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Tuberculosis vaccine BCG: the magical effect of the old vaccine in the fight against the COVID-19 pandemic

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ABSTRACT

Bacillus Calmette-Guérin (BCG) is a live attenuated *M. bovis* vaccine that was developed about 100 years ago by Albert Calmette and Camille Guérin. Many countries have been using the vaccine for decades against tuberculosis (TB). The World Health Organization (WHO) recommends a single dose of BCG for infants in TB endemic as well as leprosy high risk countries, and globally almost 130 million infants are vaccinated yearly. The role of BCG is well known in reducing neonatal and childhood death rates. Epidemiological and retrospective cross-sectional studies demonstrated that the BCG vaccination protects the children against respiratory tract infections and lowers the risk of malaria in children. In addition, BCG enhances IFN-γ and IL-10 levels, thus providing immunity against respiratory tract infection even in elderly people. The BCG is also known to provide nonspecific innate immunity against viruses and parasites, through an innate immune mechanism termed 'trained immunity' and is defined as the immunological recall of the innate immune system by epigenetic reprogramming. Based on these studies it is suggested that the BCG has the potential to act as a protective agent against COVID-19. Further proven safety records of BCG in humans, its adjuvant activity and low-cost manufacturing make it an attractive option to stop the pandemic and reduce the COVID-19 related mortality. In this review we discuss the heterologous effects of BCG, induction of trained immunity and its implication in development of a potential vaccine against COVID-19 pandemic.

Introduction

Severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) is a novel betacoronavirus that causes coronavirus disease 2019 (COVID-19), the respiratory illness responsible for the COVID-19 pandemic [1]. Currently, the COVID-19 is affecting 218 countries and territories around the world [2], and it has already infected more than 73 million people globally, with more than 1,835 million deaths as of January 2, 2021 [2]. Worldwide the available data shows a huge difference in both incidence and mortality rates due to SARS-CoV-2 infection [2]. The reasons for these differences are certainly multifactorial, and the accurate analysis of the data needs more time to understand the reasons completely.

It is believed that the childhood vaccination programs each country follows may influence the immunological status in such a way that it can modify the COVID-19 infection and death rates. Several studies have shown an association between national Bacillus Calmette–Guérin (BCG) immunization programs and a lower rate of COVID-19 infection and the related mortality, suggesting that BCG may provide protection against COVID-19. For individuals previously vaccinated, revaccination is considered safe, well-tolerated and not associated with an increased frequency or severity of local or systemic reactions compared with the primary BCG vaccination [3–7]. Interestingly, the nonspecific effects of BCG vaccination improved the effects of low-efficiency vaccines, such as the vaccine against typhus—caused by Salmonella typhi (www.clinicaltrials.gov, number NCT02175420) or the influenza vaccine. Therefore, we hypothesize that induction of trained immunity in general, and specifically by BCG vaccination, might be a potent preventive measure against SARS-CoV-2 infection and/or might

reduce disease severity. The World Health Organization (WHO) recognized the beneficial 'off-target' effects of BCG and has called for further investigation to repurpose the BCG vaccine for other life-threatening diseases [8]. Indeed, there are multiple clinical trials testing BCG for 216 conditions other than TB, including 19 studies for COVID-19 [9].

The BCG is a live attenuated strain of *Mycobacterium bovis (M. bovis)* isolated from a cow that was developed at the Pasteur Institute in Paris by Albert Calmette and Camille Guérin [10]. The vaccine development started in 1908, and the first trial in human was carried out in 1921. The BCG is a widely used vaccine to eradicate TB and was among the most broadly used vaccinations in the 20th century in neonatal and young children [11]. Three billion doses have been administered since it was developed with a remarkable long-standing safety record [12]. The strains of *M. bovis* of BCG vaccine currently used in different countries are generated by a number of passages from actual Paris strain [10].

The BCG strains that are associated with lower COVID-19 mortality, such as BCG Japan and BCG Russia, belong to the category of early strains. The late strain, the BCG Denmark, evolved from the early strain through genetic mutations [13] and appeared to be less protective against COVID-19. It has been reported that the mutations in the late strains caused them to lose expression of membrane proteins, such as MPB64, MPB70, and MPB83, in addition to the absence of cell wall-associated lipids including methoxy mycolate [14,15]. Interestingly, the BCG strains of Japan and Russia have very high bacterial count compared to the late strains and may have the bacterial components that can induce immune responses that are required for the developing trained immunity in the host [16]. Studies showed that the BCG Japan induces the production of multiple types of inflammatory cytokines more efficiently than BCG Denmark [17,18]. These results suggest a possibility that the BCG Japan strain not only induces immunity against *Mycobacterium tuberculosis* (Mtb) but also against unrelated pathogens (Figure 1).

Therefore, vaccination with BCG prepared from the original strain may have a protective role against COVID-19 in low-TB-burden countries. In the absence of a specific treatment and vaccine for COVID-19, even a small protective effect of a cheap and safe vaccine such as BCG could be a valuable tool for fighting the disease.

The protective efficiency of BCG in bacterial, viral, and other diseases

The BCG has been effectively used to protect non-specifically against viral and bacterial infections and some cancers, probably by enhancement of cell-mediated immunity. Many studies have shown that the BCG provides protection against pathogenic
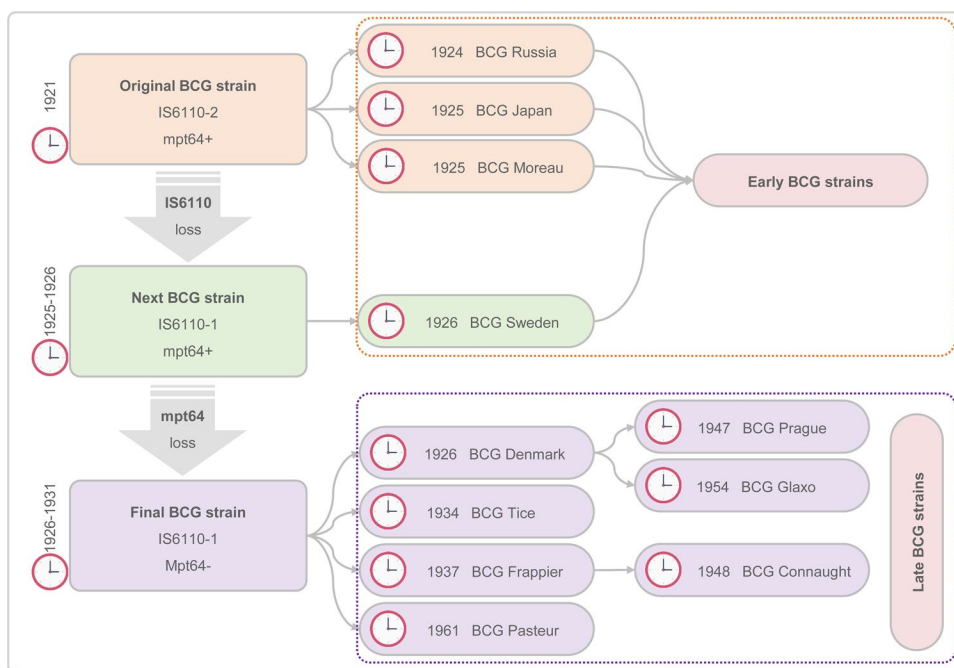

Figure 1. Evolutionary history and genetic makeup of BCG vaccine strains. The strains are based on the IS6110 and mpt64 typing. The BCG vaccine strains were produced in 1921 at the Pasteur Institute and the strains were distributed globally and used subsequently for vaccine. Figure modified from Behr and Small and Miyasaka [13, 16].

Table 1. Nonspecific effect of BCG on infectious and noninfectious diseases in humans.

Targets of human studies	Effect of BCG vaccination	References
Experimental yellow fever	Protection against experimental infection	[19]
Human papillomavirus (HPV)	Improved clearance of viral warts	[20, 21, 22]
Respiratory syncytial virus (RSV)	Fewer RSV infections in children. Lower risk of suspected ALRI in children	[23, 24]
Influenza A virus (H1N1)	Induction of antibody response against influenza	[25]
Herpes simplex virus (HSV)	Reduced episodes of clinical HSV infection	[26, 27]
Hepatitis B vaccine (HBV)	Increased cytokine production	[28]
Melanoma	Contributes to antitumor immunity	[29]
Bladder cancer	Contributes to antitumor immunity	[30]
Severe oral aphthosis	Therapeutic and prophylactic agent	[31, 32]
S. pneumoniae or virus infection	Reduces pneumonia risk	[33]
Salmonella typhi	Improves the efficiency of the vaccine	[34]

organisms and other diseases in humans (Table 1). In two randomized clinical trials in Guinea-Bissau, children vaccinated with the BCG showed reduced mortality that is attributed to the prevention from neonatal sepsis, respiratory infections, and fever [35,36]. In a community-based case-control study in Guinea-Bissau, the BCG vaccination showed a non-targeted protective effect against lower respiratory tract infection (ALRI) caused by a respiratory syncytial virus (RSV) [23]. In addition, a similar protective effect of BCG on acute upper respiratory tract infection (AURTI) was observed in 60-75-years-old people who were vaccinated with the BCG once a month for three months, and they also showed a significant increase of IFN-γ level compared to the placebo group [37]. In a clinical trial in Japan involving elderly people who were tuberculin-negative, the BCG vaccine reduced the risk of pneumonia, suggesting that the vaccination might be an effective strategy for the prevention of pneumonia of the elderly [33]. A recent study also showed a threefold reduction in respiratory tract infections (RTIs) in adolescents after BCG administration [7]. In addition to the prophylactic effect against bacterial and viral infections, the BCG vaccination has shown nonspecific immunotherapeutic effects on virus-induced clinical conditions, such as cutaneous and genital warts caused by human papillomavirus (HPV). A study involving children showed a reduction of common warts in 65% of the patients and 45% of patients with plane warts treated with viable topical BCG compared to the control group treated with saline [20]. The

study did not observe recurrences or side effects in the BCG group, suggesting that the BCG could be a topical immunotherapeutic which is both effective and safe for the treatment of common and plane warts [20].

In addition to the nonspecific effects against viral and bacterial infections, the BCG also improves the effectiveness of other vaccines targeted against viral infections. In healthy human volunteers, vaccination with BCG prior to influenza vaccination augmented the immunogenicity of the vaccine against the H1N1 influenza strain [25]. Similarly, the BCG vaccinated volunteers showed increased and longer sustained interferon (IFN)-γ production when the influenza vaccine was given compared to the control group [25]. Another study characterized the effect of BCG on hepatitis B virus (HBV) immunogenicity and showed that the BCG administered along with HBV vaccine synergistically increased the whole blood production of IL-1β and cytokines compared to either BCG or HBV alone [28]. These studies showed that vaccination with BCG increases the production of viral-specific antibodies, providing an indirect evidence of enhanced protection against viral infection upon BCG vaccination [38].

In addition to the protective role of BCG against viral and bacterial infections, the BCG is also used to treat some cancers. It has been shown that the BCG through the recruitment of natural killer (NK), CD4+, and CD8+ T cells contributes to antitumor immunity [29]. In the treatment of melanoma, the BCG has been administered either alone or in combination with other drugs. It may lead to regression of the tumor and possibly improve the patient survival, although large prospective randomized clinical trials are still lacking [39]. BCG is considered as the gold-standard immunotherapy treatment for early-stage, non-muscle-invasive bladder cancer. The preclinical and clinical studies have suggested that the inflammatory response to BCG involves several steps. It induces innate immunity by affecting cellular and cytokine milieu and initiates tumor-specific immunity, but the exact mechanisms are still poorly understood [30].

In addition to the human studies showing nonspecific effects of BCG against pathogens and cancers, the experiments in mice also provide evidence that BCG enhances protection against viral infections (Table 2). It has been shown in vitro that mouse macrophages sensitized by BCG were more effective in reducing the titers of influenza virus than control macrophages [42]. In addition, an in vivo study in mice showed that BCG confers protection against influenza virus infection, and the effect is independent

Table 2. Nonspecific effect of BCG on infectious and noninfectious diseases in mice.

Targets of mice studies	Effect of BCG vaccination	References
Herpes simplex virus 1 (HSV 1)	Enhanced survival	[40]
Herpes simplex virus 2 (HSV 2)	Enhanced survival and protection from infection	[41, 40]
Influenza A virus	Reduced viral titers, reduced inflammation and lung injury, enhanced survival	[42, 43, 40]
Murine hepatitis virus 2 (MHV)	Confers resistance to the virus	[40]
Root-and-mouth disease virus	Confers resistance to the virus	[40]
Hepatitis B virus	Enhanced antibody production against the virus	[28]
Japanese encephalitis virus	Delayed occurrence of clinical symptoms and increased survival	[44]
Encephalomyocarditis virus	Enhanced resistance (induced by nonviable M. tuberculosis)	[45, 46]
Ectromelia virus	Enhanced survival and increased IFN-γ production	[47, 48]
Vaccinia virus	Protection from infection (induced by MDP) and increased IFN-γ production	[49, 50]
Sendai virus (SeV)	Protection from infection	[51]
Candida albicans	Protection from candidiasis	[52, 53]

of IFN-γ [42]. Recent studies also showed that the use of BCG enhances the protection against *Influenza A virus* by increasing the levels of efferocytosis by alveolar phagocytes and leads to reduced inflammation and lung injury compared to the control group [43]. A single intradermal BCG dose has also been shown to protect against *Herpes simplex virus 2* (HSV2) in a controlled newborn mouse model [41]. In another study, administration of muramyl dipeptide (MDP), a component of mycobacterial cell wall and known activator of IL-1α and IL-1β [54], protected mice against *Vaccinia virus* and HSV2. Interestingly, the conferred protection was independent of IFN-γ induction and was mediated by peritoneal macrophages [49]. Similarly, the mice given the MDP component of mycobacterial peptidoglycan were protected against the infection by *Murine respirovirus* or *Sendai virus* [51].

BCG might protect mice from Japanese encephalitis infection [44]. The mice vaccinated with BCG when infected with the causative virus showed a delay in the appearance of clinical symptoms and increased survival compared with the control group mice [44]. Notably, many other studies have demonstrated that the mice vaccinated with BCG develop resistance against viral infections. These studies include the mice

injected with nonviable Mtb Jamaica cells associated with oil-droplet emulsions (WCV). These mice showed a very high resistance to the injection of *Encephalomyocarditis virus* (EMCV) [45,46]. It has been shown that BCG also protects the mice from *Ectromelia virus* by enhanced production of interferon in peritoneal exudate cells and spleen cells [47,48]. In another study, the mice were inoculated with BCG intravenously and challenged with *Encephalomyocarditis virus*, *Murine hepatitis virus 1*, *Murine hepatitis virus 2*, *Herpes simplex virus*, foot-and-mouth disease virus, and A0 and A2 influenza viruses. In most cases, the mouse inoculated with BCG exhibited significantly higher resistance to lethal infections compared to the control mice [40]. In a murine model of severe combined immunodeficiency (SCID) with disseminated candidiasis, BCG vaccination led to an increased survival, which was partially dependent on NK cells [52,53].

The relationship between BCG vaccination and incidence rate and mortality of COVID-19

The protective effect of BCG on COVID-19 morbidity and mortality

Several studies have shown an association between national BCG immunization programs and a lower rate of COVID-19 infection and the related mortality (Table 3). In the early phase of the COVID-19 pandemic, a large scale analysis was carried out in 175 countries to investigate the COVID-19 incidence and related mortality in the presence or absence of neonatal BCG vaccination [56]. The study found that the mean of COVID-19 cases per population ratio was significantly lower in countries with BCG vaccination programs (n = 138) than those without (n = 37). The same trend was observed with the deaths per population ratio, showing fewer deaths in countries with BCG-vaccination programs [56]. Similarly, a comparative study was conducted on the association of BCG, adult pneumococcal and adult seasonal influenza vaccination with COVID-19 mortality in four European countries [55]. Among the three vaccines included, the BCG vaccination score remained significantly associated with COVID-19 mortality at day 30. Another observational study on the incidence of COVID-19 or recovery rate showed that the countries with nationwide BCG policy had a lower number of COVID-19 cases or had a better recovery rate [73]. A study involving 140 countries with higher coverage of childhood live vaccines [BCG or measles- containing-vaccine (MCV)] also showed a reduced risk of COVID-19

Table 3. Studies showing relationship between BCG vaccination and COVID-19 infection rate and mortality.

	Effect	Subjects	Reference
An observational study	Significantly less cases and mortality	4 European countries	[55]
An observational study	BCG was better compared to the other vaccines on the mortality	175 countries	[56]
An Ecological Study	Associated with BCG and COVID-19 related deaths	140 countries	[57]
A cohort study	BCG prevents more severe COVID-19	120 adults	[58]
Comparative study	BCG is effective in fighting COVID-19	134 countries	[59]
Epidemiological analysis	BCG is protective against SARS-CoV-2	Countries with BCG vaccination data, ≥ 1 million inhabitants	[60]
A cross-sectional study	BCG coverage reduces the incidence of COVID-19 infections	174 countries	[61]
An Ecological Study	Associated with reduced mortality	173 countries	[62]
Longitudinal, retrospective observational study	Decreases clinical symptoms of COVID-19 in the Healthcare workers (HCW)	6,201 HCW	[63]
Correlation analysis	BCG is protective against SARS-CoV-2	Worldwide	[64]
A Multivariable Analysis	BCG is protective against SARS-CoV-2	55 Countries	[65]
Linear regression analysis	BCG is protective against SARS-CoV-2	225 countries	[66]
Meta regression analyses	Less COVID-19 infections	160 countries	[65]
A Cohort Study	The BCG is safe and reduces the sickness during the COVID pandemic		[68]
A Multivariable Analysis	BCG vaccinated countries had less mortality compared without BCG vaccination	Countries above 1 m people	[59]
Investigative study	Prevents spread of COVID-19	Japan	[67]
Exploratory study	–	67 countries	[70]
Multiple regression analyses	30-fold decrease in COVID-19 related mortality	171 countries	[71]

related mortality associated with higher cumulative BCG coverage, whereas only a marginal effect of MCV coverage was found [57].

A cohort study reviewed 120 adult patients with COVID-19 visiting a health center in Rhode Island, United States. The study found that the patients with BCG vaccination were less likely to require hospital admission than those without BCG vaccination (3.7% vs. 15.8%, $p = 0.019$) [58]. These findings suggested that the BCG has a great potential in preventing more severe COVID-19. An ecological study was conducted using the data of 61 factors along with BCG vaccine coverage from 173 countries and correlated the data with COVID-19 related morbidity and mortality [62]. The results of the analyses suggested that BCG vaccination was helpful in reducing the COVID-19 related mortality in the population, whereas no significant association was found with COVID-19 morbidity. The effect of BCG on COVID-19 was recently assessed in a longitudinal, retrospective observational study involving 6201 healthcare workers (HCWs) [63]. Among the study subjects, 29.6% reported a history of BCG vaccination, whereas 68.9% were never vaccinated with BCG. Lower seroprevalence of anti-SARS-CoV-2 IgG and decreased incidence of clinical symptoms related to COVID-19 were found in HCWs vaccinated with BCG compared to the control group without BCG vaccination. Interestingly, the decreased SARS-CoV-2 IgG seroconversion was not associated with meningococcal, pneumococcal, or influenza vaccination. These findings were in line with several others and confirmed that the BCG vaccine directed against TB confers protection against the COVID-19 infection. In contrast, the vaccines directed against other viral or bacterial diseases have no significant effect in this context.

An exciting study by Escobar and coworkers determined the correlation between BCG vaccination policies and COVID-19 mortality rates in various countries of the world [60]. They found a strong correlation between the estimated degree of universal BCG vaccination deployment in a country and COVID-19 mortality. The results also indicated that every 10% increase in the BCG index was associated with a 10.4% reduction in COVID-19 mortality, suggesting a protective effect for the BCG vaccine [60]. These results have been criticized later when the pandemic shifted toward South America, and data from more countries became available [74]. When the updated mortality data from South American countries were included, there was no longer a significant correlation between the BCG mean coverage and COVID-19 deaths. Another interesting study to investigate the potential correlation between universal BCG vaccination policy and mortality for COVID-19 was carried across the countries with more than 1 million people [69]. The countries with BCG policies were significantly less severely affected compared to the countries without a universal BCG vaccination policy. In fact, none of the other factors, such as COVID-19 testing, duration of the epidemic, median life expectancy or median income, reached statistical significance to explain the different outcomes between the analyzed countries.

In an interesting cross-sectional study, an assessment was made on the effect of TB endemicity and

BCG coverage on COVID-19 incidence across 174 countries [61]. The analyses firstly showed that the high TB-burden countries had a lower incidence of COVID-19 irrespective of the BCG vaccine status of the country. Secondly, it was also found that the COVID-19 incidence was lower in countries with high BCG vaccination coverage. The authors proposed that the association between TB and COVID-19 may be due to cross immunity between mycobacterium species and SARS-CoV-2, which is conferred either by latent TB or previous TB infection [61].

To test the hypothesis that BCG vaccination correlates with a better outcome of COVID-19 patients, the analysis was carried out in 55 countries [65]. The results indicated that the BCG coverage, especially among the recently vaccinated population, contributes to slow spread and less severe COVID-19. Another study involving three cohorts of healthy vaccinated or non-vaccinated volunteers focused on the safety of BCG vaccination and the retrospective assessment of COVID-19 and its related symptoms [68]. The results demonstrated that the BCG vaccination was safe and was associated with a decrease in the incidence of sickness during the COVID-19 pandemic.

A comparative study of countries, either mandating or not BCG vaccination, suggested that mandated BCG vaccination might be effective in the fight against COVID-19 [59]. Countries that had terminated the national BCG vaccination policy before 2000 were not significantly different from those that never mandated such as policy. A similar trend was observed in the growth rate of COVID-19-related deaths, which were significantly rarer in countries with mandated BCG vaccinations until at least the year 2000.

A meta-regression analyses of data from 160 countries was done to estimate the difference in the incidence of COVID-19 between the BCG vaccinated and non BCG vaccinated countries reported prior to May 31, 2020 [67]. BCG coverage was categorized as ≤70%, > 70% and no vaccination. The countries with ≤70% BCG coverage reported 6.5 less COVID-19 cases per 10,000 population compared to the countries with no coverage. The analyses further showed that the countries with BCG coverage of over 70% had 10.1 fewer cases of COVID-19 per 10,000 people.

In Japan, Kinoshita and Tanaka recently investigated the impact of BCG vaccination on preventing the local spread of COVID-19 [70]. The prevalence of COVID-19 varied much among 47 prefectures. On March 29, no cases were reported in five prefectures. The BCG vaccine coverage was significantly higher in these five prefectures than in prefectures with a high prevalence of COVID-19. The findings suggested that BCG vaccination had contributed to the prevention of the local spread of the COVID-19 pandemic in Japan.

In an exploratory study, the impact of environmental temperature and neonatal BCG vaccination coverage in 67 countries on the spread and mortality rate of COVID-19 was carried out [71]. This study suggested that the temperature does not have any impact on the transmission of COVID-19. However, in countries having neonatal BCG vaccination policy, the spread of the disease was significantly lower in addition to the 58% lower mortality rate due to COVID-19.

To study further the effect of universal BCG vaccination policy on COVID-19 mortality a multiple regression analyses using data from 171 countries was performed [72]. The results showed an association of the universal pediatric BCG policy with a 30-fold decrease of COVID-19 mortality per population compared to the countries without the policy.

The association between parameters indicating immunity from BCG vaccination at country level and the morbidity and mortality of COVID-19 was investigated in populations from 225 countries [66]. The study found a significant inverse correlation between the cases and deaths of COVID-19 and BCG parameters, which highlights the immunity from BCG as a potential factor contributing to the variation of COVID-19 status across the countries. In addition, many other studies have shown that BCG vaccination was associated with lower incidence of COVID-19 and related mortality [64,75–80].

Based on the results from a number of publications, BCG may be a potential tool to prevent the COVID-19 spread and its complications. The BCG vaccination policy may lower the total number of COVID-19 cases and improve the recovery rate [73]. Clinical trials are needed to evaluate the effectiveness of BCG in protection from COVID-19, its safety in the elderly and possible adverse effects.

Studies that show no impact of BCG on COVID-19 morbidity and mortality

There are also several epidemiological studies which have suggested a no association between national BCG vaccination policy and the prevalence and mortality of COVID-19 (Table 4). A lot of discussion has been raised about potential confounding factors, which may have led to spurious associations. For instance, a study by Hensel and coworkers suggested that the epidemiological analyses showing benefits of BCG vaccination against COVID-19 were not accurate as the studies

Table 4. Studies showing no relationship between BCG vaccination and COVID-19 infection rate and mortality.

	Effect	Subjects	Reference
Analyses of the epidemiological studies	No correlation	Review of literature	[81]
Analyses of the epidemiological studies	No correlation	Review of literature	[82]
Comparative analyses of the data	No correlation	Review of literature	[83]
A systematic review and meta-analysis	No correlation	Review of literature	[84]
Cohort study	No correlation	3064 people	[88]
Regression discontinuity study	No correlation	2,044,848 people	[89]
Retrospective study	No correlation	334 people	[87]
Validation study	No correlation	68 countries	[83]
A retrospective cross-sectional study	No correlation	123 adults	[89]

did not systematically correct the confounding variables [81]. These authors observed that after correction for confounding variables, such as testing rates, there was no association between BCG vaccination policy and COVID-19 spread rate or percent mortality. A review of literature carried out in April and May 2020 to study the effect of BCG vaccination on the incidence and mortality of COVID-19 found no evidence to support the role of BCG vaccination in the reduction of COVID-19 incidence and mortality in countries maintaining obligatory BCG vaccination [82].

In a small retrospective study, a total of 334 patients with bladder cancer, 167 treated with intravesical BCG and 167 without, were investigated for the occurrence of COVID-19 between March and May 2020 [87]. The COVID-19 test and/or positive chest CT scan for viral pneumonia of these groups showed the presence of the disease in 5 patients in the BCG-treated group and 4 patients in the control group. The authors concluded that intravesical BCG did not protect these patients from COVID-19 [87].

A comprehensive epidemiological study was carried out by Szigeti and coworkers to validate the observations that the universal BCG vaccination protects against the COVID-19 morbidity and mortality [88]. The authors examined the country-based association between COVID-19 mortality per million population or daily rates of COVID-19 case fatality and the presence of BCG vaccination before 1980 or the year of the establishment of universal BCG vaccination. The associations were studied in multiple regression modeling based on publicly available databases from 68 countries. No significant correlation was detected between the year of the establishment of universal BCG vaccination and mortality rates

defined as daily rates of COVID-19 case fatality (i.e. death per case/days of the endemic) than simply using death/million people. The authors of the study concluded that the global COVID-19 data was unreliable due to political, cultural, and socioeconomic biases, and therefore should be critically examined before using it for hypothesis driven scientific discoveries. They further pointed out that their results did not explicitly address the question whether BCG vaccination may acutely protect against COVID-19 infection. Nevertheless, the analysis highlighted the difficulties in drawing reliable epidemiologic conclusions from the current data on the COVID-19 pandemic.

In a retrospective cross-sectional study, relationship between BCG vaccination status and severity of COVID-19 pneumonia and the factors affecting disease severity were investigated. The small study cohort involved 123 adults visiting a state hospital in Istanbul, Turkey. The results suggested that the BCG vaccination was not associated with the severity of COVID-19 pneumonia [89]. An analogous conclusion was drawn based on a much larger study cohort involving 2 044 848 individuals from Sweden [86]. The analysis was carried out to estimate the effect of BCG without the biases associated with cross-country comparisons. The authors concluded that their study provided a strong evidence that receiving the BCG vaccine at neonatal age did not provide any protective effect against COVID-19 among middle-aged individuals [86]. It was also suggested that the effect of recent BCG vaccination must be evaluated.

A systematic review and meta-analysis were carried out on data obtained from PubMed, Scopus and medrxiv.org to study if there is a correlation between BCG vaccination and protection against COVID-19 [84]. The study concluded that analyses of publicly available data showed no convincing evidence that BCG had a protective effect against the infection with SARS-CoV-2.

Another cohort study was carried out in Israel to compare the rates of COVID-19 and PCR test positivity in the patients visiting a hospital for suspected infection [85]. The patients visiting the hospital included both who received BCG vaccination and who did not as part of routine childhood immunization in the early 1980s. The results of the study showed that there was no significant difference in the proportion of positive test results in the BCG-vaccinated group compared to the unvaccinated group. The authors concluded that the results did not support the idea that BCG vaccination in childhood had a protective effect against COVID-19 in adulthood.

The studies showing protective effect of BCG are observational, and no causal relationship has been substantiated between the vaccination and severity of COVID-19 cases. Similarly, the studies showing negative correlation between BCG and the severity of COVID-19 are not reliable as the authors chose BCG vaccination as a dichotomous variable and didn't adjust for potential confounders [8]. The results of both the above-mentioned studies are based on epidemiological data and have fundamental biases; therefore, well-designed clinical trials are needed to show the effect of BCG on COVID-19.

The emerging evidence suggests a connection between TB, BCG and SARS-CoV-2

The pathogens, Mtb and SARS-CoV-2 are typically transmitted through droplet nuclei of aerosols generated by infected people, both pathogens infect the lungs and compromise the host immunity. The Mtb has a longer incubation period of 3-9 months or 1-2 years, whereas the incubation period for SARS-CoV-2 is only a few days, and both diseases exhibit similar symptoms to some extent [90]. Interestingly, it has been demonstrated that the countries with high TB prevalence have a lower incidence of COVID-19, irrespective of the BCG vaccine status of the country [61]. The relationship between TB and COVID-19 may be explained by cross immunity between Mtb species and SARS-CoV-2, which may be conferred either by latent or previous Mtb infection or BCG vaccination. It has been shown that the COVID-19 and TB share the common Th1 immune pathway and thus, a latent or past Mtb infection could lead to a better immune response to SARS-CoV-2 [91,92].

Indeed, recent evidence suggests that SARS-CoV-2 and Mtb share unique similarities in terms of the host protein interaction partners, and both pathogens infect lung tissues [93]. Another study that involved bioinformatics analysis of the *M. bovis* (strain BCG/Pasteur 1173P2) proteome revealed four immunodominant antigens that could induce an immune response against SARS-CoV-2 [94]. The BCG antigens showed that the bacterial proteins Rv0934, Rv3763, Rv3875, and Rv2997 share common informational properties with the S1 protein of SARS-CoV-2 [94]. The BCG vaccine may also carry similar T cell epitopes with SARS-CoV-2, which was evaluated by utilizing publicly available database and computer algorithms predicting human leukocyte antigen (HLA) class I-binding peptides [95]. It was found that BCG contained 9-amino acid sequences that are similar to SARS-CoV-2 and

these peptides may possess moderate to high binding affinity for multiple common HLA class I molecules, supporting the hypothesis that cross-reactive T cells against SARS-CoV-2 could be generated by BCG vaccination [95]. Another recent study showed that the BCG vaccination might induce a specific immunity against SARS-CoV-2 that targets the viral envelope protein that is essential for infectivity [96]. This is due to the high homology of the SARS-CoV-2 envelope protein that contains 12 consecutive amino acids of the protein LytR C, which is unique to *Mycobacteria* [96]. The main finding of this study was that the BCG vaccination may offer heterologous immunity against SARS-CoV-2 infection by inducing an adaptive immunity response against a protein that is essential to virus infectivity. These results suggest that a BCG vaccination booster could induce some anti-viral protection specifically against COVID-19. The possible mechanism for such induction of adaptive immune response has been outlined in Figure 2.

The development of COVID-19 vaccines is full of challenges, but the future can be predicted

The outbreak of the COVID-19 pandemic has imposed a serious burden on the global health system and has had a profound impact on the world economy [97,98]. As of January, 2 2021, the cumulative number of confirmed cases of COVID-19 worldwide has risen to 84,382,650, including 1,835,399 deaths [2]. This trend will not change soon unless effective vaccines or drugs are available. In response to the sudden pandemic, a growing number of research institutions and pharmaceutical companies have been struggling to quickly find an effective vaccine to defeat COVID-19 [99]. However, the development of vaccines is facing many challenges, such as viral gene mutations, re-infection of recovered patients with COVID-19, short duration of neutralizing antibodies, and shortage of time to evaluate the safety and effectiveness of vaccines. Although the road of COVID-19 vaccine development is full of thorns, there are already specific vaccines tested against SARS-CoV-2, and BCG has also brought people a ray of light. It is noteworthy that the safety of BCG and its effectiveness against TB have been tested in decades of practice, and its production process is mature and standardized. If the results of clinical trials confirmed the hypothesis of the beneficial role of BCG vaccination in the fight against SARS-CoV-2 infection, enough BCG vaccine could be produced at a short time to resist the spread of COVID-19 and possibly other future pandemics.
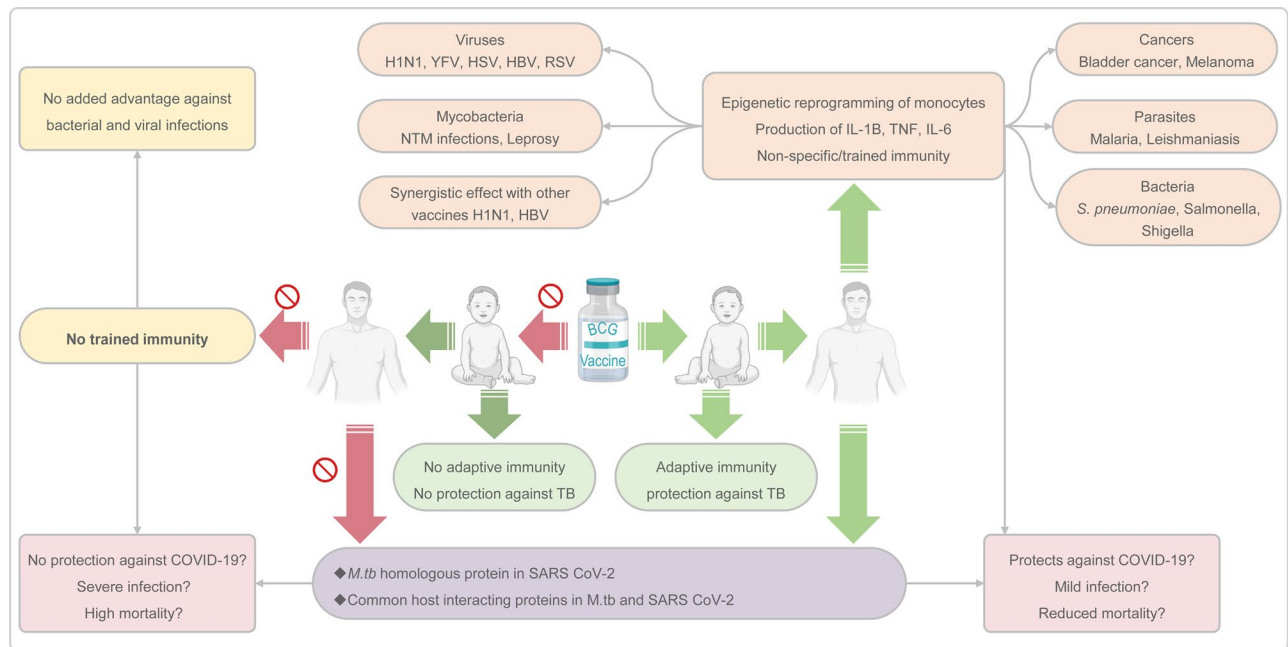
Figure 2. The childhood BCG vaccination in addition to the protection against TB though adaptive immunity has been shown to protect against different types of pathogens that include, viruses, bacteria and parasites through the nonspecific immunity. Based on the latest information, it is hypothesized that BCG also confers immunity against SARS-CoV-2 due to presence of homologous proteins in Mtb and common interacting proteins in both the pathogens.

BCG vaccine has a precedent in preventing severe infectious respiratory diseases

Although there are many challenges and obstacles on the road of COVID-19 vaccine development, the continuous advancements from clinical trials have given people the dawn of hope. According to a systematic review, 1,303 randomized clinical trials have been initiated in 71 countries to investigate 381 different single interventions [100]. The development process of these vaccines has been dramatically accelerated and compressed due to urgent needs, but in order to obtain more comprehensive and reliable vaccines, the standards of clinical trials should not be artificially lowered. This means that even if the progress of these clinical trials is fastest in history, many different vaccines and strategies are needed to conquer this and other epidemics to come [101]. Fortunately, the BCG vaccine may be able to bring people unexpected surprises during this period of anxious waiting. BCG has been used as an immunomodifier or therapeutic agent in the prevention or treatment of respiratory diseases other than TB, such as human respiratory syncytial virus (hRSV) [102] and influenza infection [25,43,103]. The mechanism by which BCG plays a key role in the prevention and treatment of these respiratory diseases is believed to be the induction of trained immunity and activation of heterologous lymphocytes, resulting in macrophage activity, T cell response, cytokine production, and the increase of antibody titers [104]. Given the similarity of respiratory virus infections, these data support the hypothesis that BCG vaccination could also protect against SARS-CoV-2 infection. Encouragingly, more than 37 clinical trials have been launched worldwide to assess this hypothesis.

BCG'S well-known safety accelerated clinical trials

When the safety and effectiveness of a vaccine have been verified in preclinical studies, clinical trials are performed. Clinical trials are generally divided into four phases: Phase I clinical trials will evaluate the preliminary clinical pharmacology, safety, and pharmacokinetics, Phase II clinical trials will focus on the preliminary efficacy and safety of vaccines, Phase III clinical trials will further verify the preventive or therapeutic effects and safety of the vaccine, and Phase IV clinical trials will investigate the efficacy and adverse reactions of the vaccine. It can be seen that the safety assessment is critical at all stages of vaccine development [105]. Since the BCG vaccine was first used in humans in 1921, it has gone through a long history of 100 years [106], and its safety has been widely confirmed. This advantage of BCG has saved precious time and accelerated the process of its clinical trials for COVID-19, which is why the vast majority

of the 37 BCG clinical trials against COVID-19 have entered Phase III and IV in just eight months.

Conclusions

The emergence of COVID-19 led to a quick pandemic, causing morbidity and mortality, huge economic losses, and humanitarian crisis. All potential preventive and treatment methods are urgently needed to control the spread of the disease and to reduce its negative impacts. High expectations have been placed on specific vaccines designed against the SARS-CoV-2 virus. However, over 1.6 million people have already been died (as of December 2020) for this pandemic, and the threat of future pandemics is real. As stated by the World Health Organization, "The best time to prevent the next pandemic is now."

Several studies over the years have shown that BCG vaccines can assert a powerful specific and nonspecific immune response, but we still do not know for sure if the vaccine can offer meaningful protection against the diseases like COVID-19. The statistical data from several studies have shown lower infection and mortality rates in the countries that follow BCG vaccination policies compared to other countries. However, a limited number of studies have shown no positive correlation between BCG vaccination and its protective effect neither against the severity nor the mortality of COVID-19. Though the studies showing an association between BCG and COVID-19 is exciting, the results of the studies do not prove a causal relationship unless the vaccine is tested in well-planned clinical trials. Randomized controlled trials using BCG vaccination are required to determine how fast an immune response develops that may provide some protection against COVID-19. Indeed, several clinical trials are underway to evaluate the ability of the BCG vaccine to modulate immunity against COVID-19. The goal of these trials is to determine if BCG vaccination reduces the incidence and severity of the SARS-CoV-2 infection. These studies will eventually help us to understand whether and to what extent BCG offers protection against SARS-CoV-2.

Author contributions

Conceptualization: Ashok Aspatwar and Wenping Gong; Software: Wenping Gong and Shuyong Wang; Funding acquisition: Wenping Gong and Ashok Aspatwar; Writing – original draft, Ashok Aspatwar, Wenping Gong, and Shuyong Wang; Writing – review & editing, Seppo Parkkila and Xueqiong Wu. All Authors have read and approved the manuscript.

Competing interests

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

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