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Accessing the significance of Bacille Calmette-Guerin (BCG) Vaccine in COVID-19 Patients: Is it still relevant?

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ABSTRACT

COVID-19 is an evolving pandemic and has spread to over 200 countries claiming approximately 3730000 deaths. While scientists work at the forefront of developing strategies to counter this deadly virus, many have identified the significance of bacille Calmette-Guerin (BCG) vaccine against conferring immune protection against COVID-19. This observation comes amidst a significantly lower mortality rate in countries where the BCG vaccine remains an integral part of the neonatal vaccination regimen. Even though the purpose of the vaccine in these countries is to protect against tuberculosis infection; the BCG vaccine has shown protective effects against non-mycobacterial infections and certain cancers. In this review, we highlight these observations and discuss the various vaccine-host immune interactions, particularly the role of cytokines that may have a role in conferring immuno-protection against COVID-19 in BCG vaccinated individuals.

Keywords: BCG vaccination, COVID-19, immunoprotective effect

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INTRODUCTION

Tuberculosis (TB) remains an endemic disease in many countries such as Pakistan, India, China, and Bangladesh among others. In these countries, Bacillus Calmette–Guérin (BCG) vaccine is intradermally given to neonates, usually on the first day of life. This live attenuated vaccine has demonstrated efficacy in not only protecting against tuberculous/non-tuberculous infections, such as leprosy but also against certain cancers, such as the bladder (1) and colorectal, and autoimmune disorders, including melanoma (2). The proposed mechanism for BCG in all these cases is an initial stimulant to a person's immune system. The ability of BCG to confer protection against such widespread diseases has persuaded scientists to hypothesize that BCG can also confer immunoprotection in COVID-19 patients. In this review, we highlight we discuss the various vaccine-host immune interactions, particularly the role of cytokines that may have a role in conferring immuno-protection against COVID-19 in BCG vaccinated individuals.

Mechanism of Action of BCG

As summarized in Figure 1, following intradermal administration, BCG antigens stimulate/prime macrophages and dendritic cells (DCs). These cells when interacting with the actual pathogens, via Pathogen Associated Molecular

Patterns within the antigen, such as peptidoglycans, mycolic acid, etc. (2-4), readily phagocytose the pathogens, followed by the immune-mediated release of several cytokines, such as IL-1β, IL-2, IL-6, IL-8, and IL-12, IFN-gamma, MCP-1 and TNF-α (3-5). The phagocytosed pathogens are processes/degraded and presented via MHC class I and II molecules to the cells of adaptive immunity, not only promoting pathogen clearance via the release of inhibitory cytokines, such as interferons, but also aid in developing immune memory (4). It is also suggested that neutrophils play an essential role in enhancing the action of DCs via the release of IL-2 (6). Th-17 cells, T-regulatory cells, and CD-1 restricted T-cells also contribute to the BCG-immune response, however to a lesser extent. The induction of CD8+ T cells also takes place, which is known to mediate toxic effects via perforins, causing cell lysis (3, 4). On the note, the significance of CD8+ T cells in controlling SARS-CoV-2 (the virus responsible for CVID-19) is increasingly being recognized. Furthermore, non-antibody secreting B cells are also found to play a key role in enhanced immune response following BCG (7).

BCG, trained immunity, Cytokines, and Anti-viral Effect

Recent pieces of evidence suggest that BCG confers nonspecific (heterologous) antiviral immunity in addition to protecting Tuberculosis (TB) (8, 9). BCG has shown to provide immune protection against viral infections such as respiratory syntactic virus, human papillomavirus, influenza virus, etc (10). Moreover, BCG is also shown to act as an adjuvant, when co-administered with virus-targeting vaccines such as the Hepatitis B vaccine, generating enhances the antibody-mediated effects against Hepatitis B virus via increased production of cytokines (11).

Several plausible explanations have been suggested with context to the role of BCG in providing innate immunity. Heterologous lymphocytes may be involved in the activation of memory B cells that are unique to non-target antigens, hence adapting the T helper cells' (subset 1 and 17), responses to secondary non-mycobacterial infections. BCG also considerably improves the generation of inflammatory chemokines, such as IL-1β and TNF-α, from mononuclear peripheral blood cells, especially when stimulated with non-similar pathogens. This reaction promotes enhanced activation of the markers CD14, TLR4, and CD11b, as well as monocyte epigenetic reprogramming, where monocytes undergo histone alteration at the promoter of genes expressing proinflammatory cytokines, resulting in longstanding improvements in their capacity to respond to novel stimuli, culminating in a significantly prompt immune response when reactivated (12).

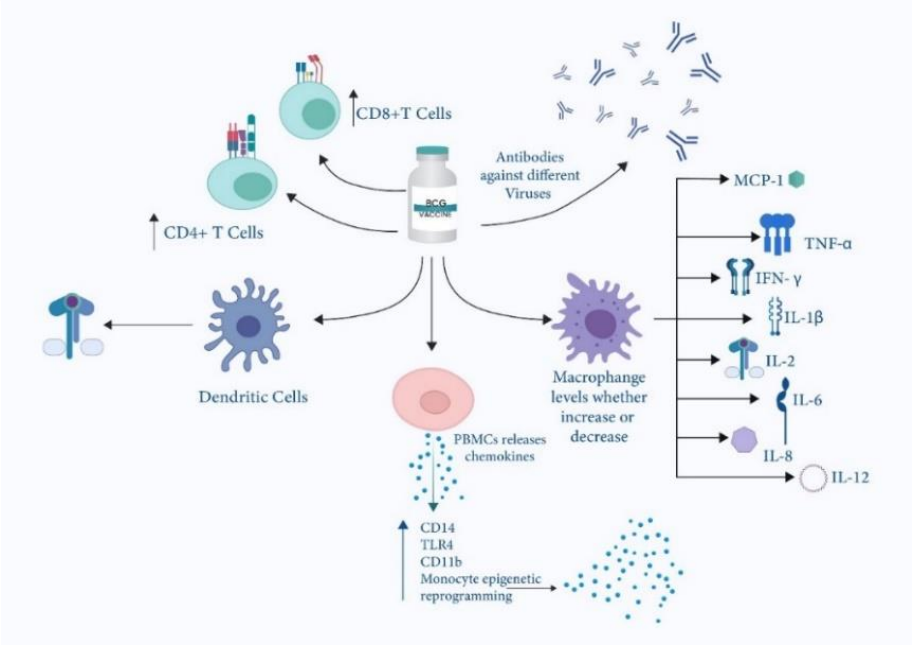

Figure 1: Proposed mechanism for immune-protective effect BCG vaccination: BCG activates macrophages and dendritic cells that induces the release of antiviral cytokines, such as IL-2, IL-1β, IFN-gamma, and TNF-α etc. BCG also stimulates the production of CD4+and CD8+ T cells and anti-viral antibodies.

An increase in monocyte-related cytokine production by the BCG vaccine plausibly confers anti-viral immunity via:

1) Generation f "cross-reactive T-cell responses" in event of viral re-exposure, leading to amplified CD4/ CD8 T-cell activity, as well as an increase in titers of functional antibodies against secondary viral infections.
2) Epigenetic reprogramming of the cells of the monocytes lineage, leading to the greater synthesis of pro-inflammatory and potent antiviral cytokines such as IL-1b, which contributes towards sustained anti-viral immunity(10-12).

Additionally, some immune studies suggest that BCG is also capable of mounting 'trained immunity', i.e. the capability of inducing innate immune memory mediated by innate cells (NK cells, macrophages, and monocytes); that produce cytokines like IL-1β and IL-6; thus developing specific cell-mediated response to protect against both viruses, i.e. DNA and RNA (10). The ability to muster trained immune response via innate cells may prove beneficial in preventing viral infections and can serve as an alternate approach for conferring immune protection against the virus. However, in presence of limited in vivo support, major clinical trials need to be performed to validate this correlation and test this alternate approach(10).

BCG-mediated lung protection and protection against respiratory viruses

Numerous trials, notably that of Wardhana and colleagues (13), have shown a significant diminution in the development of acute upper respiratory tract infection (AURTI) in individuals aged >65 years, who received monthly BCG vaccine dose for three consecutive months. Recent investigations suggest that mucosal intranasal administration of BCG confers enhanced lung protection, via an increase in antigen-specific CD4+ cells residing in lung parenchyma with enhanced proliferative capacity. Hence, it is suggested that mucosal administration of BCG has direct immune-protective effects on the lung, which can explain the synergistic role of the BCG vaccine in Influenza virus infection, where BCG enhances the removal of apoptotic cells by alveolar phagocytes, by an effect independent of IFN-Y; thus preventing lung injury (10, 14).

A similar kind of immunoprotective profile is also seen in the case of Respiratory Syncytial Virus (RSV), where BCG-mediated protection from RSV and lung pathology was mediated by the stimulation of balanced Th1 immunity (prime mechanism of action of BCG), which involves the activation of IFN-gamma secreting T cells, while significantly reducing infiltration of inflammatory cell in the airways (15, 16).

BCG coverage and COVID

The novel coronavirus outbreak began in the Wuhan city of China in 2019 as pneumonia of unknown etiology. RT-PCR testing of nasopharyngeal and oropharyngeal swabs revealed the organism as being a variant of the coronavirus (17). This viral epidemic soon converted to a WHO declared pandemic and has affected more than 218 countries worldwide. At the time of writing this article, approximately 173 million people have been infected, causing approximately 3730000 deaths.

The COVID-19 heat map has raised speculations with mortality rate significantly low in TB endemic regions where BCG forms the mandatory component of the neonatal vaccine regimen (18). A comparison, showing the distribution of countries that either has a universal BCG vaccination program, don't have one in place, or have it only for the high-risk individuals, suggests striking similarities in terms of COVID severity and BCG coverage(19, 20). Countries lacking policy of BCG vaccination, such as the USA, Spain, and Iceland, etc. reportedly have more sever COVID-19 cases as compared to countries with BCG vaccination policy such as Pakistan, Saudi Arabia, etc. These observations have been reported in other countries around the globe, including the highly developed European Union (21). However, there is no clinical evidence favoring this trend which is questionable because of an interplay by other confounding factors.

Even though published literature suggests a higher incidence of deaths from acute respiratory illnesses in low-income countries (22), the observations by Shet and colleagues (18) suggest otherwise in the case of COVID-19. The authors, after adjusting for confounders, such as a prevalent younger population structure in Low-resource countries, rate of detection of cases and time lag in deaths; the direct link between the use of BCG vaccine and lower COVID-19 attributable mortality seem to be true. While countries with sustained BCG vaccination programs seem to have lesser mortality, however, several other confounding factors could lead to the noticed variation such as low COVID-19 testing/screening rates, ratio of young vs old, ethnic distribution, backdrop of chronic diseases, and major government policies (imposing lockdown, etc.), economic conditions, availability of resources (hospitals, doctors, etc.), knowledge and awareness about preventive measures amongst the masses(23). Moreover, differences can be seen due to different strains of vaccine used by countries. Another recent retrospective cohort study by *Moorlag et al*

showed a reduction in the severity of symptoms in BCG-vaccinated COVID-19 patients, thus indicating its safe usage (24). However, further clinical trials are essential to establish the efficacy of this vaccine. Therefore, the World Health Organization (WHO) has recommended its usage only in clinical trials which are underway as specified in Table 1.

Table 1: Ongoing Clinical Trials (Registered in Clinical Trials.gov)

Study Title	Study design	Trial Identifier	Country	Phase	Population	Intervention Model description
"Outcome of COVID-19 Cases Based on Tuberculin Test: Can Previous BCG Alter the Prognosis?"	Prospective Case-control	NCT04347876	Egypt	-	100	Group 1 will comprise of COVID-19 positive individuals with a positive tuberculin test Group 2 will consist of COVID-19 positive individuals with negative tuberculin test
"BCG Vaccination to Protect Healthcare Workers Against COVID-19 (BRACE)"	Randomized Clinical trial (RCT)	NCT04327206	Australia, Spain, Netherlands	Phase 3	10078	The experimental group will receive the BCG vaccine Placebo group will receive 0.9% NaCl (saline) injection
"Reducing Health Care Workers Absenteeism in Covid-19 Pandemic Through BCG Vaccine (BCG-CORONA)"	RCT	NCT04328441	Netherlands	Phase 3	1500	The experimental group will receive the BCG vaccine Placebo group will receive 0.9% NaCl (saline) injection
"Application of BCG Vaccine for Immune-prophylaxis Among Egyptian Healthcare Workers During the Pandemic of COVID-19"	RCT	NCT04350931	Egypt	Phase 3	900	The experimental group will receive the BCG vaccine Placebo group will receive 0.9% NaCl (saline) injection
"Prevention, Efficacy, and Safety of BCG Vaccine in COVID-19 Among Healthcare Workers"	RCT	NCT04461379	Mexico	Phase 3	908	The experimental group will receive the BCG vaccine Placebo group will receive 0.9% NaCl (saline) injection

"BCG Vaccine in Reducing Morbidity and Mortality in Elderly Individuals in COVID-19 Hotspots"	Non-randomized Clinical trial	NCT04475302	India	Phase 3	2175	Interventional group: all elder individuals aged 60 - 80 years. The Control group will not be vaccinated
"COVID-19: BCG As Therapeutic Vaccine, Transmission Limitation, and Immunoglobulin Enhancement (BATTLE)"	RCT	NCT04369794	Brazil	Phase 4	1000	BCG Group (n = 500) Placebo group (n = 500): 0.9% saline solution
"Prevention Of Respiratory Tract Infection And Covid-19 Through BCG Vaccination In Vulnerable Older Adults (BCG-PRIME)"	RCT	NCT04537663	Netherlands	Phase 4	5200	BCG group Placebo group: Intradermal injection of sterile 0.9% NaCl.
"Bacillus Calmette-guérin Vaccination to Prevent COVID-19 (ACTIVATEII)"	RCT	NCT04414267	Greece	Phase 4	900	BCG group Placebo group: Normal saline
"Using BCG to Protect Senior Citizens During the COVID-19 Pandemic"	RCT	NCT04542330	-	Phase 3	1900	BCG group Placebo group: Normal saline

As a safety precaution, it is proposed that usage of BCG to combat SARS-Cov2 infection without any clinical proof can not only lead to exacerbation of immune response and worsening the disease during the stage of cytokine storm, but also a shortage of vaccine for regular immunization of newborns in TB endemic countries. While many health experts are skeptical about a vaccine administered in earlier years to have such profound protective effects in later stages of life against unrelated infections, such as SARS-COV2; this could be another reason for lower disease severity in children (25, 26).

A summary of the arguments and an explanation of differences in mortality rates between BCG immunization-laden countries has been stated in Table 2.

Table 2: Arguments in favor and denial of hypothesis and explanation of variation in mortality between BCG countries

Argument	**Support**
Favor	• Non-specific immune response mediated via trained immunity • Data supports an association between BCG vaccination and low COVID-19 cases • Protective effects against lung injury and respiratory infections in neonates • Reduced incidence of worsening symptoms in recently vaccinated BCG individuals with COVID-19 infection
Against	• No clinical trial has been done to prove the correlation • Enhanced immunity can lead to an exaggeration of inflammatory response leading to cytokine storm responsible for ARDS during SARS-COV2 infection
Explanation of differences in mortality rates between BCG immunization-laden countries	
• Different BCG vaccine strains • Different routes of administration (mucosal route seems to be more protective for lung as compared to systemic) • Use of Recombinant BCG vaccines • Boosters given or not given • Delay in BCG vaccinations (many children do not receive it right after birth) • Vaccination setting • Differences in response to BCG about the different extent of trained immunity amongst individuals • BCG scars/ marks used as a sign of vaccination if records are unavailable which might be misleading • Different timing for introduction of sustained programs	

CONCLUSION

In conclusion, the BCG vaccine which has been used for decades in conferring immunity against tuberculosis has proven time and time again that it does have other covert immune-modulatory properties that can prove beneficial in extra-tuberculous respiratory tract infection, such as SARS-CoV-2. Further clinical trials and evidence-based studies are warranted to prove correlations and explain the observations mentioned above.

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JT and MIS: Conceptualization, original draft
FN: original draft
SHA: original draft, review & editing, supervision
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