Clinical Microbiology and Infection 25 (2019) 1473–1478

Contents lists available at ScienceDirect

Clinical Microbiology and Infection

journal homepage: www.clinicalmicrobiologyandinfection.com

Narrative review

Non-specific effects of BCG vaccine on viral infections

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ARTICLE INFO

Article history:
Received 25 January 2019
Received in revised form
17 April 2019
Accepted 18 April 2019
Available online 2 May 2019

Editor: I. Gyssens

Keywords:
BCG
Innate immune memory
Non-specific effects of vaccines
Trained immunity
Viral infection

ABSTRACT

Background: Some strains of Bacillus Calmette–Guérin (BCG) vaccine not only confer protection against disseminated forms of tuberculosis, but also reduce all-cause mortality by the induction of protection against infections with non-related pathogens.
Objectives: We review evidence for non-specific protection induced by BCG vaccination against viral infections, discuss possible mechanisms of action, and summarize implications for vaccination policies and vaccine discovery.
Sources: Relevant studies retrieved from PubMed and clinicaltrials.gov.
Content: Numerous epidemiological, clinical and immunological studies demonstrate that BCG vaccination impacts the immune response to subsequent infections, resulting in reduced morbidity and mortality. Important lines of evidence indicating that BCG protects against viral pathogens comes from experimental studies in mice showing that BCG offers protection against various DNA and RNA viruses, including herpes and influenza viruses. Recently, the effect of BCG on an experimental viral infection in humans has been demonstrated. These effects are thought to be mediated via the induction of innate immune memory and heterologous lymphocyte activation, resulting in enhanced cytokine production, macrophage activity, T-cell responses and antibody titres.
Implications: The discovery of innate immune memory has greatly improved our understanding of the mechanisms underlying the non-specific effects induced by BCG vaccination. However, a full understanding of the molecular mechanisms that underlie this phenomenon is still evolving. By identifying the factors that impact the non-specific effects of BCG, we will take an important step towards novel therapeutic options and vaccination strategies, which might lead to a reduction in severe morbidity and mortality associated with viral infections. **S.J.C.F.M. Moorlag, Clin Microbiol Infect 2019;25:1473**

Introduction

The tuberculosis (TB) vaccine Bacillus Calmette-Guérin (BCG) is a live attenuated strain derived from an isolate of *Mycobacterium bovis*. BCG is currently one of the most widely used vaccines in the world, with more than 4 billion BCG-vaccinated individuals globally and an additional 100 million newborn BCG-vaccinated children every year. The vaccine offers protection against disseminated forms of TB in children, including TB meningitis and miliary TB. However, its efficacy against pulmonary TB in adults is limited and ranges from 0 to 80%, depending on various factors such as geographical location and previous exposure to environmental mycobacteria [1]. Interestingly, soon after its introduction in the 1920s, epidemiological studies demonstrated that the BCG vaccine reduces infant mortality independent of its effect on tuberculosis (reviewed in [2,3]). Several observational studies in West Africa demonstrated a 50% reduction in overall mortality in children vaccinated with BCG, an effect too big to be explained by protection against tuberculosis alone [4–6]. Similar observations were made in other countries and more recently these findings have been validated in randomized controlled trials (RCTs) and in a meta-analysis of three RCTs [7–9]. The mortality reduction in infants by BCG appeared to be due to the induction of protection against unrelated infectious agents. These beneficial effects have been called ‘heterologous’ or ‘non-specific’ effects. Here we review the evidence for the protection of BCG against viral infections, discuss

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https://doi.org/10.1016/j.cmi.2019.04.020
1198-743X/

the mechanisms responsible for this effect of BCG and summarize implications for vaccination strategies.

Impact of BCG vaccination on viral infections in humans

In humans, limited clinical evidence suggests that BCG vaccination may have non-targeted protective effects against viral infections (Table 1). In a trial in which children were randomized to receive BCG at birth or a delayed BCG vaccination, reduced mortality in the BCG-vaccinated neonates could be attributed to prevention of neonatal sepsis, respiratory infections and fever [7,9]. Although the authors do not discriminate between bacterial and viral infections, it could be that this result is at least partially explained by a reduction in viral infections. However, in a controlled study in neonates under 2000 g in India no such effect was observed [10], which might be partially explained by the use of a different BCG strain as different strains have different immunological effects [11]. A case–control study performed in young children in Guinea-Bissau investigated the impact of BCG on the incidence of respiratory syncytial virus (RSV) infection specifically, and results suggested that BCG vaccination may reduce the incidence of acute lower respiratory tract infections caused by RSV [12]. A similar effect of BCG on respiratory tract infections was found in elderly people. Wardhana et al. [13] vaccinated elderly people once a month for the three following months to examine the impact of BCG on the incidence of acute upper respiratory tract infections. They showed that BCG vaccination resulted in a significant reduction in the amount of respiratory tract infections compared with the placebo [13]. Furthermore, a clinical trial performed in Japan demonstrated the impact of BCG vaccination on the risk of pneumonia in tuberculin-negative elderly people and found a reduced risk in subjects that developed a positive tuberculin test results upon BCG vaccination [14]. In addition, a very recent study in adolescents in South Africa also reported an almost threefold reduction of respiratory tract infections by BCG vaccination [15]. However, in the studies on the effect of BCG on respiratory infections the effect could have been due to an effect of BCG on bacterial infections, as no discrimination is made between respiratory tract infections caused by bacteria or viruses.

Besides offering prophylactic protection against infections, BCG has also been shown to be effective as non-specific immunotherapy in virus-mediated clinical conditions. One known example of these effects is the positive effect of BCG against cutaneous and genital warts caused by human papillomavirus (HPV). An Egyptian placebo-controlled trial, with 40 subjects in each group, showed resolution in 65% of children with common warts that were treated with topical viable BCG; no response was seen in the group that received topical saline as control [16]. Similarly, in Indian trials a complete clearance of viral warts of 48.5% (16 out of 33) was found after three BCG vaccinations at a 4-weekly [17] interval or a 28.6% clearance after one single vaccination [18]. The study however also mentions that side effects such as pain at the injection side and scars or abscess formation were more common after repeated BCG vaccinations, and that the development of adverse effects precluded any further BCG vaccination in 57% of the subjects.

Finally, BCG can also improve the response to vaccines directed against viral infections. A randomized controlled trial in 40 human healthy volunteers demonstrated that BCG vaccination prior to influenza vaccination enhanced antibody titre against the 2009 pandemic influenza A (H1N1) vaccine strain. In addition, a trend towards an increase and longer sustained interferon (IFN)-γ production upon stimulation with influenza vaccine was observed in BCG-vaccinated individuals compared with control [19]. Moreover, effects have been observed for the impact of BCG vaccination on the responsiveness to other vaccines such as hepatitis B vaccine (HBV) [20,21]. Compared with HBV alone, BCG together with HBV enhanced *in vitro* cytokine production in human whole blood and enhanced HBV-specific antibody production in neonatal mice *in vivo* [22]. As the magnitude of antigen-specific antibody titres is seen as the primary correlate of protection against various viruses including influenza virus [23], enhanced antibody titres against viral pathogens upon BCG vaccination may provide indirect evidence of enhanced protection against infection.

Table 1
Overview of non-specific effects of BCG vaccine described for various viral infections

	Virus	Study type	Effect of BCG	References
Human studies	Yellow fever vaccine	RCT	Reduced yellow fever vaccine titres correlating with IL-1β production	Arts et al. [42]
	HPV	RCT	Improved clearance of viral warts	Salem et al. [16]
		Case series		Podder et al. [17]
		Case series		Daulatabat et al. [18]
	RSV	Case–control study	Non-significant association of fewer RSV infection in Guinea-Bissau in young children	Stensballe et al. [12]
	Influenza A (H1N1)	RCT	Enhanced antibody production	Leentjens et al. [19]
	HSV	Case series	Reduced episodes of clinical HSV infection	Anderson et al. [49]
		Case series		Hippmann et al. [50]
Animal studies	HSV 1	CD-1 mice	Enhanced survival	Floc'h et al. [35]
	HSV 2	—	Enhanced survival and protection from infection	Starr et al. [26]
		CD-1 mice		Floc'h et al. [35]
	Influenza A	—	Reduced viral titres of against influenza A virus	Spencer et al. [24]
		C57Bl/6 mice	Reduced inflammation	Mukherjee et al. [25]
		CD-1 mice	Enhanced survival	Floc'h et al. [35]
	Influenza A (H7N9)	BALB/c mice	No increased protection	de Bree et al. [29]
	Hepatitis B	C57Bl/6 mice	Enhanced antibody production	Scheid et al. [22]
	Japanese encephalitis	BALB/c mice	Delayed occurrence of clinical symptoms and increased survival	Kulkarni et al. [30]
	Encephalomyocarditis virus	C57B1/10	Enhanced resistance (induced by nonviable *M. tuberculosis*)	Lodmell and Ewalt [31], Lodmell and Ewalt [32]
	Ectromelia virus	DDN mice	Enhanced survival and increased IFN-γ production	Suenaga et al. [33]
		DDN mice		Sakuma et al. [34]
	Vaccinia	BALB/c mice	Protection from infection (induced by MDP)	Ikeda et al. [27]
		C57Bl/6 mice	Protection from infection and increased IFN-γ production	Mathurin et al. [37]

Strain of mice unknown; BCG, Bacillus Calmette–Guérin; IL, interleukin; IFN, interferon; RSV, respiratory syncytial virus; HSV, herpes simplex virus; HPV, human papilloma virus; MDP, muramyl dipeptide; RSV, randomized controlled trial.

Increased resistance of BCG-treated mice to viral infections

Experimental models have provided increasing evidence suggesting an effect of BCG on secondary viral infections (Table 1). Spencer et al. [24] showed an increased capacity of BCG-stimulated macrophages in mice in reducing viral titres of influenza A virus compared with control. Additionally, a controlled *in vivo* study in mice demonstrated that BCG vaccination protects against influenza virus infection, an effect independent of IFN-γ [24]. These findings are in line with the results found by Mukherjee et al. [25], who showed that administration of BCG protects against influenza virus A by increased levels of efferocytosis by alveolar phagocytes compared with placebo, which resulted in reduced inflammation and lung injury. Pulmonary delivery of BCG was, however, shown to have the best effect, a method of administration that is unlikely to become available for humans [25]. One single BCG vaccine intradermal or intraperitoneal also protected from herpes simplex virus type 2 in a controlled murine model with newborn mice [26]. Subcutaneous administration of muramyl dipeptide (MDP), a component of the mycobacterial cell wall, protected against vaccinia virus and herpes simplex virus type 2 in a controlled study in mice. This effect was independent of IFN-γ induction and mediated by peritoneal macrophages [27]. Similarly, it has been reported that mice that received MDP component of mycobacterial peptidoglycan are protected against Sendai virus infection [28]. A more recent controlled murine study showed that intravenous BCG induced increased cytokine production by both splenocytes and peritoneal macrophages upon *ex vivo* restimulation with various unrelated pathogens in mice. However, administration of BCG did not result in increased protection against avian influenza A (H7N9) infection, which may be due to differences in dose, route of administration or BCG strain between the studies [29].

In an additional study, Kulkarni et al. [30] showed that BCG might offer protection against Japanese encephalitis virus infection in mice. In an experimental controlled model in mice, BCG vaccination led to a delay in the occurrence of clinical symptoms and increased survival compared with Phosphate-buffered saline (PBS)-inoculated mice [30]. Furthermore, enhanced resistance against encephalomyocarditis virus [31,32], ectromelia virus [33,34], herpes simplex type 1 and influenza A2 viruses was observed in BCG-vaccinated mice compared with control mice [35]. Most of the animal studies are performed decades ago and different types of mice as well as different BCG strains are used in the various studies.

Mechanisms explaining BCG-induced protection against viral infections

Heterologous lymphocyte responses

Two possible mechanisms have been proposed as an explanation of the beneficial non-specific effects of BCG (Fig. 1). First, BCG has been shown to induce heterologous lymphocyte responses, resulting in enhanced immune responses to secondary unrelated infectious agents [36]. For example, BCG vaccination protected mice against infection with vaccinia virus via increased IFN-γ production by $CD4^+$ cells [37]. Heterologous lymphocyte responses can also involve the activation of $CD4^+$ and $CD8^+$ memory cells that are specific for non-targeted antigens, thereby modulating Th1 and Th17 responses to non-mycobacterial secondary infections. In human healthy volunteers, BCG vaccination enhanced non-specific Th1 and Th17 responses for at least 1 year after vaccination [38]. Furthermore, in patients with recurrent respiratory papillomatosis, which is caused by human papillomavirus in 90% of the cases, BCG therapy restored the efficiency of the antiviral T-cell response by stimulating Th1 cytokines and Treg induction [39].

Innate immune memory

Until less than a decade ago, only B- and T-lymphocytes were considered to be able to build memory responses. However, numerous recent studies have demonstrated that the functional programme of innate immune cells is changed upon certain

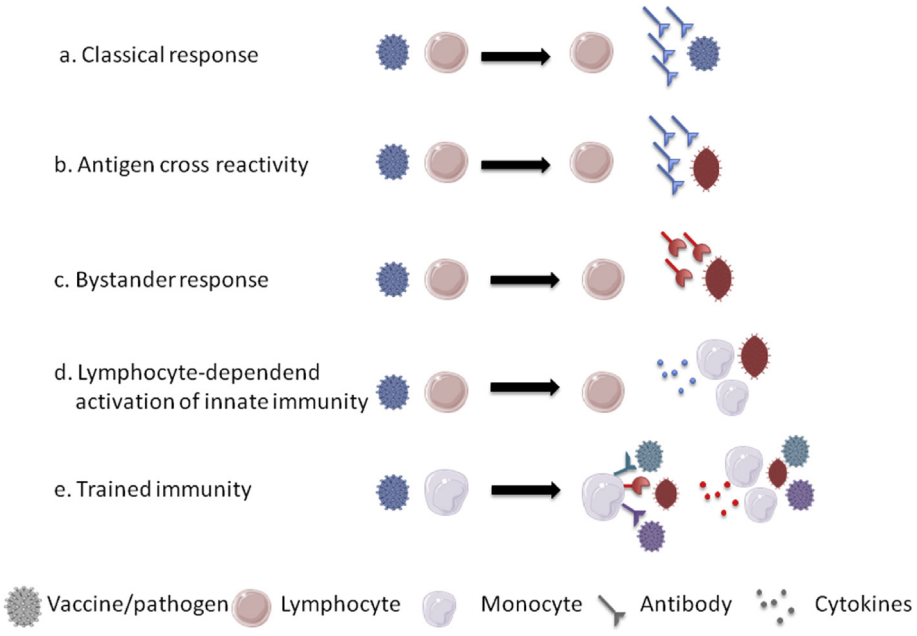

Fig. 1. Heterologous or non-specific effects of vaccination. (a) Classical vaccination response, which induces specific antibodies. (b) Antigen cross reactivity with cross-reactivation antibodies to related antigens on other pathogens. (c) Bystander response, with activation and antibody production by other lymphocytes. (d) Cytokines produced by lymphocytes after vaccination activate the innate immune system. (e) Trained immunity, with epigenetic and metabolic reprogramming of monocytes resulting in enhanced immune responses, such as enhanced cytokine and Reactive oxygen species (ROS) production, to related and non-related pathogens.

infections or vaccinations, resulting in an increased immune response when cells encounter a secondary stimulus [40]. The induction of a non-specific memory in innate immune cells was named 'trained immunity' and is mediated by epigenetic and metabolic rewiring [40,41]. Evidence suggests that the key mechanism by which BCG induces its non-specific effects is probably via the induction of immunological memory in innate immune cells, especially natural killer (NK) cells, monocytes and macrophages rather than via T-cell- and B-cell-based adaptive immune mechanisms. In severe combined immunodeficiency (SCID) mice, which lack functional T- and B-cells, BCG vaccination provided protection against a secondary non-mycobacterial challenge, demonstrating the importance of innate immune cells in mediating this effect [41].

In healthy human volunteers, BCG vaccination strongly enhanced the production of pro-inflammatory cytokines such as interleukin (IL)-1 β and tumour necrosis factor (TNF)-α by peripheral blood mononuclear cells for up to 3 months after vaccination upon *in vitro* stimulation with unrelated pathogens [41]. This was associated with an increase of the activation markers CD11b, TLR4 and CD14 and epigenetic reprogramming of human monocytes at the promoter sites of genes encoding for pro-inflammatory cytokines.

Based on these findings, we hypothesized that the BCG-induced increase in cytokine production in monocytes may contribute to a better clinical outcome during a secondary viral infection. We investigated the impact of BCG on a viral infection in human healthy volunteers using the live attenuated yellow fever vaccine (YFV) as a model of a viral human infection [42]. One month after BCG or placebo vaccination, all volunteers were vaccinated with YFV and levels of viraemia were compared between volunteers that had received BCG 1 month earlier and the volunteers who had received placebo vaccination. Volunteers who had been vaccinated with BCG showed a significantly lower amount of circulating virus than subjects that received placebo vaccination. Interestingly, this correlated with epigenetic changes induced in circulating monocytes in these volunteers. Finally, we examined whether the induction of trained immunity, as measured by the induction of *ex vivo* cytokine responses, correlated with yellow fever viraemia. No correlation was found between viraemia and post-vaccination increases in concentrations of IL-6, TNFα or heterologous T-cell responses (IFN-γ, IL-17 and IL-22). However, the induction of IL-1β production capacity after BCG vaccination was a strong predictor of lower levels of viraemia after yellow fever infection. These findings suggest that the BCG-induced enhanced IL-1β production during viral infections, rather than IFN-γ production by lymphocytes and NK cells, underlies the protection by BCG against viral infections. The protective role of IL-1β in this study is in line with recent findings indicating a crucial role for IL-1β in anti-viral immunity [43]. For example, it was shown that mice lacking the receptor for IL-1β or the inflammasome components NLRP3, ASC and caspase-1 are highly susceptible to virus infection and display reduced survival rates compared with control mice following viral challenge [44,45].

Implications and future directions

As discussed in this review, there is strong evidence that suggests that BCG vaccination reduces all-cause mortality, most likely by protection against unrelated infections. A first line of evidence indicating a protective effect against infections caused by viral pathogens resulted from various *in vitro* and *in vivo* studies in mice. More recently, we have shown that BCG vaccination protects against an experimental viral infection in human healthy volunteers. As described in this review, different immunological mechanisms might explain the non-specific effects of BCG vaccination on viral infections. First, BCG may induce cross-reactive T-cell responses leading to increased $CD4^+$ and $CD8^+$ T-cell activity upon exposure to a subsequent viral infection [37,46]. In addition, an increase in functional antibody responses against secondary viral infections upon BCG vaccination has been observed [19,21]. Since cytotoxic T-cell activity and neutralizing antibody production by B-cells are critically involved in the immune response to viruses, these effects may partially explain the non-specific beneficial effect of BCG. Second, BCG induces epigenetic changes in monocytes/macrophages, resulting in an increased production of proinflammatory cytokines such as IL-1β. As IL-1β is considered to play an important role in anti-viral immunity [44,47,48], an enhanced increase of IL-1β may contribute to protective immunity against viral infections.

The non-specific effects of BCG might have important implications for prevention and treatment of viral infections and vaccination strategies. Firstly, administration of BCG may be used as a novel strategy to protect against viral infections, especially against those infections that cause global pandemics and for which an effective vaccine is currently not available. This could be particularly important for very young children, elderly people and others who are at increased risk of illness and death from infection. For example, the administration of BCG might be an attractive strategy to decrease influenza-related morbidity and mortality. In addition, BCG administration might be considered as treatment to prevent reactivation of latent viruses, such as varicella zoster virus (VZV), cytomegalovirus (CMV) or Epstein–Barr virus (EBV). In a small trial of 15 patients, BCG vaccination reduced recurrence of genital herpes [49]. In a larger trial with 109 patients suffering from recurrent herpes simplex virus (HSV) infections, both the frequency and duration of recurrent HSV infections was significantly reduced after BCG vaccination, an effect that lasted even up to 10 years after vaccination [50]. However, the efficacy of BCG in preventing the reactivation of different herpes viruses needs to be studied in larger trials.

Murine studies showed suppression of virus growth by BCG or MDP [28,37]. Inhibition by BCG of viral replication in acutely and chronically infected cells might therefore be considered as a therapeutic option for conditions such as HIV and hepatitis-associated liver disease. However, the effect of BCG on viral replication in humans remains unknown and future studies are necessary to address this question. Furthermore, the use of effective induction of innate immune memory in combination with adaptive immune responses has potential for stimulating the development of new generations of vaccines that use BCG to enhance antibody titre and T-cell responses to other vaccines, as recently proposed with a novel HIV-1 vaccine [51], simian immunodeficiency virus vaccine [52] and hepatitis C vaccine [53]. In addition, the ability of BCG to enhance responses to viral vaccines may be used to improve immunogenicity of vaccines that currently induce suboptimal immunity, such as HBV and varicella vaccine. Finally, as BCG may reduce morbidity and mortality caused by viral infections, new TB vaccine candidates should be evaluated for their non-specific beneficial effects before the current BCG vaccine is replaced.

Although the studies that we have reviewed here suggest that BCG might offer protection against viral infections, direct clinical evidence for the prevention of viral infections *in vivo* in humans is limited. Therefore, large clinical randomized controlled trials on all-cause mortality in which the effects of BCG are specified for type of infection (e.g. bacterial/viral) are needed. In addition, further research on the immunological mechanisms underlying the non-specific effects of BCG vaccination is highly warranted in order to apply the activation of trained immunity for the prevention and treatment of viral infections.

Transparency declaration

M.G.N. was supported a Spinoza grant from The Netherlands Organization for Scientific Research (NWO). All authors have nothing to disclose.

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