy of BCG against TB in mice and non-human primates by employing alternative strategies of BCG immunization via mucosal or intravenous (IV) routes have shown considerable promise. Although BCG delivery through these routes may face technical challenges and is yet to undergo rigorous testing in human trials, these reports emphasize that the route of vaccination is a determining factor for robust and durable protection against TB.

4.2 | II. BCG revaccination

BCG vaccination at birth protects children against disseminated and meningeal TB but is not so effective in preventing TB in adults.[16,26,141] This can be attributed to the waning of BCG-induced immunity with age.[146] One of the ways to overcome this decline or dampening in immunity is BCG revaccination. Several BCG revaccination trials have been carried out yielding a mixed bag of results depending on the end point studied. In a trial in Karonga district in Malawi, between 1986 and 1989, BCG was administered to previously vaccinated individuals and this reduced the incidence of leprosy but not tuberculosis.[183] In another very extensive trial in Brazil, about 100 000 children between the ages of 7-14 were revaccinated with BCG. A similar number formed the control group which received no BCG. The rate of TB incidence was found to be similar in both groups; in other words, BCG revaccination provided no benefit.[184] Similar results were obtained in another trial in Hong Kong where previously vaccinated TST^- children between the ages of 6-9 years were revaccinated with BCG, but this did not have any impact on TB incidence.[185] However, more recent trials and studies on BCG revaccination have yielded more promising results. When BCG revaccination was compared to vaccination with *Mtb* candidate vaccine H4:IC31, it was found that the former was marginally better at preventing sustained QFT conversion in young adults in a TB-endemic area.[163] Moreover, BCG revaccination induced generation of Ag85A, TB10.4, and BCG-specific $IFN\text{-}\gamma^+$ and polyfunctional $CD4^+$ and $CD8^+$ T cell responses that were equivalent or even better than those generated by *Mtb* candidate vaccines H4:IC31 and H56:IC31.[164] Also, BCG revaccination boosts $IL\text{-}22^+CD4^+$ T cells in addition to Th1 cells.[186] Another trial conducted in South Africa in TST^+ 18-40-year-olds revealed that BCG revaccination significantly boosts pre-existing BCG-specific $cytokine^+$ (IFN-γ/IL-2/TNF-α/IL-22/IL-17) $CD4^+$ and $CD8^+$ responses at 5 weeks post-administration. Apart from adaptive T cell responses, revaccination also boosted BCG-reactive $IFN\text{-}\gamma^+$ NKT, NK, and γδ T cells.[61] Our study which examined BCG revaccination in $IGRA^-$ and $IGRA^+$ young adults also showed similar results with anti-mycobacterial Th1 and polyfunctional responses boosted at 4 weeks postimmunization.[114] In addition, we also observed increment in frequencies of Ag85A-specific $IFN\text{-}\gamma^+IL\text{-}17^+$ cells in both $IGRA^-$ and $IGRA^+$ vaccinees. The

other interesting finding was the increase in IL-10$^+$IL-17$^+$ regulatory Th17 cell frequencies in response to Ag85A, BCG and latency Ag restimulation in both IGRA$^-$ and IGRA$^+$ vaccinees at 34 weeks post-vaccination. Notably, the ability of BCG to augment regulatory Th17 frequencies has been identified as an important potential correlate of protection in non-human primates.[111] BCG revaccination also enhanced NKT, CD56$^{dim/bright}$ NK, and γδ T cells at both 4 (IGRA$^-$ and IGRA$^+$ Ag85A-reactive) and 34 weeks (IGRA$^+$ BCG-reactive) suggesting that revaccination impacts BCG-induced innate, adaptive immunity in the cohort we have studied.[114] Notably, we also found that BCG revaccination boosted anti-mycobacterial immunity irrespective of prior *Mtb* exposure as has been previously reported.[61] This is important if BCG revaccination is to be considered as a strategy in TB-endemic settings. However, it should be noted that BCG revaccination does not boost immune responses against all mycobacterial antigens. In our study, we found that while Ag85A-specific responses were boosted by BCG revaccination, there was no alteration in frequencies of TB10.4-specific cells.[114] This may be due to our reported higher pre-existing levels of TB10.4 compared to Ag85A-specific CD4$^+$ T cells.[114] Alternatively, this could also be due to the fact that Ag85A (~35.6 kDa) is larger than TB10.4 (~10.4 kDa) and therefore likely encompasses more T cell epitopes; additionally, Ag85A from *Mtb* and BCG are highly homologous.[187] Moreover, the CD4$^+$ T cell response induced by BCG has been shown to map primarily to the N-terminal region of TB10.4 gene,[188] whereas the CD4$^+$ T cell response induced by *Mtb* infection spans more broadly across TB10.4 implying that BCG vaccination may not induce a response to all T cell epitopes in TB10.4.[188] Further studies with a much wider panel of mycobacterial antigens in BCG revaccinated individuals will shed light on exactly cells of which antigenic specificity are most affected. Also, our study and those by Suliman S and Nemes E et al have not looked at BCG revaccination efficacy in terms of TB incidence or mycobacterial growth control.[61,163] This is important as previous studies have indicated that even though a second BCG dose boosts PPD-specific adaptive immunity it fails to augment the vaccinees pre-existing ability to control BCG growth as determined by an in vitro mycobacterial growth inhibition assay.[189] TB incidence and mycobacterial growth control are two end points that should be considered in future BCG revaccination studies/trials. Another important clinical end point to consider is the acquisition of new TB infection or disappearance of previous infection as measured by QFT conversion at regular intervals postrevaccination.[163,190] In conclusion, several recent studies have reported promising results from BCG revaccination studies,[61,114,163] thus providing strong evidence in support of BCG revaccination as a possible strategy to enhance BCG efficacy and TB control in absence of another candidate TB vaccine in the immediate future.

4.3 | III. Recombinant BCG and use of adjuvants

Apart from altered route of vaccination and revaccination, another approach being actively pursued to improve BCG efficacy is the use of recombinant BCG. The best example of this in recent times is the VPM1002, a urease C deficient recombinant BCG (rBCG) which expresses *Listeria monocytogenes* derived listeriolysin (rBCGΔurec::Hly).[160,191] BCG and other mycobacteria when internalized are able to prevent acidification of the phagosome and hence its maturation. The absence of urease C from VPM1002 is able to stop this; hence making the phagosome containing BCG VPM1002 acidic and triggering secretion of listeriolysin which perturbs the phagosomal membrane enabling leakage of BCG antigens into the cytosol and their cross-presentation, thereby ensuring the generation of a more robust T cell response.[162] The safety and immunogenicity trials for VPM1002 have been carried out and the vaccine was found to be safe and induced a CD4$^+$ T cell response comparable to BCG in infants. The proportion of CD8$^+$IL-17$^+$ cells was higher in the VPM1002 vaccinated infants compared to the BCG group.[108,161] This rBCG vaccine is currently under phase III clinical trials to ascertain efficacy. Another rBCG expressing the ESX-1 secretion system (BCG::RD-1) when delivered mucosally was found to be protective against aerosol *Mtb* challenge in a mouse model of type-2 diabetes.[192] Similarly, subcutaneous immunization of mice with rBCG expressing *E coli* derived heat-labile enterotoxin LTAK63 was found to be more protective than wild-type BCG against an *Mtb* challenge.[193]

Another strategy that has been pursued to boost BCG efficacy is to use adjuvants along with BCG, some examples of which are clofazimine, lactoferrin, inarigivir, and IL-15.[194-198] BCG when administered along with clofazimine, a lipophilic compound used mainly for treatment of leprosy, was more efficacious than BCG alone in reducing cfu and granuloma formation upon *Mtb* H37Rv challenge in mice.[194] The mechanism for this was found to be clofazimine's ability to boost frequencies of stem cell like memory cells, precursors of central and effector memory cells.[194] Similarly, administration of human or bovine lactoferrin along with BCG in mice was found to better protect them from challenge with virulent *Mtb* by inducing Th1 responses, reducing lung cfu, and protecting against pathogen-induced tissue damage.[195] BCG-vaccinated IL-15 transgenic mice are better protected against aerosol *Mtb* challenge and this prompted suggestions that it could be used as an adjuvant.[197] Indeed, rBCG expressing IL-15 was found to reduce cfu, lung pathology and boost Th1 responses in mouse model of aerosol *Mtb* infection.[198] Another recent example of use of an adjuvant is small molecule SB9200 or Inarigivir which is an activator of RIG-1 and NOD-2 mediated antiviral immune responses. Subcutaneous administration of Inarigivir along with BCG better protected mice against an aerosol *Mtb* challenge than just BCG alone.[196]

5 | THE EFFECTS OF BCG BEYOND TB

Beyond studies in TB, there is strong evidence of BCG vaccination being particularly effective against leprosy and non-tuberculous mycobacteria (NTM) and more broadly against respiratory viral infections (please see Table 2 for details). Among other mycobacterial diseases, BCG efficacy has been most well studied in context

of leprosy, the causative organism of which is *Mycobacterium leprae*. There is no separate, widely used vaccine for leprosy and BCG administration has been found to be protective especially in household contacts.[199] Two meta-analysis (in 2006 and 2010) of BCG trials for leprosy prevention showed that the TB vaccine provides around 26%-61% protection suggesting that BCG indeed has cross-mycobacterial effects.[200,201] In the context of NTM infections, two trials in Uganda showed BCG vaccination protects against Buruli ulcer, caused by *Mycobacterium ulcerans*.[202,203] Further, a discontinuation of universal BCG vaccination in Sweden led to an increase in NTM infections in infants.[204,205]

Apart from its cross-mycobacterial protective effects, several studies have documented the effects of BCG in completely unrelated infections and disease conditions (Table 2). Studies in Guinea-Bissau, Indonesia, Japan, and South Africa have shown that BCG vaccination reduces respiratory tract infections in children by about 73%.[163,206-208] Due to its ability to generate non-specific cross-protective immune responses, BCG vaccination appears to reduce infant mortality by about 38%.[209] This capacity of BCG to induce non-specific cross-protective immune responses is not fully understood but is being actively probed. Based on experimental evidence so far, non-specific cross-protective effects of BCG can be attributed primarily to a process known as "trained immunity" as is discussed below.[210,211]

5.1 | BCG-induced trained immunity

The biological process by which the response of innate immune cells like monocytes to a challenge is amplified due to their previous exposure to unrelated immunological agents is termed as "trained immunity" (Figure 1). It is therefore a concept that explains how like the adaptive response, the innate immune response can also be reprogrammed or "trained" to exhibit features of memory.[212] In various study systems, trained immunity has been found to be induced by exposure to LPS[213]; β-glucan,[214] muramyl dipeptide,[215] CpG,[216] flagellin,[217] controlled malaria infection,[218] and BCG.[219] In addition, BCG-induced trained immunity has now been well demonstrated to be a feature of BCG vaccination in humans.[211] Indeed, monocyte responses as measured by secretion of IL-1β, IL-6, and TNF-α to LPS, *Mtb*, and *C albicans* are higher in BCG vaccinated compared to unvaccinated individuals.[211] Also, BCG vaccinees are able to reduce yellow fever virus viremia better than non-vaccinees demonstrating very clearly that BCG vaccination indeed provides broad protection against non-related pathogenic threats.[211] For a detailed list of heterologous benefits of BCG vaccination, please refer to Table 2.

Evidence of trained immune responses has been observed in myeloid cells[220,221]; NK cells[222,223]; ILCs[224]; and even stem cells.[225] However, trained immunity in context of BCG has been most well studied in monocytes.[31,211,226] The primary mechanisms that govern BCG-induced trained immunity are epigenetic reprogramming and immune-metabolism changes influenced by BCG exposure.[211,226] What innate training by agents such as BCG is able to achieve is that during a primary exposure, there are epigenetic changes (H3K27ac, H3K4me3, etc) caused by secreted mediators such as IL-1β leading to active transcription of IL-6, IL-8, and TNF-α; upon removal of the primary stimulus, the "trained cell" goes back to a resting state while still retaining the epigenetic signatures so that upon exposure to a secondary heterologous challenge there are further epigenetic changes and more pronounced expression of cytokines that eventually contribute to a protective immune response.[212] Genome-wide epigenetic changes induced by BCG in monocytes are H3K27ac in genes of signaling pathways such as the PI3K/AKT, EGFR, FGF, and VEGF, and H3K4me3 in the promoter sites of genes such as IL-6, TNF-α, mTOR, HK2, PFKP, GLS, and GLUD.[211,226] BCG vaccination induces transcriptional changes in the bone marrow hematopoietic stem and progenitor cells (HSPCs) which boosts myelopoiesis, an effect that is visible up to 3 months postvaccination.[227] These transcriptional changes in HSPCs are accompanied by epigenetic modifications in peripheral $CD14^+$ monocytes, that is, overexpressed genes in HSPC transcriptome and genes with epigenetic modifications in $CD14^+$ monocytes overlap meaning that transcriptomic changes in HSPCs are relayed to newly formed monocytes and neutrophils in the periphery.[227] One of the other mechanisms by which BCG-induced trained immunity is brought about is through changes in cellular metabolism. BCG shifts metabolism in trained monocytes primarily to glycolysis and inhibition of glycolysis but not oxidative phosphorylation dampens trained immunity responses like secretion of TNF-α and IL-6 in $CD14^+$ monocytes.[226] This shift toward glycolysis is due to epigenetic modifications in glycolytic pathway genes and their enhanced expression in BCG-stimulated monocytes.[226] Inhibition of glycolysis also abrogates BCG-induced epigenetic modifications in the promoters of IL-6 and TNF-α; thus, demonstrating that during BCG-induced training in monocytes metabolic changes and epigenetic reprogramming regulate each other.[226] One of the chief molecular mediators of BCG-induced training in monocytes is IL-1β. Exposure of purified monocytes to recombinant IL-1β alone in vitro can induce a training response similar to BCG.[211]

BCG-induced innate trained immunity has been implicated in protection against *Mtb*.[228] Thus, BCG-vaccinated TB case contacts who persistently test interferon-γ release assay (IGRA) negative (implying that they are uninfected at the time of the IGRA test despite persistent exposure) have higher levels of TNF-α, IL-6, IL-8, and IL-1β in response to heterologous stimuli such as *E coli* and *Streptococcus pneumoniae* providing evidence for BCG-induced trained immunity in these individuals, which may also contribute to effective *Mtb* control.[229] In a large longitudinal study tracking the efficacy of BCG revaccination in S. Africa through repeated IGRA testing to identify the proportion of *Mtb*-infected individuals in the vaccine compared to the non-vaccine arm highlighted reduced incidence of respiratory infections in the BCG-vaccinated subjects implying that BCG vaccination induced trained immunity mechanisms may also be relevant in protection against *Mtb* infection.[163] More direct experimental evidence for this comes from in vitro mycobacterial growth inhibition assays (MGIA) which show that BCG outgrowth is best controlled not only by individuals with recent *Mtb* exposure but also by BCG

vaccinees.[230] Indeed, the MGIA assay has been tested as a surrogate measure of BCG vaccine-induced protective immunity mediated in part by regulation of trained immunity through epigenetic modification of key innate cytokine gene expression in macrophages.[230] Therefore, the observation that BCG-mediated protective immunity includes the induction of trained immunity as demonstrated in the MGIA assay,[230] indirectly supports the notion that the heterologous protection against respiratory infections in infants following BCG vaccination at birth, may be due to trained immunity. The peak inhibition in MGIA was observed anywhere between 4 and 12 weeks postvaccination and was dependent on CXCL9, CXCL10, and CXCL11 producing $CD14^{dim}$ monocytes.[230]

6 | CONCLUSIONS AND FUTURE DIRECTIONS

A century of BCG use has shown us that while it is protective against TB especially disseminated and meningeal in children, its efficacy in preventing TB in adults is highly variable and dependent on several factors (summarized in Table 1). What should also be noted is that the BCG-induced immune response is still not very well understood and the immune correlates of TB protection is still not well determined. For example, we still do not have a clear picture of how BCG antigens are processed, presented and which ones are responsible for generating a protective immune response. However, recent work in this direction to determine BCG antigens presented by MHC-I and MHC-II pathways has provided insight which will be crucial for development of future anti-TB vaccines.[231] In an effort to move beyond BCG and as part of the WHO End TB strategy, multiple anti-TB vaccines have been developed and tested in the recent past. Notable among these are MVA85A, H4:IC31, H56:IC31, ID-93/GLA-SE, and M72AS01. MVA85A efficacy trials showed that it did not reduce the incidence of TB in infants.[88] H4:IC31 was found to be immunogenic and efficacious in reducing sustained QuantiFERON conversion (however, efficacy of BCG revaccination was found to be higher than H4:IC31) and phase 1b trial of H56:IC31 showed that it is safe and immunogenic in individuals previously vaccinated with BCG.[163,164] Phase 1 trial of ID-93/GLA-SE established that it is safe and immunogenic[232]; while a phase 2b controlled trial of M72AS01 showed that it was 54% efficacious in preventing active TB disease in $IGRA^+$ adults.[233] Therefore, significant progress is being made in finding an alternate TB vaccine. However, this does not undermine the significance of BCG. In fact, BCG continues to be relevant for the following four reasons. Firstly, because of its wide-ranging effects specially on innate immunity, it is well established that BCG reduces instances of child-mortality by about 38%,[209] reduces respiratory infections in children by about 73%,[163,206-208] and also reduces the risk of developing melanoma when given at birth.[234] Secondly, BCG can be used as therapy in bladder cancer, type-1 diabetes, and multiple sclerosis. BCG therapy in bladder cancer has been shown to reduce tumor progression.[235] Also, BCG by virtue of its ability to influence immune cell metabolism and epigenetic changes, reduces symptoms of autoimmunity in type-1 diabetes patients and multiple sclerosis.[128,129,131] Thirdly, as BCG has the capacity to boost non-specific immunity, it can serve as an adjuvant for other vaccines. This concept has been explored in the field of cancer vaccine development[236,237] and also TB.[238] BCG boosted with subunit candidate TB vaccine ID-93/GLA-SE protects better against aerosol *Mtb* challenge in mice.[238] Fourthly, till the time that there is alternative and effective TB vaccine, BCG revaccination might be a worthwhile strategy to counter TB especially in the light of encouraging data from recent BCG revaccination trials/studies.[61,114,163] Another possible benefit of revaccination could be the ability of BCG to induce trained immunity which enhances protection against not just *Mtb* but various heterologous insults/challenges in adult life including novel pathogens such as SARS-CoV-2 (Table 2). Interim results from the Phase III ACTIVATE trial to study whether BCG vaccination in the elderly protects against infection and improves survival like it does in children, show that BCG vaccination reduces the incidence of infection, predominantly respiratory, and also increases the time to first infection.[239] Indeed, BCG-induced trained immunity might prove to be very useful in combatting challenges posed by pathogens including novel ones such as SARS-CoV-2.[240] Analysis of SARS-CoV-2 infection data showed that countries with mandatory BCG vaccination had fewer cases and also significantly lesser deaths per million from COVID-19.[241,242] While it is possible that the incidence of COVID-19 might not be different in unvaccinated individuals or those vaccinated with BCG at birth or in the recent past,[243,244] it is certainly possible that BCG-induced trained immunity might dampen the severity of infections, prevent fatality as well as infection spread in a community.[240] To test this hypothesis, several trials have been initiated worldwide, an example of which is the multi-center BRACE trial which aims to determine whether BCG vaccination can protect health workers against COVID-19.[245] Even though there are currently no definitive data available with respect to effect of BCG on COVID-19, results from two trials investigating the link between BCG vaccination history and COVID-19 look promising.[244,246] In the first study, individuals vaccinated with BCG in the past five years in the Netherlands were studied for incidence of COVID-19 and its symptoms.[244] The incidence of COVID-19 did not significantly differ between the vaccinated and unvaccinated arms but fewer vaccinated individuals self-reported sickness and fatigue (a symptom of COVID-19) during the pandemic. The authors of this study emphasized that their data only demonstrate that BCG vaccination during the COVID-19 pandemic is safe and does not provide any evidence of protective effects of BCG for which much larger trials are needed.[244] In the second observational study in Los Angeles, anti-SARS-CoV-2 IgG seroprevalence and self-reported COVID-19 clinical symptoms were significantly less in healthcare workers with a history of BCG vaccination.[246] Importantly, such protective effects were not observed for pneumococcal, meningococcal, and influenza vaccination.[246] In contrast to results from the two trials described above, BCG vaccination did not confer any protection from SARS-CoV-2 infection and disease symptoms in the most vulnerable elderly population according to unpublished data from the

BCG-PRIME study.[247] These data have raised several questions, for example, does BCG vaccination/revaccination dampen COVID-19 disease severity, reduce duration of hospital stay, and whether its efficacy is associated with age. In summary, definitive data on the efficacy of BCG vaccination in the context of COVID-19 are awaited from the large ongoing clinical trials.

Despite its availability over the past 100 years, we are yet to fully understand BCG-induced immunity, both innate and adaptive. Decoding which immune responses elicited by BCG are protective against TB and how to boost them is an important objective for future research. Also, in light of its multiple non-specific benefits, studying BCG efficacy in preventing not just TB but other infections in future vaccination/revaccination trials in infants and adults will help in framing effective BCG vaccination policies in the days to come.

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CONFLICT OF INTEREST

All authors declare no competing or conflicting interests.

AUTHOR CONTRIBUTIONS

All authors listed have made substantial, direct and intellectual contribution to the work, and approved it for publication.

DATA AVAILABILITY STATEMENT

Data sharing not applicable to this article as no datasets were generated or analysed during the current study.

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