REVIEW

BCG Vaccine-Induced Trained Immunity and COVID-19: Protective or Bystander?

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Abstract: In late 2019, a new virulent coronavirus (CoV) emerged in Wuhan, China and was named as severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2). This virus spread rapidly, causing the coronavirus disease-2019 (COVID-19) pandemic. Bacillus Calmette–Guérin (BCG) is a live attenuated tuberculosis (TB) vaccine, associated with induction of non-specific cross-protection against unrelated infections. This protection is a memory-like response in innate immune cells (trained immunity), which is caused by epigenetic reprogramming via histone modification in the regulatory elements of specific genes in monocytes. COVID-19 related epidemiological studies showed an inverse relationship between national BCG vaccination policies and COVID-19 incidence and death, suggesting that BCG may induce trained immunity that could confer some protection against SARS-CoV-2. As this pandemic has put most of Earth's population under quarantine, repurposing of the old, well-characterized BCG may ensure some protection against COVID-19. This review focuses on BCG-related cross-protection and acquisition of trained immunity, as well as the correlation between BCG vaccination and COVID-19 incidence and mortality.

Keywords: COVID-19, BCG vaccine, coronavirus, trained immunity, tuberculosis

Introduction

Coronaviruses (CoVs) have been known since the 1930s and they cause a wide variety of diseases in both animals and humans. Since the 1960s, seven human alpha- and beta-CoVs were identified. However, life-threatening pathogenic strains started to spill over from animals into humans in late 2002, causing severe respiratory disorders.[1–4] These strains are beta-CoVs such as severe acute respiratory syndrome coronavirus (SARS-CoV), which emerged in 2002/2003 in China, and ten years later, Middle East respiratory syndrome coronavirus (MERS-CoV) emerged in the Middle East region.[1–3,5–7] In late 2019, a new coronavirus strain emerged in Wuhan/China and in February 2020, it was named severe acute respiratory syndrome coronavirus-2 (SARS-CoV-2) due to the phylogenetic similarity (79.5%) with SARS-CoV.[8] This virus spread rapidly between and within other countries, causing coronavirus disease-2019 (COVID-19) and created a public health emergency. In March 2020, it was declared by the World Health Organization (WHO) that the COVID-19 outbreak was a global pandemic.[9] As of February 14, 2021, the WHO states that this global COVID-19 outbreak has resulted in around 108,246,992 confirmed cases and over 2,386,717 deaths worldwide.[10] To rapidly contain this outbreak, the genome sequence of SARS-CoV-2 was revealed within weeks of the viral emergence. Parallel to several

repositioned antiviral drugs (such as remdesivir),[11–28] several promising vaccine candidates (such as Ad5-nCoV,[29] mRNA-1273,[30] PiCoVacc and INO-4800[31]) are being clinically studied. Currently, there are available vaccines for COVID-19 as Pfizer-BioNTech COVID-19 Vaccine, Moderna COVID-19 vaccine, Janssen (Johnson & Johnson) COVID-19 vaccine and AstraZeneca/Oxford COVID-19 vaccine.[32]

Considering the urgent need to strengthen the immune response of populations and to face the rapidly spreading COVID-19 pandemic,[33,34] induction of trained immunity could be a potential protective approach against infections until developing effective therapy.[35] Trained immunity means a prolonged hyperactivation of the innate immune system (monocytes, macrophages, and natural killer (NK) cells) to unrelated infections. Bacillus Calmette–Guérin (BCG) vaccine, which has been used for decades against tuberculosis (TB), is one of the most prominent examples for induction of trained immunity. BCG shows decreased susceptibility to unrelated infectious agents, especially respiratory tract infections such as influenza A virus, respiratory syncytial virus (RSV) and herpes simplex virus type 2 (HSV2).[36–41] Interestingly, nations with mandatory BCG vaccines were shown to correlate with low number of COVID-19 confirmed cases as well as reduced mortality.[42–46] This inverse correlation may be attributed to the long-term boosting of innate immune mechanisms (BCG-induced trained immunity). However, comparison between countries where the BCG vaccine is used with countries where it is not used can be affected by different factors including demographic characteristics, socioeconomic status, COVID-19 testing rate, stage of the pandemic in each country, clinical care, infection prevention and control policies. Additionally, climate and urban differences between countries affect COVID-19 confirmed cases.[47,48] Moreover, being still in the midst of the COVID-19 pandemic and with cases still increasing even in countries with BCG vaccines, it is considered too early to have immature conclusions based only on ecological studies. Therefore, to draw conclusions regarding BCG vaccine and COVID-19, clinical studies are needed to support the ecological studies. The WHO declared that there is no evidence that BCG vaccine can protect against COVID-19 and they are still waiting for clinical trials outcomes.[49] A collection of published articles about CoVs and BCG vaccination are used in the review. This review focuses on BCG-induced cross-protection and acquisition of trained immunity, as well as the correlation between this BCG vaccine-induced trained immunity on COVID-19.

Coronaviruses, Symptoms and Prognosis of COVID-19

Coronaviruses (CoVs) are a group of viruses that infect humans and animals. There are four CoVs (229E, NL63, OC43 and HKU1), which are characterized with low pathogenicity. The more pathogenic CoVs causing fatal diseases are SARS-CoV, which emerged in China and MERS-CoV, which emerged in the Middle East region.[1–3,5–7,50,51] Currently, we live a global crisis due to SARS-CoV-2.[50,52–54] On December 2019, the first COVID-19 cases were documented by the WHO in the seafood market in Wuhan city, Hubei province, China.[8] Within a month, the virus spread from Wuhan to other areas within and outside China.

Transmission from person to person frequently occurs with close contact.[55] Initially, transmission occurs through the respiratory droplets produced by sneezing, coughing or even talking. Contaminated droplets settle in various parts of the body, such as nose, mouth, lungs and eyes.[56,57] Additionally, the virus can spread by touching contaminated surfaces or objects.[56,58] SARS-CoV-2 can infect the gastrointestinal tract and it was isolated from fecal swabs.[26] Therefore, the virus can spread via the fecal-oral route.[26,59,60] Airborne transmission may be possible in specific conditions.

COVID-19 symptoms vary among individuals, from asymptomatic infection to serious respiratory failure.[61] Fever, cough, fatigue, slight dyspnoea, sore throat, headache and conjunctivitis are common symptoms of the disease.[62–64] Gastrointestinal involvement, with diarrhoea, nausea and vomiting, was reported in a lower percentage of cases. Li et al[65] hypothesized that SARS-CoV-2 could have neuroinvasive potential, since viral entry into the central nervous system may contribute in some patients to development of respiratory failure. The reported hyposmia and hypogeusia experienced by individuals with COVID-19 could also indicate a potential neurotropism of this virus.[66] The neuroinvasive capacity of SARS-CoV-2 remains poorly understood.[67] Mortality due to COVID-19 appears to be lower than that of SARS-CoV (10%) and MERS-CoV (35%).[58,68] However, it is still too early to evaluate the actual mortality rate of the disease, considering the rapid spread of COVID-19. Old age, ischaemic

heart disease, hypertension, diabetes mellitus, chronic lung disease, cancer and patients receiving immunosuppressive medicines are the major risk factors for poor outcomes.[59]

BCG Vaccine and Its Impact on Viral Infections

BCG as a live attenuated vaccine against TB was developed from a virulent strain of *Mycobacterium bovis* (*M. bovis*) at the Institut Pasteur in Paris. In the 1950s, clinical trials on BCG vaccine were initiated in both UK and USA, and accordingly, the use of BCG vaccine was recommended by the UK, but in USA, it was restricted only to high-risk populations. Since then, most countries have followed BCG vaccination policies. The distribution of BCG vaccine to many laboratories worldwide and the repeated subculture in the different countries resulted in the emergence of phenotypically different vaccine strains.[69] Continuing studies are trying to determine the effect of the genomic diversity amongst BCG vaccine strains.

There is an available database for policies and practices of BCG worldwide, the BCG World Atlas.[70] BCG vaccine was never used in the national vaccination program of Italy. Spain, Germany and the UK stopped systematic BCG vaccination in 1981, 1998 and 2005, respectively.[71] Currently, countries that have mandatory BCG policies include Argentina, Brazil, Bulgaria, Chile, China, Egypt, Estonia, Iran, Ireland, Japan, Mexico, Poland, Singapore, South Africa, Taiwan, Thailand and Turkey.[70,71] For information about the current and past BCG vaccination policies and practices for more than 180 countries, the BCG World Atlas and interactive map are used.[70] BCG is given to newborns with high protective effect against tuberculous meningitis and miliary TB, but this effect is significantly lower against pulmonary TB. In the case of adults, BCG vaccination does not fully protect against pulmonary TB, which could explain why TB is one of the leading causes of death worldwide.

Interestingly, BCG also reduces infant mortality, which could be attributed to the non-specific cross-defence against other, unrelated pathogens.[72] BCG vaccine shows a lower risk of developing respiratory tract infections such as influenza A virus, RSV and HSV2.[36–41] Additionally, with BCG vaccination, West African studies have shown significant decreases in malaria mortality, sepsis, respiratory infections, and leprosy. Overall, decreased infant mortality due to BCG vaccination has been observed in many countries.[73] Furthermore, BCG could be used in treatment of other diseases such as bladder cancer, warts, leishmaniasis, candidiasis and asthma.[74] Table 1 highlights the non-specific effects of BCG vaccine on different viral infections. Moreover, BCG vaccine helps in the production of other vaccines against pathogenic bacteria and viruses. This is due to its safety for a long time in vaccinated neonates, children and adults and because BCG antigens can act as adjuvants, inducing innate and adaptive immune responses.[73] In humans, limited clinical evidence suggests that BCG vaccination may have non-targeted protective effects against viral infections. Many studies have been performed to explain the mechanisms behind these non-targeted protective effects of BCG.[37,75,76]

Immune Response to BCG Vaccine

Generally, vaccines work by activation of the adaptive immune response and formation of immunological memory of antigen-specific T and B cells to target the pathogens.[95] Following BCG vaccination, the bacilli are recognized and identified at the inoculation site by neutrophils, macrophages, and dendritic cells (DCs) to start the immune response (Figure 1), where pathogen-associated molecular patterns (PAMPs) expressed by mycobacteria (such as peptidoglycan, arabinogalactan, and mycolic acids) interact with pattern recognition receptors (PRRs) expressed on innate immune cells, stimulating the macrophage, maturation of DCs and pro-inflammatory cytokines release.[96–99]

PRRs, which are involved in BCG recognition and internalization, include toll-like receptors (TLRs), nucleotide oligomerization domain (NOD)-like receptors, complement receptors (CR3 and CR4), CD14 receptors, and C-type lectins such as dendritic cell-specific intercellular adhesion molecule grabbing nonintegrin (DC-SIGN).[96,99] Maturation and migration of DCs to the nearest secondary lymphoid tissues or spleen are associated with increased expression of co-stimulating molecules (CD40, CD80, CD83 and CD86).[100] Antigen (Ag) 85, which found in BCG cell wall and *M. tuberculosis*, triggers production of tumor necrosis factor-alpha (TNF-α), interleukin 1-beta (IL-1β) and IL-6, resulting in a pro-inflammatory state to activate immune cells.[101]

The adaptive immune response develops when antigen-presenting cells (DCs, macrophages, and B cells) present antigenic peptides on MHC and primary T cells in lymph nodes.[102] In lymph nodes, BCG infected DCs release IL-6, IL-12 and TNF-α as well as triggering activation of CD4$^+$ and CD8$^+$ T cells with high production of IFN-γ.[103] Ten weeks after vaccination, in the blood mycobacteria-specific CD8$^+$ T cells proliferate and release IFN-γ and express

Table 1 Overview of the Non-Specific BCG Vaccine Effects Described for Different Viral Infections (Adapted from Moorlag et al.[77])

	Virus	Study Type	Effect of BCG	Ref.
Human Studies	Yellow fever vaccine	RCT	Reduced yellow fever vaccine titres correlating with IL-1b production	[78]
	HPV	RCT	Improved clearance of viral warts	[79–81]
		Case series		
	RSV	Case control study	Non-significant association of fewer RSV infections in Guinea-Bissau in young children (girls)	[41]
	Influenza A (H1N1)	RCT	Enhanced antibody production	[82]
	HSV	Case series	Reduced episodes of clinical HSV infection	[83,84]
Animal Studies	HSV 1	CD-1 mice	Enhanced survival	[85]
	HSV 2	_	Enhanced survival and protection from infection	[37]
		CD-1 mice		[85]
	Influenza A	_	Reduced viral titres of against influenza A virus	[76]
		C57Bl/6 mice	Reduced inflammation	[86]
		CD-1 mice	Enhanced survival	[85]
	Influenza A (H7N9)	BALB/c mice	No increased protection	[87]
	Hepatitis B	C57Bl/6 mice	Enhanced antibody production	[88]
	Japanese encephalitis	BALB/c mice	Delayed occurrence of clinical symptoms and increased survival	[89]
	Encephalomyocarditis virus	C57B1/10 mice	Enhanced resistance (induced by non-viable *M. tuberculosis*)	[90,91]
	Ectromelia virus	DDN mice	Enhanced survival and increased IFN-g production	[92,93]
	Vaccinia	BALB/c mice	Protection from infection (induced by MDP)	[75]
		C57Bl/6 mice	Protection from infection and increased IFN-g production	[94]

Abbreviations: RCT, Randomized control trial; HPV, Human papillomavirus; RSV, Respiratory syncytial virus; HSV, Herpes simplex virus; CD-1 mice, Outbred mice, generally used for genetics, toxicology, pharmacology, and ageing research; C57BL/6 mice, common inbred strains of laboratory mice; BALB/c mice, an albino, laboratory-bred strain of house mouse; C57BL/10 mice, superficially identical to C57BL/6 mice in appearance and behaviour and widely used in inflammation and immunology research; DDN mice, inbred albino mice with distinctive characteristics of the central nervous system; IFN-g, Interferon gamma; MDP, Muramyldipeptide.

granzymes.[104] Additionally, IFN-γ, TNF-α, and IL-2 are released by activated TH1 CD4^{+} T cells.[105] Moreover, BCG-infected neutrophils team up with DCs (BCG-infected), stimulating the responses of antigen-specific T cells.[106] The released IFN-γ activates B cells and leads to generation of memory and plasma cells, releasing antigen-specific antibodies, which are able to opsonize BCG and *M. tuberculosis*, inhibiting intracellular bacterial growth.[107]

BCG Vaccine Reprogrammes Innate Immunity (Trained Immunity)

As a consequence of BCG vaccination, an increased non-specific response to unrelated pathogens is mediated through induction of innate immune cells including monocytes/macrophages and NK cells and independent of T and B cell responses (trained immunity) (Figure 2). Trained immunity following BCG vaccination is associated with elevated production of pro-inflammatory cytokines (e.g. TNF-α, IL-1β and IL-6), achieving significant protection against different viral infections.[108,109]

One of the molecular mechanisms behind trained immunity is the epigenetic reprogramming of monocytes through histone modifications (methylation and acetylation of histone) in regulatory elements of specific genes (such as TNF-α, IL-6, and IL-1β).[43,109,110] This histone modification results in enhanced chromatin accessibility and easier transcription of genes, which are related to increased antimicrobial responses and enhanced cell function.[110] Accordingly,
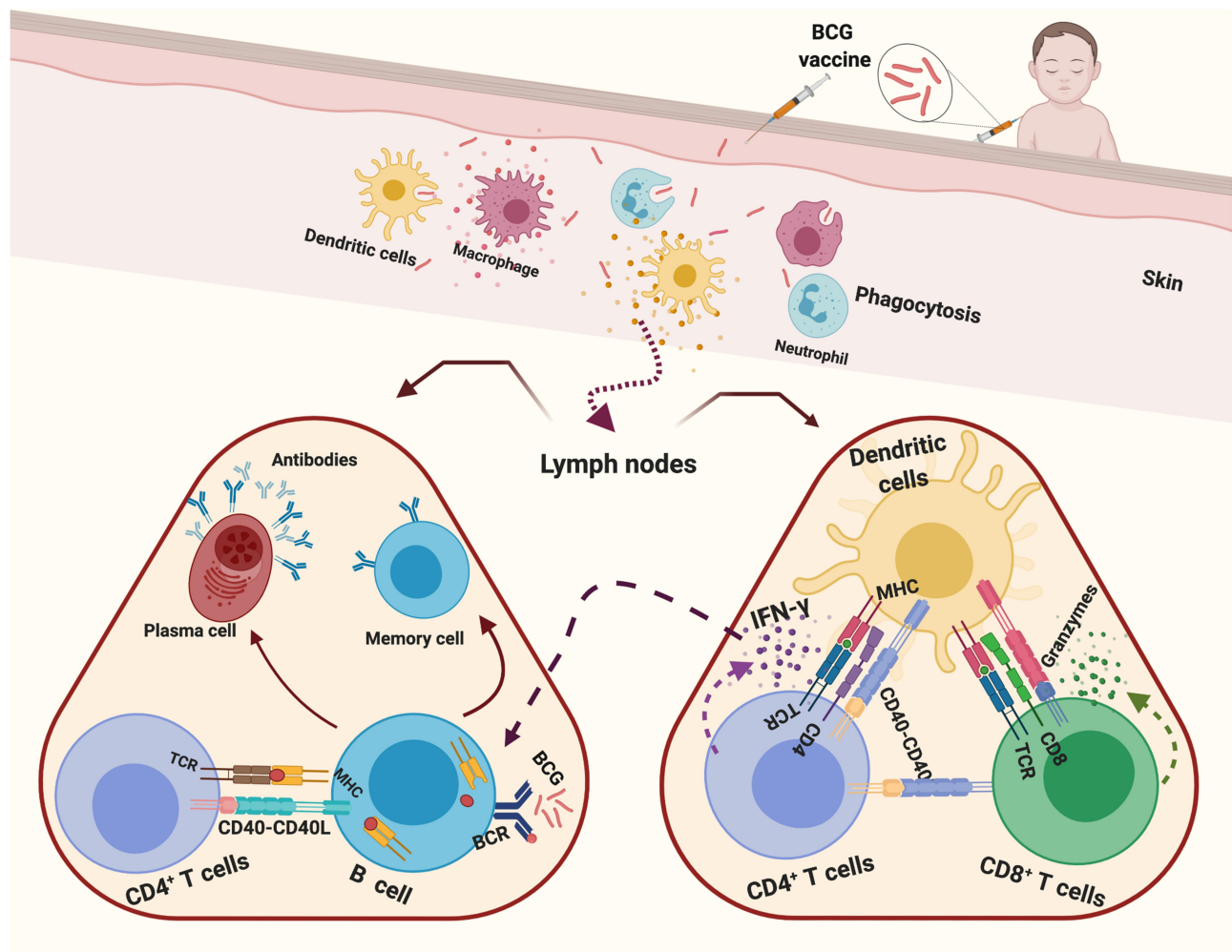
Figure 1 Schematic representation of the immune response elicited by BCG vaccination.
Notes: First, neutrophils, macrophages and dendritic cells recognize BCG at the inoculation site. Then the activated dendritic cells migrate to lymph nodes, activating the adaptive immune cells. CD4^{+} and CD8^{+} T cells are activated, releasing IFN-γ and granzymes. Activation of B cells results in production of memory and plasma cells with the release of antigen-specific antibodies.

when BCG-epigenetically trained monocytes are exposed to another pathogen (viruses and bacteria as pathogen-associated molecular patterns), PPRs easily and rapidly recognize it, leading to increased cytokine production (e.g. TNF-α, IL-1β and IL-6).[95] Additionally, metabolic reprogramming results in selective accumulation or depletion of some metabolites, which regulate epigenetic changes.

BCG Vaccine as a Tool Against COVID-19

SARS-CoV-2 is a single-stranded RNA virus. A vital encoded structural protein within its RNA chain is Spike glycoprotein (S), which consists of three heterodimers of S1-S2 that bind to the angiotensin-converting enzyme 2 (ACE2) type II pneumocyte receptor. SARS-CoV-2 enters host cells by endocytosis and then

multiplies in the cytoplasm, leading to cell apoptosis due to high protein manufacturing stress. Coronaviral RNA itself works as a PAMP and it is recognized by a PRR or TLR, resulting in a chemokinetic surge that causes migration and activation of neutrophils, leading to destruction of the alveolar-capillary walls. At the microscopic level, this results in a loss of the interface between intra-alveolar space and the surrounding stroma and subsequently, the fluids leak and fill the alveolar sacs.[111] Trained immunity could have a potential protective effect against COVID-19.

The association of some vaccines (such as BCG, adult pneumococcal and adult seasonal influenza) with COVID-19 mortality has been studied, suggesting that BCG-vaccinated individuals have reduced mortality rates.[43,112] BCG, as the most prominent example for induction of
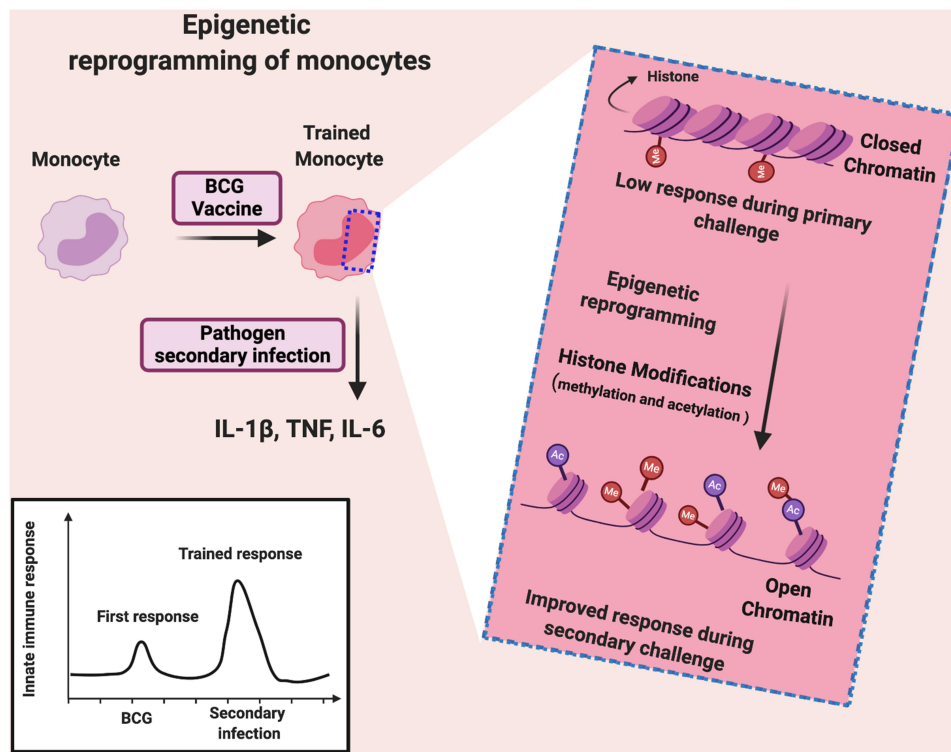
Figure 2 Trained immunity mediated by epigenetic reprogramming of monocytes.

trained immunity, shows a broad-spectrum protection, which is not only against TB, but also against unrelated infections, especially respiratory tract infections.[36–41,72] Interestingly, several ecological studies observed that there is an inverse correlation between BCG vaccination and COVID-19 prevalence and mortality, suggesting a potential protective effect of BCG against COVID-19.[42–46,113–116]

The cooperation between the innate and adaptive immune system plays a crucial role to defend against viral infections. Although the current review focuses on BCG vaccine-related trained immunity in terms of COVID-19, another mechanism of cross-protection was recently reported as BCG vaccine may generate cross-reactive T cells against SARS-CoV-2 because BCG has been shown to contain similar 9-amino acid sequences with SARS-CoV-2, and those closely related peptides have moderate to high binding affinity to common HLA class I molecules.[117]

Moreover, BCG vaccination can modulate anti-inflammatory cytokine and chemokine responses, preventing hospitalization and resulting in less severe cases of COVID-19.[118,119] This could be attributed to the suggestion that BCG vaccine modulates the innate immune system.

Based on these studies, countries with BCG in their national vaccination programmes (BCG countries) show lower numbers of confirmed COVID-19 cases/million inhabitants than countries with no BCG vaccination policy (non-BCG countries) (Figure 3).[43,116,120] Further, amount of deaths/million inhabitants in BCG countries is lower than in non-BCG countries (Figure 3C and D).[43,116,120] Escobar et al.[8] showed that each 10% increase in BCG index was associated with a decrease in COVID-19 mortality by 10.4%. Also, Gallagher et al.[112] found that 64% reduction in log (10) mortality/10 million population is associated with BCG vaccination. Moreover, a negative correlation was observed between cases and deaths of COVID-19 and the years following BCG administration.[121] A research study (published in October 2020) also revealed fewer COVID-19 cases in BCG countries including Afghanistan, India, Bangladesh, Nepal and Japan compared with non-BCG countries including the USA, UK, Canada, Italy and Spain.[122]

The negative correlation between routine infant BCG vaccination and COVID-19 spread in young people was recorded across different countries in several studies.[123–125] BCG vaccination under 25 years of age showed a protective effect against COVID-19.[121,123,125] Twenty-seven BCG countries (either at birth or during
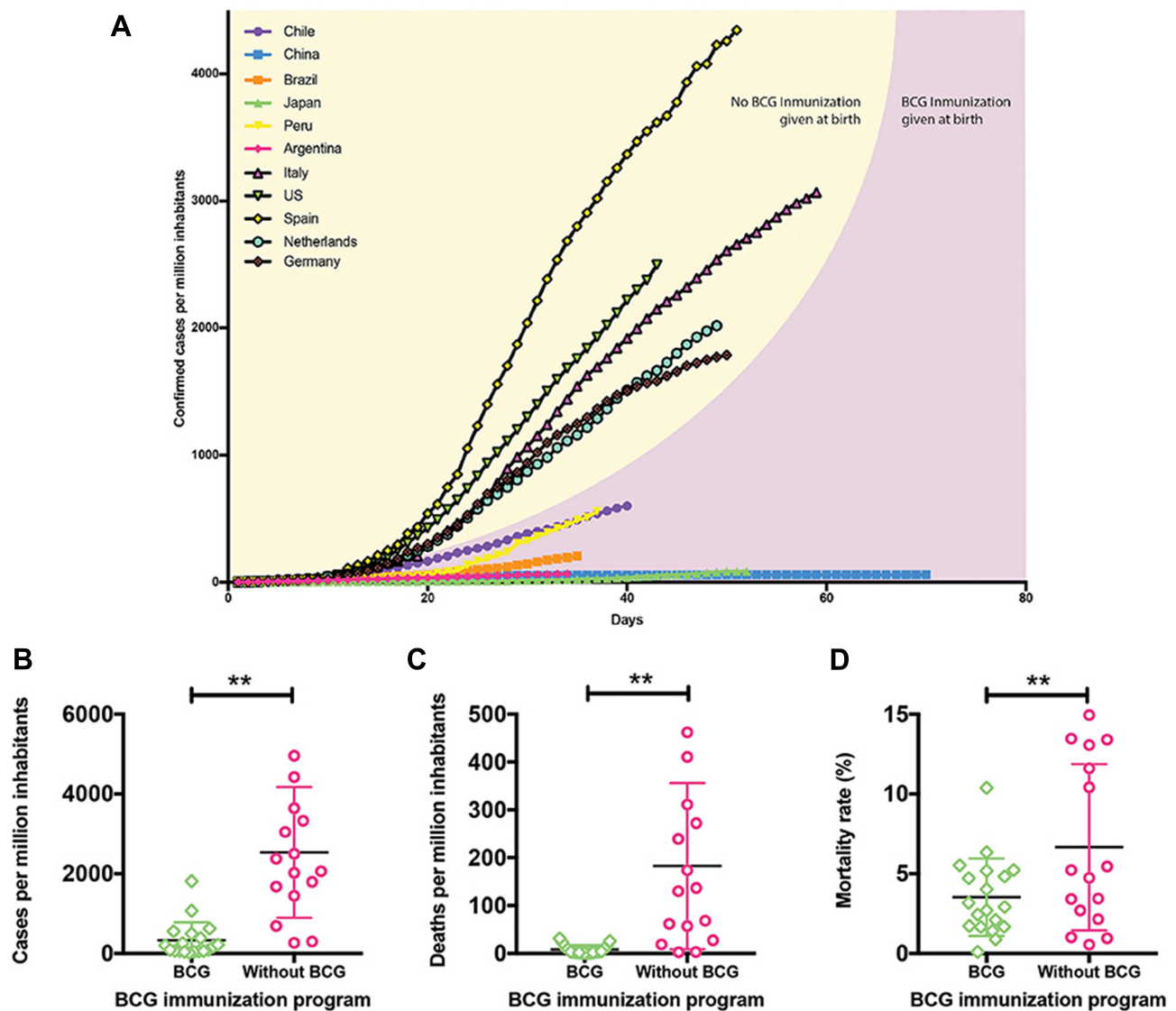
Figure 3 Protective effect of BCG vaccine against COVID-19.

Notes: (**A**) COVID-19 cases in different countries starting from the day at which the cases exceeded 2 cases/million till few months of the pandemic (April 2020). Curves of countries without BCG vaccination program are in the yellow background and curves of countries with BCG vaccination program at birth are in the pink background, (**B**) Confirmed cases/per million inhabitants in BCG- and non-BCG countries, (**C**) deaths/million inhabitants, and (**D**) mortality rates in BCG- and non-BCG countries. This figure is based on collected data till April 2020. ** Statistically significant differences between the groups. Reproduced from Covián C, Retamal-Díaz A, Bueno SM, Kalergis AM. Could BCG vaccination induce protective trained immunity for SARS-CoV-2? *Frontiers in Immunology*. 2020;11:970. Copyright © 2020 Covián, Retamal-Díaz, Bueno and Kalergis. Creative Commons Attribution License (CC BY).[43]

childhood) showed lower mortality than that for 23 non-BCG countries (P < 0.001).[123]

Most of these correlational ecological studies were carried out during the first few months of the COVID-19 pandemic.[42–46,126] As with any observational epidemiological study, the collected data (such as data in Figure 3) are interpreted as a hypothesis only, which further needs detailed studies to confirm. Thus, further investigations considering the differences between countries including demographics, socioeconomic status, climate, testing rate,

pandemic stage and infection prevention protocols are required.

Importantly, clinical studies are also essential to draw a conclusion regarding the role of BCG vaccine against COVID-19.[49] Accordingly, some countries have started clinical studies to confirm whether BCG vaccine is able to protect healthcare workers against SARS-CoV-2 infection and the recruitment in these clinical trials has started (Table 2).[127,128] Table 2 shows different clinical trials around the world, which are planned to randomize

Table 2 Characteristics of Ongoing Clinical Trials on BCG vaccine as prophylaxis for COVID-19.

Clinical Trial (Title/ID)	Status/ Country	Date	Study Design	Population	Intervention	Outcomes
Reducing Health Care Workers Absenteeism in Sars-Cov-2 Pandemic Through Bacillus Calmette-Guérin Vaccination, A Randomized Controlled Trial (BCG-CORONA) ClinicalTrials.gov Identifier: NCT04328441	Recruiting/ Netherlands	March 2020/ October 2020	Multicentre randomized controlled trial, placebo controlled	1000 workers (nurses and physicians) at emergency rooms and wards used for management of COVID- infected patients	BCG vaccine	Primary: Number of days of unplanned absenteeism. Secondary: Incidence of documented SARS-CoV-2 infection Incidence of severe respiratory symptoms, hospital admission, intensive care admission and death from SARS-CoV-2 infection Number of days of fever, respiratory symptoms Incidence of SARS- CoV-2 antibodies
BCG Vaccination to Protect Healthcare Workers Against COVID-19 (BRACE) ClinicalTrials.gov Identifier: NCT04327206 EudraCT Number: 2020–000919-69	Recruiting/ Australia	March/2020/ October 2020	Multicentre, open label randomized controlled trial	4000 healthcare workers in hospital sites	BCG vaccine	Primary: Incidence of COVID-19 disease and severe COVID-19 disease Secondary: Time to first symptom and duration of symptoms of COVID- 19 Work absenteeism, Hospital admission, critical care admission, Need for oxygen therapy, mechanical ventilation Mortality, Local and systemic adverse events to BCG
Application of BCG Vaccine for Immune-prophylaxis Among Egyptian Healthcare Workers During the Pandemic of COVID- 19 ClinicalTrials.gov Identifier: NCT04350931	Not yet recruiting/ Egypt	April 2020/ October 2020	Multicentre randomized controlled trial, placebo controlled	900 healthcare workers at emergency rooms, ICUs and wards of isolation hospitals	BCG vaccine	Primary: incidence of confirmed cases Secondary: Number of days of absenteeism Incidence of hospital admission Incidence of ICU admission Mortality
Performance Evaluation of BCG Vaccination in Healthcare Personnel to Reduce the Severity of SARS-COV-2 Infection ClinicalTrials.gov Identifier: NCT04362124	Not yet recruiting/ Colombia	April 2020/ June 2021	Multicentre randomized controlled trial	1000 healthcare workers directly involved in the care of COVID-19 patients	BCG vaccine	Primary: incidence of confirmed and probable COVID-19 cases Secondary: Incidence of severe COVID-19 infection Mortality Safety of BCG vaccination

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BCG Vaccine for Health Care Workers as Defense Against COVID 19 (BADAS) ClinicalTrials.gov Identifier: NCT04348370	Recruiting/ USA	April 2020/ May 2021	Multicentre randomized controlled trial	1800 healthcare workers involved in the care of suspected and confirmed COVID-19 patients with at least 25 hours per week of direct patient care	BCG vaccine	Primary: incidence of COVID-19 infection Secondary: Disease severity (COVID –19 Severity Scale Scoring)
Outcome of COVID-19 Cases Based on Tuberculin Test: Can Previous BCG Alter the Prognosis? ClinicalTrials.gov Identifier: NCT04347876	Recruiting/ Egypt	April 2020/ June 2020	Observational Case-Control	100 participants: Group A: COVID-19 positive with positive tuberculin test Group B: COVID-19 positive with negative tuberculin test	None	Primary: Pneumonia severity index Need for ICU admission Secondary Outcome Measures: COVID –19 test conversion Mortality
Reducing COVID-19 Related Hospital Admission in Elderly by BCG Vaccination ClinicalTrials.gov Identifier: NCT04417335	Active, not recruiting/ Netherlands	April 2020/ May 2021	Interventional Randomized controlled trial	2014 participants	BCG vaccine	Primary: SARS-CoV-2 related hospital admission Secondary Outcome Measures: duration of hospital admission due to COVID-19, cumulative incidence of SARS-CoV-2 infection, self-reported acute respiratory symptoms or fever, death due to SARS-CoV-2 infection, hospital admission for any reason, cumulative incidence of Intensive Care Admission due to SARS-CoV-2 infection
COVID-19: BCG As Therapeutic Vaccine, Transmission Limitation, and Immunoglobulin Enhancement (BATTLE) ClinicalTrials.gov Identifier: NCT04369794	Not yet recruiting/ Brazil	June 2020/ June 2021	Interventional randomized controlled trial	1000 participants	BCG vaccine	Primary: Clinical evolution of COVID-19 SARS-CoV-2 elimination Virus detection by PCR Seroconversion rate and titration Secondary Outcome Measures: Local and systemic adverse events to BCG

(*Continued*)

Table 2 (Continued).

Clinical Trial (Title/ID)	Status/ Country	Date	Study Design	Population	Intervention	Outcomes
BCG Vaccination for Healthcare Workers in COVID-19 Pandemic ClinicalTrials.gov Identifier: NCT04379336	Recruiting/ South Africa	May 2020/ April 2021	Interventional Randomized controlled trial	500 participants	BCG vaccine	Primary: Incidence of HCWs hospitalized due to COVID-19/arm. Secondary Outcome: Incidence of SARS-CoV -2 infection/arm, Incidence of upper respiratory tract infections per arm, Days of unplanned absenteeism, Incidence of hospitalization for any reason/ arm, Incidence of death/arm To compare incidence of death of HCW due to COVID-19 or any reason/arm.
Using BCG Vaccine to Protect Health Care Workers in the COVID-19 Pandemic ClinicalTrials.gov Identifier: NCT04373291	Not yet recruiting/ Denmark	May 2020/ December 2020	Interventional randomized controlled trial	1500 participants	BCG vaccine	Primary: Number of days of unplanned absenteeism Secondary Outcome Measures: cumulative incidence of COVID-19, cumulative incidence of hospital admission, The number of days of unplanned absenteeism because of COVID-19 Cumulative incidence of Hospital Admission due to COVID-19
Efficacy of BCG Vaccination in the Prevention of COVID19 Via the Strengthening of Innate Immunity in Health Care Workers (COVID-BCG) ClinicalTrials.gov Identifier: NCT04384549	Not yet recruiting/ France	May 2020/ February 2021	Interventional randomized controlled trial	1120 participants	BCG vaccine	Primary:Incidence of COVID-19 among health care workers exposed to SARS CoV-2 and vaccinated with BCG compared with placebo Secondary Outcome: Numbers of COVID-19 patients requiring hospitalization in ICU and O2, artificial ventilation or extracorporeal membrane oxygenation, or deaths in BCG-vaccinated health care workers compared with placebo. Incidence of asymptomatic SARS- CoV-2 seropositive subjects among BCG-vaccinated health care workers compared with placebo

Study	Status/Country	Dates	Design	Participants	Intervention	Outcome Measures
Bacillus Calmette-guérin Vaccination to Prevent COVID-19 (ACTIVATEII) ClinicalTrials.gov Identifier: NCT04414267	Recruiting/ Greece	May 2020/ May 2021	Interventional randomized controlled trial	900 participants	BCG vaccine	Primary Outcome Measures: Positive for the respiratory questionnaire consisted of questions concerning appearance of symptoms possibly, probably and/or definitively related to COVID-19 on visit 3 Secondary Outcome Measures: Positive respiratory questionnaire endpoint consisted of questions concerning the appearance of symptoms possibly, probably and/or definitively related to COVID-19 on visit 4 Positive respiratory questionnaire endpoint consisted of questions concerning the appearance of symptoms possibly, probably and/or definitively related to COVID-19 on visit 5 Prevalence of IgG/IgM against SARS-CoV-2
Efficacy and Safety of VPM1002 in Reducing SARS-CoV-2 (COVID-19) Infection Rate and Severity (COBRA) ClinicalTrials.gov Identifier: NCT04439045	Not yet recruiting/ Canada	June 2020/ April 2021	Double-blind, randomized controlled trial	3626 front-line police officers	Recombinant BCG	Primary: COVID-19 infection Secondary: Incidence of hospitalization, ICU admission, ARDS, mechanical ventilation, secondary infection, mortality and innate trained immunity Adverse events
Study to Assess VPM1002 in Reducing Healthcare Professionals' Absenteeism in COVID-19 Pandemic ClinicalTrials.gov Identifier: NCT04387409	Recruiting/ Germany	May 2020/ June 2021	Phase III, Double-blind, Randomized, Placebo-controlled Multicentre Clinical Trial	1200 healthcare professionals	Recombinant BCG	Primary: Number of days absent from work due to respiratory disease Secondary: Incidence of COVID-19 related symptoms, SARS-CoV-2 infection, mortality, ICU admission, hospital admission
Study to Assess VPM1002 in Reducing Hospital Admissions and/or Severe Respiratory Infectious Diseases in Elderly in COVID-19 Pandemic ClinicalTrials.gov Identifier: NCT04435379	Recruiting/ Germany	June 2020/ May 2021	Phase III, Randomized, Double-blind, Placebo-controlled, Multicentre, Clinical Trial	2038 adults aged 60 and above	Recombinant BCG	Primary: Number of days with severe respiratory disease Secondary: Cumulative incidence of hospital admissions, SARS-CoV-2 infection, respiratory symptoms, hospital admission, ICU admission, mortality

Notes: Adapted from Sanchez-Mostiero D, Melicor AF. Should Bacillus Calmette–Guérin (BCG) vaccine be used in the prophylaxis of COVID-19? *Acta Medica Philippina*. 2020;54:(Special Issue on Coronavirus Disease (COVID19)). Copyright 2020 University of the Philippines Manila. The material has been obtained with permission from the publisher of the original article.[128]

cumulatively more than 10,000 BCG-immunization healthcare workers. Different primary outcomes are measured in each study. In the case of the Australian and American designs, they assess incidence of COVID-19 and disease symptoms; however, the Dutch group is looking primarily at absenteeism of the healthcare workers. The fourth observational case-control study in Egypt, started recruitment of positive COVID-19 cases and compares the disease severity in thoracic patients.[129] Another study in Germany is done to test whether VPM1002 (a recombinant vaccine strain derived from BCG), can protect either healthcare workers or older patients from COVID-19.[130]

Based on the results of these clinical studies, we can confirm if there is a protective effect of BCG vaccination against COVID-19. However, further studies will be required to answer many questions about this protection. First, for how long does this BCG-engendered heterologous immunity last after BCG vaccination? And if this trained immunity will last for a few months after vaccination and then gradually decrease. Second, what is the best timing for BCG vaccination? And if the early life BCG vaccination (before age of nine months) results in better effects on respiratory infections and COVID-19 than later vaccinations?[43,131] Importantly, these questions should be carefully studied to answer all the raised concerns regarding insufficient evidence between BCG vaccination and COVID-19 protection.[132] Additionally, mechanistic studies are still required to decipher the mechanisms behind the correlation between BCG-induced trained immunity and COVID-19.

Until now, the WHO still recommends that BCG vaccine should be used against COVID-19 only in randomized controlled trials for a number of reasons, a) uncertain ability of BCG to protect against COVID-19, b) shortage of BCG vaccine, c) false sense of safety, d) BCG vaccine may be affected by subsequent administration of another vaccine and e) up-regulation of immunity by BCG could worsen COVID-19 in some critically ill patients.[133,134] Another important aspect relates to boosting BCG's innate immune response and complications in COVID-19 patients due to an exaggerated cytokine response. This hypothesis requires further clarification because it was noticed that BCG-vaccinated healthy individuals had induced trained immunity, which enhanced the antimicrobial properties, and reduced viral loads, resulting in less inflammation and symptoms.[44,135] On the contrary, older people as a high-risk group have defective antiviral response, resulting in high viral loads and systemic inflammation. The suggestion that BCG's induction of trained immunity may provide a defence against COVID-19 must be evaluated in randomized clinical trials.

Interestingly, inductions of qualified immunity against COVID-19 may not be restricted to BCG because oral polio vaccines are suspected to protect against unrelated viral infections, and the recombinant BCG-based vaccine (VPM1002) may also be considered for clinical trials.[136] Therefore, BCG vaccine or other trained immunity inducers, which provide non-specific protection, would be an important tool in responding to COVID-19 and future pandemics.

Conclusion

Currently, the COVID-19 pandemic has put the entire globe in an unprecedented crisis, which requires rapid development of effective vaccine or treatment. BCG, as a live attenuated vaccine, reduces infant mortality due to the non-specific cross-defence against other unrelated pathogens including respiratory tract infections. During the first months of the pandemic, several epidemiological studies revealed an inverse correlation between BCG vaccination and COVID-19 incidence and mortality. Because there is a debate around the non-specific protection of BCG, results from several ongoing clinical trials in different countries are awaited to confirm the correlation between BCG vaccination and COVID-19 and caution should be considered in the interpretation of the related results. Strong evidence about any protective role of BCG vaccination should be concluded before reflecting on practice and vaccination policies.

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Author Contributions

All authors made substantial contributions to conception and design, acquisition of data, analysis and interpretation of data; took part in drafting the article or revising it critically for important intellectual content; agreed to submit to the current journal; gave final approval of the version to be published; and agree to be accountable for all aspects of the work.

Disclosure

The authors declare no conflicts of interest for this work.

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