BCG vaccination of infants confers *Mycobacterium tuberculosis* strain-specific immune responses to leucocytes

To the Editor:

The World Health Organization (WHO) reported that tuberculosis (TB) accounted for 1.5 million deaths and 10 million new cases in 2018. At present, bacille Calmette-Guérin (BCG) is the only vaccine approved by WHO to prevent TB, and is recommended for use, particularly among children in TB endemic countries (http://www.tbfacts.org/bcg-tb-vaccine/). However, the reported protective efficacy of BCG against pediatric TB is highly variable, ranging from 0-80% [1]. Although peripheral blood mononuclear cells (PBMC) from BCG-vaccinated infants demonstrated the induction of immune activation markers, the magnitude of the T cell responses in vaccinated infants does not correlate with the risk of TB [2, 3]. Thus, the response of other immune cells, such as macrophages, and their role in TB pathogenesis needs to be elucidated. However, it is not clear exactly which components of the host immune response to BCG confer protection against progression to TB and severity of disease.

Epidemiologic studies have demonstrated a wide range of Mtb clinical strains, distributed differently in various regions of the world. In vitro studies and animal models of infection have shown differences in the host immune response and control of infection by different Mtb clinical strains [4]. For example, we have demonstrated that infection with the clinical Mtb strain HN878 induced a highly inflammatory type I IFN response that led to severe disease and accelerated mortality of animals [5]. In contrast, infection with Mtb CDC1551 activated a Th1 response that controlled the bacterial burden and disease pathology [6]. Thus, these two clinical Mtb strains display differential host responses in vitro and animal models. Here, we report the effect of BCG

vaccination on the profiles of cytokines and chemokines produced by infant PBMC in response to in vitro stimulation with Mtb HN878 or CDC1551.

This study was approved by the Institutional Review Board (IRB) of the University of Cape Town and UMDNJ/Rutgers University (Pro2012001418 and Pro0120110233). Since the enrolled participants were infants, informed consent to participate in this study was obtained from the parent(s) [7]. The study population had two cohorts of 10-week old, South African infants (n=20 each); one group received routine intradermal vaccination with BCG (Danish strain 1331, Statens Serum Institute) at birth while BCG vaccination was deferred in the other cohort until after blood collection at ten weeks.

Blood was collected in heparinized tubes, and PBMCs were isolated by density gradient centrifugation and the viability of PBMCs was determined by Trypan blue exclusion assay [7]. $1x10^5$ PBMC/well were seeded into 24 well plates and grown in complete RPMI media and either stimulated with live Mtb HN878 or CDC1551 at an MOI of 5 or left unstimulated at 37°C with 5% CO_2 supply. At 24 hrs post-stimulation, supernatants from PBMC cultures were collected, filtered through a 0.22-micron Nylon filter and used for cytokine analysis using the Bio-Plex Pro Human Cytokine 27-plex Assay kit as per the manufacturer's protocol (Bio-Rad, USA). Samples were read on Bio-Plex 200 systems and analyzed using Bio-Plex Manager software (Bio-Rad, USA). All cytokine concentrations are presented as pg/ml and each sample was tested twice and the average was used for calculations. Pair-wise comparisons (the same sample tested with two different Mtb strains) were analyzed by non-parametric Wilcoxon signed-rank test, and inter-group (BCG versus no BCG) comparisons were analyzed by non-parametric Mann Whitney U test using Prism (version 5, GraphPad). Bonferroni post-test correction for multiple group comparison was applied.

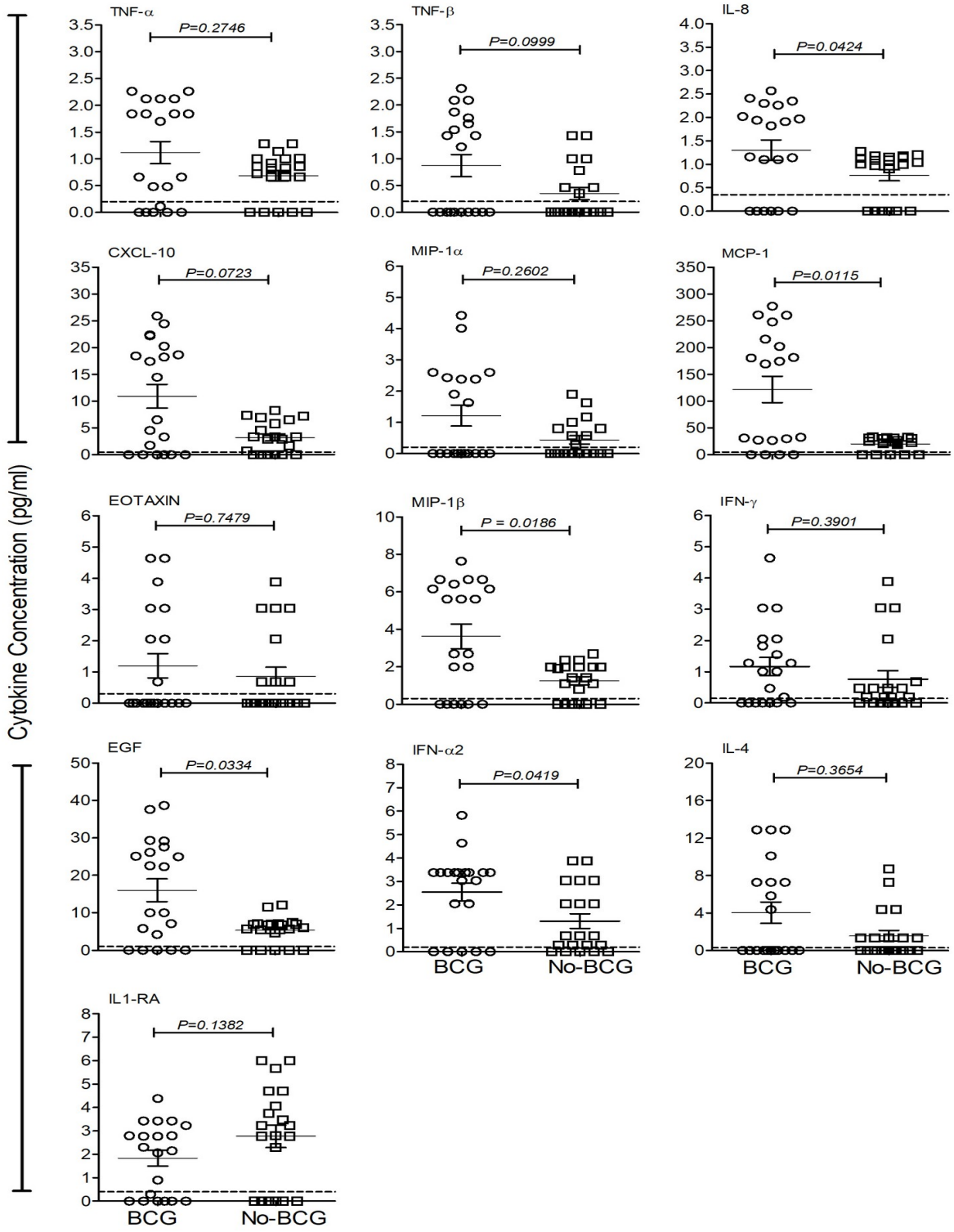
The pattern of release of selected cytokines and chemokines revealed a predominantly pro-inflammatory response in the culture supernatant of PBMCs of BCG-vaccinated infants. Although variable among tested individuals, IL-8, MCP-1, MIP-1β, EGF, IFN-α2 levels were significantly increased, while the anti-inflammatory IL-1Ra levels were substantially lower in the BCG-vaccinated, compared to non-vaccinated infant PBMCs. No statistically significant difference was noticed in TNF-α, TNF-β, CXCL-10, MIP-1α, Eotaxin, IFN-γ, and IL-4 levels between these two groups (Fig. 1).

Next, we compared the cytokine/chemokine profile of BCG-vaccinated and non-vaccinated infant PBMCs after stimulation with two different clinical Mtb strains, HN878 or CDC1551 (Fig. 2). The unstimulated values were subtracted from the HN878 or CDC1551-stimulated PBMCs. In general, the PBMCs from BCG-vaccinated infants showed a higher pro-inflammatory response to Mtb, compared to the non-vaccinated counterparts. In response to stimulation with Mtb HN878, the PBMCs of the majority of BCG-vaccinated infants showed significantly increased levels of TNF-α, CXCL-10, MCP-1, MIP-1β, EGF, and IL-1Ra, compared to the non-vaccinated counterpart. Further, the level of inflammatory mediators TNF-α, IL-8, CXCL-10, MIP-1α, MCP1, MIP-1β, and EGF was significantly higher in response to HN878, compared to CDC1551 stimulation in BCG-vaccinated infants. In contrast, exposure of PBMCs from BCG-vaccinated infants to Mtb CDC1551 significantly increased the levels of IL-1Ra (Fig.2). Moreover, the level of MCP-1 and EGF was significantly elevated by exposure of PBMC of BCG-vaccinated infants to CDC1551, compared to non-vaccinated infants. The level of TNF-β, Eotaxin, and IL-4 were not significantly different between HN878 and CDC1551 stimulated PBMCs of BCG-vaccinated infants. Similarly, none of the tested molecules showed a statistically significant difference between HN878- and

CDC1551-stimulated PBMCs of non-vaccinated infants (Fig.2). As reported previously, we observed a higher pro-inflammatory response in the leukocytes of BCG-vaccinated, compared to non-vaccinated infants [8, 9].

. However, what was not predicted from the majority of previous studies was that the profile of inflammatory mediators differed according to the strain of Mtb used to stimulate the PBMC from BCG-vaccinated and not in non-vaccinated infants. This suggests that BCG vaccination may prime the host immune system to mount an Mtb-specific response, which may result in variable protective efficacy, dependent on the nature of the infecting Mtb clinical isolate. BCG vaccination has been shown to induce variable frequencies of mycobacteria-specific CD4+ T cell responses [2]. However, the magnitude and functional profile of the polyfunctional T cell response to BCG vaccination measured at ten weeks of age did not correlate with subsequent risk of TB [3]. These data suggest that mycobacteria-specific T cell responses are not sufficient for protective immunity against Mtb and imply that additional factors that regulate the immune response may be involved in conferring protective immunity.

Our data suggest that the diversity in clinical Mtb isolates circulating in different parts of the world may contribute to the differential levels of protective efficacy of BCG vaccination reported in published studies [10]. A better understanding of the signaling pathways that underlie the disparate PBMC response of BCG-vaccinated infants to various clinical Mtb strains can help to improve our understanding of the nature of protection and facilitate the efficient development of future vaccines to control TB.



94**Figure 1:** Cytokine and chemokine profile of non-stimulated PBMCs isolated from the 95blood of non-vaccinated (No-BCG) and BCG-vaccinated (BCG) infants. Levels of

selected pro- and anti-inflammatory markers were determined in the culture supernatant and reported as pg/ml. Dotted line indicates lower level of quantification.

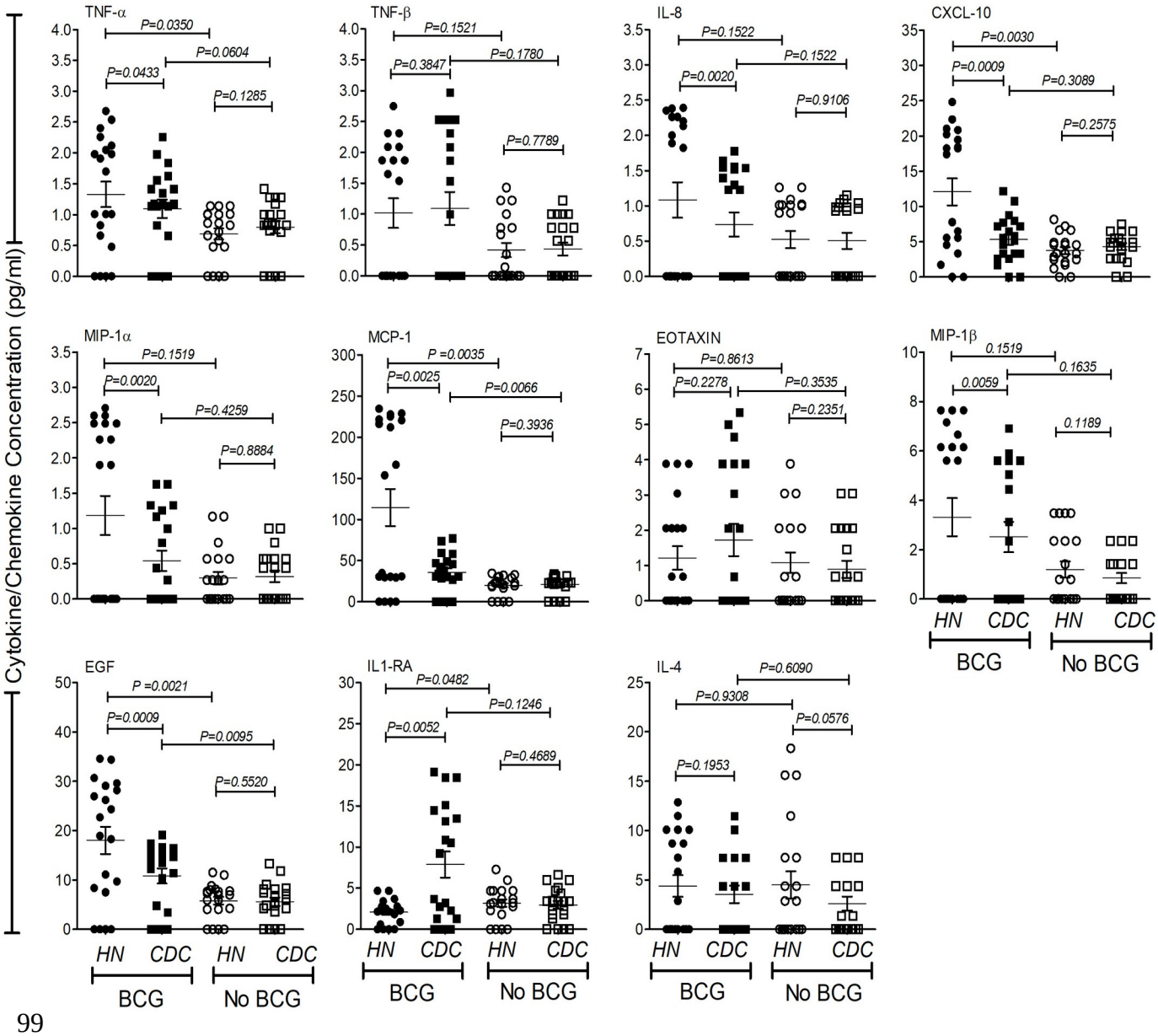


Figure 2: Clinical Mtb strains of variable lineage induce differential cytokine and chemokine expression in the PBMCs of BCG-vaccinated (BCG) and non-vaccinated (No BCG) infants. Culture supernatants of PBMCs stimulated with Mtb HN878 (HN) or

CDC1551 (CDC) for 24 hours were used to determine the level for selected cytokines and chemokines, and reported as pg/ml.

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