

University of Groningen

BCG Vaccination Protects against Experimental Viral Infection in Humans through the Induction of Cytokines Associated with Trained Immunity

Arts, Rob J. W.; Moorlag, Simone J. C. F. M.; Novakovic, Boris; Li, Yang; Wang, Shuang-Yin; Oosting, Marije; Kumar, Vinod; Xavier, Ramnik J.; Wijmenga, Cisca; Joosten, Leo A. B.

Published in:
Cell Host & Microbe

DOI:
[10.1016/j.chom.2017.12.010](https://doi.org/10.1016/j.chom.2017.12.010)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2018

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Arts, R. J. W., Moorlag, S. J. C. F. M., Novakovic, B., Li, Y., Wang, S-Y., Oosting, M., Kumar, V., Xavier, R. J., Wijmenga, C., Joosten, L. A. B., Reusken, C. B. E. M., Benn, C. S., Aaby, P., Koopmans, M. P., Stunnenberg, H. G., van Crevel, R., & Netea, M. G. (2018). BCG Vaccination Protects against Experimental Viral Infection in Humans through the Induction of Cytokines Associated with Trained Immunity. *Cell Host & Microbe*, 23(1), 89-100. <https://doi.org/10.1016/j.chom.2017.12.010>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

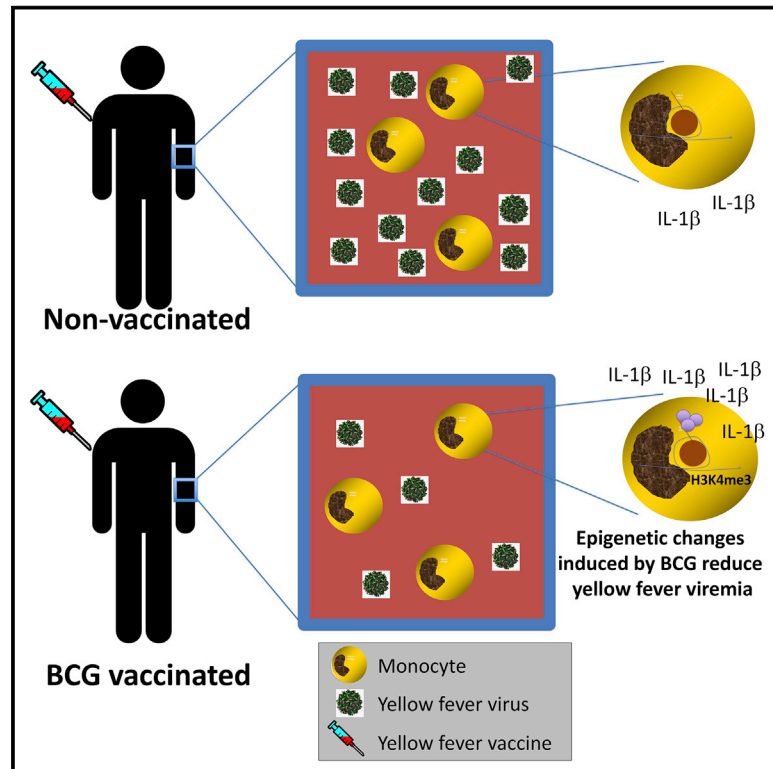
If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

Cell Host & Microbe

BCG Vaccination Protects against Experimental Viral Infection in Humans through the Induction of Cytokines Associated with Trained Immunity

Graphical Abstract



Authors

Rob J.W. Arts,
 Simone J.C.F.M. Moorlag,
 Boris Novakovic, ...,
 Hendrik G. Stunnenberg,
 Reinout van Crevel, Mihai G. Netea

Correspondence

mihai.netea@radboudumc.nl

In Brief

In this paper, Arts et al. describe that BCG vaccination induces genome-wide epigenetic reprogramming of human monocytes that correlates with protection against experimental viral infection. Reduction of viremia correlated with upregulation of non-specific IL-1 β production, and genetic polymorphisms in the IL-1 pathway affect the induction of trained immunity by BCG.

Highlights

- BCG vaccination of humans induces genome-wide epigenetic reprogramming in monocytes
- BCG-induced changes correlate with protection against experimental virus infection
- Viremia reduction correlates with IL-1 β upregulation, indicative of trained immunity
- SNPs in *IL1B* affect the induction of trained immunity by BCG

Data Resources

GSE104149



BCG Vaccination Protects against Experimental Viral Infection in Humans through the Induction of Cytokines Associated with Trained Immunity

Rob J.W. Arts,^{1,10} Simone J.C.F.M. Moorlag,^{1,10} Boris Novakovic,² Yang Li,³ Shuang-Yin Wang,¹ Marije Oosting,¹ Vinod Kumar,³ Ramnik J. Xavier,^{4,5} Cisca Wijmenga,³ Leo A.B. Joosten,¹ Chantal B.E.M. Reusken,⁶ Christine S. Benn,^{7,8} Peter Aaby,^{7,8} Marion P. Koopmans,⁶ Hendrik G. Stunnenberg,² Reinout van Crevel,¹ and Mihai G. Netea^{1,9,11,*}

¹Department of Internal Medicine and Radboud Center for Infectious Diseases, Radboud University Medical Center, Nijmegen, the Netherlands

²Department of Molecular Biology, Radboud University, Nijmegen, the Netherlands

³Department of Genetics, University Medical Center Groningen, Groningen, the Netherlands

⁴Center for Computational and Integrative Biology and Gastrointestinal Unit, Massachusetts General Hospital, Harvard School of Medicine, Boston, MA, USA

⁵Broad Institute of MIT and Harvard University, Cambridge, MA, USA

⁶Department Viroscience, WHO Collaborating Centre for Arboviruses and Viral Hemorrhagic Fever Reference and Research, Erasmus University Medical Center, Rotterdam, the Netherlands

⁷Research Center for Vitamins and Vaccines (CVIVA), Bandim Health Project, Statens Serum Institut, Copenhagen, Denmark

⁸OPEN, Institute of Clinical Research, University of Southern Denmark/Odense University Hospital, Odense, Denmark

⁹Department for Genomics & Immunoregulation, Life and Medical Sciences Institute (LIMES), University of Bonn, 53115 Bonn, Germany

¹⁰These authors contributed equally

¹¹Lead Contact

*Correspondence: mihai.netea@radboudumc.nl

<https://doi.org/10.1016/j.chom.2017.12.010>

SUMMARY

The tuberculosis vaccine bacillus Calmette-Guérin (BCG) has heterologous beneficial effects against non-related infections. The basis of these effects has been poorly explored in humans. In a randomized placebo-controlled human challenge study, we found that BCG vaccination induced genome-wide epigenetic reprogramming of monocytes and protected against experimental infection with an attenuated yellow fever virus vaccine strain. Epigenetic reprogramming was accompanied by functional changes indicative of trained immunity. Reduction of viremia was highly correlated with the upregulation of IL-1 β , a heterologous cytokine associated with the induction of trained immunity, but not with the specific IFN γ response. The importance of IL-1 β for the induction of trained immunity was validated through genetic, epigenetic, and immunological studies. In conclusion, BCG induces epigenetic reprogramming in human monocytes *in vivo*, followed by functional reprogramming and protection against non-related viral infections, with a key role for IL-1 β as a mediator of trained immunity responses.

INTRODUCTION

Bacillus Calmette-Guérin (BCG) is a live-attenuated vaccine strain of *Mycobacterium bovis* that protects against mycobacterial infections such as tuberculosis and leprosy and has now

been used for almost a century (Colditz et al., 1994; Zumla et al., 2013). Interestingly, the BCG vaccine also has heterologous protective effects against non-related infections: several epidemiological studies have shown that vaccination at birth results in reduced child mortality (Hirve et al., 2012; Kristensen et al., 2000; Moulton et al., 2005; Roth et al., 2005). These beneficial effects of BCG have been validated in randomized controlled trials, and the reduced mortality appeared to be mainly due to protection against neonatal sepsis and respiratory infections (Aaby et al., 2011; Biering-Sørensen et al., 2012; Roth et al., 2004). BCG vaccination has been also shown to protect against mortality in murine experimental models of non-mycobacterial infections (summarized in Blok et al., 2015). Lastly, BCG is used as non-specific immunotherapy in cancer, with BCG installations in bladder cancer inducing immune-stimulating effects that slow tumor progression (Han and Pan, 2006).

Two types of immunological mechanisms have been suggested to mediate these effects. First, CD4 and CD8 memory cells can be activated in an antigen-independent manner (e.g., by cytokines stimulated by a secondary infection), a process called heterologous immunity (Berg et al., 2002, 2003; Lertmongsolkolchai et al., 2001; Mathurin et al., 2009). Second, BCG vaccination induces histone modifications and epigenetic reprogramming of human monocytes at the promoter sites of genes encoding for inflammatory cytokines such as *TNFA* and *IL6*, resulting in a more active innate immune response upon restimulation, a process called trained immunity (Kleinnijenhuis et al., 2012; Netea et al., 2016). However, the extent of the effects of BCG on histone modifications at a genome-wide level remains unknown. Moreover, while the described epidemiological studies clearly suggest protective heterologous effects of BCG, controlled experimental studies in humans to establish the immunological basis for these observations remain



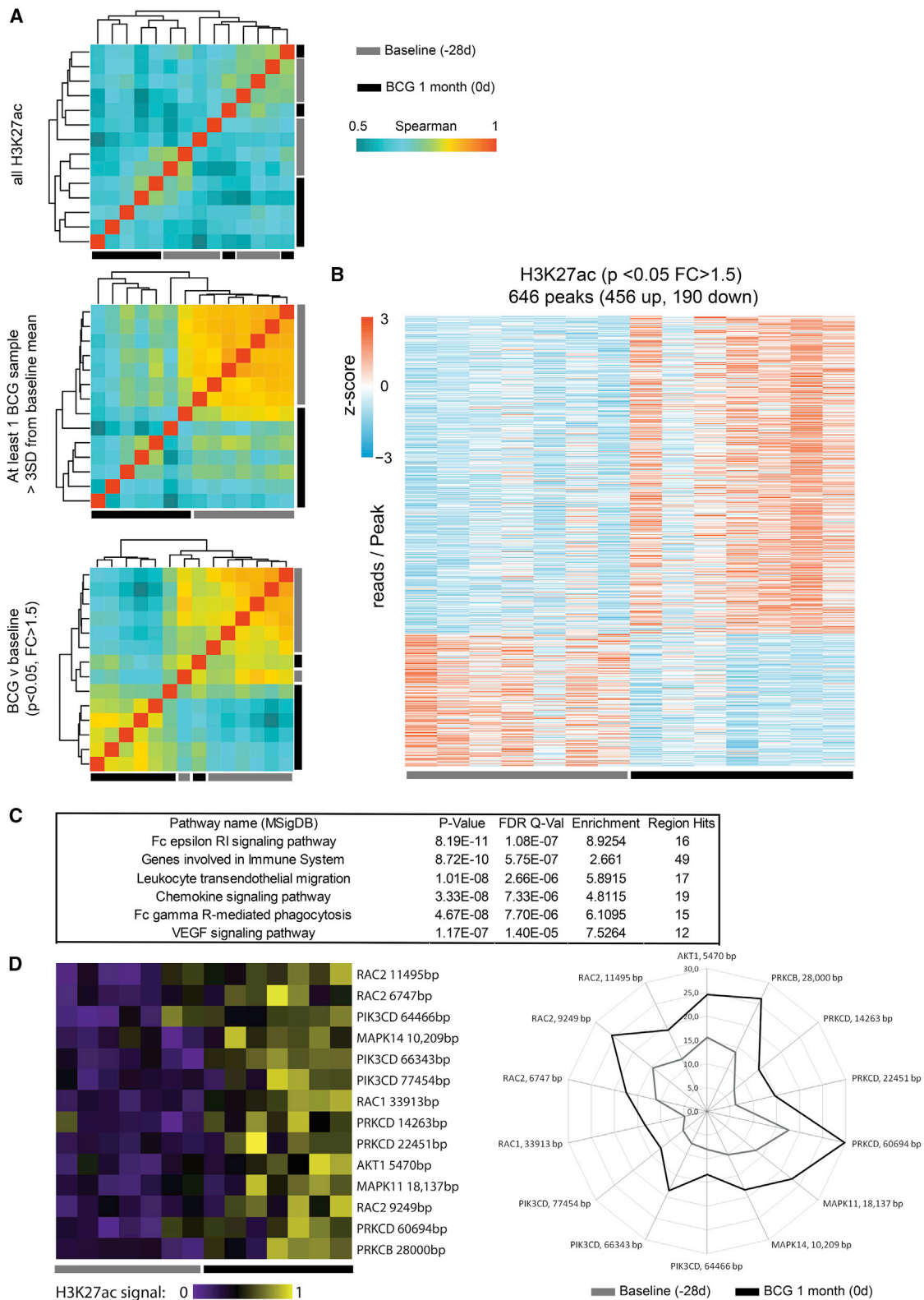


Figure 1. BCG Vaccination Induced Genome-wide H3K27ac Changes in Monocytes

(A–D) Monocytes from seven donors were analyzed by ChIP-seq to determine the distribution of H3K27ac at “baseline” (before BCG vaccination, –28d) and “BCG 1 month” (1 month after BCG vaccination, 0d).

(legend continued on next page)

outstanding, and the potential role of trained immunity in mediating these effects has not been assessed.

In this study, we examined the effects of BCG vaccination on genome-wide histone modifications induced in circulating monocytes by BCG vaccination. Subsequently, we examined the functional changes induced by BCG in monocytes and examined how BCG vaccination impacts viral, serological, and immunological parameters after yellow fever vaccine (YFV) administration. YFV is an attenuated viral strain that can be detected in the circulation after vaccination, thereby being an ideal model of experimental viral infection in humans. We show that BCG vaccination reduces the level of YFV viremia after vaccination and that this effect correlates with induction of cytokine responses indicative of trained immunity, with a crucial role for IL-1 β production and release.

RESULTS

BCG Vaccination Induces Genome-wide Epigenetic Reprogramming of Human Monocytes *In Vivo*

In vitro experiments have previously shown that the increased cytokine responses after monocyte training with BCG are the result of epigenetic changes (Arts et al., 2015). In order to examine the genome-wide rewiring of the epigenetic program by BCG, monocytes from seven donors were analyzed by chromatin immunoprecipitation sequencing (ChIP-seq) to determine genome-wide changes in the distribution of histone H3 acetylation at lysine 27 (H3K27ac), a marker of active promoters and enhancers, at “baseline” (before BCG vaccination, day -28) and “BCG 1 month” (1 month after BCG vaccination, day 0). Correlation plots of whole-genome H3K27ac sequencing reads showed a clear separation of the baseline and BCG samples, especially when analysis was limited to peaks that were highly induced (> 3 SD) in at least one donor or with a statistical significant fold change of at least 1.5 (Figures 1A and S1A). The heatmap clearly shows the separation of baseline and post-BCG samples according to 646 differential H3K27ac peaks (Figure 1B). A similar effect was seen when analysis was restricted to peaks related to promoters (Figures S1B and S1C). Finally, pathway analysis including genes near the 646 differential peaks showed differential regulation of several important signaling- and inflammatory-related pathways (Figure 1C; Tables S2 and S3).

Most changes induced by BCG vaccination in promoter regions involve G protein-coupled receptors and protein kinases, indicating the extensive effect of BCG on remodeling signal transduction molecules (Table S3). Accordingly, BCG induces increased H3K27ac in several important signaling pathways, including the PI3K/AKT (phosphatidylinositol 3-kinase) pathway, epidermal growth factor receptor (EGFR), fibroblast growth

factor (FGF), and vascular endothelial growth factor (VEGF) signaling pathways (Table S3) (upregulated genes involved in these pathways can be found in Table S4). Interestingly, genes such as AKT1, MAPKs, and PI3K-related genes that have been shown to be major regulators in β -glucan-induced trained immunity appear to be also important in BCG-induced trained immunity (Cheng et al., 2014; Saeed et al., 2014) (Figure 1D; Table S4). Genes directly involved in the inflammatory response and cytokine production also showed increased H3K27ac upon BCG vaccination. Intriguingly, the receptor of oxidized low-density lipoprotein (oxLDL), OLR1, a marker of atherosclerosis, previously shown to induce a long-term pro-inflammatory phenotype in monocytes (Bekkering et al., 2014), was also differentially regulated upon BCG vaccination (Table S3).

RNA sequencing has also been performed on the same samples, but as expected and as shown before (Novakovic et al., 2016; Saeed et al., 2014), no major differences were found. This shows that trained monocytes are epigenetically primed to respond (transcriptionally) differently to a secondary stimulus (e.g., *ex vivo* stimulation), but that in resting state they do not display a changed transcriptional program.

Epigenetic Reprogramming Is Accompanied by Functional Changes

In line with our previous finding of BCG-induced functional changes in monocytes (Arts et al., 2016; Cheng et al., 2016), we demonstrate that genes involved in the PI3K/AKT/mTOR pathway are already marked at epigenetic level. This pathway has a crucial role for the increased cytokine production capacity during induction of trained immunity (Arts et al., 2016; Cheng et al., 2016), and these epigenetic changes upon BCG vaccination were indeed accompanied by enhanced *ex vivo* cytokine responses to unrelated pathogens, as shown by increased cytokine production capacity 1 month after BCG vaccination (Figures 2A, 2B, and S2), which was not the result of differences in monocyte subpopulations or total monocyte number (Figure S3A). Heterologous T cell responses were moderately increased after BCG vaccination (Figure S2). Induction of monocyte-derived cytokine production (used here as a readout of trained immunity) varied between individuals, with unsupervised cluster analysis of increased cytokine production 1 month after BCG vaccination clearly showing a cluster of innate cytokines and a second cluster of T helper 1/Th17 lymphocyte cytokines. Within the innate cytokine cluster, the induction of IL-1 β and IL-6 production was highly correlated (Figure 2C).

BCG Vaccination Lowers Yellow Fever Viremia

To examine the clinical significance of the observed epigenetic and functional effects of BCG, we used the live-attenuated

(A) Spearman's correlation plot showing the relationship between the different donors and exposures, based on sequencing reads at (1) all H3K27ac peaks, (2) peaks dynamic in at least one donor after BCG exposure, and (3) peaks dynamic between the baseline and BCG 1 month groups ($p < 0.05$, $FC > 1.5$). Based on all H3K27ac peaks, we observe mixing of the baseline and BCG samples, which suggests little global change in H3K27ac in response to BCG. However, based on significantly different peaks (2, 3), clear separation between baseline and BCG-exposed monocytes can be observed.

(B) Heatmap of 646 peaks showing differences between baseline and BCG-exposed groups; most of these changes are increased H3K27ac signal in BCG-exposed monocytes.

(C) Top pathways associated with genes near the 646 differential peaks and names of genes associated with inflammation and cytokine signaling.

(D) Genes involved in the Fc ϵ RI signaling pathway. The heatmap shows the H3K27ac relative signal before and 1 month after BCG vaccination. The genomic locations are named by their closest gene and the distance in base pairs (bp). The spiderweb plot shows the same genes with the corresponding reads per peak ($n = 7$).

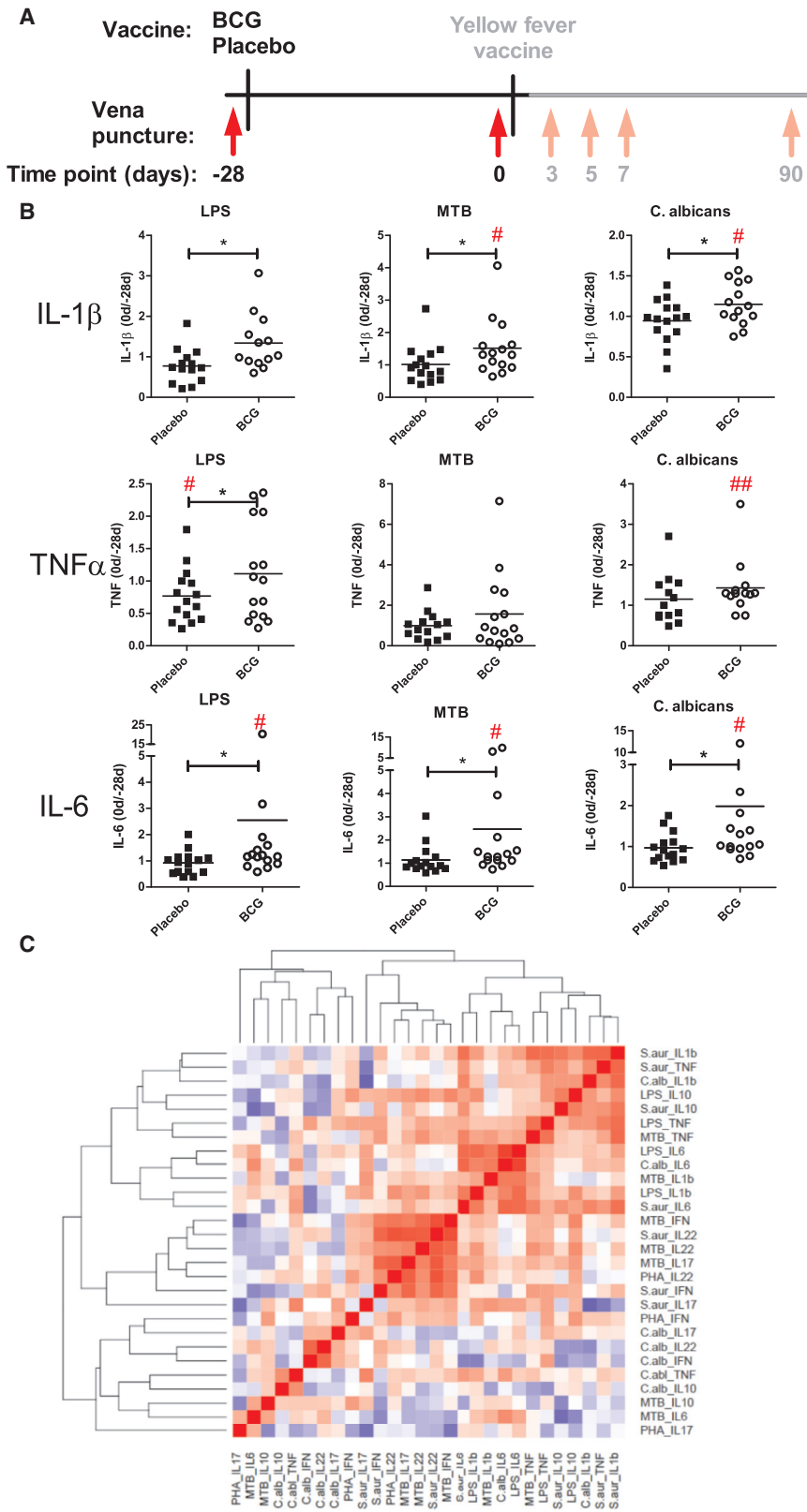


Figure 2. BCG Induces Trained Immunity In Vivo

(A) Healthy volunteers randomly received BCG or placebo vaccination. Before vaccination and 1 month later, blood was drawn and PBMCs were isolated and restimulated *ex vivo*.

(B) Fold increases (compared to baseline) of IL-1 β , TNF α , and IL-6 production to LPS, *M. tuberculosis* (MTB), and *C. albicans* are shown (mean \pm SEM, n = 15 per group, *p < 0.05 BCG versus placebo, #p < 0.01 mean versus 1, ##p < 0.01 mean versus 1, fold increase baseline versus 1 month, Mann-Whitney U test).

(C) Unsupervised clustering analysis performed on Spearman's correlations of fold induction of cytokines as presented in (B). IL-1 β , TNF α , IL-6, and IL10 were determined after 24 hr and IL-17, IL-22, and IFN γ after 7 day *ex vivo* incubation with LPS, *M. tuberculosis* (MTB), *S. aureus*, or *C. albicans*.

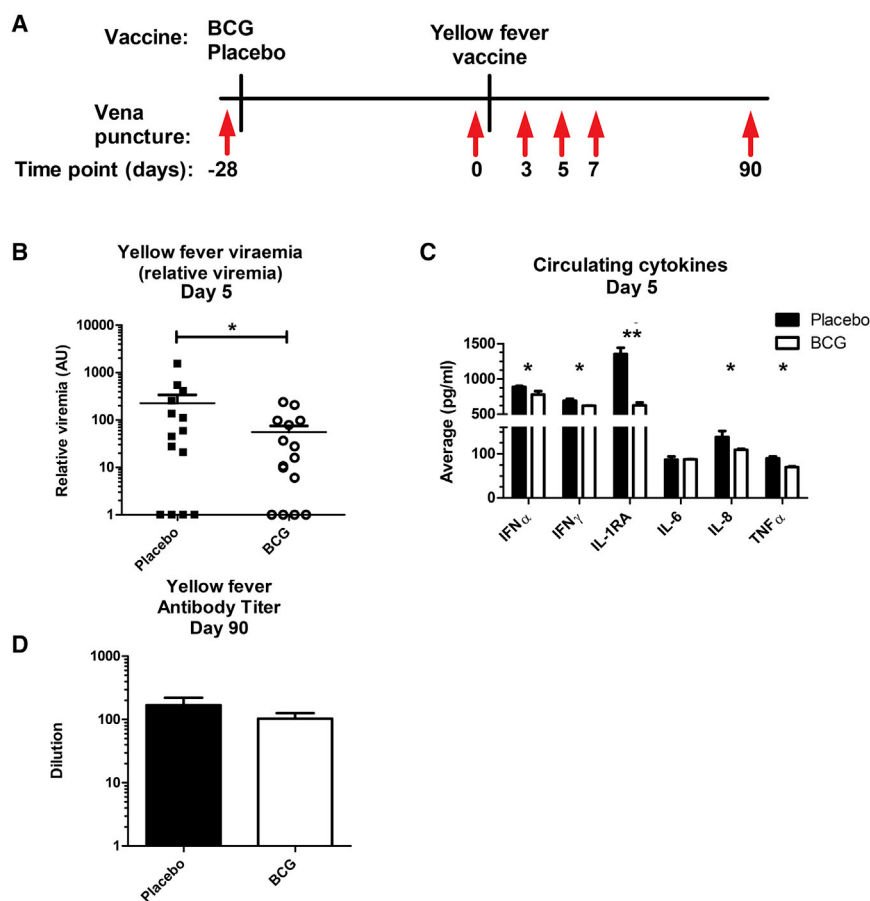


Figure 3. BCG Vaccination Lowers Yellow Fever Vaccine Viremia

(A) Post-vaccination viremia was determined by PCR 3, 5, and 7 days after YFV.

(B) Viremia at day 5 post-infection. CT values and corresponding calculated relative viremia (CT 40 is set as 1, by the formula Relative Viremia = $2^{-(40 - Ct)}$) are shown (mean \pm SEM, $n = 15$ per group, $*p < 0.05$, Mann-Whitney U test).

(C) Circulating cytokines were determined 5 days post-vaccination in plasma. IL-1 β and IL-10 were below detection limit in almost all samples (mean \pm SEM, $n = 15$ per group, $*p < 0.05$, $**p < 0.01$, Mann-Whitney U test).

(D) Yellow fever-neutralizing antibodies were determined 90 days after yellow fever vaccination.

YFV as a model for a viral human infection. YFV viremia, determined by RT-PCR, peaked on day 5 after vaccination, as previously reported by others (Edupuganti et al., 2013) (Figures 3A, 3B, and S3B). Subjects who had been BCG vaccinated 1 month prior to yellow fever vaccination showed significantly lower viremia compared to subjects who had received placebo vaccination (Figure 3B, higher CT values, thus lower viremia, for BCG-vaccinated volunteers). Lower circulating virus concentrations in BCG-vaccinated volunteers were also reflected by lower concentration of circulating cytokines (Figure 3C). Circulating IL-1 β concentrations before and after vaccination were below detection limit. Importantly, when yellow fever-neutralizing antibodies were measured at 3 months post-vaccination, no significant differences were found between the two groups (Figure 3D), suggesting that BCG vaccination has minor impact on humoral responses to yellow fever vaccination, despite the diminished viremia. In addition, BCG vaccination did not change the specific cellular response to YFV as measured by IFN γ production by PBMCs (Figure S3C).

Non-specific IL-1 β Production Increase after BCG Vaccination Is a Correlate of Protection against Yellow Fever Viremia

We next determined whether the induction of *ex vivo* cytokine responses associated with trained immunity, as noted earlier, correlated with yellow fever viremia. Post-vaccination increases in TNF α and IL-6 production did not correlate with viremia

(Figures 4A and S4A). In contrast, increased IL-1 β production capacity after BCG vaccination strongly predicted lower viremia after subsequent yellow fever infection (Figures 4A and 4B). Among the other cytokines, induction of IL-10 upon stimulation with *Candida albicans* also displayed a negative correlation with viremia (Figures 4A, 4B, and S4A). Interestingly, viremia did not correlate with induction of specific cellular responses to YFV by BCG as measured by IFN γ production by PBMCs (Figures 4C and S4B). In addition, no correlation was found between viremia and concentrations of the Th17 cytokines IL-17 and IL-22 (Figure S4A). This is remarkable, as we and others have found an induction of CD8+ and polyfunctional CD4+ responses after BCG vaccination (Figure S2) (Boer et al., 2015; Kleinnijenhuis et al., 2012). Concentrations of yellow fever-neutralizing antibody titers also did not correlate with induction of cytokines (neither innate nor adaptive) (Figure 4D).

Epigenetic Differences Influence Yellow Fever Viremia

Apart from the correlation with cytokine induction, correlation of epigenetic changes induced by BCG and yellow fever viremia were also determined. In order to do so, volunteers presented in Figure 1 were divided into a group of responders to BCG vaccination (R, maximum yellow fever viremia CT > 36) and non-responders (NR, CT < 36). Pre-existing H3K27Ac epigenetic differences (at baseline, before BCG vaccination) at the gene region level were determined between R and NR, and we identified important differences between these two groups of volunteers, suggestive of the pathways important for the post-vaccination response. Interestingly, the top-ranked genomic region in this analysis was 7.6 kb upstream of the gene coding for NOD2 (FC = 6.3, adjusted p value = 2.42×10^{-8}), the innate immune receptor recognizing muramyl dipeptide: an earlier study demonstrated that monocytes isolated from individuals with NOD2 mutations leading to a defective receptor cannot be trained *in vitro* with BCG (Arts et al., 2015; Kleinnijenhuis et al., 2012) (Figures 5A and 5B).

Fold increase of cytokines vs yellow fever viraemia

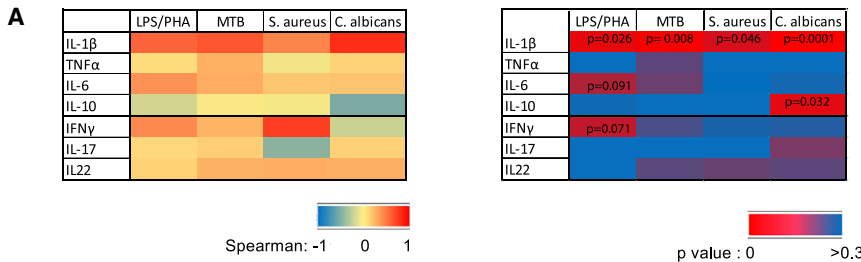


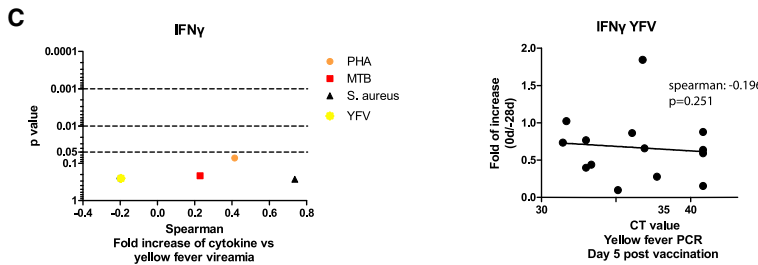
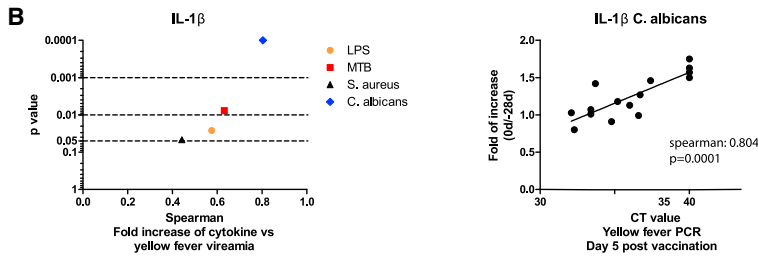
Figure 4. Induction of IL-1 β Negatively Correlates with Yellow Fever Viraemia

(A) Fold induction of cytokine production 1 month after BCG vaccination (as shown in Figure 2B) compared with yellow fever viraemia at day 5 post-vaccination (as shown in Figure 3B). Spearman's correlation coefficients and their p values are shown in two separate matrices. Induction of IL-1 β shows high correlations with suppression of yellow fever viraemia for all stimuli used.

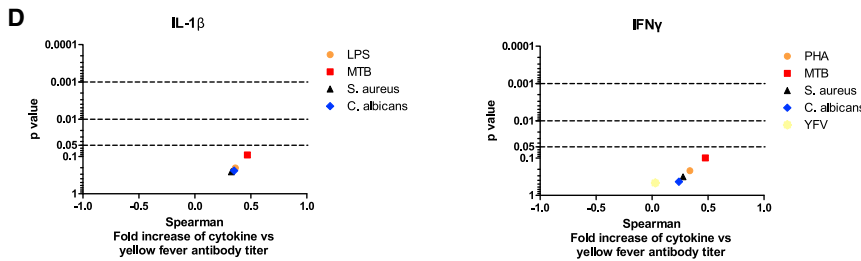
(B) An example is shown of fold increase (a month after BCG vaccination) of IL-1 β by *C. albicans* stimulation, which correlated with yellow fever viraemia (CT values, high CT value corresponds with low levels of viraemia).

(C) On the other hand, induction of IFN γ production did not correlate with yellow fever viraemia suppression. Even IFN γ production to YFV stimulation did not correlate.

(D) Fold induction of cytokine production 1 month after BCG vaccination (as shown in Figure 1B) for IL-1 β and IFN γ compared with yellow fever antibody titer 90 days post-vaccination (as shown in Figure 3D). Spearman's correlation coefficients and their p values are shown (n = 15 per group). All correlations with other cytokines can be found in Figure S4.



Fold increase of cytokines vs yellow fever antibody titer



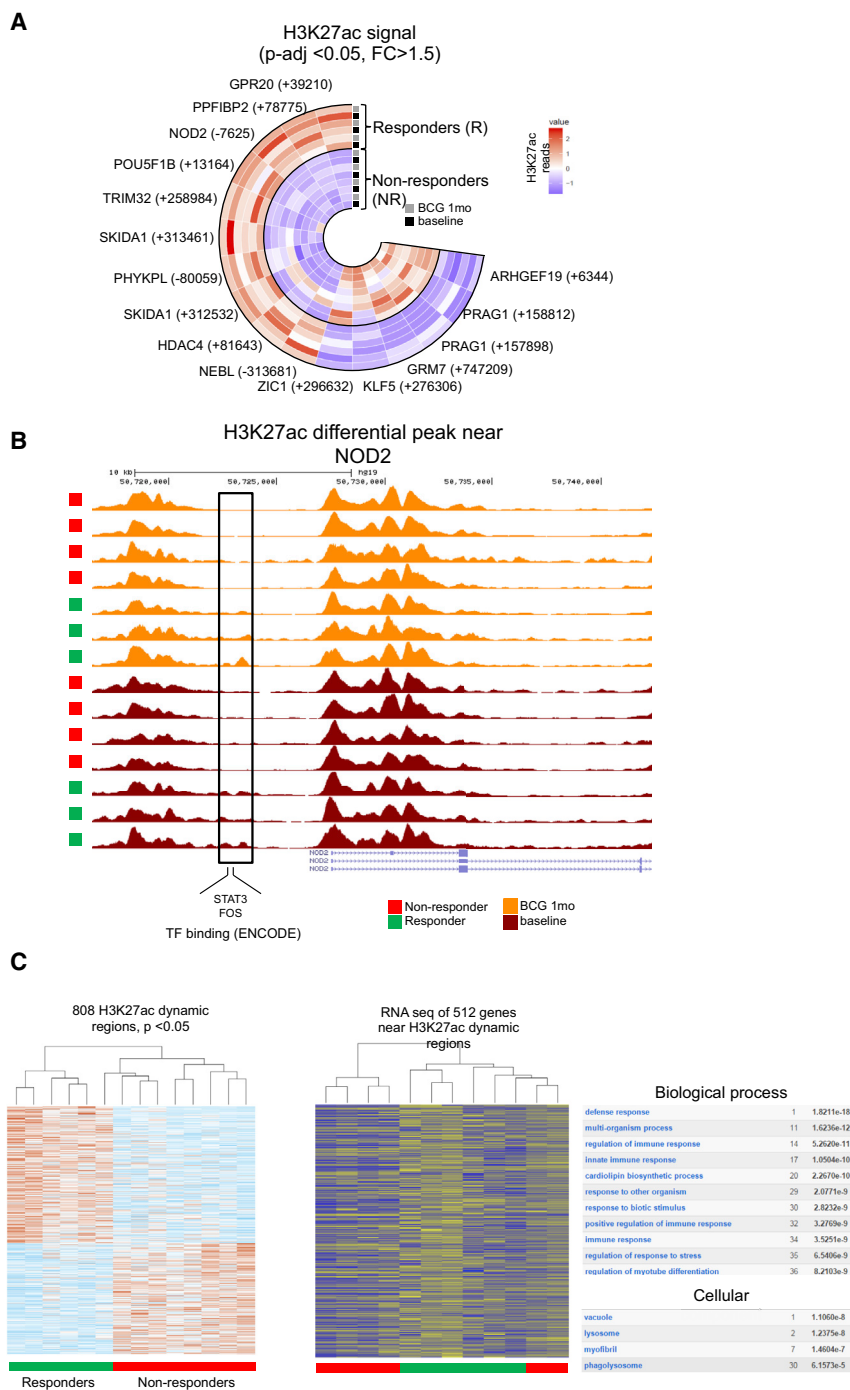
Furthermore, pathway analysis of H3K27ac profiles between R and NR reveals several interesting results. Once more, RNA-seq analysis did not show a clear pattern, as expected, as cells were not re-stimulated prior to analysis (Figure 5C). Among the main differentially regulated pathways at the epigenetic (but not RNA) level was "TB latent infection," which is in line with the BCG vaccination, as well as general immunological pathways such as "Immune Response" and "Response to Cytokine Stimulus" (Figure S5A), showing the epigenetic correlates of protection to yellow fever viraemia.

Genetic Polymorphisms in Genes of IL-1 β Pathway Modulate Trained Immunity Response

The role of IL-1 β as a trained immunity correlate of protection against yellow fever viraemia was supported by additional

genetic and immunological validation studies. First, we examined the effect of genetic variations in *IL1B* on BCG-induced trained immunity cytokine responses in the 200FG cohort of healthy volunteers (Li et al., 2016). Strikingly, the cytokine responses indicative of trained immunity that were induced by BCG were modulated by a known polymorphism located in the promoter region of the gene encoding IL-1 β (*IL1B*; rs16944) (Figure 6A). The presence of two copies of the A allele of rs16944 was associated with significantly impaired cytokine production associated with trained immunity responses. Interestingly, *in vitro* studies

have shown that the *IL1B* rs16944 A allele is associated with decreased IL-1 β production (Wen et al., 2006; Wójtowicz et al., 2015). In line with these findings, we demonstrated reduced mRNA expression of IL-1 β in individuals having the AA genotype compared to individuals who inherited the GG genotype (Figure S5B). As further support for the role for IL-1 β in BCG-induced trained immunity, polymorphisms in several additional IL-1 family genes, encoding IL-1 and IL-18 receptors as well as the inflammasome component PYCARD/ASC, showed a strong impact on the induction of cytokines associated with trained immunity (Figure 6B). In contrast, polymorphisms in the gene encoding TNF α did not modulate *in vitro* trained immunity responses, and only one polymorphism in *IL6* was found to weakly affect training (data not shown).



Epigenetic Changes Mediate IL-1 β -Induced Trained Immunity

To obtain further insight into the role of IL-1 β in mediating trained immunity responses, we next assessed whether IL-1 β itself can induce trained immunity *in vitro*. Using the previously described *in vitro* model of trained immunity (Bekkering et al., 2016; Klein-nijenhuis et al., 2012), priming of monocytes with IL-1 β (1–10 ng/mL) for 24 hr (Figure 6C) enhanced production of the pro-inflammatory cytokines IL-6 and TNF α upon LPS restimula-

Figure 5. H3K27ac Whole-Genome Analysis of Monocytes between Responders and Non-Responders

(A–C) Monocytes from seven donors were analyzed by ChIP-seq to determine the distribution of H3K27ac at “baseline” (before BCG vaccination, –28d) and “BCG 1 month” (1 month after BCG vaccination, 0d). Based on yellow fever viremia, BCG responders (R) and BCG non-responders (NR) were defined, respectively, by yellow fever CT values above or below 36.

(A) Peaks dynamic between the BCG responders (R) and non-responders (p adjusted < 0.05 , $FC > 1.5$) were defined and named after their closest gene (gene name, number of base pairs distance from gene).

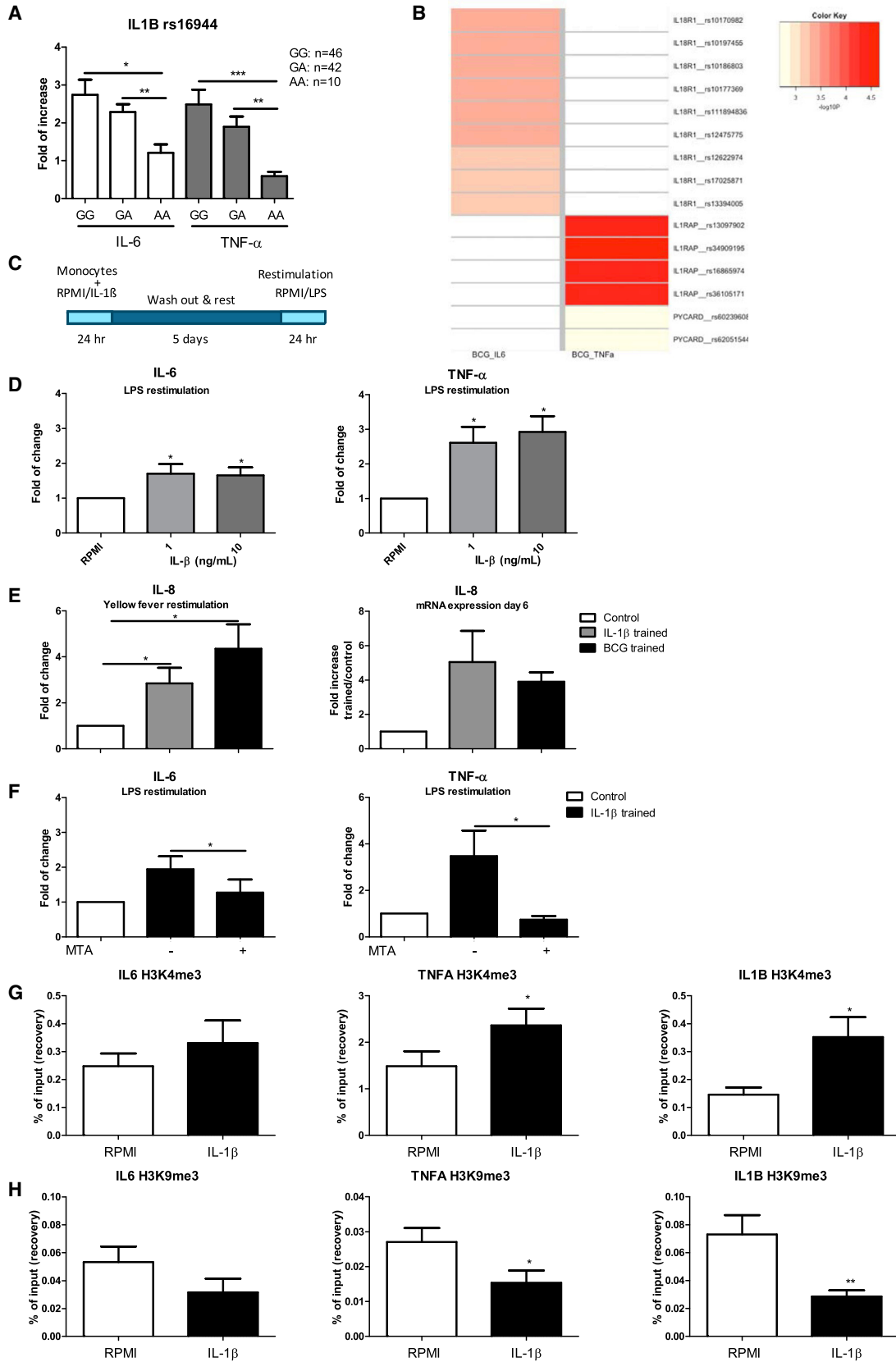
(B) Track of the H3K27ac peaks near the NOD2 gene.

(C) RNA sequencing (right panel) and H3K27ac peak analysis (left panel, $p < 0.05$, $FC > 1.5$) are shown between responders and non-responders. GO term analysis is shown in the table. Pathway analysis of these genes can be found in Figure S5A.

tion on day 6 (Figure 6D). We also observed enhanced IL-8 production by monocytes that were trained with IL-1 β or BCG and were restimulated with YFV. mRNA expression analysis of IL-8 demonstrated an enhanced upregulation of IL-8 in both BCG and IL-1 β trained monocytes compared to control (Figure 6E). Restimulation with YFV on day 6 did not lead to IL-6 or TNF α production (data not shown). Pre-treatment with the histone methyltransferase inhibitor MTA (5'-deoxy-5'-methylthio-adenosine) abrogated the IL-1 β -induced trained immunity response, suggesting that the *in vitro* training effect of IL-1 β is mediated via epigenetic reprogramming of monocytes (Figure 6F). This was confirmed by IL-1 β -mediated induction of epigenetic changes 6 days after IL-1 β treatment in promoter regions of *TNFA*, *IL6*, and *IL1B*, with increased trimethylation of lysine 4 at histone 3 (H3K4me3; Figure 6G), a marker of increased gene transcription, and reduced trimethylation of lysine 9 at histone 3 (H3K9me3; Figure 6H), a repressor mark. The increase of H3K4me3 and the decrease of H3K9me3 induced by IL-1 β was most pronounced at the promoter of *IL1B*.

DISCUSSION

Recent studies demonstrated that functional adaptation of innate immune cells after an infection or vaccination, which is induced through epigenetic reprogramming, is responsible for



(legend on next page)

a *de facto* innate immune memory, also termed trained immunity (Netea et al., 2016). BCG vaccination has long been known to be accompanied by lower mortality in vaccinated children, an effect that cannot be fully ascribed to protection against tuberculosis, but that is probably due to protection against neonatal sepsis and respiratory tract infections. Although it has been reported that this heterologous protection is due at least in part to induction of trained immunity (Kleinnijenhuis et al., 2012), no comprehensive assessment of the epigenetic programs induced by BCG has been done until now. Also, evidence for protective effects of BCG-induced trained immunity on unrelated infections in humans is outstanding.

In this study, we describe the epigenetic changes induced at a genome scale by BCG in human monocytes. We chose to study the H3K27ac histone mark because it is associated with an “active” chromatin state at both gene promoters and enhancers. Moreover, we previously showed a strong correlation between H3K27ac and H3K4me3 changes during trained immunity, with H3K27ac being the most dynamic mark (Saeed et al., 2014). Among the genes displaying increased H3K27ac, we identified enrichment in genes encoding for proteins involved in cellular signaling as well as inflammation: cytokines and chemokines, the EGFR and VEGF pathways. In addition, the PI3K/AKT pathway is also shown to be upregulated, and this is supported by the recent studies demonstrating its involvement in the induction of trained immunity by both BCG and β -glucan (Arts et al., 2016; Cheng et al., 2014). The epigenetic profile induced by BCG shows close similarities to the trained immunity induced by β -glucan *in vitro* (Saeed et al., 2014), suggesting there is a core trained immunity response induced by different stimuli.

The epigenetic reprogramming of monocytes induced by BCG vaccination is accompanied by significantly altered responses of innate immune cells. This process is clearly illustrated here by higher pro-inflammatory cytokine production (TNF α , IL-1 β , IL-6) of PBMCs from BCG-vaccinated volunteers, compared to placebo-treated individuals. We have in this case opted for PBMC stimulation (and not highly purified monocytes) to mimic as closely as possible the *in vivo* clinical situation. In addition, while the epigenetic changes observed in highly purified monocytes are indicative of the induction of trained immunity programs by BCG vaccination, the functional consequences

measured in PBMCs may be also influenced by the presence of lymphocytes. Differences in cytokine production after BCG vaccination were seen in earlier studies both in children (Jensen et al., 2015) and in adults (Kleinnijenhuis et al., 2012). The amplitude of the cytokine responses indicative of trained immunity induction in the current study is somewhat less strong compared to previous studies, probably due to BCG batch differences (Biering-Sørensen et al., 2015).

Unequivocally, demonstration of a correlation between the induction of trained immunity and protection against a controlled infection in humans has been outstanding. Our proof-of-principle study showed that BCG vaccination protects against a subsequent, non-related viral infection in a human controlled model of YFV infection. This model has the advantage of the use of a live virus, which has no deleterious effects on the host and which can be quantitatively monitored by PCR. The demonstration of a significantly lower peak of viremia in BCG-vaccinated individuals compared to individuals injected with placebo clearly demonstrates the capacity of BCG to protect against non-related infections in humans. The lower viremia was also accompanied by lower circulating inflammatory mediators in the BCG-vaccinated individuals. This result is expected, as the high cytokine production capacity upon BCG vaccination will lead to a rapid local antimicrobial response and subsequent elimination of the pathogen, thereby preventing a systemic reaction and high levels of circulating cytokines (Netea et al., 2003; van der Poll et al., 2017). Importantly, despite the lower virus load, BCG vaccination did not affect generation of protective anti-yellow fever antibodies and did not affect the specific effect of YFV. This suggests that BCG might improve the antigen-presenting capacity and adaptive responses, in line with a study showing beneficial effects on the response to influenza vaccine (Leentjens et al., 2015). The observed effects that are indicative of trained immunity induction are clinically very relevant, as epidemiological studies have shown that BCG vaccination results in lower all-cause mortality in the first month after birth (Aaby et al., 2011; Roth et al., 2004). However, long-term studies with broader analysis of monocyte function are warranted in order to determine the duration of the BCG effect. Importantly, significant differences in epigenetic markers were apparent on monocytes from BCG responders (with low yellow fever viremia) and BCG

Figure 6. The Role of IL-1 β for Trained Immunity

(A) Genetic variations in IL-1 β were assessed in DNA samples from 98 healthy volunteers from the 200FG cohort by a genome-wide Illumina Infinium SNP array. Monocytes were isolated and stimulated *in vitro* with culture medium (negative control) or BCG (10 μ g/mL) for 24 hr. On day 6, cells were restimulated with culture medium or LPS (10 ng/mL). After 24 hr, supernatants were taken and cytokine concentrations were assessed using ELISA. Medium-restimulated cells did not produce detectable cytokine production. A SNP in IL-1 β (*IL1B*; rs16944) was found to affect the trained immunity response induced by BCG (mean \pm SEM, * p < 0.05, ** p < 0.01, *** p < 0.001, Mann-Whitney U test).

(B) Additional genetic analysis in genes of the IL-1 pathway identify polymorphisms in the IL-1 and IL-18 receptors, as well as the inflammasome component PYCARD/ASC, that significantly modulate BCG-induced trained immunity.

(C) Schematic outline of the *in vitro* training experiments. Human monocytes were primed for 24 hr with culture medium or different concentrations of IL-1 β . On day 6, cells were restimulated for 24 hr with LPS, and levels of IL-6 and TNF α were assessed (mean \pm SEM, n = 7, * p < 0.05).

(D) Human monocytes were primed for 24 hr with culture medium or different concentrations of IL-1 β . On day 6, cells were restimulated for 24 hr with LPS, and levels of IL-6 and TNF α were assessed (mean \pm SEM, n = 7, * p < 0.05).

(E) Human monocytes were incubated with either culture medium, IL-1 β (10 ng/mL), or BCG (5 μ g/mL). At day 6, cells were restimulated with YFV (1,000 IU/mL). Concentrations of IL-8 in the supernatants were measured after 24 hr (mean \pm SEM, n = 6), and mRNA expression of IL-8 was determined after 4 hr restimulation (mean \pm SEM, n = 3 in duplo, * p < 0.05, Wilcoxon signed-rank test).

(F) Monocytes were stimulated for 24 hr with culture medium or IL-1 β (100 ng/mL) in the presence or absence of MTA (1 mM). On day 6, after restimulation with LPS for 24 hr, IL-6 and TNF α were measured in the supernatants (mean \pm SEM, n = 7, * p < 0.05, Wilcoxon signed-rank test).

(G and H) Human monocytes were stimulated for 24 hr with culture medium or IL-1 β (100 ng/mL). Chromatin was fixed on day 6 and ChIP-qPCR was performed. H3K4me3 and H3K9me3 were determined at promoters of *IL6*, *TNFA*, and *IL1B* (mean \pm SEM, n = 8, * p < 0.05, Wilcoxon signed-rank test).

non-responders (with higher viremia). H3K27ac at the level of the NOD2 receptor (which is essential for the induction of BCG-induced trained immunity [Kleinnijenhuis et al., 2012]) correlated best with response to BCG. Additional immune pathways related to cytokine production and innate immune responses were shown to be of importance.

One of the most remarkable observations was made when the correlates of protection against viremia were investigated. It was previously suggested that development of a scar after BCG vaccination could be used as a marker for non-specific effects and childhood survival (Garly et al., 2003; Roth et al., 2005). However, all BCG-vaccinated volunteers developed a scar with a comparable size (0.5–0.7 cm), and we observed no correlation of this with protection. In addition, the fold change of heterologous T cell responses (IFN γ , IL-17, and IL-22 production) upon BCG vaccination did not show any correlation with the YFV viremia. In contrast, the post-BCG fold increase of IL-1 β production strongly correlated with lower viremia after YFV administration, while production of other monocyte-derived cytokines showed less correlation. This suggests that induction of IL-1 β production stimulated by non-related pathogens, which is an indicator of trained immunity responses, rather than adaptive cellular responses as assessed by IFN γ production induced by specific stimuli, is responsible for the protection induced by BCG against the viral infection.

The fact that the increase in the capacity of innate immune cells to release IL-1 β is the stronger correlate of protection suggests that this cytokine is a crucial component of trained immunity. This finding is highly relevant due to the direct protective effect of higher IL-1 β levels in viral infections (Azuma et al., 1992; Iida et al., 1989; Sergerie et al., 2007), although other mechanisms are possible as well. The role of IL-1 β is supported by complementary genetic and immunological studies. By studying *in vitro* training of monocytes induced by BCG in cells isolated from healthy volunteers of the 200FG cohort (Li et al., 2016), we demonstrate that genetic polymorphisms in the gene encoding for IL-1 β , but also other genes of the IL-1 pathway such as the inflammasome component ASC/PYCARD and genes encoding for the IL-1 and IL-18 receptors, are associated with the magnitude of the individual trained immunity response induced by BCG. In follow-up experiments, we found that IL-1 β itself can induce trained immunity, and that this effect is accompanied by epigenetic changes at the level of histone methylation (H3K4me3 and H3K9me3). This may explain earlier studies that have shown that administration of one dose of IL-1 β to mice before infection can protect against lethal bacterial and fungal sepsis (van der Meer et al., 1988). In addition, IL-1 β is well known to exert strong effects on myelopoiesis (Dinarello, 2002), and one may hypothesize that IL-1 β represents the endogenous mediator between the peripheral stimulation of monocytes and macrophages by BCG and long-term functional reprogramming at the level of bone marrow progenitors. Indeed, accompanying studies from the International Trained Immunity Consortium (INTRIM) in an upcoming issue of *Cell* demonstrate that effects at the level of myeloid cell progenitors are crucial for the induction of trained immunity by β -glucan (Mitroulis et al., 2018), BCG (Kaufmann et al., 2018), and Western-type diet (Christ et al., 2018) and identify IL-1 β and the inflammasome pathway as key mediators of this process (Mitroulis et al., 2018;

Christ et al., 2018). Mitroulis et al. show that the adaptations in hematopoietic stem and progenitor cells (HSPCs) are associated with an increase in IL-1 β (and not other cytokines) in the bone marrow fluid, while inhibition of IL-1 β prevented the β -glucan-dependent expansion of HSPCs and the effects on myeloid cell progenitors. Similarly, Christ et al. demonstrate that a Western-type diet is able to induce trained immunity in mice, and IL-1 plays an important role in this effect. Thus, Western-type diet did not induce trained immunity and myeloid progenitor reprogramming in NLRP3 knockout mice that have defects in IL-1 β processing. Similarly, genetic analysis revealed that polymorphisms in *NLRP3* influenced oxLDL-induced trained immunity in human cells.

As dysregulated IL-1 β production plays a pivotal role in the etiology of many autoinflammatory diseases, the capacity of IL-1 β to induce trained immunity may represent a central event in the pathophysiology of these disorders. This needs further investigation, as it may represent a therapeutic target for these diseases. Indeed, the accompanying study by Bekkering et al. of the INTRIM Consortium shows a dysregulated trained immunity phenotype in monocytes isolated from patients with hyper IgD syndrome (Bekkering et al., 2018), a well-known autoinflammatory syndrome.

In conclusion, in the present study we report the broad epigenetic program induced by BCG vaccination in humans, which results in increased activation of circulating monocytes. Furthermore, we show that BCG vaccination can protect against a non-related viral infection in an experimental model of human infection and report that IL-1 β -mediated responses, which are indicative of the induction of trained immunity, are the most reliable correlate of this protection. Additional genetic and immunological validation studies demonstrate that IL-1 pathway is crucial for an efficient induction of trained immunity in humans, and this may have important implications for both vaccination and the pathophysiology of autoinflammatory diseases.

STAR★METHODS

Detailed methods are provided in the online version of this paper and include the following:

- KEY RESOURCES TABLE
- CONTACT FOR REAGENT AND RESOURCE SHARING
- EXPERIMENTAL MODEL AND SUBJECT DETAILS
- METHOD DETAILS
 - PBMC and Monocyte Isolation and Stimulation
 - *In Vitro* Training Experiments
 - Cytokine Measurements
 - mRNA Extraction and RT-PCR
 - *In Vitro* Assessment of Specific Histone Marks
 - *In Vivo* Assessment of Genome-Wide Epigenetic Profiles
 - Library Preparation for Sequencing
 - Yellow Fever RT-qPCR and Antibody Titers
 - Genetic Analysis
 - ChIP-Sequencing Analysis
- QUANTIFICATION AND STATISTICAL ANALYSIS
- DATA AND SOFTWARE AVAILABILITY
 - Data Resources

SUPPLEMENTAL INFORMATION

Supplemental Information includes five figures and four tables and can be found with this article online at <https://doi.org/10.1016/j.chom.2017.12.010>.

ACKNOWLEDGMENTS

M.G.N. was supported by an ERC Consolidation Grant (310372) and a Spinoza grant from the Netherlands Organization for Scientific Research (NWO). M.O. was supported by a VENI grant 016.176.006 from the Netherlands Organization for Scientific Research (NWO). B.N. was supported by an NHMRC (Australia) C.J. Martin Fellowship. C.S.B. and P.A. were supported by the Danish National Research Foundation (DNRF108).

AUTHOR CONTRIBUTIONS

Conceptualization, R.v.C., M.G.N.; Methodology, R.J.W.A., S.J.C.F.M.M., B.N., Y.L., M.O., V.K., S.-Y.W., C.B.E.M.R.; Investigation, R.J.W.A., S.J.C.F.M.M., B.N., Y.L., M.O., V.K., C.B.E.M.R.; Writing – Original Draft, R.J.W.A., S.J.C.F.M.M.; Writing – Review & Editing, B.N., C.W., L.A.B.J., C.S.B., P.A., M.P.K., R.J.X., H.G.S., R.v.C., M.G.N.; Supervision, C.W., M.P.K., H.G.S., R.J.X., R.v.C., M.G.N.

DECLARATION OF INTERESTS

The authors declare no conflict of interest.

Received: April 18, 2017

Revised: September 25, 2017

Accepted: December 19, 2017

Published: January 10, 2018

REFERENCES

- Aaby, P., Roth, A., Ravn, H., Napirna, B.M., Rodrigues, A., Lisse, I.M., Stensballe, L., Diness, B.R., Lausch, K.R., Lund, N., et al. (2011). Randomized trial of BCG vaccination at birth to low-birth-weight children: beneficial nonspecific effects in the neonatal period? *J. Infect. Dis.* *204*, 245–252.
- Anders, S., and Huber, W. (2010). Differential expression analysis for sequence count data. *Genome Biol.* *11*, R106.
- Arts, R.J., Blok, B.A., Aaby, P., Joosten, L.A., de Jong, D., van der Meer, J.W., Benn, C.S., van Crevel, R., and Netea, M.G. (2015). Long-term in vitro and in vivo effects of γ -irradiated BCG on innate and adaptive immunity. *J. Leukoc. Biol.* *98*, 995–1001.
- Arts, R.J.W., Carvalho, A., La Rocca, C., Palma, C., Rodrigues, F., Silvestre, R., Kleinnijenhuis, J., Lachmandas, E., Gonçalves, L.G., Belinha, A., et al. (2016). Immunometabolic Pathways in BCG-Induced Trained Immunity. *Cell Rep.* *17*, 2562–2571.
- Azuma, I., Ishihara, C., Iida, J., Yoo, Y.C., Yoshimatsu, K., and Arikawa, J. (1992). Stimulation of host-defense mechanism with synthetic adjuvants and recombinant cytokines against viral infection in mice. *Adv. Exp. Med. Biol.* *319*, 253–263.
- Barnett, D.W., Garrison, E.K., Quinlan, A.R., Strömberg, M.P., and Marth, G.T. (2011). BamTools: a C++ API and toolkit for analyzing and managing BAM files. *Bioinformatics* *27*, 1691–1692.
- Bekkering, S., Quintin, J., Joosten, L.A., van der Meer, J.W., Netea, M.G., and Riksen, N.P. (2014). Oxidized low-density lipoprotein induces long-term proinflammatory cytokine production and foam cell formation via epigenetic reprogramming of monocytes. *Arterioscler. Thromb. Vasc. Biol.* *34*, 1731–1738.
- Bekkering, S., Blok, B.A., Joosten, L.A., Riksen, N.P., van Crevel, R., and Netea, M.G. (2016). In vitro experimental model of trained innate immunity in human primary monocytes. *Clin. Vaccine Immunol.* *23*, 926–933.
- Bekkering, S., Arts, R.J.W., Novakovic, B., Kourtzelis, I., van der Heijden, C.D.C.C., Li, Y., Popa, C.D., ter Horst, R., van Tuijl, J., Netea-Maier, R.T., et al. (2018). Metabolic induction of trained immunity through the mevalonate pathway. *Cell* *172*. Published online January 11, 2018. <https://doi.org/10.1016/j.cell.2017.11.025>.
- Berg, R.E., Cordes, C.J., and Forman, J. (2002). Contribution of CD8+ T cells to innate immunity: IFN-gamma secretion induced by IL-12 and IL-18. *Eur. J. Immunol.* *32*, 2807–2816.
- Berg, R.E., Crossley, E., Murray, S., and Forman, J. (2003). Memory CD8+ T cells provide innate immune protection against *Listeria monocytogenes* in the absence of cognate antigen. *J. Exp. Med.* *198*, 1583–1593.
- Biering-Sørensen, S., Aaby, P., Napirna, B.M., Roth, A., Ravn, H., Rodrigues, A., Whittle, H., and Benn, C.S. (2012). Small randomized trial among low-birth-weight children receiving bacillus Calmette-Guérin vaccination at first health center contact. *Pediatr. Infect. Dis. J.* *31*, 306–308.
- Biering-Sørensen, S., Jensen, K.J., Aamand, S.H., Blok, B., Andersen, A., Monteiro, I., Netea, M.G., Aaby, P., Benn, C.S., and Haslov, K.R. (2015). Variation of growth in the production of the BCG vaccine and the association with the immune response. An observational study within a randomised trial. *Vaccine* *33*, 2056–2065.
- Blok, B.A., Arts, R.J., van Crevel, R., Benn, C.S., and Netea, M.G. (2015). Trained innate immunity as underlying mechanism for the long-term, nonspecific effects of vaccines. *J. Leukoc. Biol.* *98*, 347–356.
- Boer, M.C., Prins, C., van Meijgaarden, K.E., van Dissel, J.T., Ottenhoff, T.H., and Joosten, S.A. (2015). *Mycobacterium bovis* BCG vaccination induces divergent proinflammatory or regulatory T cell responses in adults. *Clin. Vaccine Immunol.* *22*, 778–788.
- Cheng, S.C., Quintin, J., Cramer, R.A., Shepardson, K.M., Saeed, S., Kumar, V., Giamarellos-Bourboulis, E.J., Martens, J.H., Rao, N.A., Aghajani-Refah, A., et al. (2014). mTOR- and HIF-1 α -mediated aerobic glycolysis as metabolic basis for trained immunity. *Science* *345*, 1250684.
- Cheng, S.C., Scicluna, B.P., Arts, R.J., Gresnigt, M.S., Lachmandas, E., Giamarellos-Bourboulis, E.J., Kox, M., Manjeri, G.R., Wagenaars, J.A., Cremer, O.L., et al. (2016). Broad defects in the energy metabolism of leukocytes underlie immunoparalysis in sepsis. *Nat. Immunol.* *17*, 406–413.
- Christ, A., Günther, P., Lauterbach, M.A.R., Duetz, P., Biswas, D., Pelka, K., Scholz, C.J., Oosting, M., Haendler, K., Baßler, K., et al. (2018). Western diet triggers NLRP3-dependent innate immune reprogramming. *Cell* *172*. Published online January 11, 2018. <https://doi.org/10.1016/j.cell.2017.12.013>.
- Colditz, G.A., Brewer, T.F., Berkey, C.S., Wilson, M.E., Burdick, E., Fineberg, H.V., and Mosteller, F. (1994). Efficacy of BCG vaccine in the prevention of tuberculosis. Meta-analysis of the published literature. *JAMA* *271*, 698–702.
- Dinarello, C.A. (2002). The IL-1 family and inflammatory diseases. *Clin. Exp. Rheumatol.* *20* (5, Suppl 27), S1–S13.
- Drosten, C., Götting, S., Schilling, S., Asper, M., Panning, M., Schmitz, H., and Günther, S. (2002). Rapid detection and quantification of RNA of Ebola and Marburg viruses, Lassa virus, Crimean-Congo hemorrhagic fever virus, Rift Valley fever virus, dengue virus, and yellow fever virus by real-time reverse transcription-PCR. *J. Clin. Microbiol.* *40*, 2323–2330.
- Edupuganti, S., Eidex, R.B., Keyserling, H., Akondy, R.S., Lanciotti, R., Orenstein, W., del Rio, C., Pan, Y., Querec, T., Lipman, H., et al.; YF-Ig Study Team (2013). A randomized, double-blind, controlled trial of the 17D yellow fever virus vaccine given in combination with immune globulin or placebo: comparative viremia and immunogenicity. *Am. J. Trop. Med. Hyg.* *88*, 172–177.
- Garly, M.L., Martins, C.L., Balé, C., Baldé, M.A., Hedegaard, K.L., Gustafson, P., Lisse, I.M., Whittle, H.C., and Aaby, P. (2003). BCG scar and positive tuberculin reaction associated with reduced child mortality in West Africa. A non-specific beneficial effect of BCG? *Vaccine* *21*, 2782–2790.
- Han, R.F., and Pan, J.G. (2006). Can intravesical bacillus Calmette-Guérin reduce recurrence in patients with superficial bladder cancer? A meta-analysis of randomized trials. *Urology* *67*, 1216–1223.
- Heinz, S., Benner, C., Spann, N., Bertolino, E., Lin, Y.C., Laslo, P., Cheng, J.X., Murre, C., Singh, H., and Glass, C.K. (2010). Simple combinations of lineage-determining transcription factors prime cis-regulatory elements required for macrophage and B cell identities. *Mol. Cell* *38*, 576–589.

- Hirve, S., Bavdekar, A., Juvekar, S., Benn, C.S., Nielsen, J., and Aaby, P. (2012). Non-specific and sex-differential effects of vaccinations on child survival in rural western India. *Vaccine* 30, 7300–7308.
- Huang, W., Sherman, B.T., and Lempicki, R.A. (2009). Systematic and integrative analysis of large gene lists using DAVID bioinformatics resources. *Nat. Protoc.* 4, 44–57.
- Iida, J., Saiki, I., Ishihara, C., and Azuma, I. (1989). Protective activity of recombinant cytokines against Sendai virus and herpes simplex virus (HSV) infections in mice. *Vaccine* 7, 229–233.
- Jensen, K.J., Larsen, N., Biering-Sørensen, S., Andersen, A., Eriksen, H.B., Monteiro, I., Hougaard, D., Aaby, P., Netea, M.G., Flanagan, K.L., and Benn, C.S. (2015). Heterologous immunological effects of early BCG vaccination in low-birth-weight infants in Guinea-Bissau: a randomized-controlled trial. *J. Infect. Dis.* 211, 956–967.
- Kaufmann, E., Sanz, J., Dunn, J.L., Khan, N., Mendonça, L.E., Pacis, A., Tzelepis, F., Pernet, E., Dumaine, A., Grenier, J.-C., et al. (2018). BCG educates hematopoietic stem cells to generate protective innate immunity against tuberculosis. *Cell* 172. Published online January 11, 2018. <https://doi.org/10.1016/j.cell.2017.12.031>.
- Kleinnijenhuis, J., Quintin, J., Preijers, F., Joosten, L.A., Iffrim, D.C., Saeed, S., Jacobs, C., van Loenhout, J., de Jong, D., Stunnenberg, H.G., et al. (2012). Bacille Calmette-Guérin induces NOD2-dependent nonspecific protection from reinfection via epigenetic reprogramming of monocytes. *Proc. Natl. Acad. Sci. USA* 109, 17537–17542.
- Kleinnijenhuis, J., Quintin, J., Preijers, F., Benn, C.S., Joosten, L.A., Jacobs, C., van Loenhout, J., Xavier, R.J., Aaby, P., van der Meer, J.W., et al. (2014). Long-lasting effects of BCG vaccination on both heterologous Th1/Th17 responses and innate trained immunity. *J. Innate Immun.* 6, 152–158.
- Kristensen, I., Aaby, P., and Jensen, H. (2000). Routine vaccinations and child survival: follow up study in Guinea-Bissau, West Africa. *BMJ* 321, 1435–1438.
- Leentjens, J., Kox, M., Stokman, R., Gerretsen, J., Diavatopoulos, D.A., van Crevel, R., Rimmelzwaan, G.F., Pickkers, P., and Netea, M.G. (2015). BCG vaccination enhances the immunogenicity of subsequent influenza vaccination in healthy volunteers: a randomized, placebo-controlled pilot study. *J. Infect. Dis.* 212, 1930–1938.
- Lertmoungkolchai, G., Cai, G., Hunter, C.A., and Bancroft, G.J. (2001). Bystander activation of CD8+ T cells contributes to the rapid production of IFN-gamma in response to bacterial pathogens. *J. Immunol.* 166, 1097–1105.
- Li, H., and Durbin, R. (2009). Fast and accurate short read alignment with Burrows-Wheeler transform. *Bioinformatics* 25, 1754–1760.
- Li, H., Handsaker, B., Wysoker, A., Fennell, T., Ruan, J., Homer, N., Marth, G., Abecasis, G., and Durbin, R.; 1000 Genome Project Data Processing Subgroup (2009). The Sequence Alignment/Map format and SAMtools. *Bioinformatics* 25, 2078–2079.
- Li, Y., Oosting, M., Deelen, P., Ricaño-Ponce, I., Smeekens, S., Jaeger, M., Matzaraki, V., Swertz, M.A., Xavier, R.J., Franke, L., et al. (2016). Inter-individual variability and genetic influences on cytokine responses to bacteria and fungi. *Nat. Med.* 22, 952–960.
- Mathurin, K.S., Martens, G.W., Kornfeld, H., and Welsh, R.M. (2009). CD4 T-cell-mediated heterologous immunity between mycobacteria and poxviruses. *J. Virol.* 83, 3528–3539.
- McLean, C.Y., Bristor, D., Hiller, M., Clarke, S.L., Schaar, B.T., Lowe, C.B., Wenger, A.M., and Bejerano, G. (2010). GREAT improves functional interpretation of cis-regulatory regions. *Nat. Biotechnol.* 28, 495–501.
- Mitroulis, I., Ruppova, K., Wang, B., Chen, L.-S., Grzybek, M., Grinenko, T., Eugster, A., Troullinaki, M., Palladini, A., Kourtzelis, I., et al. (2018). Modulation of myelopoiesis progenitors is an integral component of trained immunity. *Cell* 172. Published online January 11, 2018. <https://doi.org/10.1016/j.cell.2017.11.034>.
- Moulton, L.H., Rahmathullah, L., Halsey, N.A., Thulasiraj, R.D., Katz, J., and Tielsch, J.M. (2005). Evaluation of non-specific effects of infant immunizations on early infant mortality in a southern Indian population. *Trop. Med. Int. Health* 10, 947–955.
- Netea, M.G., van der Meer, J.W., van Deuren, M., and Kullberg, B.J. (2003). Proinflammatory cytokines and sepsis syndrome: not enough, or too much of a good thing? *Trends Immunol.* 24, 254–258.
- Netea, M.G., Joosten, L.A., Latz, E., Mills, K.H., Natoli, G., Stunnenberg, H.G., O'Neill, L.A., and Xavier, R.J. (2016). Trained immunity: a program of innate immune memory in health and disease. *Science* 352, aaf1098.
- Novakovic, B., Habibi, E., Wang, S.Y., Arts, R.J., Davar, R., Megchelenbrink, W., Kim, B., Kuznetsova, T., Kox, M., Zwaag, J., et al. (2016). β -Glucan reverses the epigenetic state of LPS-induced immunological tolerance. *Cell* 167, 1354–1368.e14.
- Roth, A., Jensen, H., Garly, M.-L., Djana, Q., Martins, C.L., Sodemann, M., Rodrigues, A., and Aaby, P. (2004). Low birth weight infants and Calmette-Guérin bacillus vaccination at birth: community study from Guinea-Bissau. *Pediatr. Infect. Dis. J.* 23, 544–550.
- Roth, A., Gustafson, P., Nhaga, A., Djana, Q., Poulsen, A., Garly, M.L., Jensen, H., Sodemann, M., Rodrigues, A., and Aaby, P. (2005). BCG vaccination scar associated with better childhood survival in Guinea-Bissau. *Int. J. Epidemiol.* 34, 540–547.
- Saeed, S., Quintin, J., Kerstens, H.H., Rao, N.A., Aghajani, A., Matarese, F., Cheng, S.C., Ratter, J., Berentsen, K., van der Ent, M.A., et al. (2014). Epigenetic programming of monocyte-to-macrophage differentiation and trained innate immunity. *Science* 345, 1251086.
- Sergerie, Y., Rivest, S., and Boivin, G. (2007). Tumor necrosis factor- α and interleukin-1 β play a critical role in the resistance against lethal herpes simplex virus encephalitis. *J. Infect. Dis.* 196, 853–860.
- Turro, E., Su, S.Y., Gonçalves, Â., Coin, L.J., Richardson, S., and Lewin, A. (2011). Haplotype and isoform specific expression estimation using multi-mapping RNA-seq reads. *Genome Biol.* 12, R13.
- van der Meer, J.W., Barza, M., Wolff, S.M., and Dinarello, C.A. (1988). A low dose of recombinant interleukin 1 protects granulocytopenic mice from lethal gram-negative infection. *Proc. Natl. Acad. Sci. USA* 85, 1620–1623.
- van der Poll, T., van de Veerdonk, F.L., Scicluna, B.P., and Netea, M.G. (2017). The immunopathology of sepsis and potential therapeutic targets. *Nat. Rev. Immunol.* 17, 407–420.
- Wen, A.Q., Wang, J., Feng, K., Zhu, P.F., Wang, Z.G., and Jiang, J.X. (2006). Effects of haplotypes in the interleukin 1 β promoter on lipopolysaccharide-induced interleukin 1 β expression. *Shock* 26, 25–30.
- Wójciszewski, A., Gresnigt, M.S., Lecompte, T., Bibert, S., Manuel, O., Joosten, L.A., Rüeger, S., Berger, C., Boggian, K., Cusini, A., et al.; Swiss Transplant Cohort Study (STCS); Swiss Transplant Cohort Study STCS (2015). IL1B and DEFB1 polymorphisms increase susceptibility to invasive mold infection after solid-organ transplantation. *J. Infect. Dis.* 211, 1646–1657.
- Wouthuyzen-Bakker, M., Knoester, M., van den Berg, A.P., GeurtsvanKessel, C.H., Koopmans, M.P., Van Leer-Buter, C., Oude Velthuis, B., Pas, S.D., Ruijs, W.L., Schmidt-Chanasit, J., et al. (2017). Yellow fever in a traveller returning from Suriname to the Netherlands, March 2017. *Euro Surveill.* 22, 30488.
- Zhang, Y., Liu, T., Meyer, C.A., Eickhout, J., Johnson, D.S., Bernstein, B.E., Nusbaum, C., Myers, R.M., Brown, M., Li, W., and Liu, X.S. (2008). Model-based analysis of ChIP-Seq (MACS). *Genome Biol.* 9, R137.
- Zumla, A., Raviglione, M., Hafner, R., and von Reyn, C.F. (2013). Tuberculosis. *N. Engl. J. Med.* 368, 745–755.

STAR★METHODS

KEY RESOURCES TABLE

REAGENT or RESOURCE	SOURCE	IDENTIFIER
Antibodies		
Rabbit polyclonal anti-H3K4me3	Diagenode	pab-003-050
Rabbit polyclonal anti-H3K27Ac	Diagenode	pab-196-050, C15410196
Rabbit polyclonal anti-H3K9me3	Diagenode	pab-193-050, C15410193
Chemicals, Peptides, and Recombinant Proteins		
Bacille Calmette-Guérin Vaccin (BCG)	Statens Serum Institute, Copenhagen	2016-04
Yellow fever vaccine	Sanofi Pasteur	Stamaril
Lipopolysaccharide	Sigma-Aldrich	From <i>E. coli</i> serotype 055:B5, L2880
<i>Mycobacterium tuberculosis</i>	Gift	H27Rv
<i>Candida albicans</i>	Gift	UC820
<i>Staphylococcus aureus</i>		Clinical isolate
Recombinant human Interleukin-1 β	R&D Systems	201-LB-005
5'-Deoxy-5'-(methylthio) adenosine (MTA)	Sigma-Aldrich	D5011
Percoll	Sigma-Aldrich	P1644
Ficoll-Paque	GE Healthcare	17-1440-03
Roswell Park Memorial Institute medium (RPMI)	Invitrogen	22406031
Bovine Serum Albumin (BSA)	Sigma-Aldrich	A7030
CD14+ beads	MACS Miltenyi	130-050-201
iScript reverse transcriptase	Bio-Rad	1708840
TRIzol reagent	Life Technologies	15596018
SYBR Green	Applied Biosciences	4368708
16% Formaldehyde	Fisher Scientific	28908, 11835835
Protein A/G Magnetic beads	Diagenode	C03010021-150
Critical Commercial Assays		
Illumina Infinium SNP array	Illumina	
Human IL-1 β ELISA	R&D Systems	DY201
Human TNF α ELISA	R&D Systems	DY210
Human IL-17 ELISA	R&D Systems	D1700
Human IL-22 ELISA	R&D Systems	D2200
Human IL-6 ELISA	Sanquin	M1916
Human IL-8 ELISA	Sanquin	M1918
Human IFN γ ELISA	Sanquin	M1933
Milliplex human TH17 panel	Merck	HTH17MAG-14K
Bradford Concentration Assay	Thermo Fisher Scientific	23246
iScript cDNA Synthesis Kit	Bio-Rad	1708891
KAPA library preparation kit	Kapa Biosystems	KK8400
riboZero gold rRNA removal kit	Illumina	MRZG12324
Nextera DNA Library Prep Kit	Illumina	FC-121-1031
TruSeq SBS KIT v3 - HS (50 cycles)	Illumina	FC-401-3002
NextSeq 500/550 High Output v2 kit (75 cycles)	Illumina	FC-404-2005
NEBNext High-Fidelity 2 \times PCR Master Mix	New England Biolabs	M0541
iQ SYBR Green Supermix	Bio-Rad	1708880
100 \times SYBR Green I Nucleic Acid Gel Stain	Thermo Fisher Scientific	S7563
SPRIselect reagent kit	Beckman Coulter	B23218

(Continued on next page)

Continued

REAGENT or RESOURCE	SOURCE	IDENTIFIER
E-Gel SizeSelect Agarose Gels, 2%	Thermo Fisher Scientific	G661002
dNTP set 100 mM	Life Technologies	10297-018
dUTP 100 mM	Promega	U119A
Glycogen (20 mg/mL)	Life Technologies	10814-010
Random Hexamer primers	Sigma-Aldrich	11034731001
Second Strand Buffer	Life Technologies	10812-014
Superscript III Reverse Transcriptase	Life Technologies	18080-044
DNA polymerase I, <i>E. coli</i>	New England Biolabs	M0209S
USER enzyme	New England Biolabs	M5505L
<i>E. coli</i> Ligase	New England Biolabs	M0205L
Rnasin Plus Rnase Inhibitor	Promega	N2615
Ribonuclease H	Life Technologies	AM2293
T4 DNA polymerase	New England Biolabs	M0203L
Sodium Acetate (3M)	Life Technologies	AM9740
DNase I	QIAGEN	79254
Qubit RNA HS assay kit	Life Technologies	Q32852
Ribozero Gold Kit	Illumina	MRZG12324
Rneasy Mini Kit	QIAGEN	74106
Deposited Data		
BCG in vivo trained monocytes CHIP-seq and RNA-seq data	This paper	GEO: GSE104149
Experimental Models: Organisms/Strains		
Human genome assembly hg19	NCBI	Version 37
200FG cohort (Human Functional Genomics project)		http://www.humanfunctionalgenomics.org
Oligonucleotides		
Primers for qPCR	Table S1	Biogelio. Primers designed by BLAST
Software		
Image Lab	Bio-Rad	
CXP analysis software v2.2	Beckman Coulter	
GraphPad Prism	Graphpad Software	https://www.graphpad.com
MMSEQ	Turro et al., 2011	https://github.com/eturro/mmseq
DEseq	Anders and Huber, 2010	http://bioconductor.org/packages/release/bioc/html/DESeq.html
Bwa	Li and Durbin, 2009	http://bio-bwa.sourceforge.net/
Samtools and Bamtools	Barnett et al., 2011 ; Li et al., 2009	https://github.com/kundajelab/phantompeakqualtools , http://samtools.sourceforge.net/ , https://github.com/pezmaster31/bamtools
MACS2	Zhang et al., 2008	http://github.com/taoliu/MACS/
HOMER	Heinz et al., 2010	http://homer.salk.edu/homer/motif/
DAVID	Huang et al., 2009	https://david.ncifcrf.gov/

CONTACT FOR REAGENT AND RESOURCE SHARING

Further information and requests for resources and reagents should be directed to and will be fulfilled by the Lead Contact, Mihai Netea at the Radboud University Medical Center, Nijmegen, the Netherlands (mihai.netea@radboudumc.nl).

EXPERIMENTAL MODEL AND SUBJECT DETAILS

Between February and November of 2015, we randomly assigned 30 healthy Dutch male subjects (age: 19–37 years) to receive either BCG (SSI, Denmark) or placebo (the diluent used to dissolve BCG) in a double-blind fashion. Volunteers were BCG naive and had not lived in a tuberculosis endemic area. One month after placebo or BCG vaccination, all volunteers received a single dose of YFV (Stamaril, Sanofi Pasteur). Blood was drawn before BCG/placebo and yellow fever vaccination, and 3, 5, 6, 14, and 90 days after yellow fever vaccination. One of the volunteers in the BCG group already had a protective yellow fever titer before yellow fever vaccination; this volunteer was excluded from yellow fever-related analyses. The study was approved by the Arnhem-Nijmegen Medical Ethical Committee, NL50160.092.24. Inclusion of volunteers and experiments were conducted according to the principles expressed in the Declaration of Helsinki. All volunteers gave written informed consent before any material was taken.

METHOD DETAILS

PBMC and Monocyte Isolation and Stimulation

Isolation and stimulation was performed as described before (Kleinnijenhuis et al., 2014; Kleinnijenhuis et al., 2012). Briefly, PBMCs were isolated by density centrifugation of Ficoll-Paque (GE healthcare, UK). Cells were washed twice in PBS and resuspended in RPMI culture medium (Roswell Park Memorial Institute medium; Invitrogen, CA, USA) supplemented with 50 $\mu\text{g}/\text{mL}$ gentamicin, 2 mM Glutamax (GIBCO), and 1 mM pyruvate (GIBCO) and diluted to a concentration of $5 \times 10^6/\text{mL}$. 100 $\mu\text{L}/\text{well}$ was added to 96-well plates and cells were incubated for 24 hr or 7 days with RPMI, sonicated *Mycobacterium tuberculosis* H37Rv (5 $\mu\text{g}/\text{mL}$), heat-killed *Candida albicans* ($1 \times 10^6/\text{mL}$, strain UC820) or *Staphylococcus aureus* ($1 \times 10^6/\text{mL}$ clinical isolate). Supernatants were collected after 24 hr or 7 days and stored at -20°C .

Monocyte isolation was performed after the PBMC isolation step, by MACS isolation with CD14 positive magnetic beads (MACS Miltenyi) according to the protocol of the manufacturer. Monocytes were fixed in 1% methanol-free formaldehyde and stored in PBS before sonication, after which chromatin was stored at -80°C .

In Vitro Training Experiments

Monocytes were adjusted to 1×10^6 cells/mL. A 100 μL volume was added into flat-bottom 96-well plates (Corning) and cells were incubated at 37°C . After 1 hr, cells were washed once with 200 μL warm PBS to remove non-adherent cells. Subsequently, monocytes were incubated with culture medium only (negative control), or 1, or 10 ng/mL IL-1 β (R&D Systems) for 24 hr. Cells were washed once with 200 μL warm PBS and incubated for 5 days in culture medium supplemented with 10% human pooled serum. The medium was changed once on day 3 of incubation. On day 6, cells were restimulated for 24 hr with culture medium, 10 ng/mL *Escherichia coli* LPS (serotype O55:B5, Sigma-Aldrich) or 1,000 IU/mL YFV (Stamaril, Sanofi Pasteur). Subsequently, supernatants were collected and stored at -20°C until cytokine concentrations were assessed. In inhibition experiments, cells were pre-incubated for 30 min with 1 mM of a methyltransferase inhibitor MTA (5'-deoxy-5'-methylthio-adenosine, Sigma) prior to adding the stimuli.

Cytokine Measurements

Cytokine measurements were performed in the supernatants using commercial ELISA kits from R&D Systems (TNF α , IL-1 β , IL-8, IL-17 and IL-22; Minneapolis, MN, USA) or Sanquin (IL-6, IFN γ ; Amsterdam, the Netherlands). Circulating cytokines after yellow fever vaccination were determined in plasma. Ethylenediaminetetraacetic acid (EDTA) anticoagulated blood was centrifuged at $2000 \times g$ at 4°C for 10 min immediately after withdrawal, and plasma was stored at -20°C until analysis. Concentrations of TNF α , IL-6, IL-8, IL-10, IL-1 β , IL-1 receptor antagonist (IL-1Ra), IFN α , and IFN γ were measured in plasma using a Luminex assay according to the manufacturer's instructions (Milliplex; Millipore, Billerica, MA, USA).

mRNA Extraction and RT-PCR

For measurement of IL-1 β expression in individuals with a genetic variant in *IL1B*, 2×10^6 PMBCs were added to flat bottom 24-well plates (Corning, NY, USA) and cells were let to adhere for 1 hr at 37°C . Subsequently, cells were washed three times with warm PBS and incubated for 1 hr with culture medium only as a negative control or LPS (10 ng/mL). To determine mRNA expression of cytokines in training experiments, monocytes were isolated as described above and incubated with either culture medium, IL-1 β (10 ng/mL), or BCG (5 $\mu\text{g}/\text{mL}$). At day 6, cells were restimulated with YFV (1,000 IU/mL) for 4 hr. After the indicated incubation times, mRNA was extracted by TRIzol (Life technologies), according to the manufacturer's instructions. cDNA was synthesized with iScript reverse transcriptase (Invitrogen) and relative expression was measured using SYBR Green method (Invitrogen) on an Applied Biosciences Step-one PLUS qPCR machine. Primers are listed in Table S1.

In Vitro Assessment of Specific Histone Marks

Monocyte isolation was performed by density gradient centrifugation according to the procedure described above. Isolated cells were counted in a Coulter counter (Coulter Electronics) and adjusted to 1×10^6 cells/mL. A 10 mL volume was added into 10-cm Petri dishes (Corning) and cells were trained as described. On day 6, cells were harvested and fixed in 1% methanol-free formaldehyde. Fixed cell preparations were sonicated using a Diagenode Bioruptor Pico sonicator using 5 cycles of 30 s on, 30 s off and ChIP was performed using antibodies against H3K4me3 and H3K9me3 (Diagenode). A MinElute PCR purification Kit (QIAGEN) was used

for DNA isolation. Afterward, qPCR analysis was performed using the SYBR Green method and samples were analyzed by a comparative Ct method. Primers can be found in [Table S1](#).

In Vivo Assessment of Genome-Wide Epigenetic Profiles

For genome-wide chromatin assessment we chose the histone 3 lysine 27 acetylation (H3K27ac) mark, which is generally associated with an “active” chromatin state at both gene promoters and distal (enhancer) regions, thereby extending the epigenetic profiles beyond promoter regions. Moreover, we previously showed a high correlation between H3K4me3 and H3K27ac change in response to exogenous stimuli, with H3K27ac being the more dynamic mark of the two ([Saeed et al., 2014](#)). Monocytes from BCG vaccinated volunteers were isolated and chromatin was crosslinked with 1% formaldehyde as described. Chromatin was sonicated and immunoprecipitated using an antibody for H3K27ac (Diagenode) as described previously ([Novakovic et al., 2016](#)).

Library Preparation for Sequencing

Illumina library preparation was performed using the Kapa Hyper Prep Kit (Kapa Biosystems). DNA was incubated with end repair and A-tailing buffer and enzyme and incubated first for 30 min at 20°C and then for 30 min at 65°C. Sample-specific NextFlex adapters were ligated by adding 30 µL ligation buffer, 10 Kapa I DNA ligase, 5 µL diluted adaptor in a total volume of 110 µL and incubated for 15 min at 15°C. Post-ligation cleanup was performed using Agencourt AMPure XP reagent at a bead:DNA ratio of 0.8:1 selecting sizes of > 150 bp. Libraries were amplified by adding 25 µL 2x KAPA HiFi Hotstart ReadyMix and 5 µL 10x Library Amplification Primer Mix and PCR, 10 cycles. Samples were purified using the QIAquick MinElute PCR purification kit and 300 bp fragments selected using E-gel. Correct size selection was confirmed by BioAnalyzer analysis. Paired-end sequencing was performed using the Illumina NextSeq 500 machine.

Yellow Fever RT-qPCR and Antibody Titers

RT-qPCR of YF-17D was performed at the department of Viroscience of the Erasmus Medical Center as described before ([Wouthuyzen-Bakker et al., 2017](#)). Briefly, viral RNA was isolated using MagnaPure LC (Roche) and reverse transcribed (Taqman Reverse Transcription Reagents, Applied Biosystems International). cDNA synthesis was performed in a J Mini Gradient Thermal Cycler (BioRad, Netherlands) for real-time PCR, the following YF specific primers and probe were used YFV-1 (forward) 5'-AATCGAGTTGCTAGG CAATAAACAC-3', YFV-2 (reverse) 5'-TCCCTGAGCTTTACCAGA-3', YFV-P (probe) 5'-FAM-ATCGTTGAGCGATTAGCAG-BHQ-3 ([Drosten et al., 2002](#)). Real-time qPCR was monitored on ABI Prism 7500 Seq. Detection System (Applied Biosystems International). Viremia is presented as relative viremia, by setting CT value 40 at 1, or by the formula $Relative\ Viremia = 2^{-(40 - Ct-value)}$.

For YFV virus micro-neutralization tests 100 TCID₅₀ of YFV vaccine strain 17D 204 (Stamaril, Sanofi-Pasteur) were incubated with two-fold serum dilutions (starting dilution serum 1:8) for 1 hr at 37°C. Subsequently inoculum was allowed to bind to Vero cells for 1 hr at 37°C, after which cells were incubated for 5 days at 37°C. YFV infection was read out by cytopathic effect.

Genetic Analysis

For the genetic validation study, we assessed genetic variation in the genes of the IL-1 pathway in healthy individuals of Western European decent from the 200FG cohort ([Li et al., 2016](#)) by genome-wide Illumina Infinium SNP array. The genotype data were imputed and filtered using criterion of minor allele frequency $\geq 5\%$ and other standard quality filters. The volunteers were between 23 and 73 years old, and consisted of 77% males and 23% females ([Li et al., 2016](#)). Monocytes were isolated as described earlier and stimulated *in vitro* with culture medium (negative control) or BCG (10 µg/mL) for 24 hr. On day 6, cells were restimulated with culture medium or LPS (10 ng/mL). After 24 hr, supernatants were collected and IL-6 and TNF α production were measured. Raw cytokine levels were first log-transformed then ratios relative to the measurements in culture medium (negative control) were computed. The ratio data were mapped to genotype data using a linear regression model with age and gender as covariates. The genetic variants located within the IL-1 family genes or in a ± 250 kb window were tested for genetic association with BCG-induced trained immunity.

ChIP-Sequencing Analysis

ChIP-sequencing reads were aligned to human genome assembly hg19 (NCBI version 37) using bwa. Duplicate reads were removed after the alignment with the Picard tools and BAM files were first filtered to remove the reads with mapping quality less than 15, followed by fragment size modeling (<https://code.google.com/archive/p/phantompeakqualtools/>). MACS2 (<https://github.com/taoliu/MACS/>) was used to call the peaks. Data (H3K27ac reads/peak) were normalized using the R package DESeq2 and then pairwise comparisons were performed. Differential H3K27ac peaks were identified as those with fold change > 1.5, p value < 0.05 and reads/peak ≥ 15 , between the baseline (-d28) and BCG vaccinated groups, similar to ([Novakovic et al., 2016](#)). Additionally, to identify possible donor-specific BCG responses, we identified H3K27ac peaks that showed a > 3 standard deviation change from mean level at baseline in any of the BCG vaccinated individuals, similar to ([Saeed et al., 2014](#)). H3K27ac peaks were assigned to the nearest gene within 1Mb and gene ontology analysis on dynamic enhancer clusters was performed using GREAT ([McLean et al., 2010](#)).

QUANTIFICATION AND STATISTICAL ANALYSIS

Statistical parameters including the exact value of n, the definition of center, dispersion and precision measures (mean \pm SEM), and statistical significance are reported in the figures and figure legends. Differences were analyzed using Wilcoxon signed-rank test or

Mann-Whitney U test where applicable. For cytokine production before and after BCG vaccination, fold increases (1 month after BCG divided by baseline cytokine production by PBMCs) are presented in order to increase clarity. The statistical analyses presented are performed on these ratios. The analyses did not show major differences with the raw data analysis. All calculations were performed in Graphpad prism 5 (CA, USA). $p < 0.05$ was considered statistically significant and in figures, asterisks denote statistical significance (* $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$). Data are shown as means \pm SEM.

DATA AND SOFTWARE AVAILABILITY

Data Resources

Raw data files for the BCG *in vivo* trained monocytes ChIP sequencing and RNA sequencing analysis have been deposited in the NCBI Gene Expression Omnibus under accession number GEO: GSE104149.