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ABSTRACT

Leprosy is an infectious disease caused by *Mycobacterium leprae* leading to irreversible disabilities along with social exclusion. Leprosy is a spectral disease for which the clinical outcome after *M. leprae* infection is determined by host factors. The spectrum spans from anti-inflammatory T helper-2 (Th2) immunity concomitant with large numbers of bacteria as well as antibodies against *M. leprae* antigens in multibacillary (MB) leprosy, to paucibacillary (PB) leprosy characterised by strong pro-inflammatory, Th1 as well as Th17 immunity. Despite decades of availability of adequate antibiotic treatment, transmission of *M. leprae* is unabated. Since individuals with close and frequent contact with untreated leprosy patients are particularly at risk to develop the disease themselves, prophylactic strategies currently focus on household contacts of newly diagnosed patients.

It has been shown that BCG (re)vaccination can reduce the risk of leprosy. However, BCG immunoprophylaxis in contacts of leprosy patients has also been reported to induce PB leprosy, indicating that BCG (re)vaccination may tip the balance between protective immunity and overactivation immunity causing skin/nerve tissue damage.

In order to identify who is at risk of developing PB leprosy after BCG vaccination, amongst individuals who are chronically exposed to *M. leprae*, we analyzed innate and adaptive immune markers in whole blood of household contacts of newly diagnosed leprosy patients in Bangladesh, some of which received BCG vaccination. As controls, individuals from the same area without known contact with leprosy patients were similarly assessed.

Our data show the added effect of BCG vaccination on immune markers on top of the effect already induced by *M. leprae* exposure. Moreover, we identified BCG-induced markers that differentiate between protective and disease prone immunity in those contacts.

1. *Introduction:*

Leprosy is a debilitating, neglected tropical disease (NTD) that is caused by *Mycobacterium leprae* (*M. leprae*), which separated from the *M. tuberculosis* lineage over 60 million years ago [1]. It ranks second after tuberculosis (TB) in the order of severe human mycobacterial diseases [2] and currently millions of people suffer from disabilities due to leprosy [3]. Like TB, leprosy is a poverty-associated disease mostly afflicting marginalized populations in low- and middle-income countries (LMICs) in their most productive stage of life. Unlike TB, leprosy affects mainly the skin and peripheral nerves due to *M. leprae*'s tropism for macrophages and Schwann cells and preference for body parts with lower temperatures [4]. Due to its unique incubation period, leprosy can be considered a chronic "iceberg" disease [5], taking years to manifest and including many undetected, subclinical cases which probably perpetuate transmission leading to the almost stable 200,000 new cases annually during the past decade [3]. Consequently, patients diagnosed outside these endemic areas are usually not autochthonous cases [6].

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Early diagnosis of leprosy before signs and symptoms appear, is considered vital to prevent leprosy-associated disabilities. *M. leprae* transmission is generally considered to be mainly from human to human via aerosol droplets spread by the respiratory route [7]. Due to the massive gene decay in *M. leprae* compared to *M. tuberculosis* [8], its virulence is reduced, and disease development requires close and frequent contact with a human, animal or environmental source of transmission [9-11]. Thus, contacts of untreated leprosy patients are at higher risk of contracting leprosy [12] and national health care services are specifically targeting this group at risk of developing leprosy in chemo- and immuno prophylactic strategies [13,14].

M. bovis Bacillus Calmette-Guérin (BCG), known as a vaccine against TB, is provided routinely to neonates as part of the immunization scheme in high TB-burden countries worldwide (http://www.bcgatlas.org). In countries with lower overall TB incidence, governments have largely discontinued universal BCG vaccination in favor of targeting only specific TB risk groups, like children from families immigrating from high TB burden countries [15]. Additionally, BCG vaccination is also reported to protect against leprosy [16,17]. In various vaccination trials utilizing BCG, its protective effect against leprosy was shown [16,18-20]. Moreover, the Brazilian Ministry of Health has officially recommended a booster to the routine BCG-vaccination against TB at birth since the early 1970s for household contacts of newly diagnosed leprosy patients and since 1991, this recommendation was extended to two doses of BCG. Assessment of this policy in a Brazilian cohort study [20] showed 56% protection against leprosy by a booster BCG-vaccination, whereas a more recent study from Brazil in a 33 year contact cohort of leprosy patients found that a lower risk was detected for contacts with BCG scar who were revaccinated and a second dose of the BCG vaccination can exert extra protection [21]. Of note is that the risk of paucibacillary (PB) leprosy, which is characterized by strong cellular responses against *M. leprae*, during the initial months after BCG vaccination was high among BCG-vaccinated contacts in the Brazilian study [20]. More recently we conducted a randomized controlled BCG vaccination trial among 5,196 contacts of leprosy patients in Bangladesh which confirmed this finding displaying development of PB leprosy within 12 weeks after vaccination in 12 contacts (0.4%) receiving BCG as immunoprophylaxis [22,23]. In addition, leprosy reactions have also been reported to be induced by BCG vaccination [24].

Due to the delicate balance of activating and regulating immunity, we hypothesize that BCG (re)vaccination can in some instances, like in contacts of leprosy patients who are frequently/intensely exposed to *M. leprae*, lead to overactivation of immunity causing PB leprosy. Two mechanisms are proposed that could lead to the induction of PB leprosy following BCG re-vaccination [19]:

1. Boosting of pre-existing M. leprae-specific T-cells by homologs of *M. leprae* present in BCG leading to overactivation of this T-cell population.
2. Unwarranted boosting of the innate immune response leading to overactivation of innate immune cells (similar to observations for patients with COVID-19 after infection with SARS-CoV-2 [25-27]).

This overactivation is characterized by strong pro-inflammatory immunity that controls bacterial growth but, also causes collateral damage in the form of destruction of the body's own cells by the vigorous T cell response, thereby mimicking autoimmunity [28-30].

In view of BCG's beneficial, immunoprophylactic capacity as well as its long lasting effects compared to chemoprophylaxis, we have investigated in this study whether certain host proteins in contacts of untreated leprosy patients can indicate the risk of PB after BCG administration.

In this study, we have cross-sectionally compared BCG-vaccinated contacts (n = 50) to contacts (n = 54) that did not receive BCG as immunoprophylaxis. In addition, longitudinal cytokine profiles in whole blood assays of a selection of 16 contacts of this field trial in Bangladesh at week 4 and week 8 after BCG vaccination was performed. More insight into the mechanism of action of BCG in preventing leprosy but also in sporadically causing PB disease, will allow better targeting those who will benefit from BCG (re)-vaccination to prevent leprosy.

2. Materials and methods

2.1. Study setting

During this study the leprosy prevalence in the four rural districts in Bangladesh (Nilphamari, Rangpur, Panchagar and Thakurgaon; population 8,190,035) was 0.9 per 10,000 and the new case detection rate 1.18 per 10,000 (Rural health program, The Leprosy Mission Bangladesh, yearly district activity report 2018).

2.2. Study participants

Household contacts (HC) of leprosy patients were recruited on a voluntary basis between January 2013 and 2018 in leprosy endemic areas in Bangladesh as described previously [14]. At intake, all contacts are screened by trained and experienced health workers to ensure they had no apparent signs of leprosy at that time. Leprosy was diagnosed based on clinical and bacteriological observations and classified as multibacillary (MB) or PB as described by the WHO [3] and using the Ridley-Jopling classification system [31]. Clinical and demographic data were collected in a database. After intake, HC of leprosy patients were followed-up yearly: Individuals suspected to have leprosy at any of the follow-up time points or who presented to a health clinic between follow-ups were sent to the specialized leprosy hospital in Nilphamari or a local clinic for confirmation of their disease by a specialist clinician and for treatment [23]. As a reference group healthy individuals without known contact to leprosy patients from the same area (EC) were assessed for the absence of clinical signs and symptoms of leprosy and TB at intake, and after 2 and 4 years. Samples were collected from 8 villages spread randomly across the study area (2 representative villages for each of the 4 districts).

2.3. Study cohorts

Two cohorts originating from the same study area were tested (supplementary Fig. S1 and Supplementary Table S1). In the first cohort HC receiving BCG as immunoprophylaxis (n = 50) were cross-sectionally compared to HC who were not (re-)vaccinated (n = 54) with BCG and EC (n = 51), which were described previously [32,33]. In the BCG vaccinated group, blood was collected 8–10 weeks after vaccination. 0.1 ml of BCG vaccine was given by intradermal injection. The BCG vaccine is produced at the Japan BCG Laboratory and is a freeze-dried glutamate BCG vaccine (Japan), composed of 0.5 mg/ampule live bacteria of Bacille Calmette-Guérin (as approximately 70% moist bacteria) and 2.0 mg/ampule sodium glutamate (as a stabilizer). Vaccines are stored at the State Immunisation Programme facilities. In the second cohort HC were longitudinally sampled after BCG vaccination (n = 16). Blood was collected just before BCG was administered (week 0) and 4 and 8 weeks after vaccination. As controls, HC who did not receive BCG during the study were sampled at the

same timepoints (n = 16). Alongside these 32 individuals, eight BCG-vaccinated individuals who developed leprosy during follow-up (leprosy progressors) were included. Their blood was collected eight weeks after vaccination. Leprosy was diagnosed one year (n = 4), two years (n = 2), four years (n = 1) or five years (n = 1) after vaccination.

2.4. Whole Blood Assay (WBA) samples

Venous blood was drawn and 1 ml was applied directly to a microtube pre-coated with 10 μg *M. leprae* whole cell sonicate (WCS), 10 μg ML2478 and 10 μg ML0840 recombinant proteins (combined designated as Mlep) [3] or without stimulus (Nil). After 24 h incubation at 37 °C, microtubes were frozen at −20 °C, shipped to the LUMC and stored at −80 °C until further analysis.

2.5. Ethics

This study was performed according to the Helsinki Declaration (2008 revision) and the study protocol was approved by the National Research Ethics Committee (Bangladesh Medical Research Council) (Ref no. BMRC/NREC/2010-2013/1534). Participants were informed about the study objectives, the samples and their right to refuse to take part or withdraw from the study without consequences for their treatment. Written informed consent was obtained before enrolment. All patients received treatment according to national guidelines.

2.6. Multiplex bead arrays (MBA)

BCA-1 (CXCL13), C1q, C3b, CCL17, CFH, CTACK (CCL27), sCD40L, EGF, ENA-78 (CXCL5), Eotaxin (CCL11), FGF, Flt3L, Fraktalkine (CX3CL1), G-CSF, GM-CSF, GRO (CXCL1), GZMA, GZMB, I309 (CCL1), sICAM1, IFN-α2, IFN-γ, IL-1α, IL-1β, IL-1ra, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-8, IL-9, IL-10, IL-12(p40), IL-12(p70), IL-13, IL-15, IL-16, IL-17A, IL17F, IL-20, IL-21, IL-22, IL-23, IL-27, IL28A, IL-33, IP-10, MCP-1 (CCL2), MCP-3 (CCL7), MDC (CCL22), MIP-1α (CCL3), MIP-1β (CCL4), MMP2, MMP9, Myoglobin, PDGF-AA, PDGF-AB/BB, PRF, P-selectin, RANTES (CCL5), SAA, SAP, SCF, SDF-1, TGF-α, TNF-α, TNF-β, TPO, TRAIL, TSLP, sVCAM1 and VEGF were measured using the Milliplex magnetic bead kit (Merck, USA) as described previously [34]. CCL17, I309, IL-3, IL-16, IL-17F, IL-20, IL-21, IL-22, IL-23, IL-27, IL-28A, IL-33, SCF, SDF-1 and TSLP were uniquely measured in the cross-sectional cohort. C1q, C3b, CFH, GZMA, GZMB, sICAM1, MMP2, MMP9, Myoglobin, PRF, P-selectin, SAA, SAP and sVCAM1 were uniquely measured in the longitudinal cohort. For each analyte a standard curve was generated, providing information on the upper and lower limit of detection.

2.7. PGL-I ELISA

ELISAs were performed to detect anti-Phenolic glycolipid-I (PGL-I) specific antibodies as described previously [35]. Samples with an optical density at 450 nm (OD_{450}) after correction for background OD (0,1%BSA in coating buffer) above 0.2 were considered positive. This threshold was determined by a threefold multiplication of an average non-endemic control value.

2.8. Statistical analysis

Statistical analysis was performed using GraphPad Prism version 8.1.1 (GraphPad Software, San Diego, CA, USA; http://www.graphpad.com) and R Version 3.3.0 (R, Vienna, Austria; http://www.R-project.org). Volcano plots were computed using R, by plotting the log2 fold change against the -log10 (p-value) for each cytokine, chemokine or growth factor (CCGF). Mann-Whitney U-tests (2 group comparison), Kruskal-Wallis (unpaired; multiple groups) or Friedman with Dunn's correction for multiple testing (paired; multiple timepoints) were performed using GraphPad Prism.

3. Results

3.1. BCG immunoprophylaxis in leprosy contacts enhances the response to M. leprae antigens

The levels of 60 CCGFs and IgM antibody levels against *M. leprae* phenolic glycolipid (PGL-I) in household contacts (n = 50) of leprosy patients receiving BCG vaccination as immunoprophylactic measure were cross-sectionally compared to previously published data of HC without an intervention (n = 54) and EC (n = 51) [33]. The CCGFs were assessed in WBA with three different conditions; without stimulus, stimulated with *M. leprae* WCS or stimulated with two specific *M. leprae* proteins (Mlep; ML0840/ML2478).

Anti-PGL-I IgM levels which were determined as a proxy for the *M. leprae* infection rates in the test groups [35], were similar for both HC groups and EC (Supplementary Fig. S2). In contrast, comparison of the average group levels showed a clear pattern in that several CCGFs were elevated in HC compared to EC, especially in the contacts who received BCG 8–10 weeks before blood collection (Fig. 1A). Indeed, CCGFs differed less significantly between HC and EC (n = 3) than BCG-vaccinated HC (n = 8) (Fig. 1B, C). Among the most significantly different CCGFs in BCG-vaccinated HC vs EC were IL-10_{WCS}, $GCSF_{WCS}$ and TNF-α, similar to the HC [33]. In contrast, IP-10_{WCS} (p = 0.0012) and IFN-γ_{WCS} (p = 0.0153) in BCG-vaccinated HC was significantly higher compared to those in HC (Fig. 1D; Fig. 2). In addition, IP-10_{Nil} as well as IP-10_{Mlep} were only significantly different compared to EC in the BCG-vaccinated contacts, despite slightly elevated median levels in HC compared to EC (Fig. 2). A similar pattern was observed for IL-6_{WCS}, $CCL4_{WCS}$ and IL-$1Ra_{Mlep}$. These data clearly show increased levels of several immune markers in response to *M. leprae* stimuli in HC compared to EC, which was augmented by BCG-vaccination. Thus, it is plausible that the continuous exposure to *M. leprae* enhances the response to mycobacterial antigens. Our finding that the hallmark cytokines of trained immunity, IL-6 and TNF-α, could discriminate HC from EC, led us to hypothesize that a similar mechanism induced by *M. leprae* may predispose contacts to the observed response.

3.2. Longitudinal response in BCG-vaccinated leprosy contacts

In addition to the cross-sectional comparison, longitudinal profiling of BCG-vaccinated contacts was performed (n = 16) using blood collected just before BCG vaccination (week 0) and 4 and 8 weeks postvaccination. Both supernatants from unstimulated and *M. leprae* WCS stimulated WBA were assessed for the presence of 46 CCGFs (Fig. 3). As indicated by the median log2FC difference in concentration, BCG induced more than two-fold differential responses in few CCGFs (Fig. 3). Responses were predominantly increased upon *in vitro* stimulation with *M. leprae* WCS. Of note, IL-10 and GCSF showed the highest log2FC in longitudinal comparison and were also significantly different in (BCG-vaccinated) HC compared to EC in the cross-sectional comparison discussed above. However, only minor differences with the control HC group were observed. Based on the observations in this small-sized cohort, BCG vaccination in individuals who are already heavily exposed to *M. leprae*, has limited added effect on CCGF levels if assessed intra-individually at 4 and 8 weeks postvaccination.

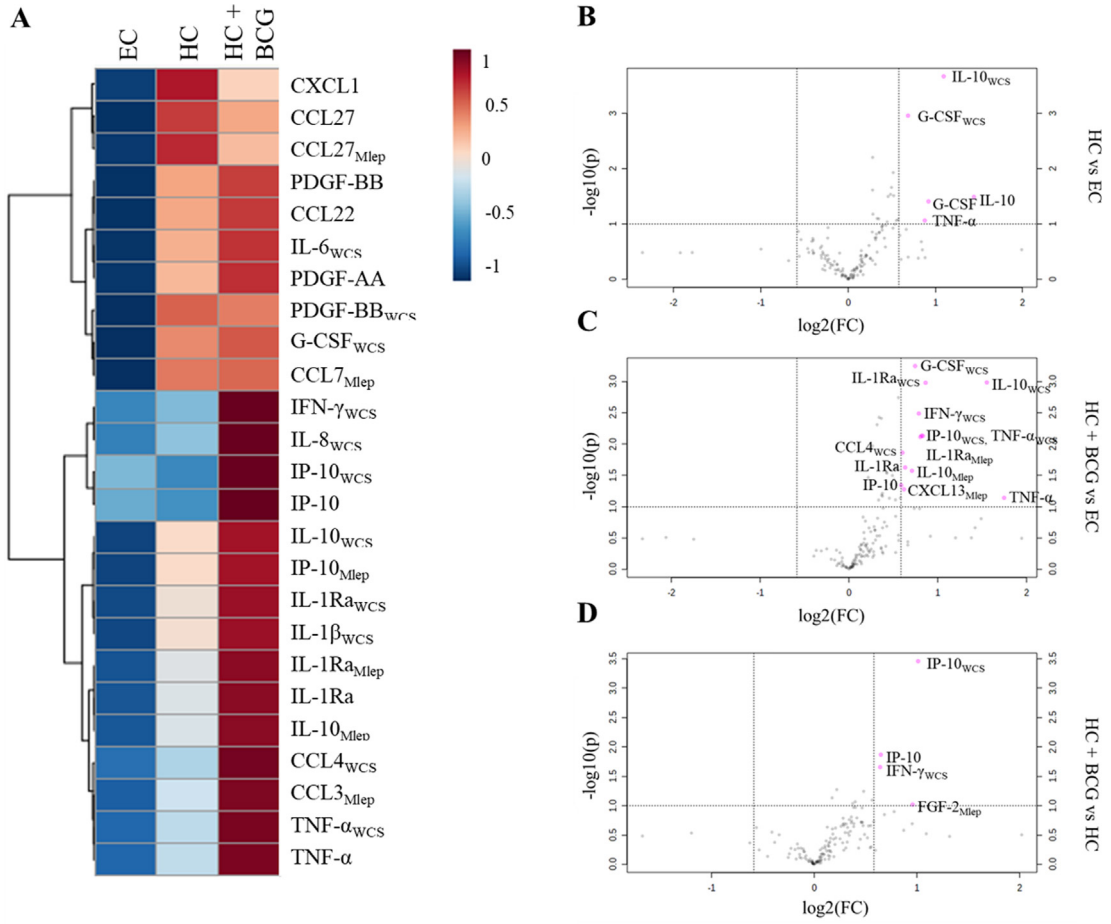

Fig. 1. Cross-sectional analysis of cytokines, chemokines and growth factors (CCGFs) in household contacts with and without BCG (re)vaccination. Sixty CCGFs were analyzed in whole blood assay (WBA) supernatants of household contacts (HC) receiving BCG as immunoprophylaxis against leprosy (HC + BCG; n = 50), HC of leprosy patients without any intervention (HC; n = 54) and endemic controls (EC; n = 51). All samples were tested in the same experiment and randomly distributed over multiple 96-wells plates. Blood was collected 8 to 10 weeks after vaccination in the BCG group and 60 CCGFs were measured in response to *M. leprae* whole cell sonicate (WCS) and two specific *M. leprae* proteins (Mlep; ML0840, ML2478). (**A**) Heatmap displaying the top 25 differently produced CCGFs comparing HC + BCG, HC and EC. The color code indicates whether the average group concentration is higher (red) or lower (blue) compared to the overall mean concentration. (**B–D**) Volcano plot displaying the log2 fold changes in concentration (*x*-axis) of CCGFs for HC compared to EC (**B**), HC + BCG compared to EC (**C**) and HC + BCG compared to HC (**D**). The *y*-axis indicates the -log10 (p-value). Red circles represent features selected by volcano plot with fold change threshold (x) 1.5 and t-tests threshold (y) 0.1 as indicated by the dotted lines. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

3.3. Leprosy progressors induce a differential response after BCG vaccination

After intake, HC of leprosy patients were followed-up annually to check for signs and symptoms of leprosy. In this study, eight individuals who received BCG developed leprosy during follow-up (leprosy progressors; Supplementary Fig. S1). Progressors were diagnosed with PB leprosy (n = 7) or MB leprosy (n = 1). All progressors (8/8) had a bacterial index of 0 (Supplementary Table S2). Their blood was collected 8 weeks after BCG vaccination, allowing to assess the BCG response in these individuals compared to BCG-vaccinated contacts who did not progress to leprosy at the same timepoint (n = 16). The level of *M. leprae*-specific IgM antibodies in progressors was similar to that in the control HC (Supplementary Fig. S2). Interestingly, progressors produced significantly less IL-6 (p = 0.04), CCL3 (p = 0.037), CXCL1 (p = 0.037) and GZMB (p = 0.034) after BCG vaccination compared to HC (Fig. 4). In contrast, the sVCAM1 (p = 0.016) and IL12p40 (p = 0.036) levels induced by BCG vaccination were increased in progressors compared to HC. These data indicate that CCGF responses 8 weeks after BCG vaccination provide information on who are at risk of progressing to leprosy.

4. Discussion

In this study we have identified differences in CCGF levels between individuals who are intensely exposed to *M. leprae* and those without known contact with leprosy patients. Moreover, we identified host proteins that differentiate between protective responses and immunity leading to PB disease after BCG vaccination of those contacts. Biomarkers (or combinations thereof) that can assist identifying who is prone to develop disease after BCG vaccination, will be of great use in the field, particularly if they can be measured at point-of-care before immunoprophylactic interventions [36].

Although only a small percentage (estimated 5%) of individuals infected with *M. leprae* actually develop disease [37], leprosy continues to be a significant health problem in endemic tropical countries, with hundreds of thousands new cases reported each year from more than 155 WHO member states and territories. In order to achieve elimination of leprosy, strategies involving early diagnosis through active case finding and contact tracing combined with postexposure prophylactic (PEP) treatment [13,38-41] are essential to interrupt transmission and prevent development of leprosy in high-risk contacts [42,43]. Reduction in incidence has been associ-

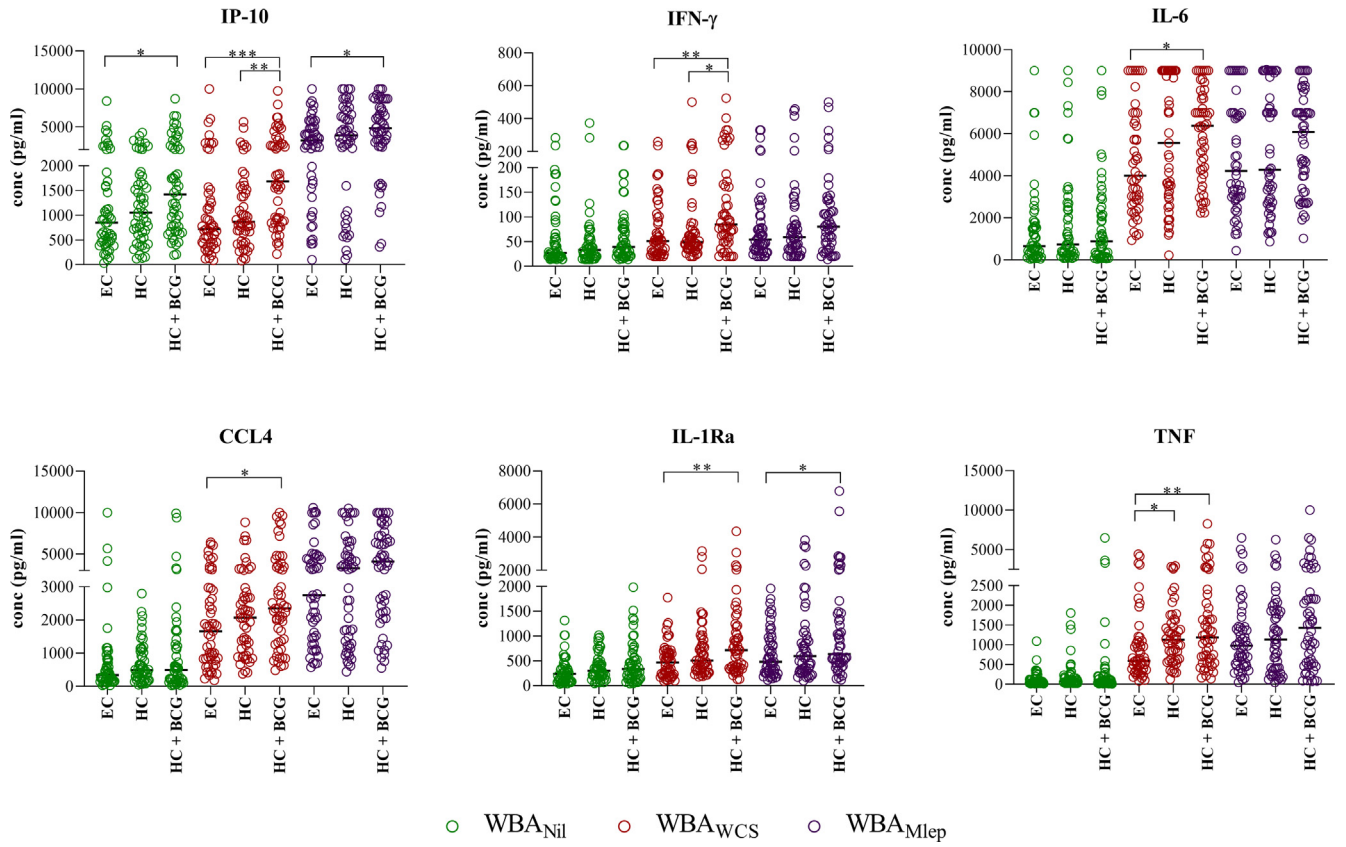

Fig. 2. CCGFs differentially produced in BCG-vaccinated household contacts compared to endemic controls. Levels of six CCGFs in whole blood assay (WBA) supernatant of household contacts who received BCG (HC + BCG; n = 50) compared to endemic controls (EC; n = 51). Blood was collected 8–10 weeks after vaccination in the BCG group. The levels of HC of leprosy patients without an intervention were plotted for comparison (HC; n = 54). All samples were tested in the same experiment and randomly distributed over multiple 96-wells plates. Each graph indicates the concentrations (*y*-axis) per CCGF in unstimulated WBA supernatant (Nil; green), in response to *M. leprae* whole cell sonicate (WCS; red) or in response to two specific *M. leprae* proteins (Mlep; purple) for the three different groups. P-values were determined per stimulus by Kruskall-Wallis test with Dunn's correction for multiple testing. *$p \leq 0.05$, **$p \leq 0.01$, ***$p \leq 0.001$. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

ated with the introduction of novel approaches, like chemo- (single dose rifampicin) or immune-prophylaxis [20-22,44]. However, the direct immunological effects of BCG vaccination in individuals who have been intensely and chronically exposed to *M. leprae* are not studied in detail.

It has been widely documented that BCG vaccination alters the innate immune system by a mechanism referred to as trained immunity [5-7], which leads to protection against TB but also to heterologous pathogens. Trained immunity is characterized by decreased anti-inflammatory cytokine responses, but increased IL-6 and TNF-α in response to non-specific innate immunity stimuli [8]. Individuals in a leprosy endemic area in Bangladesh who developed (skin) complications after BCG vaccination also showed higher proinflammatory cytokines but decreased CXCL1 and CCL4 [45]. Thus, the neuroprotective role for CXCL1 and regulatory effect of CCL4 could well be consistent with the onset of complications upon its reduction after *M. leprae* stimulation as observed in this study. Interestingly, the IL-6 response to *M. leprae* specific stimuli was more profound in individuals without complications [45], suggesting that a sufficient innate immune response may prevent such adverse effects after BCG vaccination. Potentially this could be effected by increased killing of bacteria before causing overreactive "autoimmune-like" adaptive immunity as encountered in PB leprosy.

In the BCG-vaccination trial in Bangladesh it was also observed that PB leprosy was diagnosed in contacts of leprosy patients within 12 weeks after BCG vaccination [22]. Among these patients 56% lived with MB index cases with a long average duration of symptoms before diagnosis, indicating that these contacts experienced a high level of *M. leprae* exposure for a long time before receiving BCG. This could indirectly be detected by the CCGF profile in HC as the IL-6, CCL4 and TNFα responses to *M. leprae* WCS were increased compared to those in endemic controls without known contact to leprosy patients. This enhanced innate immune response to *M. leprae* in these continuously exposed individuals is in line with previous observations in households where *M. leprae* DNA was detected in nasal swabs and SSS [46]. BCG (re)vaccination further augmented the response against *M. leprae* antigens, and additionally enhanced IP-10, a chemokine that has been identified as a trained immunity marker in addition to IL-6, TNFα and IL-1β [47]. These data suggest that BCG vaccination in individuals heavily exposed to *M. leprae* augments the innate immune response initiated by exposure alone, providing a 'trained' response to secondary stimuli. Alternatively, since *M. leprae* and BCG contain homologous antigens, responses after BCG vaccination may also be caused by cross-reactive immunity. The type of assessment of whole blood in this study does not allow the identification of the cell types producing the CCGF, therefore it cannot be concluded that the markers associated with trained innate immunity are the sole result of production by innate immune cells. Follow-up studies utilizing flow cytometry or single-cell RNAseq could provide additional insight in the cell-types responsible for the observed upregulation of CCGF in (BCG-vaccinated) contacts in this study.

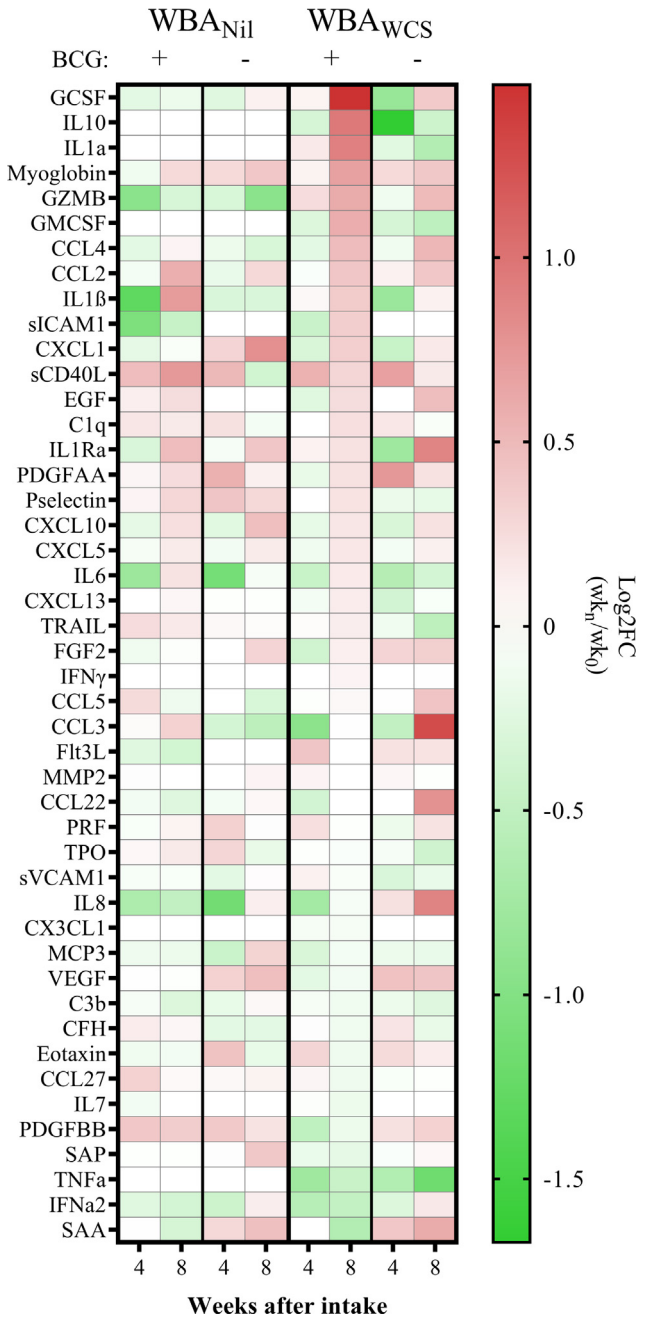

Fig. 3. Longitudinal immune profiling in BCG-vaccinated household contacts of leprosy patients. Forty-six CCGFs were determined in WBA from household contacts (HC) receiving BCG (n = 16) and HC of leprosy patients without an intervention (n = 16). Blood was collected at intake (just before vaccination in the BCG group) and 4 and 8 weeks after intake. Levels of the 46 CCGFs were measured in unstimulated whole blood assay supernatant and in response to *M. leprae* whole cell sonicate (WCS). All samples were tested in the same experiment and randomly distributed over multiple 96-wells plates. The heatmap displays the median Log2FC of the concentration level at the respective weeks (wk_n) compared to the level at intake (wk_0). An increased level over time compared to wk_0 is indicated in red and an decreased level in green. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Eight BCG (re)vaccinated individuals included in our study developed leprosy during follow-up. Interestingly, markers of innate immunity (IL-6, CCL3 and GZMB) were not strongly induced in these individuals after BCG vaccination compared to HC that did not progress to disease. Similar to the observation in BCG complications [45], CXCL1 levels were also lower in the progressors. One remark regarding the progressors that developed leprosy years after BCG vaccination is that it cannot be determined whether these individuals were already infected at the time of vaccination, and re-exposure cannot be ruled-out. Still, all of the progressors presented with few bacilli and mostly presented with PB leprosy, similar to the contacts that developed leprosy quickly (within 12 weeks) after BCG vaccination [23].

The importance of IL-6, CCL3 and GZMB to contain *Mtb* infection, was previously shown by the presence of an *Mtb*-specific cell subset in which expression of these CCGFs was upregulated upon stimulation in latently infected individuals [48]. In addition, IL-6, CCL3 and CXCL1 have been shown to contribute to effective tumor immunotherapy with BCG in bladder cancer by attracting effector cells to the bladder [49]. The lower levels of these innate immune markers in contacts progressing to leprosy are indicative of an insufficient protective innate response after BCG vaccination.

In contrast, sVCAM-1 and to a lesser extent IL12p40, were higher in progressors after BCG vaccination. sVCAM-1 is an indicator of endothelial activation and its membranous form mediates the adhesion and *trans*-endothelial migration of leukocytes. VCAM-1 has been shown to modulate IL12p40, thereby regulating the activation of dendritic cells which is critical for the initiation of the adaptive immune response [50]. As PB leprosy is characterized by a strong cell-mediated immune response, the increased endothelial activation upon BCG vaccination might be a precursor to the pathogenic immune response to *M. leprae* causing damage to skin and nerve tissue. Thus, *M. leprae* exposure alone already activates immunity towards a, still benign, pro-inflammatory immune profile which is mainly kept in balance but can be tipped over to a malign immune profile causing PB leprosy. This pathogenicity coincides with increased activation of the adaptive immune system and decreased IL-6, CCL3, GZMB and CXCL1. In order to investigate which genetic markers may precede development of leprosy instigated by BCG, DNA methylation associated with trained immunity can be analyzed in these study cohorts [51]. The lack of sufficient training after BCG vaccination in progressors, stresses the importance of a functional innate immune system in combatting *M. leprae* infection [52].

Based on our results we hypothesize that HC who progress to leprosy after BCG vaccination are not able to kill *M. leprae* bacteria sufficiently using their innate immune response, as evidenced by the lower levels of various innate immune markers. Once the infection is established in these individuals, adaptive immune responses to *M. leprae* are vigorous and cause pathogenicity resulting in PB leprosy. Additional (mechanistic) studies are required to evaluate this hypothesis and elucidate the mechanism of pathogenic immunity to *M. leprae* after BCG vaccination.

Since, vaccination in general induces long lasting protection and does not imply the risk of drug resistance, administration of BCG to those at risk of developing leprosy has shown beneficial effects, but can induce PB leprosy in a minority of contacts. Host proteins (CCGFs) such as identified in this study may help monitoring those at risk of developing disease while taking advantage of the beneficial effects of BCG vaccination.

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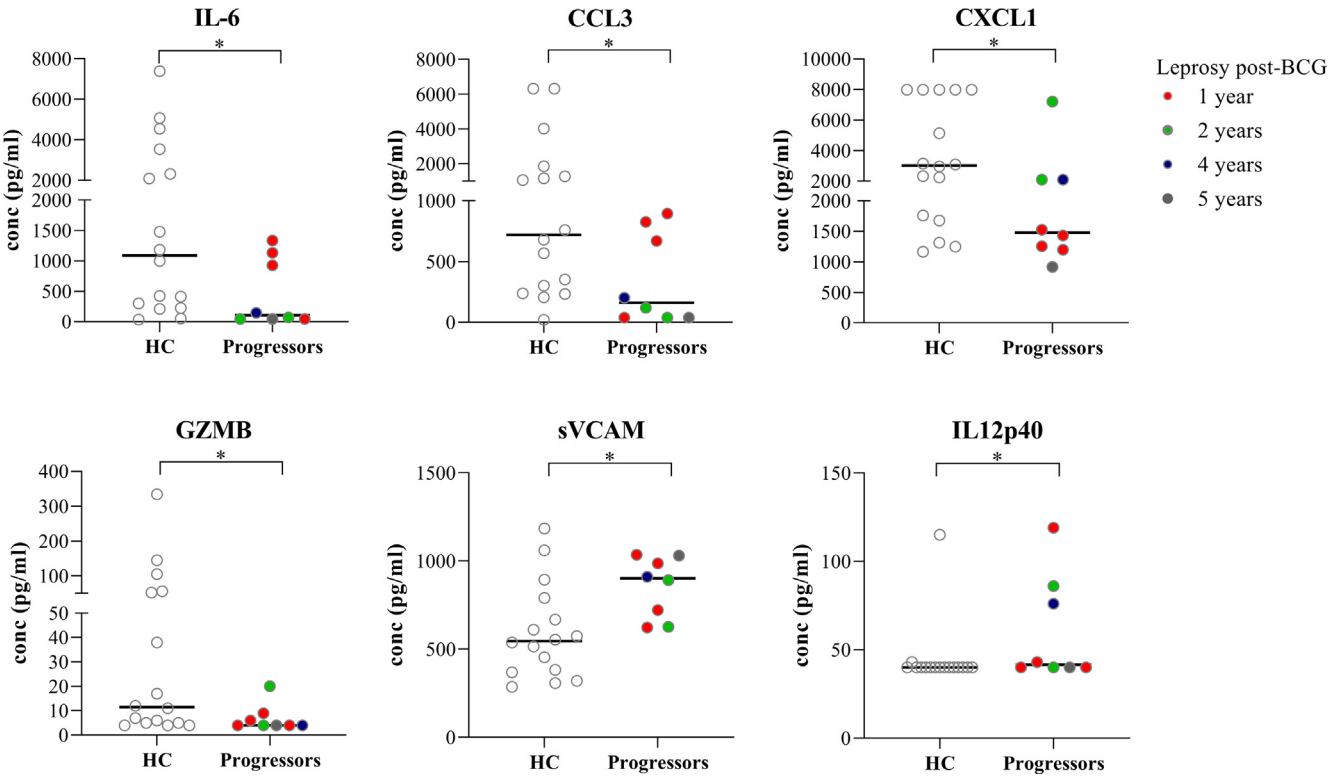

Fig. 4. Differential response in BCG-vaccinated household contacts who progress to leprosy. BCG-vaccinated household contacts (HC) of leprosy patients were followed up over time for signs and symptoms of leprosy. The response to the BCG vaccine in HC who did not develop leprosy (HC; n = 16) were compared to HC progressing to leprosy (progressors; n = 8). All samples were tested in the same experiment and randomly distributed over multiple 96-wells plates. In the progressor group leprosy was diagnosed one year (n = 4; red dots), two years (n = 2; green dots), four years (n = 1; blue dot) or five years (n = 1; grey dot) postvaccination. The graphs indicate the concentration in pg/ml (*y*-axis) of CCGFs that showed a significant difference between the two groups (*p-value <0.05). (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

Data statement

The raw data supporting the conclusions of this article will be made available by the authors upon request.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.vaccine.2021.10.027.

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