Research progress on specific and non-specific immune effects of BCG and the possibility of BCG protection against COVID-19

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Bacille Calmette-Guérin (BCG) is the only approved vaccine for tuberculosis (TB) prevention worldwide. BCG has an excellent protective effect on miliary tuberculosis and tuberculous meningitis in children or infants. Interestingly, a growing number of studies have shown that BCG vaccination can induce nonspecific and specific immunity to fight against other respiratory disease pathogens, including SARS-CoV-2. The continuous emergence of variants of SARS-CoV-2 makes the protective efficiency of COVID-19-specific vaccines an unprecedented challenge. Therefore, it has been hypothesized that BCG-induced trained immunity might protect against COVID-19 infection. This study comprehensively described BCG-induced nonspecific and specific immunity and the mechanism of trained immunity. In addition, this study also reviewed the research on BCG revaccination to prevent TB, the impact of BCG on other non-tuberculous diseases, and the clinical trials of BCG to prevent COVID-19 infection. These data will provide new evidence to confirm the hypotheses mentioned above.

KEYWORDS

Bacille Calmette-Guerin (BCG), COVID-19, specific and non-specific immunity, tuberculosis (TB), trained immunity

1 Introduction

Tuberculosis (TB) is still the most common cause of death for single hereditary pathogens. According to the Global Tuberculosis Report 2022 released by the World Health Organization (WHO), there were an estimated 10.6 million new TB cases and an estimated 1.6 million deaths in 2021 [1]. China remains one of the 30 high TB burden countries, with an estimated 842,000 new TB cases in 2020, ranking second and accounting for about 9% of new cases globally [2].

Bacille Calmette-Guérin (BCG), developed by Calmette and Guerin by attenuating the virulence of *Mycobacterium bovis* in 1908, is a century-old vaccine for TB prevention (3). BCG has an excellent protective effect on miliary tuberculosis and tuberculous meningitis in children or infants, but its protective efficacy in adults varies between 0% - 80% (4, 5). In 1995, the WHO did not recommend BCG revaccination because of the lack of sufficient scientific evidence on the protective efficiency induced by BCG revaccination, and the Chinese Health Commission also stopped the work related to BCG revaccination in adults in 1997 (6). Interestingly, a growing number of studies have demonstrated that BCG could induce a nonspecific memory immunity termed "trained immunity" in innate immune cells by activating higher frequencies of innate immune cells to secrete interleukin 1β (IL-1β), tumor necrosis factor α (TNF-α), and IL-6 cytokines (5, 7–12). Therefore, the hypothesis has been proposed that BCG-induced trained immunity can protect against infection with microorganisms other than *Mycobacterium tuberculosis* (M. tb) (13, 14).

The coronavirus disease 2019 (COVID-19) pandemic, caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2), has been raging worldwide for nearly three years. As of 13 January 2023, there were 661,545,258 confirmed cases of COVID-19 and 6,700,519 deaths worldwide (https://covid19.who.int/). More than ten COVID-19-specific vaccines have been approved for emergency use worldwide, such as Sinovac, Sinopharm BBIBP-CorV, Ad5-nCoV vaccine, ZF2001, Janssen's Ad26.COV2.S, Pfizer-BioNTech's BNT162b2, Moderna mRNA-1273, Gam-COVID-Vac (Sputnik V), EpiVacCorona vaccine, AstraZeneca's ChAdOx1 nCoV-19 (AZD1222), CureVac, and BBV152 vaccines (10, 12, 15–26). However, with the emergence of new SARS-CoV-2 variants of concern (VOC), the protective efficiency of these vaccines is facing unprecedented challenges (16). Previous studies have suggested that BCG might be a potential candidate to compensate for the shortcomings of COVID-19-specific vaccines (4, 10–12, 27–29).

In this study, we summarized specific and nonspecific immune responses induced by the BCG vaccine, the effect of BCG revaccination on the incidence of TB and other diseases, and the effects of BCG vaccination against COVID-19 infection.

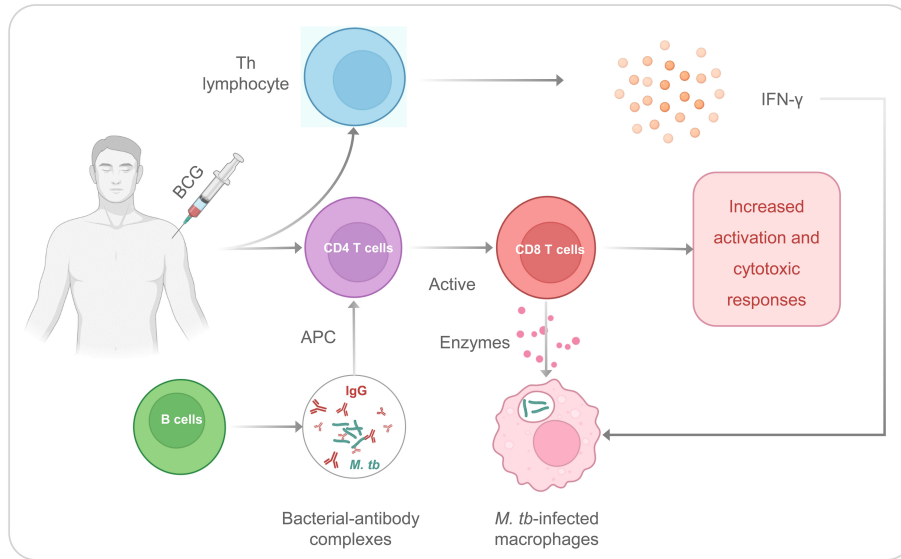# 2 Immune responses induced by the BCG

BCG is the only approved vaccine for preventing TB, and the target population is infants and newborns. BCG can reduce the risk of developing the disease by 50% and has a better preventive effect on severe tuberculosis (30). In addition, it can effectively prevent tuberculous meningitis and disseminated tuberculosis in infants. BCG is a live vaccine made from Mycobacterium bovine tuberculosis bacilli. After vaccination, it can activate T lymphocytes to produce specific immune responses (31). Since 1921, BCG has been used to prevent TB in humans, and since 1974, the WHO has included it in the Expanded Program on Immunization (EPI) (31). In recent years, research has found that the BCG vaccine can

stimulate our body to produce atopic immunity, mediate innate immunity, and provide non-specific immunity. Innate immunity is the initial barrier against infection by pathogenic microorganisms, which can produce nonspecific immune responses to different pathogens, such as chemotaxis, phagocytosis, and secreted cytokines. It has long been believed that innate immunity differs from adaptive immunity and does not have immune memory. However, more and more studies have shown that innate immune cells and their stem cells also have memory characteristics (32), indicating that the innate immune responses have been activated after receiving the first infection. Then it can produce a more robust immune responses after being infected again to provide a better protective effect in fighting against pathogens (32). After being activated by homologous or heterologous pathogens, the innate immune system can produce a more robust immune response, and this phenomenon is called "trained immunity" (33). It is worth noting that not all heterologous/homologous exposures lead to innate immune training, on the contrary, some exposures can induce the immunoparalysis of innate immune cells (34).

2.1 Specific immune responses induced by the BCG

Although BCG remains the world's only vaccine against TB, the duration of its preventive effect on TB is limited. Its efficacy in children with tuberculous meningitis and miliary tuberculosis is consistent, but its effectiveness in adults with pulmonary TB is variable (35). Possible reasons are as follows: 1) Genetic variation in the BCG strains, such as Denmark/Copenhagen strain 1331, Russian/Moscow, and Japanese/Tokyo 172; 2) The immune response to the BCG vaccine is weakened in the elderly due to immune aging; 3) Non-tuberculosis mycobacteria (NTM) infection masked the true effect of the BCG vaccine against M.tb; 4) Continuous stimulation of BCG can only induce more short-lived effector memory T cells and effector T cells (36–38).

It has been observed that BCG could induce specific or adaptive immunity to fight against infection. The T helper (Th) lymphocyte response and CD4/CD8 T cells are contributed to the adaptive immune response induced by the BCG (39, 40) (Figure 1). The Th cells can secrete interferon γ (IFN-γ), and the BCG immunization after birth induces the production of IFN-γ by γδ T cells. It is necessary for protective immunity from tuberculosis because this cytokine increases antimycobacterial activity in macrophages (41). In addition, CD4 and CD8 T cells would be considered primary mediators for long-term immunity for M. tb infection. CD8 T cells could identify mycobacterium-infected macrophages, and these macrophages are attacked by enzymes secreted by CD8 T cells, which directly protect against M. tb (40). After immunization with BCG, B cell activation in serum increases, and IgG against M. tb increases significantly (42, 43). These antibodies can also form bacterial-antibody complexes that can induce increased M. tb processing and antigen presentation by antigen-presenting cells (APCs) to CD4T cells, leading to increased CD8 T cell activation and cytotoxic responses to M. tb (43, 44).

FIGURE 1
The Th and CD4/CD8 T cells are contributed to the adaptive immune responses induced by the BCG.

2.2 Nonspecific immune responses induced by the BCG

BCG is a multifunctional vaccine with nonspecific or heterologous immunomodulatory effects, which can promote nonspecific immunomodulatory effects (45) (Figure 2). Our previous studies have indicated that mature myeloid cells, such as macrophages or monocytes, play an essential role in nonspecific immunity induced by the BCG vaccine against unrelated pathogens

(4, 12, 27). Although it has previously been shown that BCG-induced immunity is compartmentalized, trained innate immunity induced at a bone-marrow level in their respective hematopoietic stem cells, recent studies have indicated that BCG vaccination can also induce trained innate immunity in tissue-resident macrophages such as alveolar macrophages and peritoneal macrophages (46). BCG primary immunization induces more open chromatin structure in macrophages or monocytes, increases transcriptional active histone modifications such as H3K4m3 (histone H3 trimethylation at lysine
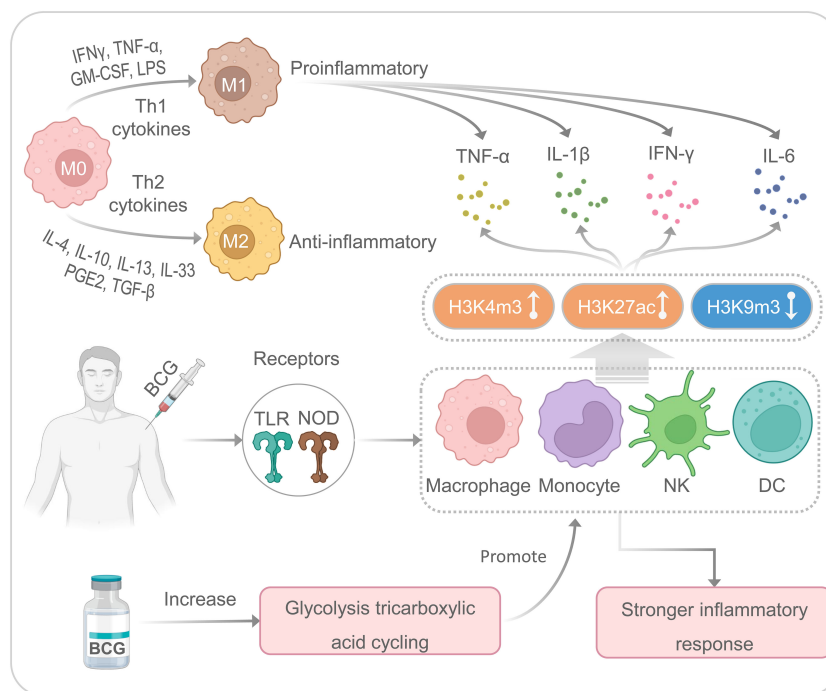
FIGURE 2
The nonspecific immune responses induced by the BCG. M, macrophages; NK, natural killer cells; DC, dendritic cells.

4) and H3K27ac (histone H3 acetylation at lysine 27), and decreases transcriptional inhibitory histone modifications such as H3K9m3 (histone H3 trimethylation at lysine 9), which in turn activates the production of IL-1β, TNF-α, and IL-6 (9, 12, 33, 47). Interestingly, when infected with an unrelated pathogen such as SARS-CoV-2, these trained nonspecific immune cells can induce a quicker and more substantial increase of H3K27ac and H3K4m3 and a decrease of H3K9m3, resulting in a higher level of IL-1β, TNFα, and IL-6 (33, 48). It also has been reported that BCG-mediated nonspecific immunity involves toll-like receptors (TLR) on the cell membrane surface and nucleotide-binding oligomerization domain (NOD) receptors located in the cytoplasm of some nonspecific immune cells, such as macrophages, monocytes, natural killer (NK) cells, and dendritic cells (DCs) (49, 50). The activation of TLRs and NOD receptors initiates the inflammatory cascade mainly through the secretion of TNF-α, IL-1β, and IL-6 (48, 51, 52). These cytokines aggregate inflammatory cells and provide signals to innate immune cells (53).

Moreover, enhanced glycolysis and glutamine-driven tricarboxylic acid cycling have been proven to be an essential metabolic pathway for trained immunity induced by BCG (54). There are two main types of macrophage polarization: classical polarization (M1) and alternative polarization (M2) (55). M1 macrophages are usually involved in eliminating pathogens and limiting tumor growth, while M2 macrophages are mainly involved in pathological processes such as anti-inflammatory response, tissue healing, fibrosis, and tumor survival (56). It has been suggested that lipopolysaccharide (LPS) and Th1 cytokines (IFN-γ, TNF-α, and GM-CSF) can stimulate M1-type polarization to produce pro-inflammatory cytokines (IL-1β, IL-6, IL-12, and IL-23), while Th2 cytokines, such as IL-4 and IL-13, can stimulate M2-type polarization to secrete anti-inflammatory cytokines (IL-10 and TGF-β) (57, 58). Interestingly, macrophages are highly plastic innate immune cells, and M1 and M2 macrophages can transform into each other based on different microenvironmental stimuli (59). In addition, M1 macrophages exert robust cytotoxic and antiproliferative effect activity by producing reactive oxygen species and nitric acid reactive substances (60, 61). However, recent studies have shown enhanced glycolysis and increased lactic acid production during M1 polarization (62). Several studies suggest that BCG can induce macrophages to secrete cytokines (such as IL-6, IL-12, and TNF-α) and a novel macrophage activated associated protein 1 (NMAAP1) (59, 63). Furthermore, BCG immunization can induce enhanced glucose consumption and lactate production *via* Akt/mTOR pathway (54).

3 Effect of BCG revaccination on the incidence of TB

Although the immune effects of BCG are not lifelong, BCG is still an important measure to control TB. The WHO recommends that BCG should be given as early as possible after the baby's birth in countries with an endemic and high incidence of TB. It has been suggested that BCG revaccination could induce a more robust trained immunity defense against TB or other diseases (64) (Table 1). A previous study assessed the effectiveness and cost-effectiveness of BCG revaccination among adolescents, and the results showed that revaccination reduced the likelihood of TB transmission, with the risk of infection falling from 5.7% per year to 4.8% per year (65). In addition, in cost-benefit evaluations, if the cost of revaccination per person is set at $1-10, the effectiveness of the vaccine is between 10% and 80%, the term of protection is ten years, and the cost of treatment for the vaccinated population to restore healthy life (disability-adjusted life year, DALY) per year is $116 to $9237. The intervention was about twice as cost-effective as preventing transmission, with recovery costs of US $52 to US $4,540 per DALY at 80% effectiveness and 17% of cases averted with revaccination. According to the survey, BCG revaccination is cost-effective compared to international benchmarks (65).

TABLE 1 The research of revaccinating BCG to protect against tuberculosis.

Authors (Countries)	Study Design	Results
Christopher Dye South Africa	9290 new cases observed in a cohort	(1) Reduce the likelihood of TB transmission (2) Risk of infection falling from 5.7% to 4.8% per year (3) Cost-effectiveness: about twice as cost-effective as preventing transmission
Rakshit S, Ahmed A, Adiga V, Sundararaj BK, Sahoo PN, Kenneth J, et al. India	Two hundred healthy adults, BCG vaccinated at birth, were tested for their IFN-γ release assay (IGRA) status. Of these, 28 IGRA+ and 30 IGRA– were BCG revaccinated, and 24 IGRA+ and 23 IGRA– subjects served as unvaccinated controls.	(1) IFN-γ and/or IL-2 Ag85A- and BCG-specific CD4+ and CD8+ T cell responses were boosted by revaccination at 4 and 34 weeks (2) CD4+ T cells expressing up to 8 cytokines were also significantly enhanced in both IGRA+ and IGRA- vaccinees relative to unvaccinated controls, most markedly in IGRA+ vaccinees. (3) Compared with control group, the innate IFN-γ+ NK/γδ/NKT cell responses were higher in both IGRA+ and IGRA- vaccinees. (4) the immunogenicity of BCG was not affected even in patients with LTBI
Nemes E, Geldenhuys H, Rozot V, et al. South Africa	Randomized, three-arm, placebo-controlled, partially-blinded clinical trial aimed to enroll 990 healthy, HIV-uninfected, QFT-negative, 12- to 17-year-old adolescents, BCG- vaccinated in infancy	An effectiveness of 45.4% for continuous QFT conversion by BCG reinoculation

Furthermore, BCG revaccination may benefit individuals with latent tuberculosis infection (LTBI). An Indian study showed that after BCG revaccination, the response of Ag85A and BCG-specific CD4^{+} and CD8^{+} T cells were significantly enhanced. Furthermore, the re-inoculated BCG was immunogenic in both positive and negative subjects with interferon release tests, suggesting that the immunogenicity of BCG was not affected even in patients with LTBI (66). It was also shown that Ag85A-, BCG-and LTG-specific CD4^{+} and CD8^{+} T cell responses were more enhanced in individuals with positive interferon release tests than in individuals with negative interferon release tests (66). Therefore, BCG revaccination may benefit people with LTBI who are more likely to develop the disease. Another randomized controlled trial conducted in South Africa employed the QuantiFERON-TB Gold In-tube test to assess the protective effects of BCG revaccination, and the result showed an effectiveness of 45.4% for continuous QFT conversion by BCG reinoculation (67).

BCG revaccination is linked to a trained innate immune mechanism. For example, a clinical trial conducted in South African adults with latent tuberculosis infection (LTBI) showed that BCG revaccination promoted BCG-specific CD4^{+}CD8^{+}γδ T cells and enhanced the responses of BCG-reactive CD3^{+}CD56^{+} NKT-like cells and CD3^{-}CD56^{dim}IFN-γ^{+} or CD3^{-}CD56^{hi} IFN-γ^{+} NK cells (68). Similarly, a phase 1b randomized study performed in South African adolescents observed the same outcome that BCG revaccination elicited robust, polyfunctional BCG-specific CD4^{+} T cells (69).

Although these studies suggest that BCG revaccination can protect against TB and LTBI, other studies have yielded different results. A randomized controlled trial assessed the effect of BCG revaccination on all-cause mortality in 46,889 individuals in Malawi, and the results showed that, with a 30-year follow-up, BCG revaccination did not have any beneficial effects on all-cause mortality (70). Therefore, whether BCG revaccination can bring benefits needs to be further studied and verified.

4 Effect of BCG vaccine on other diseases

4.1 Effect of BCG on respiratory diseases

The trained immunity of BCG vaccination showed a more robust and longer-lasting effect (9). These functions effectively prevent TB and protect against other infectious diseases, especially some respiratory diseases (9) (Table 2). Studies have demonstrated that BCG plays a protective role in fighting against respiratory syncytial virus (RSV) infection. RSV is the most common cause of lower respiratory diseases. It is the leading cause of hospitalization, morbidity, and mortality from respiratory infections, usually causing bronchiolitis and pneumonia in infants under six months, as well as rhinitis and colds in adults (71). RSV infections typically occur in 3% – 7% of the elderly, with respiratory symptoms more severe than influenza in this population (72, 73). Unfortunately, there is no licensed effective vaccine for RSV worldwide. Animal experiments have shown that a recombinant BCG vaccine (rBCG-

N-hRSV) has endowed mice with protective immunity and enhanced their ability to challenge infection (74). On day 28 after BCG immunization, it was found that the mice in the BCG immunized group lost less body weight, revealed less neutrophil infiltration, and showed a lower viral load in bronchoalveolar lavage fluid than those in the control group (74). In addition, activated banks of T cells that release IFN-γ and IL-1 were found in spleen T cells, suggesting that this recombinant BCG vaccine could induce a mixed CD8 and CD4 T cell response to inhibit the viral spread and prevent lung damage (74, 75). Therefore, this recombinant BCG vaccine (rBCG-N-hRSV) is a promising candidate for preventing RSV infection.

The influenza A virus epidemic that occurred in 2009 brought a huge disease burden on global health. A randomized, placebo-controlled pilot study conducted in 2015 showed that the combination of BCG and influenza vaccine effectively prevented influenza virus infection (76). In brief, the individuals vaccinated with the BCG vaccine had a significantly stronger response to H1 antibodies against the influenza A (H_1N_1) vaccine strain and had a more rapid seroconversion trend than those in the placebo group (76). Furthermore, the pro-inflammatory leukocyte response is enhanced after BCG vaccination, and a non-specific effect of influenza vaccines has also been observed, which can modulate cytokine responses to unrelated pathogens, suggesting that BCG vaccination before influenza vaccination may improve influenza vaccination efficiency (76, 77). Another animal experiment showed that the BCG vaccine could significantly enhance the efferocytosis effect of alveolar phagocytes (AP) (77). AP is crucial in maintaining lung health, and its funerary effect prevents pathogenic acute lung injury caused by lung infections. The efferocytosis effect of AP was significantly enhanced in mice immunized with the BCG vaccine, which increased the uptake and digestion of AP by apoptotic cells and initiated the rapid clearance of alveolar apoptotic cells (77).

Recently, a clinical trial was conducted on elderly people to evaluate the protective efficacy of BCG against respiratory infections (78). The primary endpoints of the study were time to first infection and incidence of new infections (respiratory infections caused by viruses, community-acquired pneumonia, hospital-acquired pneumonia, intra-abdominal infections, urinary tract infections, bloodstream infections, and acute bacterial infections of skin and skin structures). The results showed that the time to first infection in the BCG group was significantly delayed compared to the placebo group (16 weeks after BCG vaccination and 11 weeks in the placebo group). The incidence of new infections also showed a noticeable reduction, with 42.3% in the placebo group and 25.0% in the BCG group. The risk of new infections was reduced by 45% in the BCG group compared to the placebo group. Especially in preventing respiratory infections caused by viruses, participants in the BCG group had a 79% lower risk of viral pneumonia infection (78).

4.2 Effect of BCG on other infectious diseases

In addition to respiratory infectious diseases, BCG may also have a specific protective effect on other contagious diseases. For example,

TABLE 2 Effect of BCG on other diseases rather than TB.

Disease	Authors	Research	Result
Respiratory Syncytial Virus (RSV)	Céspedes PF, Rey-Jurado E, Espinoza JA, et al.	A single, low dose of a cGMP recombinant BCG vaccine elicits protective T cell immunity against the human respiratory syncytial virus infection and prevents lung pathology in mice	(1) Mice showed less weight loss and less infiltration of neutrophils and less viral load in bronchoalveolar lavage compared with the unimmunized controls. (2) Activated banks of T cells that release IFN-γ and IL-1 were found in spleen T cells. (3) This recombinant BCG vaccine (rBCG-N-hRSV) is a promising vaccine candidate for the prevention of RSV infection.
H_1N_1	Leentjens J, Kox M, Stokman R, et al.	BCG Vaccination Enhances the Immunogenicity of Subsequent Influenza Vaccination in Healthy Volunteers: A Randomized, Placebo-Controlled Pilot Study	(1) The combination of BCG vaccine and influenza vaccine was effective in preventing influenza virus infection. (2) Compared with the placebo group, the BCG-vaccinated trial group had a significantly stronger response to H1 antibodies to the influenza A(H1N1) vaccine strain and had a more rapid seroconversion trend.
	Mukherjee S, Subramaniam R, Chen H, Smith A, Keshava S, Shams H	Boosting efferocytosis in alveolar space using BCG vaccine to protect host against influenza pneumonia	(1) BCG vaccine could significantly enhance the efferocytosis effect of alveolar phagocytes (AP). (2) The efferocytosis effect of AP in mice after receiving BCG immunization was significantly enhanced. (3) All mice that received BCG injections were able to survive the deadly influenza A virus.
Bladder Cancer	Petar B, J WA, N GG	Old instillations and new implications for bladder cancer: the urinary microbiome and intravesical BCG	BCG immunotherapy has been the "gold standard" for the treatment of non-muscle invasive bladder cancer.
Leprosy	Glynn JR, Fielding K, Mzembe T, Sichali L, Banda L, McLean E, et al.	BCG revaccination in Malawi: 30-year follow-up of a large, randomized, double-blind, placebo-controlled trial	BCG vaccines were about 40% protected against leprosy in 30 years.
HPV	(1) Salem A, Nofal A, Hosny D (2) Podder I, Bhattacharya S, Mishra V, Sarkar TK, Chandra S, Sil A, et al.	(1) Treatment of Common and Plane Warts in Children with Topical Viable Bacillus Calmette-Guerin (2) Immunotherapy in viral warts with intradermal Bacillus Calmette-Guerin vaccine versus intradermal tuberculin purified protein derivative: A double-blind, randomized controlled trial comparing effectiveness and safety in a tertiary care center in Eastern India	(1) 65% of children with common warts were effectively treated with BCG as compared to the placebo group. (2) BCG vaccinations were given at 4 weeks intervals, and after treatment, the complete clearance of viral warts was 48.5%.
Type 1 Diabetes	(1) Keefe RC, Takahashi H, Tran L, Nelson K, Ng N, Kühtreiber WM, et al. (2) Kühtreiber WM, Faustman DL	(1) BCG therapy is associated with long-term, durable induction of Treg signature genes by epigenetic modulation (2) BCG Therapy for Type 1 Diabetes: Restoration of Balanced Immunity and Metabolism	Patients with type 1 diabetes who received at least 2 BCG vaccinations returned to normal blood glucose levels after about 3 years, and therapeutic effects were observed even in patients with advanced diabetes who had been ill for more than 20 years, and the therapeutic effect lasted for more than 5 years.
Atherosclerosis	van Dam AD, Bekkering S, Crasborn M, van Beek L, van den Berg SM, Vrieling F, et al.	BCG lowers plasma cholesterol levels and delays atherosclerotic lesion progression in mice	BCG reduced plasma total cholesterol levels in mice in the experimental group, reduced foam cell formation in peritoneal macrophages, delayed the progression of atherosclerotic lesions at the root of the aortic, and reduced the severity of lesions.

it was reported that BCG has a positive therapeutic effect on skin and genital warts caused by the human papillomavirus (HPV). A comparative study performed in Egypt showed that 65% of children with common warts were effectively treated with BCG compared to the placebo group (79). In a similar clinical trial conducted in India, three doses of BCG vaccinations were given at four weeks intervals, and after treatment, the complete clearance of viral warts was 48.5% (80). At the same time, some animal experiments have confirmed that the BCG vaccine can also resist various DNA and RNA virus infections (81). One study showed that children who received BCG immunization at birth had lower mortality rates than children who delayed BCG vaccination, which can be attributed to the BCG vaccine's prevention of neonatal sepsis, respiratory infections, and fever (82, 83).

4.3 Effect of BCG on noninfectious diseases

Besides infectious diseases, BCG has also shown a protective effect against noninfectious diseases, such as non-muscle-invasive bladder cancer (NMIBC), type 1 diabetes mellitus (T1DM), and atherosclerosis (Table 2). Since the 1970s, BCG immunotherapy has been the "gold standard" for treating NMIBC (84). A randomized, double-blind, randomized controlled clinical trial showed that the protection rate of BCG against leprosy was approximately 40% over 30 years (85). In addition, an observational study evaluated adverse events in patients with NMIBC receiving adjuvant treatment with BCG, mitomycin C (MMC), and chemohyperthermia (86). The results demonstrated no significant clinical differences among BCG, MMC, and chemohyperthermia in patient quality of life and side

effects. In contrast, another study found a rapid and marked reduction in bladder volume in one patient after BCG instillation (87). However, a study compared the efficacy of chemotherapeutic drugs and BCG's efficacy in treating bladder cancer in mice and found that cyclophosphamide significantly reduced tumor volume in mice compared with the control group, but the BCG vaccine did not enhance the anti-tumor effect of cyclophosphamide, and BCG treatment alone promoted tumor growth (88). These results indicate that although BCG has been used to treat NMIBC for decades, its therapeutic effect is still controversial, and a more in-depth understanding of its mechanism is needed (89).

Moreover, infection with M. tb is accompanied by increased circulating Treg cells (90). BCG can be used to treat autoimmune type 1 diabetes mellitus (T1DM) due to its ability to induce immunosuppressive T cells (Tregs) to restore the immune balance, and this effect can last for 2-3 years (91). In a Phase I clinical trial, patients with T1DM who received at least two doses of the BCG vaccine could return to normal glucose levels after three years, and similar treatment effects were observed even in patients with advanced diabetes for more than 20 years (92). Interestingly, the therapeutic effect of BCG can last for more than five years (92). In addition, one study found that postnatal BCG vaccination was protective in females with T1DM but not in those who did not receive BCG (93). The underlying mechanisms of BCG vaccination on T1DM may be: 1) BCG vaccination up-regulates Myc, activates nearly 24 Myc target genes under four metabolic pathways, and finally improves glucose metabolism in T1DM patients (94); 2) BCG vaccination induces an increase in the methylation levels of H3K4me3 (Histone 3 Lysine 4) and H3K36me3 (Histone 3 Lysine 36me2), which activates cytokines (TNF, IL-6, and TLR4) related to BCG therapy in T1DM patients (95). These findings suggest that BCG treatment of T1DM improves glycemic control by altering metabolism and persistence. However, a systematic review and meta-analysis revealed that although there was a trend toward improvement in glycated hemoglobin levels after BCG treatment, there was still a lack of strong evidence to support BCG's efficacy in treating T1DM (96).

Besides, BCG can regulate the progression of atherosclerosis. For example, one study evaluated the effect of subcutaneous BCG vaccination on the development of atherosclerosis in ApoE $^{-/-}$ mice. It was found that BCG vaccination reduced plaque number and macrophage content but increased lipid content in a mouse model of atherosclerosis (97). Similarly, in another animal study, the treatment of BCG killed by extended freeze-drying significantly reduced the size of atherosclerotic lesions, upregulated IL-10 production, and downregulated the expression of pro-inflammatory cytokines (IL-6, IL-13, and TNF-α) in mice (98). Furthermore, BCG can reduce plasma total cholesterol levels in mice in the experimental group, reduce foam cell formation in peritoneal macrophages, delay the progression of atherosclerotic lesions at the root of the aortic, and decrease the severity of lesions (99).

5 The effect of BCG on coronavirus infections

5.1 Amino acid sequence similarity between HSP65 protein of BCG strain and spike or nuclear protein of SARS-CoV-2

In the past 20 years, coronavirus (CoV) has triggered three major epidemic outbreaks worldwide, including severe acute respiratory syndrome (SARS), Middle East respiratory syndrome (MERS), and coronavirus disease 2019 (COVID-19) (100). On January 30, 2020, the WHO declared the severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) outbreak as a public health emergency of international concern. On February 11, 2020, the WHO officially named the pandemic as COVID-19 (101). SARS-CoV-2 is a new type of β coronavirus with the characteristics typical of the coronavirus family (102). Although SARS-CoV-2 infection does not cause as high a fatality rate as SARS-CoV or MERS-CoV, it is more transmissible (100). HSP 65, a major immunogenic component in BCG, is responsible for inducing efficient antigen-specific cell activation (103). A previous study comprehensively compared and analyzed the sequence similarity between the HSP65 protein of BCG strain and the spike and nuclear proteins of SARS-CoV-2, and the results show that the HSP65 protein sequence has a high similarity with the sequence of the spike and nuclear proteins of SARS-CoV-2 (104). This fundamental similarity suggests that BCG can induce cross-immune responses with SARS-CoV-2 antigens to reduce the susceptibility and severity of SARS-CoV-2 infection.

Additionally, studies have shown that BCG contains amino acid sequences similar to SARS-CoV-2 and has moderate to a high binding affinity for multiple common the human leukocyte antigen (HLA), suggesting that BCG vaccination can produce cross-reactive T cells against SARS-CoV-2 (105). HLA class I molecules typically bind peptides (mainly nine amino acids) in the length of 8-11 amino acids. Therefore, one study utilized two computer algorithms to analyze similar 9-amino acid sequences between SARS-CoV-2 and Mycobacterium bovis. The results showed that there were six distinct but closely related groups of peptides between SARS-CoV-2 and *Mycobacterium bovis*, and *M. bovis* contained many seven amino acid sequences that were identical to SARS-CoV-2 (106). Two computer algorithms analyzed the amino acid sequences of SARS-CoV-2 and *M. bovis*, and analysis using IEDB showed that six of the seven distinct but closely related peptides between SARS-CoV-2 and Mycobacterium bovis had moderate to a high binding affinity for multiple common HLA class I molecules. Analysis using NetMHCpan 4.1 showed that four groups of these similar peptides had weak to high binding affinities for common HLA class I molecules. HLA-binding affinity analysis in the computer showed that these very similar 9-mer peptides could be T-cell epitopes. BCG's similar amino acid sequence to SARS-CoV-2 has the potential to induce cross-reactive T cells against SARS-CoV-2.

5.2 The potential protective role of BCG in fighting against COVID-19

5.2.1 COVID-19 Vaccine and its challenges

As of 29 November 2022, a total of 13,042,112,489 COVID-19 vaccine doses have been administered (https://covid19.who.int/). Currently, 199 COVID-19-specific vaccines are evaluated in animal models, and 175 COVID-19-specific vaccines are assessed in clinical trials (https://www.who.int/publications/m/item/draft-landscape-of-covid-19-candidate-vaccines). COVID-19 vaccines approved for emergency use can be grouped into four categories: inactivated vaccines, subunit vaccines, mRNA vaccines, and viral vector-based vaccines. A meta-analysis found that the overall effectiveness of inactivated vaccine, subunit vaccine, mRNA vaccine, and viral vector vaccine against SARS-CoV-2 was 73.11%, 89.33%, 94.29%, and 79.56%, respectively (107). Although these COVID-19 vaccines have shown excellent performance in protecting individuals from SARS-CoV-2 infection, there are concerns about their adverse effects in the population. A previous study has indicated that joint pain, muscle pain, and fever were common adverse events, about 50% of people experience these conditions after receiving the COVID-19 vaccine (107). Other common complications are headaches, chills, and fatigue (22, 108–112). In addition to these common adverse events, rare and severe adverse events have also been reported. For example, myocarditis or pericarditis and thromboembolism cases were reported in people who received viral vector and mRNA vaccines, and such adverse events might cause death (113–116).

In addition, acceptance is also one of the essential factors affecting the global distribution of COVID-19 vaccines. As early as 2021, Jeffrey V Lazarus and colleagues conducted a global survey of potential acceptance of a COVID-19 vaccine among 13,426 people in 19 countries (117). It was found that 71.5% of the participants were willing to receive COVID-19 vaccines, but up to 48.1% of the participants had concerns about the safety of COVID-19 vaccines (117). It is worrisome that COVID-19 vaccine acceptance is not only low among the general population but also among healthcare workers (HCWs). A systematic review and meta-analysis involving 23,739 African HCWs found that COVID-19 vaccine acceptance among African HCWs was only 56.59 (95%CI; 46.26-66.92; I2 = 99.6%, p = 0.000) (118).

The low COVID-19 vaccine acceptance reflects people's hesitations and refusal to receive COVID-19 vaccines (119). As mentioned above, most of the new COVID-19 vaccines are still in preclinical studies or clinical trials, and the evidence of their effect and complications is unclear. According to a survey, one of the clearest reasons for refusing to be vaccinated is that a vaccine developed in a hurry is considered dangerous, and its efficacy and safety have not been confirmed (120). In addition, people with a medical background are generally expected to be more receptive to COVID-19 vaccines, but a European study suggests contrary results, showing no significant differences in COVID-19 vaccine acceptance between HCWs and non-HCWs (121). Unlike vaccine hesitance faced by COVID-19-specific vaccines, BCG has been used to prevent tuberculosis for more than 100 years, and its safety is widely recognized. Once BCG has been shown to be useful for COVID-19 prevention, it could be adopted by many more people.

5.2.2 The potential of BCG to prevent COVID-19

Based on BCG-induced trained immunity and the advantages of BCG mentioned above, a hypothesis has been proposed that BCG vaccination may reduce the morbidity and mortality of patients with COVID-19. Previous studies have shown that in countries without a universal BCG vaccination, such as Italy, the Netherlands, and the United States, populations were more likely to be infected by COVID-19 than in countries with universal and long-term BCG vaccination and countries that introduced BCG vaccine late to young children, such as Iran, have reported higher death rates from COVID-19 (122). Furthermore, it has been reported that BCG vaccination may reduce viremia after SARS-CoV-2 exposure, decrease the severity of COVID-19, and recover more quickly (123). Interestingly, BCG is a suitable vector for expressing the SARS-CoV-2 antigens. Furthermore, the trained immunity induced by the recombinant BCG vaccine and the SARS-CoV-2 specific immune response could cause a robust protective effect against COVID-19 (124). A study analyzed the association between BCG vaccination coverage and the incidence and mortality of COVID-19 (11). This study divided the countries into three groups according to their BCG coverage (coverage ≥90%, BCG has been recommended but coverage <90%, and BCG has never been introduced). The result showed that countries with higher BCG coverage showed a significantly lower incidence of COVID-19, and the mortality was much lower in countries with BCG coverage ≥90% (11). However, it is essential to note that these data are based on epidemiological and statistical analyses, and can be confounded by many factors, such as race, region, medical and economic level, weather changes, and different stages of COVID-19 (13). Therefore, a study that collected data in 171 countries and adjusted socioeconomic and weather changes showed a 30-fold reduction in COVID-19 mortality in countries with universal BCG vaccination compared to countries without universal BCG vaccination (125).

5.2.3 Landscape of clinical trials evaluating BCG vaccination against COVID-19

In recent years, a growing number of clinical trials have been conducted to evaluate the BCG vaccine's effectiveness in preventing COVID-19 (Table 3). According to the data on ClinicalTrials.gov (https://clinicaltrials.gov/), 31 clinical trials have been conducted to investigate the immune effects of the BCG vaccine on COVID-19. We found that these clinical trials mainly focused on HCWs and older adults (≥60 years old) at high risk of COVID-19. Although some evidence has suggested that the BCG vaccine can beneficially influence the incidence and severity of COVID-19, the study of the long-term effects of BCG on COVID-19 are insufficient. A clinical trial, a 5-year cohort study, in older adults was conducted by Radbond University Medical Center to explore the long-term effects of BCG for infectious and inflammatory diseases, including COVID-19 (NCT05387655). Additionally, as mentioned before, the BCG vaccine has non-specific effects on the immune system, and WHO did not recommend BCG revaccination. Therefore, a clinical trial (NCT04347876) aimed to demonstrate how the previous BCG vaccination could protect people against infection with COVID-19, which mainly focused on altering the prognosis of COVID-19. Similarly, another clinical trial (NCT04369794) was developed to evaluate the impact of previous BCG vaccination and BCG

TABLE 3 Clinical Trials of BCG fighting against COVID-19.

Trial ID	Countries	Status	Sample size	Intervention	Primary outcome
NCT05387655	Netherlands	Enrolling by invitation	500 older people	Case Control, BCG and Placebo	Incidence of infectious and inflammatory disease
NCT04347876	Egypt	Unknown	100 participants 12–80 years old	Case Control (Observational)	Pneumonia severity index and need for ICU admission
NCT04648800	Poland	Recruiting Phase 3	1000 participants, 25 years and older	Interventional clinical trial, BCG-10 vaccine and 0.9% saline	Death and life- or health-threatening condition
NCT04369794	Brazil	Active, not recruiting Phase 4	400 participants	Dose of 0.1 mL of BCG (2 and 8 x 1.000.000 CFU) and 0.1 ml of 0.9% NaCl saline solution	1. Clinical evolution of COVID-19, classified as mild, moderate and severe 2. SARS-CoV-2 elimination, virus detection by PCR 3.Seroconversion rate and titration, titration of anti SARS-CoV-2 IgA, IgM and IgG
NCT04659941	Brazil	Active, not recruiting Phase 2	753 participants	Dose of 0.1 mL of BCG and 0.1 ml of 0.9% NaCl saline solution	1. Compare the cumulative incidence of SARS-CoV-2 infection. 2. Compare the cumulative incidence of severe forms of COVID-19. 3. Assess the BCG vaccine-mediated immune response in health care workers.
NCT04379336	South Africa	Complete Phase 3	1000 adults	BCG and 0.9% Sodium Chloride	Incidence of HCWs hospitalized due to COVID-19 per arm
NCT04641858	Denmark	Active, not recruiting Phase 4	668 adults	BCG-Denmark and 0.9% Sodium Chloride	Days of unplanned absenteeism due to illness, unplanned absenteeism is defined by being absent from work due to causes other than holidays, parental leave, and other planned leaves, family assistance (including mourning leave) and quarantine measures.
NCT04327206	Australia	Complete Phase 3	6828 adults	BCG (Danish strain 1331) and 0.9% Sodium Chloride	Symptomatic COVID-19 by 6 months Severe COVID-19 incidence over 6 months
NCT04461379	Mexico	Active, not recruiting Phase 3	908 adults	BCG (Tokio 172 strain) and 0.9% Sodium Chloride	1. Demonstrate COVID- 19 disease incidence among Health care workers 2. Demonstrate cumulative incidence of hospitalization for COVID-19 among Health care workers 3. Demonstrate the Incidence of specific Antibodies against SARS-CoV-2 at 3 and 6 months in health care workers 4. Hospitalization of severe disease COVID-19 5. Oxygen supplementation in severe disease COVID-19 6. Need for intubation or non-invasive ventilation for the patient 7. Critical care admission with SARS-CoV-2 8. Mortality associated to progressive pulmonary disease
NCT04373291	Denmark	Complete Phase 3	1293 adults	BCG-Denmark and 0.9% Sodium Chloride	Number of days of unplanned absenteeism for any reason
NCT04648800	Poland	Recruiting Phase 3	1000 adults	BCG-10 vaccine and 0.9% Sodium Chloride	Death and life- or health-threatening condition
NCT04537663	Netherlands	Recruiting Phase 4	5200 Elderly	BCG (Danish strain) and 0.9% Sodium Chloride	The trial has an adaptive primary endpoint. Based on predefined objective and quantitative criteria the primary endpoint will be either a clinically relevant respiratory tract infection, or COVID-19
NCT04384614	Tunisia	Withdraw	N/A	Cross-sectional	1. Differences related to epidemiological demographic characteristics 2. Biospecimen Retention: Samples With DNA
NCT04475302	India	Complete Phase 3	2175 Elderly	BCG	Mortality due to COVID-19 disease
NCT04384549	France	Unknown Phase 3	1120 participants	BCG vaccine (AJ Vaccine) and 0.9% Sodium Chloride	Incidence of documented COVID-19 among health care workers exposed to SARS- CoV-2 and vaccinated with BCG compared to placebo.
NCT04542330	Denmark	Active, not recruiting Phase 3	1700 Elderly (65-110 years old)	BCG-Denmark and 0.9% Sodium Chloride	Acute infection, acute infection identified either by a doctor, antibiotics use, hospitalization or death due to infection.

(Continued)

TABLE 3   Continued

Trial ID	Countries	Status	Sample size	Intervention	Primary outcome
NCT04414267	Greece	Complete Phase 4	301 Participants (50 Years and older)	BCG and 0.9% Sodium Chloride	Positive for the respiratory questionnaire consisted of questions concerning the appearance of symptoms possibly, probably and/or definitively related to COVID-19 on visit 3
NCT04439045	Canada	Completed Phase 3	122 participants	VPM1002 is a recombinant BCG (rBCG) and 0.9% Sodium Chloride	COVID-19 infection, to compare the self-reported incidence of SARS-CoV-2 infection (confirmed by positive test) following vaccination with either VPM1002 or placebo.
NCT04348370	United States	Active, not recruiting Phase 4	1800 Participants (18 Years to 75 Years)	BCG Vaccine And Placebo Vaccine	Incidence of COVID 19 Infection
NCT02081326	United States	Active, not recruiting Phase 2	150 Participants (18 Years to 65 Years)	BCG and 0.9% Sodium Chloride	1. Number of Type 1 Diabetics with COVID-19 symptomatic infections 2. Impact of COVID-19 (severity, duration of symptoms, absence from work) 3. Reported Rates of Infectious Diseases
NCT04826718	Cape Verde	Enrolling by invitation	400 Participants (18 Years and older)	Observational Questionaire Capillary blood collection Collection of peripheral venous blood	1. Total number of days absent from work due to COVID-19 2. Unplanned Absenteeism 3. Symptomatology after infection by SARS-CoV-2 4. Presence or absence of anti-SARS-CoV-2 Acs 5. Duration of anti-SARS-CoV-2 Acs
NCT04387409	Germany	Active, not recruiting Phase 3	59 Participants (18 Years and older)	VPM1002 is a recombinant BCG (rBCG) and 0.9% Sodium Chloride	Number of days absent from work due to respiratory disease
NCT04435379	Germany	Completed Phase 3	2038 Participants	VPM1002 is a recombinant BCG (rBCG) and 0.9% Sodium	Number of days with severe respiratory disease at hospital and/or at home

revaccination on SARS-CoV-2 at different phases and disease phenotypes. These clinical trials focused on assessing the effect of BCG on reducing the incidence and severity of COVID-19. In contrast, an early Phase 1 clinical trial (NCT02403505) was conducted to evaluate COVID-19 Antigen Presentation Therapeutic Biological Product Mis for treating multiple gene mutation COVID-19 virus strains and activating human COVID-10 antigen presentation reaction. This clinical trial will recruit 20 participants with COVID-19, and the intervention was Ad26 COVID-19 Spike 1.0mL plus TICE®BCG Organism 50mg.

However, studies have also shown that BCG vaccination does not significantly protect against COVID-19. In a multicenter, randomized, double-blind, placebo-controlled phase III clinical trial in Poland in which investigators revaccinated HCWs with BCG, the results showed no significant difference in the incidence of COVID-19 between placebo and BCG groups (126). Results from another double-blind, randomized, controlled, phase 3 trial of HCWs in South Africa showed that vaccinating HCWs with BCG did not reduce the risk of COVID-19 and hospitalization for severe COVID-19 (127). In terms of the reasons contrary to other research findings, firstly, the success of BCG vaccination has not been verified. It can be seen that these two studies did not clarify whether TST-negative participants turned positive after receiving BCG, so this does not determine the protective efficacy of BCG. In addition, LTBI should receive significant concern. An animal study showed that Mycobacterium tuberculosis infection inhibited the trained immune processes in the bone marrow. Therefore, LTBI may have negatively influenced the non-specific role of BCG (128). However, these two studies did not address the confounding factor of LTBI.

6 Conclusions

The century-old BCG vaccine remains the only vaccine approved for TB prevention. BCG can induce trained immunity against the invasion of pathogens other than *Mycobacterium tuberculosis*, such as respiratory viruses. Based on this theory, it is speculated that BCG may potentially prevent COVID-19 morbidity and mortality. To verify this hypothesis, scientists worldwide have carried out a large number of epidemiological studies and statistical analyses, but the results of these studies are highly heterogeneous due to the interference of a variety of confounding factors. Therefore, more than 30 clinical trials have been conducted to evaluate the immune effect of BCG on COVID-19, and some clinical trials are exploring the long-term immune effects of BCG and the clinical benefits of COVID-19 antigen presentation therapeutic biological product mixed BCG on COVID-19. The protective effect of BCG on newborns and children is obvious, but the immune effect of BCG is not lifelong, and it is a question worth discussing how to produce immune effects on adults, especially adults without antibodies.

Author contributions

Conceptualization: JD and WG. Methodology: JD, YS, RW, ED, YC, WZ, and WG. Data Analysis: JD and YS. Software: YS and WG. Writing Original Manuscript: JD and YS. Review and revising manuscript: JD and WG. Funding Acquisition: JD and WG. All authors reviewed and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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