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The impact of BCG dose and revaccination on trained immunity

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ABSTRACT

The innate immune system can display heterologous memory-like responses termed *trained immunity* after stimulation by certain vaccinations or infections. In this randomized, placebo-controlled trial, we investigated the modulation of Bacille Calmette-Guérin (BCG)-induced trained immunity by BCG revaccination or high-dose BCG administration, in comparison to a standard dose. We show that monocytes from all groups of BCG-vaccinated individuals exerted increased TNFα production after ex-vivo stimulation with various unrelated pathogens. Similarly, we observed increased amounts of T-cell-derived IFNγ after *M. tuberculosis* exposure, regardless of the BCG intervention. NK cell cytokine production, especially after heterologous stimulation with the fungal pathogen *Candida albicans,* was predominantly boosted after high dose BCG administration. Cytokine production capacity before vaccination was inversely correlated with trained immunity. While the induction of a trained immunity profile is largely dose- or frequency independent, baseline cytokine production capacity is associated with the magnitude of the innate immune memory response after BCG vaccination.

1. Introduction

Traditionally, immunological memory has been a trait exclusively attributed to the acquired immune system. However, in the last decade, extensive research has revealed that our innate immune system is also equipped with adaptive characteristics and can respond to secondary infection with a more effective antimicrobial response. This phenomenon is termed *trained immunity* or innate immune memory, and has been mainly associated with the administration of live attenuated vaccines [1].

Bacille Calmette-Guérin (BCG), the tuberculosis vaccine, has garnered a lot of interest for its ability to induce trained immunity. BCG can mount a broad immune response not only against its target disease, but also against a wide variety of non-related infectious agents. This process is accompanied by epigenetic and metabolic rewiring of innate immune cells, the hallmarks of trained immunity [2–5]. Earlier observational studies already supported the notion that BCG reduced overall childhood mortality independently of its specific effect on tuberculosis (TB) [6–8]. These findings were substantiated by several randomized trials showing that BCG administration at birth resulted in decreased overall neonatal mortality, which was mainly explained by a lower incidence of sepsis and respiratory infections [9,10]. In adults, BCG decreased peak yellow fever vaccine viraemia and improved resistance to malaria infection [11,12]. The vaccine has been thoroughly researched during the COVID-19 pandemic as a potential "bridging" vaccine against SARS-CoV-2 [13]. Additionally, BCG increases antibody responses of concomitantly or subsequently administered vaccines such as the hepatitis B, *H. influenza* type B, pneumococcus, tetanus toxoid, and influenza vaccines [14–16]. Altogether, BCG can be considered as a training agent for the immune system by inducing long-term imprinting of innate immune cells, creating a state of readiness so that potential novel infections can be handled more effectively.

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There are arguments to believe that these beneficial, non-specific effects of BCG and other live attenuated vaccines may be enhanced through revaccination. Reanalysis of a trial conducted in Algiers between 1935 and 1947 indicated that BCG revaccination may have conferred strong additional non-specific effects reducing overall childhood mortality [17]. The same outcome was observed in a small clinical trial in Guinea-Bissau [18]. BCG revaccination also resulted in a minor effect on the prevention of tuberculosis (TB) among school-aged children [19]. Although revaccination is not recommended by the World Health Organization (WHO), there are many countries where this is routine [20]. Whether trained immunity responses might be boosted by revaccination or by increasing the dose of BCG is currently unknown.

Here we assessed the effect of different BCG vaccination regimens such as the standard dose of BCG (SD), a high dose of BCG (HD), and a BCG revaccination (RV) on the induction of trained immunity in healthy adult volunteers. We measured pro-inflammatory cytokine responses in peripheral blood mononuclear cells (PBMC) and natural killer (NK) cells upon various microbial stimuli, to determine whether BCG boosting was superior to standard BCG administration. In addition, we analyzed the possible association between the amounts of live BCG (*M. bovis* mycobacteria) in the vials and cytokine production capacity.

2. Materials and methods

2.1. Study design

This randomized placebo-controlled trial, depicted in Fig. 1, was designed to compare different BCG vaccination methods for identifying their efficacy to establish trained immunity. Accordingly, participants were 1:3:3:3 allocated to receive either a placebo *vaccination* (P, 0.1 ml of vaccine diluent), a single standard dose of BCG (SD, 0.1 ml, 0.75 mg/ml, *M. bovis,* BCG Denmark, AJ Vaccines), a high dose of BCG (HD, 0.1 ml 1.5 mg/ml *M. bovis,* BCG Denmark, AJ vaccines), or a revaccination with a single standard dose of BCG (RV), occurring three months after the first standard vaccination. The placebo group was intentionally kept small as the most important comparator is the SD group and to minimize the burden of the trial. To ensure that the groups ran parallel as to minimize seasonal effects, an extra placebo vaccination was introduced before the actual BCG vaccination in the SD and HD groups. Vaccination was performed intradermally in the left upper arm and, in case of revaccination, first in the right upper arm and then in the contralateral arm. Despite being generally safe and well-tolerated even in high dosages and after revaccination, the vaccine can cause localized skin reactions, which are usually mild and self-limiting [21,22]. Seven days and fourteen days after receiving their last BCG vaccination, the injection site of the participants was examined. Blood was drawn at baseline, after the first vaccination at three months and after the last vaccination at six months. The trial protocol registered under NL58219.091.16 in the Dutch trial registry, was approved in 2019 by the Arnhem-Nijmegen Ethics Committee. All experiments were conducted in accordance with the Declaration of Helsinki and no adverse events were recorded.

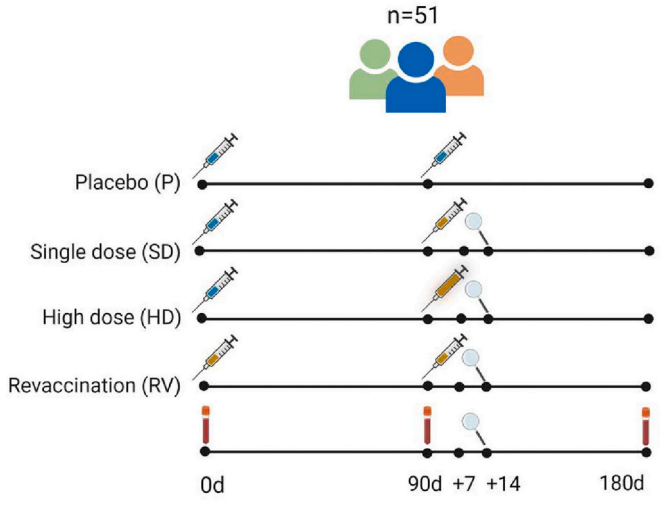

Fig. 1. Clinical study design comparing the effect of various BCG vaccination regimes on trained immunity.
Fifty-one healthy adults were randomized to either one of the four intervention groups: placebo vaccination (P), single dose BCG (SD), high dose BCG (HD) or BCG revaccination (RV). Blood was drawn at baseline, 90d and 180d. Injection sites were inspected twice after the second vaccination, indicated by the magnifying glass.

2.2. Subjects

Fifty-one healthy, non-smoking, adult volunteers were recruited between October 2019 and February 2020. Subjects with a history of BCG vaccination, a receipt of a vaccination three months prior to the start of the study, or plans to receive any other vaccination during the study period were not eligible. Acute illness within two weeks prior to the study initiation or the use of drugs, including non-steroidal anti-inflammatory drugs (NSAIDs) less than four weeks before the start of the trial, but with the exception of oral contraceptives, also resulted in exclusion. Female subjects were screened for pregnancy with a urinary test and excluded if positive. All participants gave written informed consent.

2.3. Ex-vivo trained immunity model

Venous blood was drawn from study subjects and peripheral blood mononuclear cells (PBMC) were isolated using Ficoll-Paque (VWR) density gradient isolation. PBMCs were resuspended in RPMI 1640 Dutch modified culture medium (Invitrogen) supplemented with 50 mg/ml gentamicin, 2 mM glutamax and 1 mM pyruvate (Gibco). NK cells were purified from the freshly isolated mononuclear fraction using MACS microbeads (negative selection), according to the manufacturer's protocol (Miltenyi Biotec). NK cells were counted, and purity was checked with Sysmex (Sysmex Corporation, Japan) and was >90%. NK cell suspensions were supplemented with IL15 (10 ng/ml). Subsequently, $5*10^5$ PBMCs or $1*10^5$ NK cells in 100 μl volume were added to 96-wells round bottom plates (Corning). Cells were incubated with RPMI (supplemented as previously mentioned, and with 20% human pooled serum), lipopolysaccharide derived from *E. coli* 10 ng/ml (LPS O55:B5, Sigma-Aldrich), heat-killed *E. coli* $1*10^6$/ml (ATCC35218), *S. aureus* $1*10^6$/ml (ATCC25923), *M. tuberculosis* 5 μg/ml (*M. tb,* strain H37Rv), *C. albicans* $1*10^6$/ml (strain UC 820) and phytohemagglutinin (PHA, Sigma-Aldrich) 10 μg/ml. Cells were incubated at 37 °C with 5% CO^2 for 24 h, 48 h (NK cells), and 7 days, after which supernatants were collected and stored at −80 °C.

2.4. Cytokine measurements and analysis

Cytokine concentrations were determined in 24 h (TNFα, IL6, IL1β), and 7-day supernatants (IFNγ) after stimulation of PBMCs, and after 48 h (TNFα, IFNγ, IL6, IL1β) after stimulation of NK cells, using commercial ELISA kits (R&D systems, Bio-Techne, USA) according to the instructions of the manufacturer. Differences between cytokine production before and after vaccination were analyzed using the Wilcoxon signed-rank test. Differences between the four intervention groups were analyzed with Kruskal-Wallis tests. When two intervention groups were compared, the Mann-Whitney *U* test was utilized. All calculations were performed in GraphPad Prism 8. A *p*-value lower than 0.05 was considered statistically significant.

2.5. CFU counts and cytokine correlations

To count the number of CFUs of viable mycobacteria per ml of vaccine, a 0.1 ml sample of each lot of regular and high dose vaccine was transferred to a 96-wells plate and was 10-fold serial diluted up to 10^{-6}. From each dilution, three 10 μl samples were plated on Middlebrook 7H11 plates (BD Bioscience, Erembodegem, Belgium). Plates were incubated for up to 21 days at 37 °C before counting the number of colonies. The mean of the three samples was then calculated and expressed as CFU/ml. Correlations between cytokines and CFUs were calculated with Spearman's correlation test using GraphPad Prism 8.

3. Results

3.1. The effect of BCG regimen on monocyte- derived cytokine production capacity in PBMCs

To investigate the effect of a high dose BCG and a revaccination on the induction of pro-inflammatory cytokines in reference to a regular BCG vaccination, we performed several stimulation assays using specific and heterologous microbial agents in 51 healthy volunteers. Baseline characteristics were not significantly different between groups (Table 1). All BCG vaccinated participants developed a blister. BCG vaccination, whether SD, HD or RV, led to significantly higher monocyte-derived TNFα production upon stimulation with LPS, *S. aureus and E. coli* (Fig. 2). However, there was no significant difference in the magnitude of the increase between either of the three BCG vaccination groups. Stimulation with *C. albicans* and *M. tb* did not alter TNFα production in vaccinated individuals compared to the baseline secretion before vaccination (supplementary fig. 1). Similarly, there were no differences in IL6 or IL1β production between the SD, HD, and RV groups after 6 months, although overall IL6 production followed a decreasing trend and overall IL1β production was moderately increased (supplementary fig. 2 and 3). Of note, similar cytokine production tendencies after BCG vaccination were observed in separate analyses for male and female participants, but these did not reach statistical significance due to group size (supplementary fig. 4). This indicates that a standard dose of BCG, as well as a high dose and BCG revaccination, induces increased pro-inflammatory cytokine production from PBMCs upon bacterial stimuli; surprisingly however, the magnitude of this increase is comparable, irrespective of dosage and number of doses.

3.2. The effect of BCG regimen on T-cell -derived cytokine production capacity in PBMCs

T-cell-derived IFNγ, produced by PBMCs upon stimulation with *M. tb,* was higher in the revaccination group compared to placebo vaccination, but not compared to a single dose of BCG. Although BCG vaccination reduced IFNγ production stimulated by *C. albicans* in all BCG groups, no significant differences between groups were observed in the overall reduction at 6 months (Fig. 3). This indicates that heterologous T-cell responses as well as specific immune responses are not stronger following revaccination or high dose BCG in reference to a standard BCG vaccination.

Table 1
Baseline characteristics of BCG-vaccinated individuals and controls.

Characteristic*	Placebo	Single dose	High dose	Revaccination	*P*-value†
	n = 5	n = 13	n = 16	n = 17	
Sex, female	4 (80)	6 (46.2)	8 (50)	13 (76.5)	0.245
Sex, male	1 (20)	7 (53.8)	8 (50)	4 (23.5)	
Age, years	22.8 ± 3.1	25.3 ± 8.2	22.8 ± 3.4	24.5 ± 7.6	0.733
BMI, kg/m^2	21.9 ± 3.9	23.1 ± 2.4	24.5 ± 3.2	22.9 ± 2.5	0.251

* Mean ± SD for continuous variables and n (%) for categorical variables.
† ANOVA for continuous variables and Fisher's exact test for categorical variables.

3.3. The effect of BCG regimen on cytokine production capacity in NK cells

Next, in isolated NK cells, we determined the effect of a single dose BCG, a high dose BCG and revaccination on the modulation of the two major NK cell cytokines IFNγ and TNFα. Following *C. albicans* stimulation, both cytokines were significantly elevated in the HD group, as was the case for TNFα production upon LPS exposure. Surprisingly, revaccination caused significantly lower IFNγ responses upon LPS and *M. tb* stimulation (Fig. 4). This indicates that the boosting methods have different effects on NK cells than on T-cells. However, at 6 months, no differences in cytokine production were observed between SD, HD, and RV. IL1β and IL6 production in general was low and showed a slight decrease upon *M. tb* stimulation, but there were no significant differences between the groups after 6 months (supplementary fig. 5).

3.4. Lack of correlation between BCG CFUs and cytokine production capacity

We also investigated whether the amount of viable BCG administered to the study subjects was associated with modified cytokine production in PBMCs and NK cells. To this end, we counted the number of CFUs from the remaining material in the vials and correlated these numbers with the cytokine production from PBMCs and NK cells upon stimulation. We did not find any correlation between BCG CFUs and monocyte-derived TNFα production upon stimulation with LPS, *S. aureus* or *E. coli* (Fig. 5A). We also did not see any association between the amount of live BCG and T-cell or NK cell-derived IFNγ production following exposure to specific and non-specific stimuli (Fig. 5B, C).

3.5. Negative correlation between baseline cytokine production and cytokine production capacity

Lastly, we analyzed whether baseline cytokine production in the individuals was correlated with cytokine production capacity at 6 months after BCG vaccination. Stimulation assays showed that participants who had low cytokine release at baseline were more likely to have greater TNFα fold changes (PBMCs), as well as greater IFNγ fold changes (T-cells and NK cells) after BCG vaccination (Fig. 6). Thus, indicating a significant inverse correlation between cytokine release at day 0 and cytokine fold change at 6 months.

4. Discussion

The discovery of trained immunity has propelled immunological research into a new direction, shedding a different light on existing vaccines in the pursuit of novel therapeutic strategies [23]. The 100-year-old vaccine, BCG, has been widely studied for this purpose and the mechanisms underlying BCG- induced trained immunity have been partially unraveled. In the present study, we have analyzed several vaccination regimens in order to enhance the trained immunity response. Cytokine induction in PBMCs and NK cells after a high dose BCG and a BCG revaccination were compared to a standard BCG dose and a placebo vaccination. Collectively, our data indicate that, although there is a considerable variation in live mycobacteria in BCG vials, a regular BCG vaccination dose contains a sufficient amount of stimulus to achieve the maximum effect on cytokine production capacity in monocytes, T-cells and NK cells after exposure to various stimuli. Revaccination also did not prove to be superior in eliciting pro-inflammatory cytokines compared to the other BCG interventions. In other words, BCG boosting, applied as a single vaccination with a higher dose or two doses of standard dose BCG, does not correlate with and does not favor an

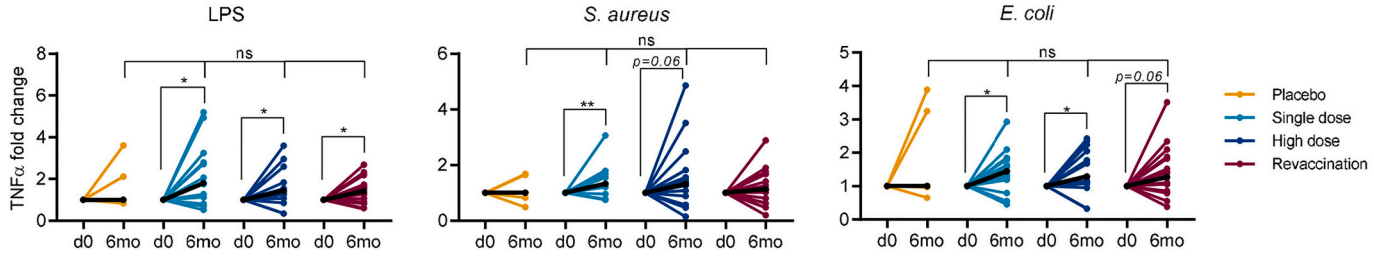

Fig. 2. Monocyte-derived cytokine responses before vaccination and at 6 months.
TNFα production was quantified in PBMCs after 24 h stimulation with LPS, *S. aureus* and *E. coli*. Black lines represent medians. Wilcoxon signed-rank test was performed to compare cytokine release before and after vaccination. Differences between the four intervention groups were analyzed with Kruskal-Wallis tests; $n = 5$ (P), $n = 13$ (SD), $n = 16$ (HD), $n = 17$ (RV); $^{*}p < 0.05$, $^{**}p < 0.01$.

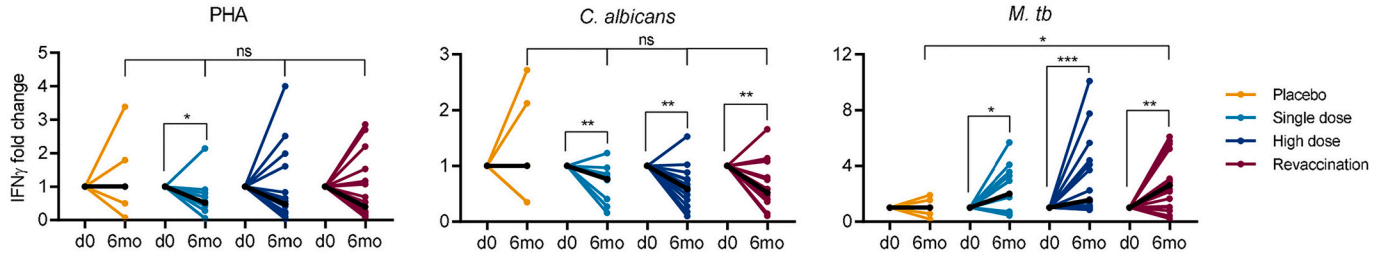

Fig. 3. T-cell-derived cytokine responses before vaccination and at 6 months.
IFNγ production was measured in PBMCs after 7d stimulation with PHA, *C. albicans* and *M. tb*. Black lines represent medians. Wilcoxon signed-rank test was performed to compare cytokine release before and after vaccination. Differences between the four intervention groups were analyzed with Kruskal-Wallis tests; $n = 5$ (P), $n = 13$ (SD), $n = 16$ (HD), $n = 17$ (RV); $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$.

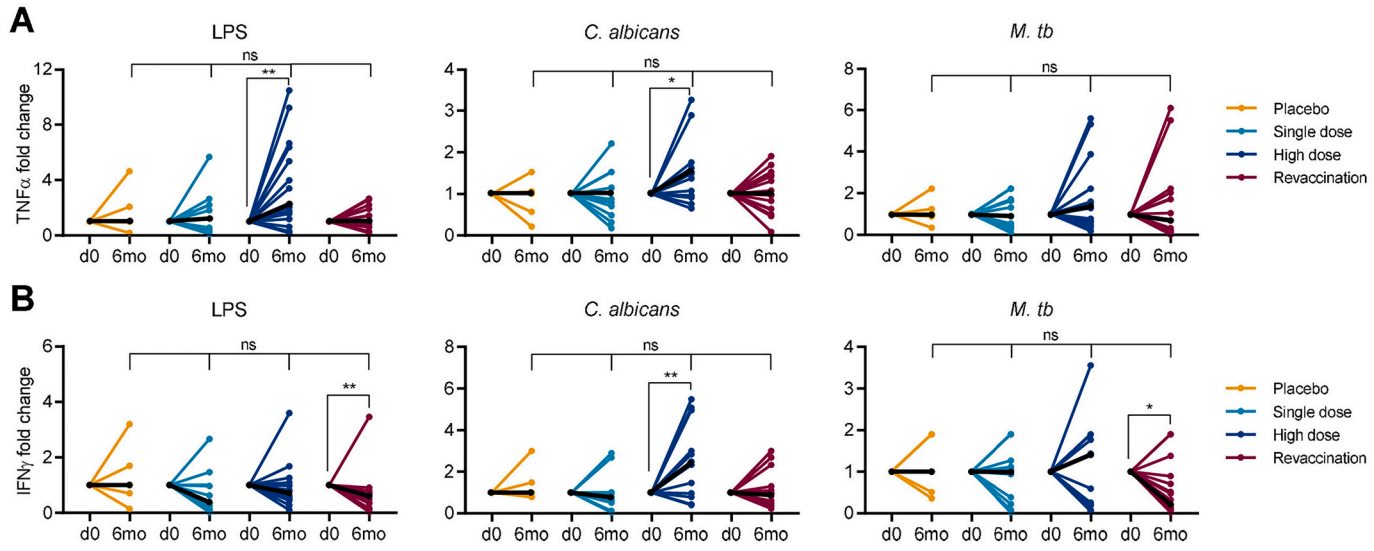

Fig. 4. NK cell-derived cytokine responses before vaccination and at 6 months.
NK cells were isolated from the PBMC fraction and stimulated with LPS, *C. albicans* and *M. tb* for 48 h. Black lines represent medians. (A) TNFα, (B) IFNγ. Wilcoxon signed-rank test was performed to compare cytokine release before and after vaccination. Differences between the four intervention groups were analyzed with Kruskal-Wallis tests; n = 5 (P), n = 13 (SD), n = 16 (HD), n = 17 (RV); $^{*}p < 0.05$, $^{**}p < 0.01$.

enhanced trained immunity profile in the majority of cases. On the other hand, the inverse correlation between baseline release and fold change production suggests that the strength of trained immunity is in part dependent on the subject's baseline cytokine induction.

Our finding that cytokine production capacity before vaccination is a predictor for BCG effectiveness in recipients is in line with recent research on determinants of population variation of trained immune reactions upon BCG administration. Genotype made the largest contribution to the breadth of changes in immune function after vaccination, a large part of which was however under genetically-encoded epigenetic control. Subjects who had little chromatin accessibility for genes involved in trained immunity at the start of the study, which in turn was reflected by low cytokine induction before vaccination, were more likely to mount a strong immune response after BCG (*Moorlag* et al., *2022, submitted*). This confirms our finding that not all individuals are equally 'trainable' with BCG and that people with a low innate immune profile are best targets for trained immunity. Moreover, it seems that BCG works to create homeostasis, promoting immune function in individuals with diminished cytokine production capacity, but not causing hyper-inflammation when immune responses are already activated before

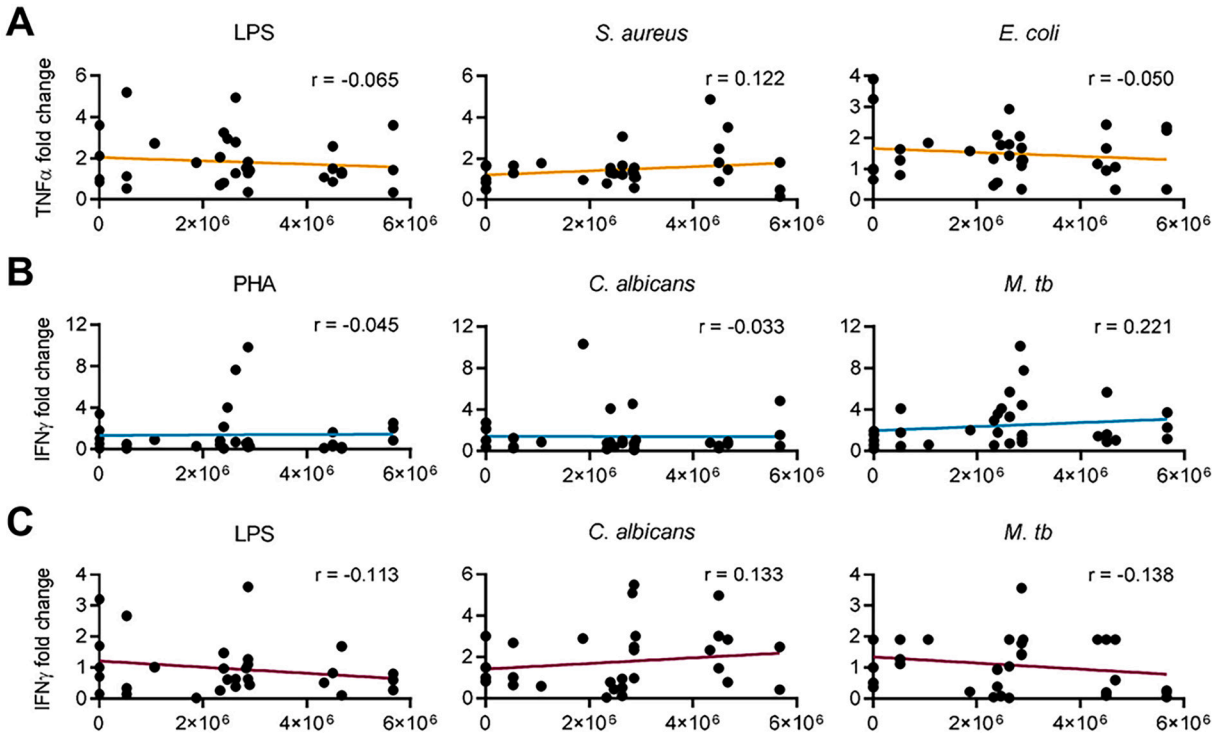

Fig. 5. Correlations between cytokine production capacity and BCG viability.
Viability in left-over material from BCG vials was measured by colony forming units (CFU). Colonies were counted after 21d and expressed as mean CFUs per ml. Spearman correlations were performed between BCG CFUs and cytokine production after stimulation with various pathogens in SD and HD individuals at 6 months, $n = 29$. (A) monocyte-derived TNFα production upon 24 h incubation with *LPS, S. aureus* and *M. tb* (B) T-cell-derived IFNγ after 7d incubation with PHA, *C. albicans* and *M. tb*. (C) NK cell- derived IFNγ after 48 h incubation with LPS, *C. albicans* and *M. tb*.

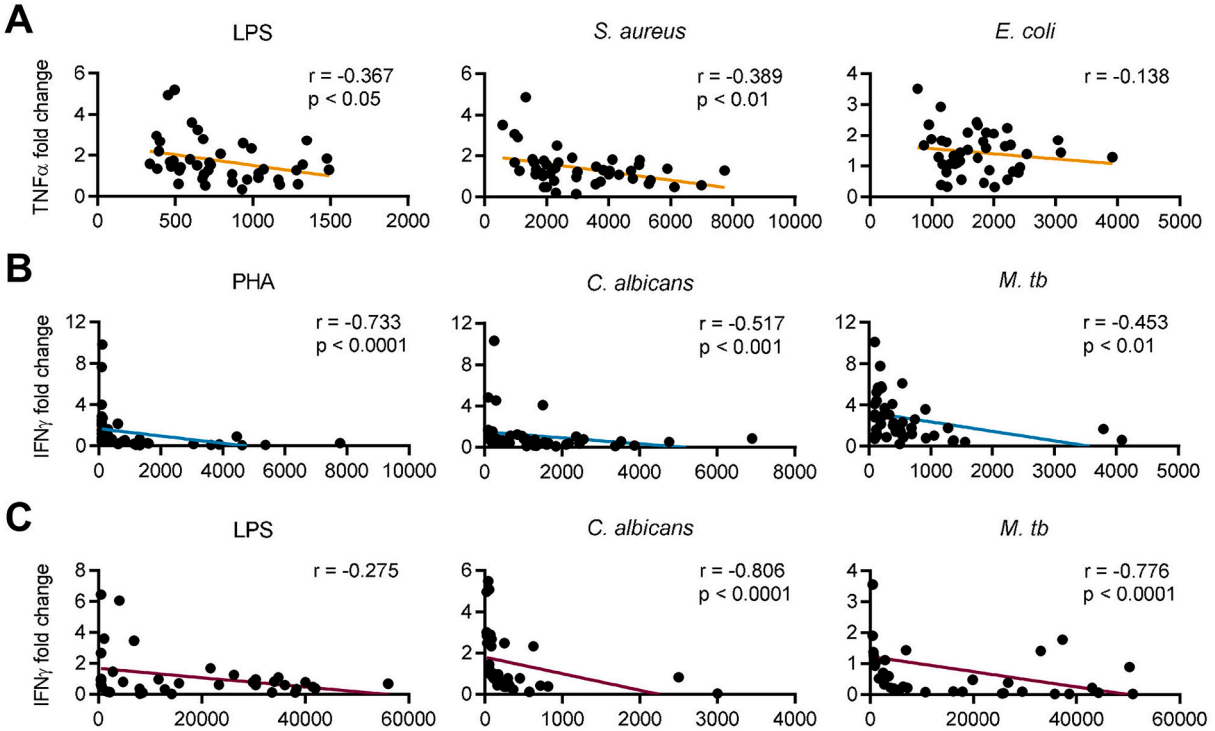

Fig. 6. Correlations between baseline cytokine production and cytokine production capacity at 6 months.
The association between baseline cytokine production and cytokine production capacity at 6 months in SD, HD and RV vaccinated individuals was assessed using Spearman correlations, $n = 46$. (A) Baseline monocyte-derived TNFα production upon 24 h incubation with *LPS, S. aureus* and *M. tb* (B) Baseline T-cell-derived IFNγ after 7d incubation with PHA, *C. albicans* and *M. tb*. (C) Baseline NK cell- derived IFNγ after 48 h incubation with LPS, *C. albicans* and *M. tb*.

vaccination.

A few aspects are important to underline. In the group vaccinated with a high dose of BCG, NK cells exhibited an increased cytokine production capacity after stimulation with *C. albicans*. Surprisingly, in response to stimulation with mycobacteria, T-cell-derived IFNγ and monocyte-derived cytokines were less strong compared with the standard BCG vaccination. This might suggest that in the case of fungal infections, a higher dose of BCG could be beneficial specifically to drive a NK cell response at the cost of downregulating T-cell and monocyte responses. This conclusion should be however interpreted with caution, and independent validation in future studies is warranted. If validated however, this finding could have considerable importance. Studies in experimental mouse models showed that NK cells are directly engaged in the antifungal immune response. The depletion of NK cells in these models prompted susceptibility to aspergillosis, candidiasis, and *C. neoformans* infection, leading to a rise in mortality [24–26]. BCG improvement of anti-fungal host defense may thus prove to have clinical potential.

Some studies have reported that revaccination with BCG could harbor more protection against TB than a single dose [17–19]. We could not provide an immunological explanation for these observations based on T-cell derived IFNγ production upon ex-vivo challenge with *M. tb*. Although a BCG revaccination caused a somewhat greater increase in cytokine production than a single BCG vaccination, this difference was not statistically significant. We cannot exclude that this was due to the relatively small number of test subjects in our study, and additional studies are warranted. This is in contrast to other, albeit few, studies using immunologic markers as a biomarker of the effects of BCG revaccination. In particular, revaccination boosted BCG-specific Th1 responses in CD4, CD8, and γδT-cells and improved IFNγ release from NK cells [27]. However, it is currently debated whether a Th1 response with elevated IFNγ activity following exposure to mycobacterial antigens is an accurate correlate of protection [28]. Furthermore, expanded cell frequencies and a Th1 dominant cytokine expression profile in CD4, CD8 and γδT-cells in children are not associated with BCG-induced protective immunity against TB, although in adults T-cell proliferation seems to have some predictive value [29–31]. In the absence of a solid immune correlate of protection it cannot be inferred that a revaccination with BCG is completely redundant in the prevention of active TB disease or *M. tb* infection. To overcome this, Nemes et al. used sustained seroconversion in adolescents, resembled by positive QuantiFERON-TB Gold (QFT) tests that remained positive for 6 months, as a surrogate for *M. tb* infection. They found a sustained QFT conversion of 6.7% due to BCG revaccination, as opposed to 11.6% in primary vaccinated individuals [32]. Interestingly, this study also conveyed evidence for non-specific protection of BCG revaccination. The authors reported significantly fewer upper respiratory tract infections among the revaccination group as compared to the single dose group (2.1% and 7.9% respectively) [32]. This partial protection against respiratory tract infections could indicate the possible role of trained immunity.

Only one study on BCG revaccination and non-specific beneficial effects was conducted in adults, however with a sub-optimal design. Glynn et al. assessed all-cause mortality in a double-blind, randomized, placebo-controlled trial over the course of 30 years in Malawi. They found no difference between BCG revaccination or placebo, arguing against beneficial non-specific effects [33]. It must be stressed however that the advantages of trained immunity are best appreciated in a time period of one to two years from vaccination. Additionally, trained immunity protects against communicable diseases, which indeed accounts for reduced mortality in children, but in adults simply results in fewer infections and thereby a decline in morbidity, rather than mortality [34].

Our study also has limitations that should be considered. The intervention groups in our trial are relatively small, which makes it difficult to extrapolate our findings to the general public. The placebo group was smaller than the other groups, as it only aimed to ensure that the differences observed were due to BCG vaccination alone, and not to seasonal effects on cytokine production. Subsequently, the most informative comparator to examine the superiority of BCG booster regimens to induce trained immunity is represented by the single dose BCG group. The distribution of sex among some intervention groups, although not significantly different, is somewhat uneven. Similar tendencies in cytokine production capacity were obtained in sex-stratified comparisons, not reaching statistical significance because of the group size. Nonetheless, it cannot be excluded that potential sex- dependent effects remained undetected because of limitations in group size. It has been reported that protective as well as deleterious non-specific effects of vaccines can behave in sex-dependent ways and should be further explored [35,36]. Also, the population that would benefit most from trained immunity is presumably composed of people with a frail immune system, such as the immunocompromised and the elderly. Since recent trials have shown that BCG in elderly is generally safe, it would be interesting to see whether the purported effects are similar among people of older age [37]. Finally, it is essential to point out that, although we did not find immunological basis to support double vaccination regimes, this study was not conducted in a TB endemic area and therefore, these results should be interpreted cautiously before independent validation.

In conclusion, we provide immunological data suggesting that BCG revaccination or high dose BCG administration do not contribute to an enhanced trained immunity program beyond that induced by a standard BCG dose. Our results suggest that, in most cases, a single dose BCG is sufficient for achieving an effective trained immunity response. There are some arguments that if BCG is used to improve host defense against infections that are more contingent upon NK cell activity, it may be beneficial to increase the amount of BCG administered. Furthermore, the strength of the trained immunity response upon BCG vaccination seems to be influenced by baseline cytokine induction. More research is warranted to explore modulation of trained immunity by BCG vaccination, and the potential benefit of other schemes of vaccination.

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Institutional review board statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Arnhem-Nijmegen Ethics Committee of CMO Radboudumc (protocol code NL58219.091.16, date of approval November, 11, 2019).

Informed consent statement

Informed consent was obtained from all subjects involved in the study.

Data availability statement

The data presented in this study are available on reasonable request from the corresponding author.

CRediT authorship contribution statement

Priya A. Debisarun: Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing – original draft, Visualization, Project administration. **Gizem Kilic:** Conceptualization, Methodology, Software, Formal analysis, Investigation, Data curation, Writing – review & editing, Visualization. **L. Charlotte J. de Bree:**

Conceptualization, Methodology, Writing – review & editing. **Lian J. Pennings:** Conceptualization, Formal analysis, Investigation, Writing – review & editing. **Jakko van Ingen:** Conceptualization, Formal analysis, Investigation, Writing – review & editing. **Christine S. Benn:** Conceptualization, Methodology, Writing – review & editing. **Peter Aaby:** Conceptualization, Methodology, Writing – review & editing. **Helga Dijkstra:** Conceptualization, Validation, Formal analysis, Writing – review & editing. **Heidi Lemmers:** Conceptualization, Validation, Formal analysis, Writing – review & editing. **Jorge Domínguez-Andrés:** Conceptualization, Writing – review & editing, Supervision. **Reinout van Crevel:** Conceptualization, Writing – review & editing. **Mihai G. Netea:** Conceptualization, Methodology, Resources, Writing – review & editing, Supervision, Funding acquisition.

Declaration of Competing Interest

The authors declare no conflict of interest. The funders had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Data availability

Data will be made available on request.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clim.2022.109208.

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