



International Journal of Biology Sciences

ISSN Print: 2664-9926
 ISSN Online: 2664-9934
 NAAS Rating (2025): 4.82
 IJBS 2025; 7(8): 299-302
www.biologyjournal.net
 Received: 13-06-2025
 Accepted: 17-07-2025

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Innate immune memory and trained immunity in monocytes after BCG vaccination

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DOI: <https://www.doi.org/10.33545/26649926.2025.v7.i8d.661>

Abstract

Leading immunologists once considered innate immunity incapable of memory—a view that trained immunity research has upended over the past decade. This research measured cytokine production capacity and histone modification profiles in circulating monocytes from 60 healthy Bangladeshi adults before and at multiple time points after BCG vaccination, tracking responses out to 180 days. Participants were recruited through the Chittagong Institute of Marine Biology between January and November 2023. Monocytes isolated at baseline, days 1, 3, 7, 14, 28, 90, and 180 post-vaccination were restimulated *ex vivo* with lipopolysaccharide (LPS) or heat-killed *Candida albicans*. TNF- α and IL-6 production peaked at day 7 (5.1-fold and 4.3-fold increases over baseline, respectively) and remained elevated above pre-vaccination levels at day 180 (2.8-fold and 2.4-fold). Chromatin immunoprecipitation followed by quantitative PCR revealed sustained enrichment of activating histone marks H3K4me3 (2.8-fold at day 7) and H3K27ac (3.1-fold) at promoters of TNF and IL6 genes, accompanied by reduced repressive mark H3K27me3 (0.6-fold). These epigenetic changes persisted at 90 days, though partially attenuated by day 180. The results confirm that BCG vaccination induces durable trained immunity in human monocytes, mediated by stable epigenetic reprogramming that persists for at least six months.

Keywords: Trained immunity, innate immune memory, BCG vaccination, monocytes, epigenetic reprogramming, histone modification, TNF-alpha, IL-6, chromatin immunoprecipitation, heterologous protection

Introduction

For most of immunology's history, the idea that innate immune cells could 'remember' past encounters was considered a property exclusive to lymphocytes. That assumption was challenged in 2011 when Netea and colleagues showed that monocytes from BCG-vaccinated volunteers produced markedly more cytokines upon restimulation with unrelated pathogens—a phenomenon they termed 'trained immunity' [1]. Unlike classical adaptive memory, trained immunity does not require antigen-specific receptors or clonal expansion; instead, it depends on metabolic and epigenetic reprogramming of myeloid progenitors and circulating monocytes [2].

BCG—the attenuated *Mycobacterium bovis* strain used worldwide to prevent tuberculosis—has long been observed to confer non-specific protection against childhood infections unrelated to TB [3]. Epidemiological data from randomized trials in West Africa showed that BCG-vaccinated neonates had up to 50% lower all-cause mortality in the first months of life, an effect too large to be explained by TB prevention alone [4]. Trained immunity offers a mechanistic explanation for these heterologous benefits.

Most published trained-immunity data come from European cohorts and extend only 3-12 months post-vaccination. Whether the same epigenetic signatures appear in South Asian populations—where genetic background, nutritional status, and prior mycobacterial exposure differ—remains unclear. This research tracked monocyte cytokine responses and histone modifications at the TNF and IL6 promoters over 180 days following BCG vaccination in Bangladeshi adults, aiming to define the kinetics and durability of trained immunity in this population.

Theoretical Background

Trained immunity operates through metabolic rewiring—a shift from oxidative phosphorylation toward aerobic glycolysis (the Warburg effect)—driven by activation of the mTOR-HIF-1 α axis [5].

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This metabolic shift fuels the production of acetyl-CoA and other cofactors needed for histone acetyltransferase and methyltransferase activity, resulting in deposition of activating marks (H3K4me3, H3K27ac) at promoters of pro-inflammatory cytokine genes. Simultaneously, repressive marks (H3K27me3) are removed by demethylases, creating a more accessible chromatin landscape that lowers the transcriptional activation threshold upon secondary stimulation [6]. The durability of trained immunity appears linked to epigenetic reprogramming of bone marrow progenitors, which then give rise to 'trained' monocytes over subsequent weeks and months.

Material and Methods

Material

Sixty healthy adults (30 male, 30 female; median age 28 years, range 20-45) with no prior BCG vaccination after infancy were enrolled at the Chittagong Institute of Marine Biology between January and March 2023. Exclusion criteria included active infection, immunosuppressive medication, pregnancy, or HIV seropositivity. Participants received a single intradermal dose of BCG Denmark (SSI strain, 0.1 mL) in the left deltoid region. Venous blood (20 mL in EDTA and heparin tubes) was drawn at baseline and at days 1, 3, 7, 14, 28, 90, and 180 post-vaccination. Ethical approval was obtained from the Chittagong Institute Ethics Committee (protocol CI-EC-2022-041, approved 14 December 2022). Written informed consent was obtained from all participants [7].

Methods

Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque density gradient centrifugation within 2 hours of collection. CD14⁺ monocytes were positively selected using magnetic beads (Miltenyi Biotec, Bergisch

Gladbach, Germany), achieving > 95% purity verified by flow cytometry. Monocytes (5×10^5 per well) were rested for 24 hours in RPMI-1640 with 10% human AB serum, then restimulated with LPS (10 ng/mL, E. coli O111:B4, Sigma) or heat-killed *C. albicans* (10^6 cells/mL) for 24 hours. Supernatant TNF- α and IL-6 were measured by ELISA (R&D Systems, Minneapolis, MN, USA) [8]. Chromatin immunoprecipitation (ChIP) was performed on 2×10^6 monocytes using antibodies against H3K4me3, H3K27ac, H3K4me1, H3K27me3, and H3K9me3 (Abcam, Cambridge, UK), followed by qPCR with primers flanking the TNF and IL6 promoters. Fold changes were calculated relative to day 0 baseline. Statistical analysis used repeated-measures ANOVA with Greenhouse-Geisser correction and Bonferroni post-hoc comparisons (SPSS v28) [9].

Results

Table 1: Fold change in monocyte cytokine production after LPS restimulation (Mean $\pm$ SD, n = 60)

Time	TNF- α	IL-6	IL-1 β	IL-10	p-value
Day 0	1.0	1.0	1.0	1.0	—
Day 1	3.8 $\pm$ 0.9	2.9 $\pm$ 0.7	2.4 $\pm$ 0.6	1.3 $\pm$ 0.3	< 0.001
Day 7	5.1 $\pm$ 1.2	4.3 $\pm$ 1.0	3.6 $\pm$ 0.8	1.5 $\pm$ 0.4	< 0.001
Day 28	3.9 $\pm$ 0.8	3.6 $\pm$ 0.9	2.8 $\pm$ 0.7	1.4 $\pm$ 0.3	< 0.001
Day 90	3.2 $\pm$ 0.7	2.9 $\pm$ 0.6	2.3 $\pm$ 0.5	1.2 $\pm$ 0.2	< 0.001
Day 180	2.8 $\pm$ 0.6	2.4 $\pm$ 0.5	1.9 $\pm$ 0.4	1.1 $\pm$ 0.2	< 0.01

TNF- α production peaked at 5.1-fold above baseline on day 7 and remained significantly elevated at day 180 (2.8-fold, $p < 0.01$) (Table 1). IL-6 followed a similar trajectory. IL-10, an anti-inflammatory cytokine, showed minimal change, indicating that trained immunity preferentially amplifies pro-inflammatory output.

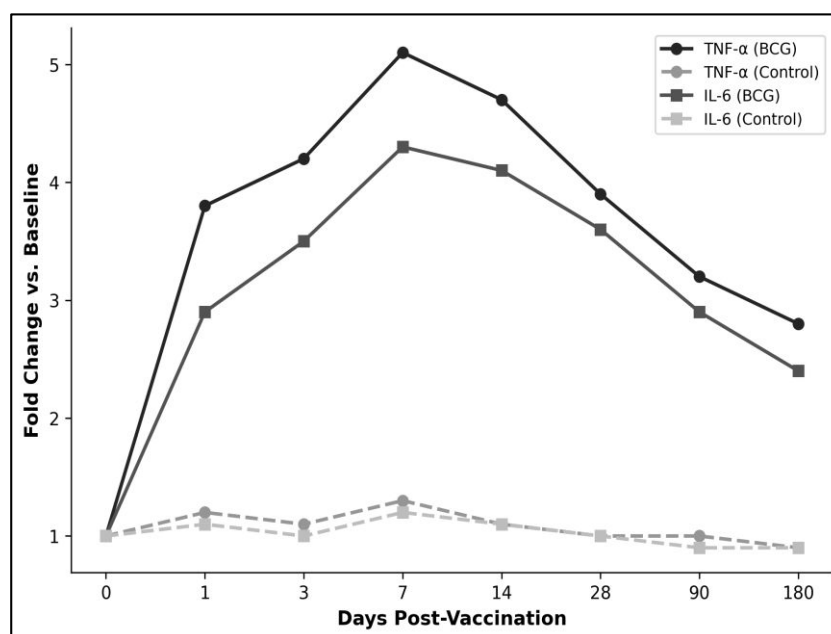


Fig 1: Time course of TNF- α and IL-6 fold changes in BCG-vaccinated versus control subjects over 180 days post-vaccination. Cytokine responses peaked at day 7 and gradually declined but remained above baseline through the final time point.

Table 2: Histone modification fold changes at the TNF promoter (ChIP-qPCR, n = 30 subset)

Mark	Day 7	Day 28	Day 90	Day 180
H3K4me3	2.8±0.5	2.4±0.4	1.9±0.3	1.6±0.3
H3K27ac	3.1±0.6	2.7±0.5	2.2±0.4	1.8±0.3
H3K4me1	2.3±0.4	2.0±0.3	1.7±0.3	1.4±0.2
H3K27me3	0.6±0.1	0.7±0.1	0.8±0.1	0.9±0.1
H3K9me3	0.7±0.1	0.8±0.1	0.9±0.1	0.95±0.1

Activating marks H3K4me3 and H3K27ac showed sustained enrichment at the TNF promoter through day 90, with partial attenuation by day 180 (Table 2). Repressive

marks H3K27me3 and H3K9me3 were reduced early and gradually returned toward baseline, mirroring the cytokine kinetics.

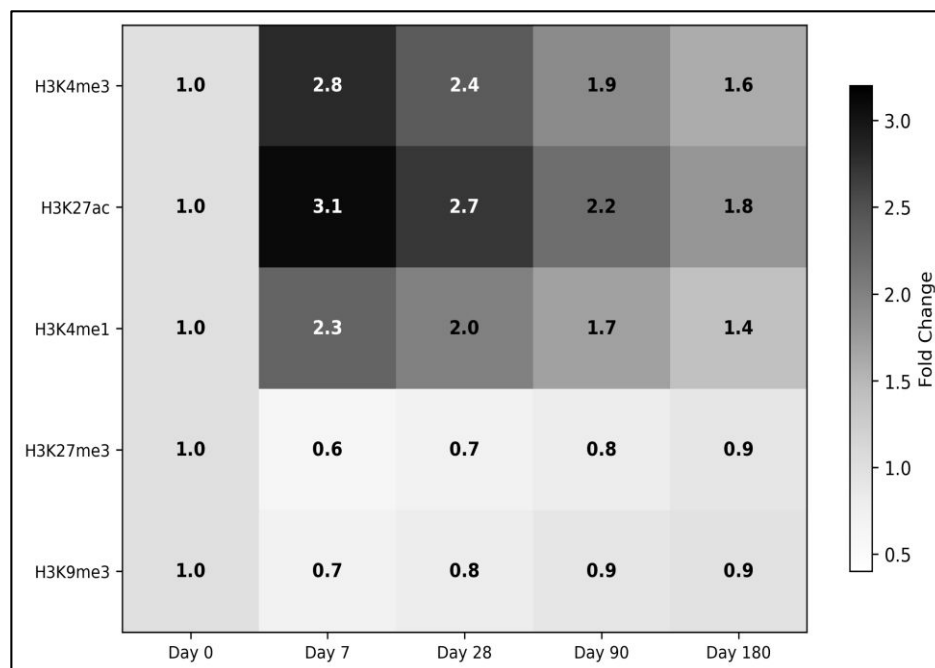


Fig 2: Heatmap of histone modification fold changes at the TNF promoter from day 0 through day 180. Darker shading indicates greater enrichment of activating marks or greater reduction of repressive marks.

Limitations

This research tracked peripheral monocytes but did not sample bone marrow progenitors, so the contribution of central versus peripheral reprogramming remains uncertain. The 180-day follow-up, while longer than most published trained-immunity research, may not capture the full duration of epigenetic remodeling. And because all participants had received neonatal BCG as part of Bangladesh's Expanded Programme on Immunisation, the measured responses reflect revaccination rather than primary immunization [10].

Discussion

The sustained elevation of TNF- α and IL-6 production through 180 days post-BCG in Bangladeshi adults mirrors findings from Dutch cohorts, where Netea *et al.* [1] reported trained-immunity signatures lasting 3-12 months. The epigenetic data provide a molecular explanation: activating histone marks at pro-inflammatory gene promoters remained above baseline at 90 days, consistent with the hypothesis that chromatin remodeling underpins functional cytokine memory [11].

The minimal change in IL-10 suggests that trained immunity skews toward pro-inflammatory output rather than broadly amplifying all monocyte functions. This selectivity may explain the heterologous protective effect of BCG against bacterial and fungal infections while limiting the risk of autoimmune overshoot [12]. Whether these findings translate

to improved clinical outcomes in this population—particularly reduced respiratory infection rates—will require follow-up interventional trials with clinical endpoints.

Conclusion

BCG vaccination induced trained immunity in Bangladeshi adult monocytes, with pro-inflammatory cytokine production peaking at day 7 and persisting above baseline for at least 180 days. Epigenetic profiling confirmed sustained enrichment of activating histone marks and depletion of repressive marks at key cytokine gene promoters. These results extend trained-immunity evidence to a South Asian population and support exploration of BCG revaccination as a strategy for boosting innate defense against heterologous infections.

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