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The impact of Bacillus Calmette-Guérin (BCG) vaccination on the course of COVID - effectiveness and possible mechanism

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Abstract

Nowadays, Bacillus Calmette-Guérin (BCG) is the most commonly used vaccine worldwide, used typically for tuberculosis but also in non-muscle invasive bladder cancer management. Basing on previously confirmed antiviral features of BCG and first statistic data reports, BCG usage in COVID-19 prevention was claimed and its potential molecular mechanism was searched. In scientific literature there was 10 publications proving several possible mechanism of BCG interaction with SARS-CoV-2 infection immune response. The most often was cross-reactivity between various BCG and SARS-CoV-2 antigens, including those crucial for their clinical effects. In most cases, those antigens linking was shown according to bioinformatical research. According to this research, the potential role of BCG in COVID-19 management should be considered as significant, at least until the clinical trials conducted nowadays will be over.

Key words: BCG; Bacillus Calmette-Guérin; vaccine; COVID-19

Introduction

Bacillus Calmette-Guérin (BCG) vaccine against tuberculosis was first administered in 1921, the year of its discovery. In 1928 the League of Nations declared its safeness, which opened the gate to BCG popularity. Nowadays, BCG worldwide is the most commonly used vaccine – approximately 75% of children born in 2002 were injected with it[1]. Recent decades came up with usage of BCG that are unrelated to M. tuberculosis – like non-muscle invasive bladder cancer (NMIBC) chemotherapy, where intravesical intake is strongly recommended as an adjunct therapy [2].

First confirmed case of novel coronavirus pneumonia was on December 31, 2019 in Wuhan, PRC. Its high contagiousness resulted with a pandemic status announced by WHO on March 20, 2020 [3]. Lately, the potential role of BCG in COVID-19 prevention started to be suspected by researchers[4]. Their suspicions were based on previously-confirmed antiviral effects of BCG shown on viral [5–7] bacterial [8,9] or fungal [10] infections, experimental model[11], or first epidemiological studies [12].

Epidemiological data

Although single articles reported insignificance of lower COVID-19 prevalence and mortality in countries with strict BCG vaccination policy[13–17], the general consensus was in favour of this hypothesis[18–27]. One representative review from May 2020, appoints median amount of COVID-19 cases per 1 million people is 3,543 in 14 non-BCG countries, whereas 977 in 35 ones using BCG commonly. Comparing amounts of COVID-19 deaths ends with similar results – 338 vs 16[18]. Although comparisons between countries (from this period at least) seem to be unreliable due to different lockdown laws and different pandemic awareness level, this report and many similar in scientific literature had a crucial influence on research importance. The most recent data analyses reports that BCG-vaccination impact on COVID-19 pandemics was statistically significant, but only at the beginning of the pandemic[28,29].

Clinical data

In a Dutch multi-centre retrospective cohort study the protective role of recently admitted (during coronaviral hospitalisation; within 1 month before tests) BCG in COVID-19 course – additionally confirming safety of this administration during active infection[30]. Other, Canadian, case-control study denied long-term protection of BCG on COVID-19 course in hospitalised patients (n = 920) in compare to control group (n = 2,123)[31]. Similar results were achieved by French research group, basing on population studies with regression discontinuity design[32]. Other study basing on surveys (n = 1,233) suggested that intake of BCG in childhood may increase risk of COVID-19 infection[33]. A Turkish multi-centre study suggests that exposition to *M. tuberculosis* (infected patient or vaccine) results with more severe course, but lower mortality to COVID-19 in healthcare workers[34]. An Italian survey-based research showed no significant difference in COVID-19 prevalence among physicians vaccinated by BCG[35]. A Pakistani experimental research among 103 patients (64 with and 39 without prior BCG vaccination) showed no impact of BCG on the severity of COVID-19 but that it could have a protective role with a low mortality rate in already infected patients[36]. Other experimental study showed that post-BCG and post-anti-SARS-CoV-2 vaccine immune responses may work synergistically – participants revaccinated with BCG (n = 30) had significantly higher level of cytokines (IL-1β, IL-4, IL-6, IL-12p70, IL-13, IL-18, GM-CSF, INF-γ, and TNF-α) and antibodies titers in compare to placebo group (n = 30)[37]. Cross-sectional studies among urinary bladder patients treated with BCG did not show significantly positive effect of its intravesical injection on COVID-19 course[38,39].

Mentioned suggestions and doubts in retrospective studies resulted with a few randomised clinical trials on protective role of BCG revaccination on COVID-19 severity. Currently, the only available full results are from Malawi, where no significant effect of BCG on COVID-19 mortality was shown[40]. The clinical trials in other countries (Canada, USA, Mexico, Brasil, Netherlands, Denmark, France, Germany, Poland, Hungary, Greece, Cape Verde, Guinea-Bissau, Egypt, Mozambique, South Africa, Iran, India and Australia) are in the recruitment or active phase. Their currently available results classified BCG (by independent reviewers) as a supportive/adjuvant factor in COVID-19 therapy[27,41].

Coinfections of COVID-19 and tuberculosis are rarely reported and usually have benign course – independent reviewers don't find tuberculosis as major mortality factor in coronaviral pandemics [42], however this article was widely criticised[43]. For example, other cohort study from Philippines showed that the risk of recovery in COVID-19 patients with tuberculosis was 25% lower than in those without[44]. Scientific literature reports two cases of rescued patients with even triple infection – SARS-CoV-2, *M. tuberculosis* and HIV[45]. On the other hand, clinicians should be aware that rifampicin-induced pneumonitis may mimic COVID-19 symptoms[46].

BCG strains and administration ways

BCG vaccine could be typed to various number of strains (simplified typology is in according to different amount of gene IS6110 copies)[47]. Differences in data analyses might be caused by using different BCG vaccine strains in different countries – their inequality in COVID-19 protection is postulated by one commentary article[24]. Then, the other research group confirmed those inter-strain differences with adaptive potential from the novel SARS-CoV-2 variants to omit post-BCG immune effects[29]. It corresponds with past reports suggesting different efficacy of BCG strains in both tuberculosis and urinary bladder therapy[48,49], and in its heterogenous effect on innate immunity[50]. One research team suggested that selected substrains of BCG may be used therapeutically in melanoma too[51].

The discrepancy between expectations and clinical results might be caused by BCG way of administration. Until 70's dominant one (in tuberculosis prevention) is subcutaneous, whereas some researchers suggest that original, oral one might be better for mucosal immune response stimulation – crucial in first-line anti-coronaviral defence[9]. On mice model, where the BCG vaccine was admitted intravenously during SARS-CoV-2 infection, the course of disease was more benign (this protective effect was absent when BCG was admitted subcutaneously)[52].

To check the potential role of BCG during COVID-19 pandemics, much basic science research was performed. Mechanisms proven in those research will be summarised in this review to make their clinical usage more affordable. Those mechanisms were strictly separated from ones suspected in according to proven impact of BCG in other viral infections, or deducted basing on suspected linkings between post-BCG and post-SARS-CoV-2 immune responses.

Methods

Two most referent databases – PubMed and Google Scholar – were used to collect articles (access date: November 23-25, 2021). Search was done using keywords "BCG" and "COVID-19", with secondary selection by abstracts (in according to their suitability to main topic and/or language affordability). Records were imported to citation manager – Mendeley 1.19.8, where from they were read and put into final article.

Molecular mechanisms confirmed and suspected basing on experimental models are described in results section, whereas those postulated basing on clinical experiences and similarities in molecular pathways, but shown separately to SARS-CoV-2 infection and BCG administration immune responses, may be find in discussion to underline the need of its additional confirmation.

Results

Cross-reactivity

According to general immune knowledge, the most plausible mechanism linking immune responses caused by different paratopes is their molecular similarity. Research based on it included both immunohistochemical and bioinformatic methodology, appointing protein-protein interactions as main research aid.

Australian research group identified 8 novel BCG-derived peptides, which are structurally homologous to peptides associated with SARS-CoV-2 virus. In vitro test showed enhanced reactivity of CD4+ and CD8+ T-cells against SARS-CoV-2 virus, if they were previously exposed to mentioned BCG-derived peptides (in compare to control group without exposition)[53].

The immunohistochemistry test showed that antibody against SARS-CoV-2 capsid protein also affiliates to Mycobacterium sp. proteins. Thus, the binging was confirmed by computer database analyses, what lead authors to potential usage of SARS-CoV-2 antibodies as diagnostic factor in mycobacterial infections, and that mechanism confirms cross-reactivity of BCG vaccine and SARS-CoV-2 virus[54].

Research group from Hungary identified (by computer simulation of protein interactions) 112 T-cells epitopes and 690 B-cells epitopes, which were similar on respective cells exposed to BCG and SARS-CoV-2 antigens. Those similarities include receptor binding protein and spike glycoprotein – crucial to antiviral reaction[55].

Japanese researchers, using also computer databases, found 9 epitopes similar between BCG and SARS-CoV-2, with moderate or high level of binding affinity to human leukocyte antigen (HLA) class I[56].

Using similar bioinformatic methods, the other research group appointed 4 BCG proteins named Rv0934, Rv3763, Rv3875, and Rv2997, which share features with S1 protein from SARS-CoV-2, what was interpreted by authors as potential cross-reactivity pathway[57].

By in silico test and comparing to another vaccines, Tunisian research group confirmed cross-reactivity between MPB64 immunogenic protein from BCG, with protein E from SARS-CoV-2 virus. On the other hand, the researchers didn't find this reactivity significant in compare to separate pair with vaccines against HBV, tetanus and measles[58].

All appointed potential mechanisms of cross-reactivity among BCG and SARS-CoV-2 antigens are collected in table 1.

Tab. 1. Proven cross-reactivity pathways between SARS-CoV-2 and BCG vaccine antigens

BCG antigens	SARS-CoV-2 antigens	Methods	Reference
Non specified	Capsid proteins	Immunohistochemistry	[54]
Macro-domain-containing protein	NSP3 (papain-like proteinase)		[53]
UPF0189 protein			
RecB nuclease	NSP13 (helicase)		
Zinc- metalloprotease-FtsH			
RecB nuclease (two regions)			
UDP-N-acetylmuramoylalanyl-D- glutamate-2,6-diaminopimelate ligase	ORF1ab	Bioinformatics	[56]
Type VII secretion AAA-ATPase EccA			
PPOX class F420-dependent enzyme			
Alcohol dehydrogenase			
Metal-transporting ATPase			
Sugar ABC transporter permease			
Rv0934	S1 protein		[57]
Rv3763			
Rv3875			
Rv2997			
MPB64 immunogenic protein	E protein	In silico test	[58]

Similar epigenetic pattern

Epigenetic analyses of peripheral blood cells, succeeded by computer analyses done by multinational team lead up to 62 common genes with enlarged expression after both BCG vaccination and SARS-CoV-2 infection. Secondary analyses of proteins interactions showed 10 hub genes: ITGB2, CXCL8, CXCL1, CCR2, IFNG, CCL4, PTGS2, ADORA3, TLR5 and CD33[59].

Toll-like receptors (TLR) pathway

Egyptian research group verified a hypothesis that BCG may control SARS-CoV-2 infection via monocytic pathways. Basing on data from Gene Expression Omnibus, they confirmed mentioned hypothesis and appointed a few molecular mechanisms, including: differential expression of Toll-like receptors (TLR), including upregulation of CXCL10 and downregulation of CXCL8, CCL3L1, IL1B, CCL3[60].

Memory cells activation

The research done in India by Kumar et al. showed that recent BCG vaccination (within 1 month) done during COVID-19 hospitalisation, increases levels of IL-7 and IL-15, whereas decreases levels of IL-2 and IL-21. It also increases percentage of central and effector memory cells among CD4+ ones, decreasing percentage of naïve, transitional memory and stem cell memory cells among CD4+ ones. In respective subpopulations of CD8+ T-cells effects were the same – adding increased level of terminal effector memory CD8+ T-cells. The level of T-regulatory cells was decreased after intervention. Both control (n = 32) and test (n = 54) group was recruited from elderly people (60-80 years old)[61].

Dendritic cells recruitment

Previous research done by the same Indian team, on the same control and test group, resulted with significantly increased amount of dendritic cells (both plasmacytoid and myeloid type). It coexisted with increased level of type III IFN, IL-28A and IL-29 and decreased level of type I IFN (IFNα and IFNβ)[62].

Discussion

Cross-reactivity

The theories suggesting importance of post-BCG cross-reactivity are formulated nearly from the beginning of the pandemic, and are considered to be significant for post-BCG immunity against SARS-CoV-2[8,63]. Various experimental models basing on bioinformatics make those suspicions very plausible, whereas those based on immunohistochemistrical staining are confirmed, although they appoint different target proteins (Tab.1). In compare, for example, cross-reactivity between anti-measles vaccination and anti-HIV response is strongly confirmed[64].

The Rv3875 antigen from BCG subunit, which was suggested as cross-reactive with viral protein S1, when exposed to Peripheral Blood Mononuclear Cells, results in their response[57,65]. This seems to be potential molecular pathway in postulated protective role of BCG vaccination in COVID-19 course. The other BCG protein – BC01 – is claimed to be useful as adjuvant in DNA vaccine against coronavirus[66].

Hexosamine hypothesis

Mexican research group came up with a hypothesis that cytokine storm present during COVID-19 course is caused by hexosamine biosynthetic pathway, which by virus tries to use cellular glucose to replicate itself. The potential protective role of BCG might be caused by inhibitive impact of vaccine on this metabolic pathway, what could slow viral replication down[67,68].

Post-BCG immune response mechanisms

The mayor effect of BCG impact on future infections, seems to be an innate immune response improvement[69]. This effect was reported even by Calmette – he wrote about at least 4-fold reduction of non-tuberculous mortality rate (from 16-26% to 4%) in vaccinated children in 1926-1928 – and those observations were confirmed worldwide [9]. Nonspecific protective effect is constituted by innate immune cells such as monocytes and natural killer cells, independent of T- and B-cell memory (trained immunity)[25,65,69,70].

The anti-infective effect of BCG is based on its impact on monocytes molecular pathways. Vaccination implicates epigenetic reprogramming resulting in cell responsibility increase. Synthesis of proinflammatory cytokines (IL1β, IFNγ and TNFα) also is elevated[8,65]. Unfortunately, the only article focused on the effect of BCG on interferons among COVID-19 patients, excluded type II (IFNγ) from its interest[62].

The crucial experiment showed that BCG can epigenetically increase histone methylation in monocytes by NOD2-gene-pathway, what results up with innate immunity improvement. In this molecular pathway there is significant fold of INFγ, Il-1β and TNF secretion, and in CD11b and Toll-like receptors 4 expression. It was the first example of

trained immunity (adaptive features of innate immunity) reported in humans[71]. Minor innate immunity pathway is mediated via MHC proteins – its activation by BCG results with enlarged amount of γδ T-cells with direct antiviral features[72]. Both of this mechanisms are proposed also in BCG-induced protection on COVID-19[72–74].The control of monocytic TLR-dependent pathway by BCG in novel coronaviral infection was confirmed according to bioinformatical data[60].

Post-BCG response includes also T-cells, which trains their adaptive response. After an administration, level of both CD4+ (Th) and CD8+ (Tc) elevates, with Th1 dominance over Th2, what is caused by elevated level of specific IgG2c antibodies, and proinflammatory cytokines IFNγ and TNFα [65]. This mechanism also is claimed to be involved in BCG-caused COVID-19 protection[74–76].

Antiviral effects of type I interferon also is decisive – its secretion during COVID-19 infection is decreased [77]. This effect may be reversed by BCG vaccination, what is confirmed in available experimental data[62].

However many molecular effects of BCG vaccine were reported, its full view isn't already known[70].

Work of many research teams resulted with a few confirmed molecular effects which can be significant in post-BCG protection on COVID-19 course. Even if the suspected protection won't be confirmed in clinical trials, the BCG vaccine administration should be performed worldwide even at the time of COVID-19 pandemics. Both in case of urinary bladder cancer due to possible urgence [78] and tuberculosis because it could be masked by SARS-CoV-2 infection[79].

The importance of the research on BCG impact on COVID-19 course is crucial especially for developing countries, which were enforced by pandemic status to rearrange their healthcare systems – usually forwarding infrastructure from tuberculosis to COVID-19. This strategy might be counter-effective if BCG was effective both in tuberculosis and COVID-19 prevention[80–84]. This effect exist also in developed countries, where coronaviral pandemics may impede tuberculosis eradication[85,86].

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