MAJOR ARTICLE

BCG Vaccination Enhances the Immunogenicity of Subsequent Influenza Vaccination in Healthy Volunteers: A Randomized, Placebo-Controlled Pilot Study

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Background. Influenza-related morbidity and mortality remain high. Seasonal vaccination is the backbone of influenza management but does not always result in protective antibody titers. Nonspecific effects of BCG vaccination related to enhanced function of myeloid antigen-presenting cells have been reported. We hypothesized that BCG vaccination could also enhance immune responses to influenza vaccination.

Methods. Healthy volunteers received either live attenuated BCG vaccine (n = 20) or placebo (n = 20) in a randomized fashion, followed by intramuscular injection of trivalent influenza vaccine 14 days later. Hemagglutination-inhibiting (HI) antibodies and cellular immunity measured by ex vivo leukocyte responses were assessed.

Results. In BCG-vaccinated subjects, HI antibody responses against the 2009 pandemic influenza A(H1N1) vaccine strain were significantly enhanced, compared with the placebo group, and there was a trend toward more-rapid seroconversion. Additionally, apart from enhanced proinflammatory leukocyte responses following BCG vaccination, nonspecific effects of influenza vaccination were also observed, with modulation of cytokine responses against unrelated pathogens.

Conclusions. BCG vaccination prior to influenza vaccination results in a more pronounced increase and accelerated induction of functional antibody responses against the 2009 pandemic influenza A(H1N1) vaccine strain. These results may have implications for the design of vaccination strategies and could lead to improvement of vaccination efficacy.

Keywords. BCG; influenza vaccination; vaccination strategy; trained immunity; innate immune memory; heterologous immunity.

Annually, influenza virus infection leads to millions of cases of severe illness worldwide and up to 500 000 deaths [1]. The potential for the sudden emergence of pandemic influenza virus strains represents an incessant threat on even a larger scale: it is estimated that if a strain with a virulence similar to that of the 1918 Spanish influenza strain emerged today, it could kill 50 million–80 million people [2]. With very few therapeutic options available, seasonal vaccination is the backbone of influenza management. High-affinity antibodies play a key role in the protective immune response against influenza virus infection [3]. However, antibodies generated by vaccination most often do not effectively neutralize emergent strains, owing to the high mutation rate of the influenza virus genome [4]. In addition, vaccination is not always effective, as 85% of healthy adults and only 40%–60% of elderly people mount a protective antibody response, owing to original antigenic sin [5] and an age-related decline in immune function (ie, immunosenescence) [6]. As a result, particularly in high-risk groups, the protective effects of influenza vaccination are limited, and strategies to improve host immune defenses against influenza virus

Received 25 February 2015; accepted 28 May 2015; electronically published 12 June 2015.

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The Journal of Infectious Diseases® 2015;212:1930–8

DOI: 10.1093/infdis/jiv332

infection and the response to influenza vaccination are highly warranted [7].

In addition to protection against tuberculosis, BCG vaccination provides protection against other infectious diseases [8]. Murine studies have shown that BCG vaccination results in protection against secondary infections with *Candida albicans* [9], *Schistosoma mansoni* [10], and influenza virus [11]. Moreover, nonspecific beneficial effects of BCG vaccination on mortality among young children were demonstrated in observational studies [12], and several randomized studies demonstrated reduced overall mortality among BCG-vaccinated neonates, which could not be explained by tuberculosis prevention [13, 14]. The underlying immunologic mechanisms responsible for the nonspecific effects of BCG are currently being unraveled, and they may be mediated by both induction of trained innate immunity and heterologous adaptive immune responses. Assessment of trained immunity in BCG-vaccinated individuals has recently shown that monocytes undergo epigenetic reprogramming toward an enhanced proinflammatory phenotype [15, 16]. This results in increased production of proinflammatory cytokines, such as interferon γ (IFN-γ), tumor necrosis factor α (TNF-α), and interleukin 1β (IL-1β) upon ex vivo stimulation with unrelated pathogens, even up to 1 year after BCG vaccination [15, 16].

As the nonspecific immunomodulatory effects of BCG vaccination increase the function of myeloid immune cells with antigen-presenting properties, we hypothesized that BCG vaccination could enhance immune responses to other vaccines in general and to influenza vaccination in particular. In the present randomized trial, we investigated the effects of BCG vaccination on the immunogenicity of a trivalent influenza vaccine in healthy volunteers.

MATERIALS AND METHODS

Subjects

This study was registered at ClinicalTrials.gov as NCT02114255. After approval from the Arnhem-Nijmegen Ethics Committee, 40 healthy, nonsmoking, male volunteers gave written informed consent to participate in the study, which occurred from May to July 2014. All experiments were conducted in accordance with the Declaration of Helsinki. Subjects were screened before the start of the experiment and had normal findings on physical examination. Subjects who received BCG vaccine before, received influenza vaccination in the previous year, or had febrile illness during the 2 weeks before the experiment were excluded. Subjects were not allowed to use prescription drugs.

Study Design

The design of this placebo-controlled randomized trial is depicted in Figure 1. Briefly, subjects were randomized using the sealed-envelope method to receive intradermal injections of either 0.1 mL of live attenuated BCG vaccine (BCG vaccine SSI/Danish strain 1331; Bilthoven Biologicals, Bilthoven, the Netherlands; n = 20) or placebo (NaCl 0.9%; n = 20) in a double-blinded fashion. Fourteen days later, all subjects received an intramuscular injection of 0.5 mL of trivalent 2013–2014 seasonal influenza vaccine containing A/California/7/2009 (A[H1N1] pdm09)–derived strain, Victoria/361/2011-related strain derived from A/Texas/50/2012 (A[H3N2]2012), and B/Massachusets/2/2012 (B/2012)–derived strain surface antigens and no adjuvants (Batrevac; Abbot Biologicals, Weesp, the Netherlands). Adverse effects were recorded after day 0, and antibody titers and cytokine production capacity were assessed before BCG vaccination (on day −14), before influenza vaccination (on day 0), and on days 7, 14, and 28 after influenza vaccination. The primary study end point was the difference in hemagglutination-inhibiting (HI) antibody titers over time after influenza vaccination. Secondary end points were the proportion of participants in each group who achieve seroconversion (defined by a ≥ 4-fold rise in antibody titer) over time after influenza vaccination and cytokine responses of leukocytes stimulated ex vivo with various influenza-related and unrelated stimuli over time after BCG vaccination.

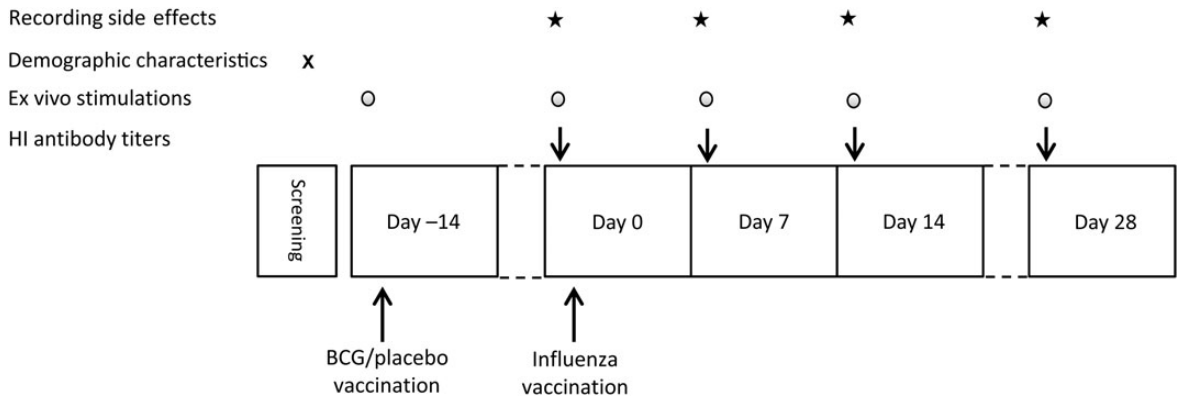

Figure 1. A schematic representation of the study design. Abbreviation: HI, hemagglutination inhibition.

HI Assay

HI assays were performed according to standard procedures, which are detailed in the Supplementary Materials. Every sample was run in duplicate, and geometric mean titers were determined by calculating the mean of the log-transformed duplicate titers followed by back transformation (calculated as 10^x, where x is defined as the mean log-transformed titer). Seroconversion was defined as ≥4-fold titer increase, compared with baseline. Antibody titers were similar between day −14,(before BCG/placebo vaccination) and day 0 (before influenza vaccination) within groups for all 3 vaccine strains in the placebo group and for 2 vaccine strains in the BCG vaccination group (a significant difference in the A[H3N2]2012 strain was found; Supplementary Table 1). This variability could due to assay variation or from a nonspecific boosting effect of BCG vaccine on plasma cells. We calculated relative increases in antibody titers as compared to titers at day 0, just before influenza vaccination.

Peripheral Blood Mononuclear Cell (PBMC) Stimulation and Cytokine Measurements

Venous blood was drawn into ethylenediaminetetraacetic acid tubes, PBMCs were isolated and stimulated with various stimuli, and cytokine levels were determined in supernatants. A detailed description is provided in the Supplementary Materials.

Statistical Analyses

All data were not normally distributed (determined using Kolmogorov-Smirnov tests). Demographic data were analyzed using Mann–Whitney *U* tests. Differences between the 2 groups over time were calculated using Mann–Whitney *U* tests of areas under the curve (AUCs) calculated from the fold-change data (to correct for baseline differences). Within-group differences in cytokine production over time were calculated using Friedman tests. Within-group differences in cytokine production between day 0 and day 14 and antibody titers between day −14 and day 0 were calculated using Wilcoxon matched-pairs tests. Stratified HI assay analysis according to baseline titers was based on routine dilutions used to assess HI titers. Finally, differences in the seroconversion rate between groups over time were calculated using log-rank tests. A *P* value of <.05 was considered statistically significant. We calculated that 20 subjects per group were needed to detect a 2-fold difference in A(H1N1) titer increase between the BCG vaccine and placebo groups with a power of 80%, an SD of 113% [17], and a 2-sided α of 0.05. Calculations and statistical analyses were performed using GraphPad Prism, version 5.0 (GraphPad Software, San Diego, California).

RESULTS

Demographic Characteristics and Side Effects

Baseline characteristics were similar in both groups (Table 1). No serious adverse events occurred during the study. None of the subjects in the placebo group and 10 subjects in the BCG group reported a local inflammatory reaction at the injection site, which resolved in all cases within 4 weeks after injection. After influenza vaccination, 6 placebo-vaccinated subjects and 6 BCG-vaccinated subjects reported mild complaints (including fatigue, headache, malaise, and muscle pain at the injection site), which resolved within 2 days after vaccination in all cases.

Table 1. Demographic Characteristics of Subjects Who Received Placebo or BCG Vaccine

Characteristic	Placebo Vaccine (n = 20)	BCG Vaccine (n = 20)	*P* Value
Age, y	20.5 (20.3–25)	21 (20–24)	.35
Height, cm	178 (175–188)	183 (180–190)	.13
Weight, kg	74.4 (66.2–82.8)	80.2 (72.3–93.4)	.06
BMI[a]	22.3 (21.1–24.7)	24.5 (22.2–27.0)	.08

Data are medians (interquartile ranges). *P* values were calculated using Mann–Whitney *U* tests.

[a] Body mass index (BMI) is calculated as the weight in kilograms divided by the height in meters squared.

Influenza Virus Antibody Titers

There were no baseline differences in antibody titers between groups for the 3 influenza virus strains (Table 2). In BCG-vaccinated subjects, HI antibody responses against the A(H1N1) pdm09 vaccine strain was markedly enhanced, compared with the placebo-treated group, and there was a trend toward more-rapid seroconversion (Figure 2*A*). No significant differences between groups were observed regarding HI antibody responses against the A(H3N2) and B/2012 vaccine strains (Figure 2*B* and 2*C*). Stratified analyses according to baseline antibody titers revealed similar patterns, compared with the overall analysis presented in Figure 2 (Supplementary Figures 1–3). As expected, HI antibody responses induced by influenza vaccination were much more pronounced in subjects with low baseline antibody titers. Accordingly, subjects with high baseline antibody titers in both groups barely attained seroconversion, defined as a ≥4-fold increase from baseline. These data indicate that differences between groups were mainly based on responses of subjects

Table 2. Baseline (Day 0) Antibody Titers Against Influenza Virus Strains Among Subjects Who Received Placebo or BCG Vaccine

Strain	Placebo (n = 20)	BCG (n = 20)	*P* Value
A(H1N1)pdm09	132.7 (68.71–257.04)	42.31 (22.5–103.3)	.13
A(H3N2)2012	66.4 (32.1–137.4)	115.9 (57.9–231.2)	.33
B/2012	39.0 (20.5–74.3)	40.9 (20.9.78.0)	.78

Data are geometric mean titers (95% confidence intervals). Descriptions of each strain are specified in "Materials and Methods" section. *P* values were calculated using Mann–Whitney *U* tests.

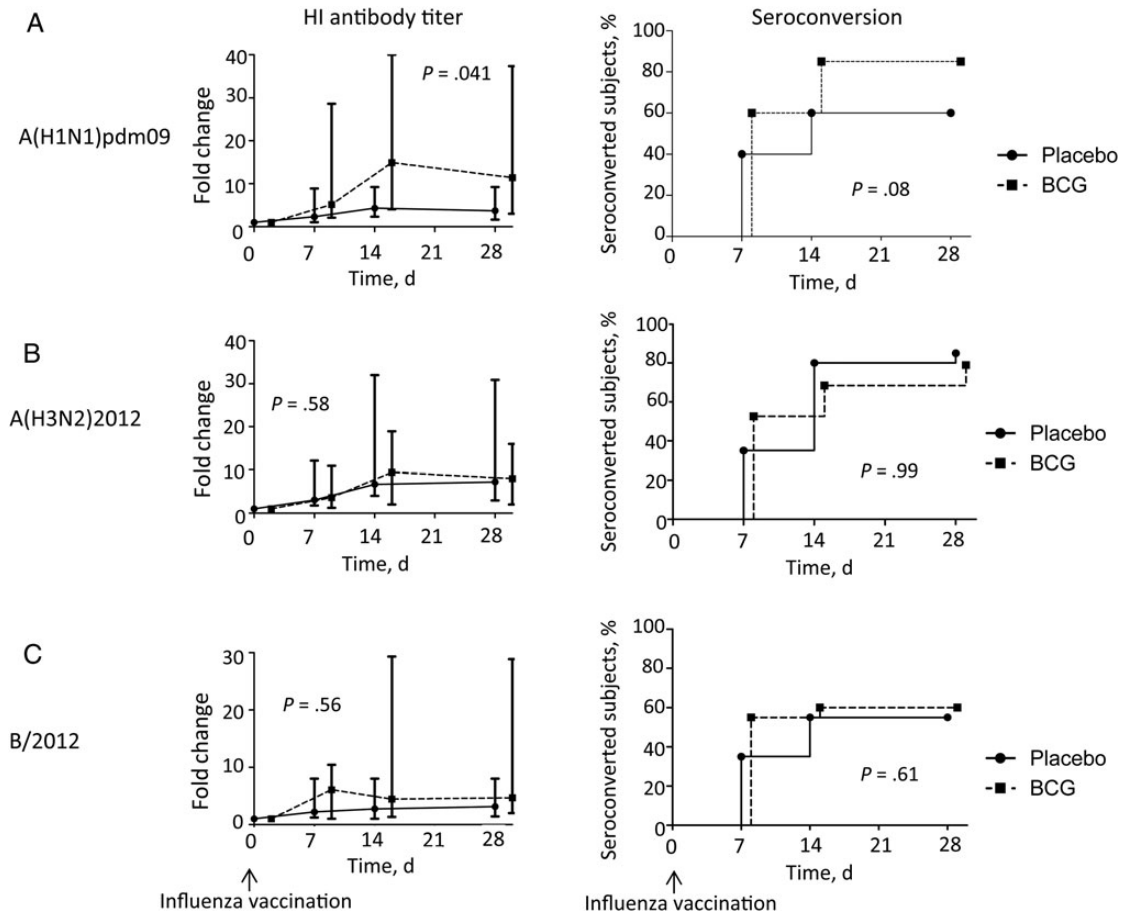

Figure 2. Hemagglutination-inhibiting (HI) antibody titers (left panels) and percentage seroconversion (right panels) over time for 2009 pandemic influenza A(H1N1) (A[H1N1]pdm09), 2012 influenza A(H3N2) (A[H3N2]2012), and 2012 influenza B (B/2012) in subjects who received BCG or placebo vaccine followed by influenza vaccine. There were no differences at baseline (defined as day 0) between groups. *Left*, Baseline titers are plotted as 1, and titers at subsequent time points are plotted as fold changes from baseline values. *P* values were calculated using Mann–Whitney *U* tests of areas under the curve for subjects in both groups. *Right*, Percentage of subjects who attained seroconversion (defined as a ≥4-fold titer increase, compared with baseline) over time. *P* values were calculated using log-rank tests.

with low baseline antibody titers. When subjects with high baseline antibody titers (defined as the titer for which ≤ 25% of subjects in both groups attained seroconversion) were excluded, the potentiating effect of BCG vaccination on antibody responses against A(H1N1)pdm09 became more apparent (Supplementary Figure 4*A*). In addition, a trend toward enhanced antibody responses against B/2012 in the BCG vaccination group also emerged when groups were stratified (Supplementary Figure 4*C*), while antibody responses against A(H3N2) remained similar between groups (Supplementary Figure 4*B*).

Cytokine Responses Upon Ex Vivo Stimulation of PBMCs With Influenza-Related Stimuli

There were no differences in baseline ex vivo cytokine responses between groups (data not shown). In both groups, the production of IFN-γ upon stimulation with influenza vaccine was enhanced after influenza vaccination (Figure 3*A*). Likewise, IFN-γ production upon stimulation with live influenza virus increased in both groups after vaccination, although this did not reach statistical significance in the placebo group (Figure 3*B*). No effect was observed on TNF-α production upon stimulation with influenza vaccine in both groups (Figure 3*C*), whereas production of this cytokine upon stimulation with live influenza virus was enhanced in both groups, although this did not reach statistical significance in the BCG group (Figure 3*D*). No significant differences between treatment groups were observed, apart from a trend toward enhanced and more-sustained IFN-γ production upon stimulation with influenza vaccine in the BCG vaccine group.

Cytokine Responses Upon Ex Vivo Stimulation of PBMCs With Stimuli Unrelated to Influenza

There were no differences in baseline ex vivo cytokine responses between groups (data not shown). As expected, enhanced

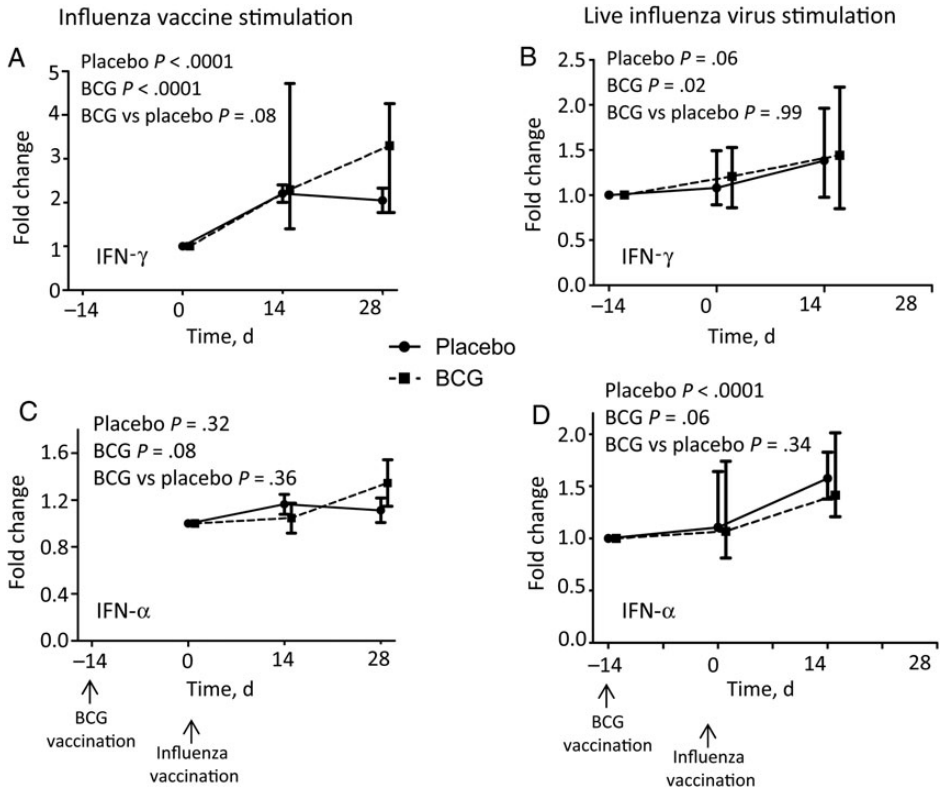

Figure 3. Cytokine responses of peripheral blood mononuclear cells (PBMCs) stimulated ex vivo with live influenza virus or trivalent influenza vaccine. There were no differences at baseline (defined as day −14 for live influenza stimulations and as day 0 for influenza vaccine stimulations) between groups. Baseline responses are plotted as 1, and titers at subsequent time points are plotted as fold changes from baseline values. Within-group differences in cytokine production over time were calculated using Friedman tests. *P* values of differences between groups (BCG vs placebo vaccination) were calculated using Mann–Whitney *U* tests of areas under the curve for subjects in both groups. Abbreviations: IFN-α, interferon α; IFN-γ, interferon γ.

production of IFN-γ and IL-6 was found upon stimulation with *Mycobacterium tuberculosis* in BCG-vaccinated subjects, compared with the placebo group (Figure 4). This enhanced cytokine production was already present at day 0 (just before influenza vaccination), thereby indicating that these effects are mediated by BCG vaccine. For *M. tuberculosis*–induced TNF-α and IL-1β production, a trend toward enhanced responses was observed in BCG-vaccinated subjects (Figure 4), while no effects were observed for the antiinflammatory cytokine interleukin 10 (IL-10; data not shown).

To investigate the effects of influenza vaccination on ex vivo cytokine responses to unrelated pathogens, we assessed changes in cytokine responses within the placebo-group between day 0 (before influenza vaccination) and day 14 (Figure 5*A*). Influenza vaccination on its own resulted in enhanced TNF-α and IL-6 production upon stimulation with lipopolysaccharide (LPS). Furthermore, upon stimulation with *C. albicans,* enhanced production of TNF-α and reduced production of IL-10 was observed. However, production of IFN-γ and IL-1β was also decreased upon stimulation with C. *albicans*. Stimulation with S. *aureus* also resulted in reduced expression of IFN-γ, IL-1β, and IL-10, which was also the case for IL-1β and IL-10 production upon stimulation with *M. tuberculosis*.

Furthermore, we assessed how BCG vaccination modulates influenza vaccination–induced nonspecific cytokine responses (Figure 5*B*). BCG vaccination potentiated the influenza vaccination–induced increase in TNF-α and IL-6 production upon LPS stimulation. Also, BCG vaccine enhanced production of IL-1β upon stimulation with LPS in the volunteers vaccinated with influenza vaccine. In accordance with these findings, the influenza vaccination–induced attenuation of IFN-γ and IL-1β production upon stimulation with *C. albicans* was less pronounced in BCG-vaccinated subjects, although the influenza vaccination–induced increase in TNF-α was also attenuated. Proinflammatory effects of BCG vaccination were also observed for ex vivo stimulation with *S. aureus*: in BCG-vaccinated subjects, the influenza vaccination–induced attenuation of IL-1β production was abrogated, and production of IL-6 was enhanced. Finally, BCG vaccination resulted in abrogation of the influenza vaccine–induced attenuation of IL-1β upon stimulation with *M. tuberculosis* and resulted in enhanced production of TNF-α in response to this pathogen (Figure 5).

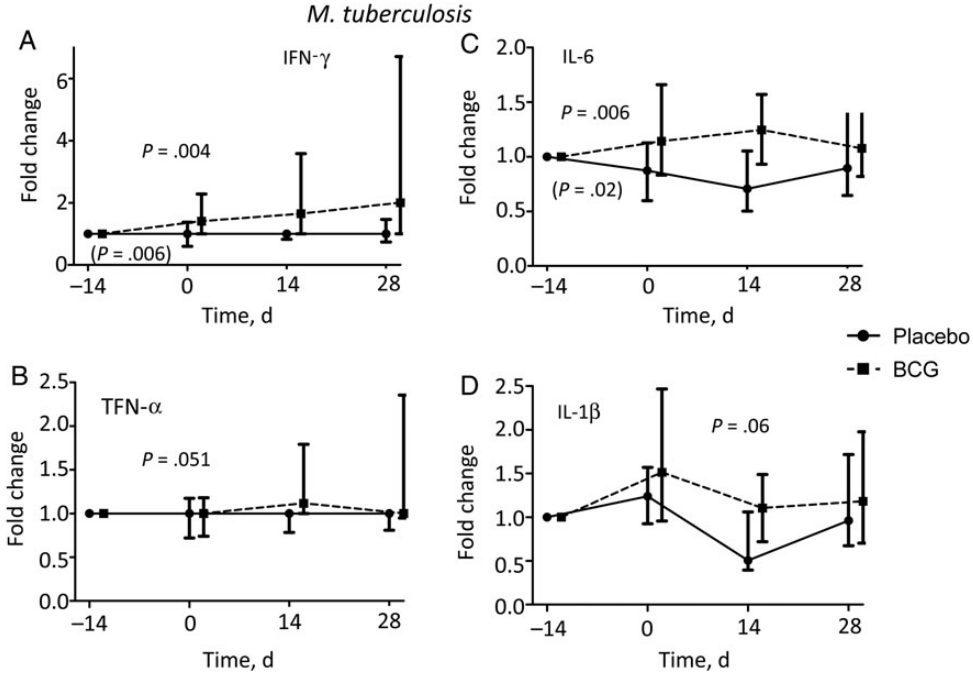

Figure 4. Cytokine responses of peripheral blood mononuclear cells (PBMCs) stimulated ex vivo with *Mycobacterium tuberculosis*. There were no differences at baseline (defined as day −14) between groups. Baseline responses are plotted as 1, and values at subsequent time points are plotted as fold changes from baseline values. *P* values were calculated using Mann–Whitney *U* tests of areas under the curve for subjects in both groups. Changes in cytokine responses between day −14 (before BCG or placebo vaccination) and day 0 (before influenza vaccination) were calculated using Mann–Whitney *U* tests between 2 groups (*P* values between brackets). Abbreviations: IFN-γ, interferon γ; IL-1β, interleukin 1β; IL-6, interleukin 6; TNF-α, tumor necrosis factor α.

DISCUSSION

In the present study, we investigated the capacity of BCG vaccination to modulate the immune response to subsequent vaccination with a trivalent influenza vaccine. We demonstrate that BCG vaccination not only modulates innate immune responses upon ex vivo stimulation with unrelated pathogens, as previously reported [15, 16], but also enhances functional antibody responses against A(H1N1)pdm09 induced by subsequent influenza vaccination, reflected by a more pronounced increase in antibody titers and a trend toward more-rapid seroconversion.

In addition to its effects on the severe clinical forms of tuberculosis, BCG vaccination also beneficially influences morbidity and mortality due to other infections [18]. This is accompanied by nonspecific stimulatory effects on the function of both myeloid and lymphoid cells [15, 19]. These epidemiological and immunological data formed the basis of the hypothesis that BCG vaccination may also potentiate the function of antigen-presenting cells and thus improve the response to other vaccines. This hypothesis is supported by the increase in the titers of neutralizing antibodies against A(H1N1)pdm09, while a similar tendency was observed for the responses to B/2012, especially in individuals with initial low antibody titers. A potentiating effect of BCG vaccine on the response to other vaccines is also supported by observational studies in infants, in which BCG vaccination increased heterologous responses to poliovirus vaccination [20]; responses to antipneumococcus, anti–*Haemophilus* type B, and anti–tetanus toxoid vaccines [21]; and responses to hepatitis B vaccine [22].

BCG vaccination influenced both humoral and cellular responses to influenza vaccination. The magnitude and quality of antigen-specific antibody titers is considered to be the primary correlate of protection against most pathogens/viruses that infect the host through mucosal surfaces, such as influenza virus [23, 24]. The effects of BCG vaccination on antibody responses to subsequent influenza vaccination observed in this study demonstrate that the immunological history affects the humoral immune response to subsequent infections/vaccinations in a clinically relevant manner. Moreover, a trend toward enhanced and more-sustained IFN-γ production upon ex vivo stimulation with influenza vaccine was also observed in the BCG-vaccinated group.

The percentage subjects who achieve a 4-fold increase in antibody levels, compared with baseline levels, in this study is in line with the influenza vaccination–induced increase previously observed in healthy male volunteers [25]. Not surprisingly, subjects with low baseline anti–influenza virus antibody titers

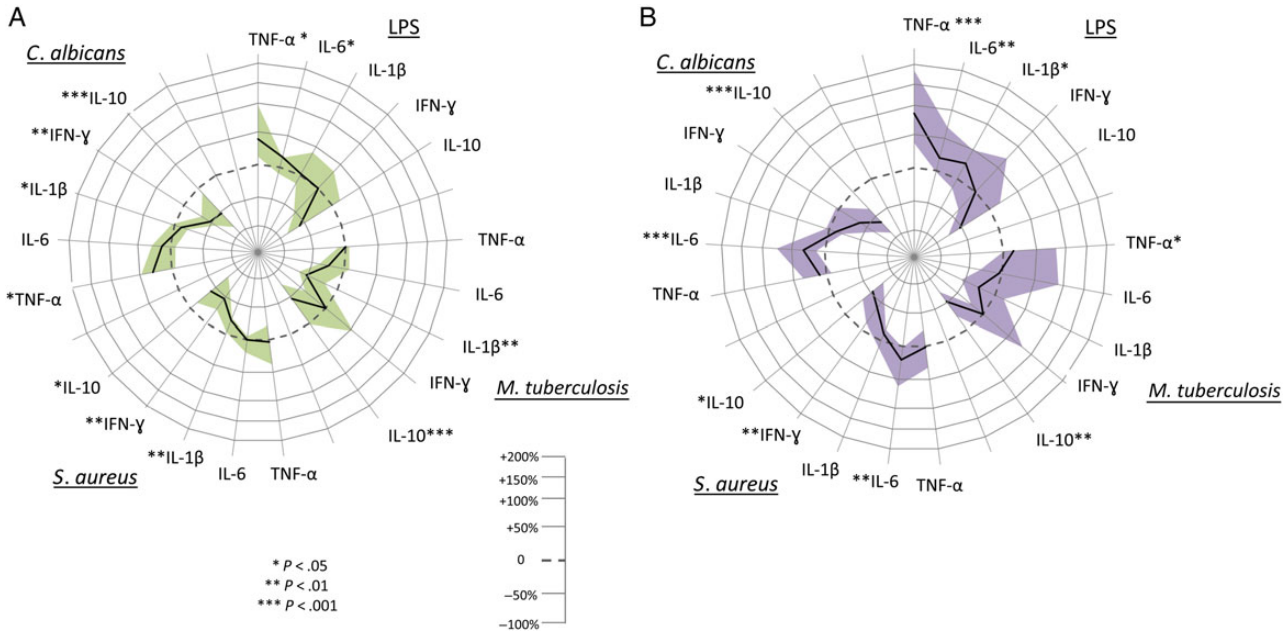

Figure 5. Spider plots of effects of influenza vaccination on cytokine responses of peripheral blood mononuclear cells stimulated ex vivo with influenza-unrelated pathogens in the placebo (*A*) and BCG vaccine (*B*) groups. Cytokine responses on day 0 (before influenza vaccination) are set as 0 (dashed lines), and cytokine responses 14 days later are plotted as fold changes. Data are represented as medians (solid lines) and interquartile ranges (hatched areas). **P* < .05, ***P* < .01, and ****P* < .001, by Wilcoxon matched-pairs tests between responses at day 0 and those at day 14. Abbreviations: *C. albicans*, *Candida albicans*; IFN-γ, interferon γ; IL-1β, interleukin 1β; IL-6, interleukin 6; IL-10, interleukin 10; LPS, lipopolysaccharide; *M. tuberculosis*, *Mycobacterium tuberculosis*; *S. aureus*, *Staphylococcus aureus*; TNF-α, tumor necrosis factor α.

displayed the strongest increase in antibody titers following influenza vaccination. Nevertheless, an increase in antibody titer was also observed in subjects with baseline antibody titers greater than 1:40 [26]. Moreover, in these subjects, BCG vaccination still exerted a potentiating effect on antibody responses. Earlier studies demonstrated that a progressive increase in protection is reached with increased titers, rather than that protection is attained above a discrete threshold that is applied to each individual [26]. The increase in protection is particularly important for titers up to 1:160 [26], which applies to our study.

The reason for the significant effects of BCG vaccination on the response to A(H1N1)pdm09 but not to A(H3N2) remains unknown. Previous studies have reported differential effects of BCG vaccine on different antigens from the same vaccination, as well [21], but no clear mechanism has been proposed. The absence of BCG-induced effects on A(H3N2) antibody titers (also discussed below) could be explained from an immunological point of view. Previous work has shown differences in the immunopathology caused by pandemic and seasonal strains, related to the strain virulence and the localization of the inflammatory response [27, 28]. In this respect, infections with A(H1N1)pdm09 result in expression of viral antigens in mucosal epithelial cells of the airways (from the nasopharynx to the bronchioles), but also in alveolar macrophages and pneumocytes [27, 28]. In contrast, infections with A(H3N2) show viral antigens primarily in mucosal epithelial cells of the larger airways [27, 28]. It is tempting to hypothesize that the potentiating effects of BCG vaccination on antibody responses against A(H1N1)pdm09 but not A(H3N2) may be due to differences in cells responsible for the recall response caused by the different localization of the primary infection, but this remains to be demonstrated by future studies.

In addition to the effects of antibody titers, BCG vaccination also modulated the effects on cytokine production capacity induced by influenza vaccination. Interestingly, our data indicate that trivalent influenza vaccine exerts nonspecific effects, as well, although they differ from those observed following BCG vaccination [15]. BCG vaccination exerted an overall immunostimulatory effect on the cytokine production modulated by influenza vaccine. In contrast, influenza vaccination results in enhanced responses against certain pathogens but impaired responses against others. These findings support the hypothesis that infection and vaccine histories affect immune status in a clinically relevant manner.

This study has several limitations. First, the relatively small sample size could be a reason for a type 2 error, resulting in the fact that some of the effects observed did not reach statistical significance, most importantly the enhanced antibody production against B/2012 in the BCG-vaccinated subjects, when stratified according to baseline antibody titers. Nevertheless, the

significant effects on antibody titers to the pandemic strain and the ex vivo cytokine responses illustrate the extent to which BCG vaccination influences unrelated adaptive and innate immune responses. Second, although BCG vaccination potentiated antibody responses, humoral immunity is not the only mechanism involved in the protection against influenza virus infection. Upon vaccination, generation of specific memory cytotoxic T lymphocytes (CTLs), in addition to an adequate antibody response, contribute to protection against influenza virus [29]. It has even been suggested that, in elderly individuals, CTL immune responses are better predictors of immunity than antibody titers [30, 31]. We only studied IFN-γ responses as a surrogate of T-cell function, and direct CTL assays could be also considered in future studies to understand how BCG affects the response to influenza vaccination. Finally, we based our 14-day interval between BCG vaccination and influenza vaccination on previous studies in mice, in which viral challenges were performed 14–49 days after BCG vaccination [11], and in humans, in whom nonspecific effects of BCG vaccination were demonstrated ex vivo 14 days after vaccination [15]. However, there is little knowledge on the optimal timing of BCG vaccinations in the context of influenza vaccination, and it is possible that a different interval between BCG vaccination and influenza vaccination could prove to be even more effective.

In conclusion, in the present study, we demonstrated that BCG vaccination followed by trivalent influenza vaccination significantly improves the magnitude and, possibly, the swiftness of the antibody responses against A(H1N1)pdm09 in humans in vivo. In addition, this study validates the previously observations that vaccination exerts nonspecific effects on cytokine responses against unrelated pathogens. In line with this, our data indicate that modulatory effects on innate immunity are not restricted to BCG vaccination but that trivalent influenza vaccination also exerts nonspecific effects on cytokine responses elicited by various pathogens. Overall, our data further support the concept that trained immunity effects on myeloid antigen-presenting cells can influence the specific response to other vaccines and the hypothesis that vaccination history affects immune status in a clinically relevant manner. This is the first randomized trial showing that BCG vaccination can potentiate the responses to other vaccines. As such, these results open the door to improve vaccination strategies in at-risk groups such as neonates or elderly individuals.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

Acknowledgments. We thank Ruud van Beek, for technical assistance with the hemagglutination-inhibiting assay, and Trees Jansen, for technical assistance with cytokine measurements.

Financial support. This work was supported by the Netherlands Organization for Scientific Research (Vici grant to M. G. N.) and the European Research Council (consolidator grant 310372 to M. G. N.).

Potential conflicts of interest. All authors: No reported conflicts.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed.

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