UNIVERSITY OF BIRMINGHAM

University of Birmingham
Research at Birmingham

The association between parental and neonatal BCG vaccination and neonatal T helper 17 cell expansion

Schaltz-buchholzer, Frederik; Toldi, Gergely

[Link to publication on Research at Birmingham portal](#)

The association between parental and neonatal BCG vaccination and neonatal T helper 17 cell expansion

Frederik Schaltz-Buchholzer [a,b], Gergely Toldi [c,d,e,*]

[a] Bandim Health Project, INDEPTH Network, Bissau, Guinea-Bissau
[b] Bandim Health Project, OPEN, Institute of Clinical Research, Uni. Southern Denmark and Odense University Hospital, Odense, Denmark
[c] Department of Neonatology, Birmingham Women's and Children's NHS Foundation Trust, Birmingham, UK
[d] Institute of Immunology and Immunotherapy, University of Birmingham, Birmingham, UK
[e] Liggins Institute, University of Auckland, Auckland, New Zealand

ABSTRACT

Background: Bacille Calmette-Guérin (BCG) vaccination reduces the severity of neonatal infections; this effect appears enhanced if the mother has received BCG. We performed immunophenotyping of the T-cell subset and characterized T-cell proliferation responses to assess possible immune response pathways.
Methods: Healthy BCG-vaccinated (n = 8) and unvaccinated (n = 9) neonates born by elective caesarean section were sampled 3 weeks after birth. We compared a wide panel of intracellular cytokine and cell surface expression markers as well as proliferation response in T-cells between BCG-vaccinated and unvaccinated neonates, stratified by parental BCG status.
Results: For all BCG-vaccinated neonates and 3 of 9 unvaccinated neonates that served as controls, both parents had a BCG scar. Th17 (CD4 + IL-17+) prevalence as percentage of total CD4 + T-cells was expanded 4-fold in BCG-vaccinated compared to unvaccinated, being 11.6% [3.6–19.6%] vs 2.8% [1.0–6.6%]. Th17 counts for 3 unvaccinated neonates born to BCG-vaccinated parents was comparable to vaccinated neonates, and higher than remaining controls, parental BCG = 8.5% [4.4–8.9%] vs 1.8% [0.8–3.3%] for no parental BCG (median [interquartile range] for all data).
Conclusion: Among neonates born to BCG-vaccinated parents, the prevalence of Th17 cells, important in the response against bacterial infections, was substantially elevated. The interaction between neonatal and parental BCG for Th17 responses and the importance remains to be further investigated.
© 2022 The Authors. Published by Elsevier Ltd. This is an open access article under the CC BY license (http://creativecommons.org/licenses/by/4.0/).

1. Introduction

A series of observational studies[1] and randomized controlled trials (RCTs)[2,3] from low-income countries indicate that vaccination with Bacille Calmette-Guérin (BCG) against tuberculosis (TB) is associated with beneficial non-specific effects (NSE), providing protection against a broad range of infections. A systematic review commissioned by the WHO thus concluded that BCG is associated with a halving of subsequent all-cause mortality, encouraging more research.[1] In an RCT conducted in Denmark, however, receiving BCG within 7 days after birth did not provide overall protection against hospital admissions due to infections.[4] This was true for the 82% (n = 3453) of the cohort born to BCG-naïve moth-

ers; the BCG vs control Hazard Ratio (HR) being 1.10 (95% CI: 0.93–1.29), but not for the 18% (n = 740) born to BCG-vaccinated mothers, the same HR being 0.65 (0.45–0.94).[4] Similarly, a study has reported the all-cause mortality effects for children of having a scar stratified by the maternal BCG scar status; while there was no effect in the absence of maternal scars, all-cause mortality was reduced 66% (33–83%) if both the child and mother had a scar, compared to only the mother having a scar.[5] One retrospective study reported a reduction of 60% (4–83%) in all-cause mortality by 6 weeks of age if the mother had a BCG scar and 89% (13–99%) if both parents had a scar.[6] In a recent prospective study, the 0–6 week case-fatality rate among hospitalized BCG-vaccinated neonates whose mother had a BCG scar was 60% (9–83%) lower for males but unchanged for females, when compared to neonates born to mothers with no scar; the protective effect was particularly evident against sepsis.[7] Interestingly, a recent study has indicated that BCG vaccination increases lymphocyte

counts in both sexes, while neutrophil counts are increased in males only.[8]

The potential immunological mechanisms contributing to BCG's NSE in neonates include "*trained innate immunity*", induction of emergency granulopoiesis and/or induction of heterologous T-cell immunity.[9,10] However, possible immunological pathways of parental priming with BCG in the offspring are not well described; a study from Uganda has associated maternal BCG scars with an increased proinflammatory immune profile.[11] In a small cohort of healthy neonates from the UK, we conducted an explorative study to investigate immune phenotype and responses associated with the triad of offspring and parental BCG vaccination.

2. Materials and methods

2.1. Participants

Peripheral venous blood samples were collected at 3 weeks of age from a cohort of healthy neonates that were born at term by elective caesarean section before labor onset as part of a larger study focusing on the immunological effects of breastfeeding.[12] The study was reviewed and approved by the East Midlands – Nottingham 2 NHS Research Ethics Committee (reference 16/EM/0379). Seventeen of these neonates had detailed immunophenotyping data and BCG vaccination history available and were included in the current retrospective analysis. Maternal peripheral blood samples were also collected prior to the caesarean section. Participant characteristics are summarized in Table 1. Exclusion criteria included multiple pregnancy, clinical risk factors for maternal sepsis (especially maternal fever, chorioamnionitis), Group B Streptococcus positivity in the current pregnancy, known genetic conditions of the fetus or the mother, and maternal HIV, TB, new-onset viral infection, hypertensive disorder, endocrine condition/diabetes, asthma, autoimmune conditions, and medication use aside from pregnancy supplements.

Vaccination of participating neonates with BCG-Denmark (AJ Vaccines) was provided based on individual TB-risk, as per national UK guidelines (National Institute for Health and Care Excellence guideline NG33), namely if one or more of the neonate's parents or grandparents were born in a country where TB is widespread. The parental BCG scar status was assessed at telephone interviews (parental recall), since the possibility of an interference on offspring immune responses associated with parental BCG only became evident after the neonatal blood samples had been collected. There were no social or socioeconomic concerns or discrepancies documented in the hospital notes in relation to any of the participating families. Informed written consent to participate in the study was obtained from all women prior to delivery.

2.2. Immunophenotyping

Peripheral blood mononuclear cells (PBMCs) from blood samples were isolated and stored as described earlier and used in two flow cytometry panels.[12] Panel 1 was designed to study intracellular cytokine production following stimulation, using unstimulated cells as controls. Cells were stained with FITC-conjugated CD107a (Biolegend, San Diego, CA, USA) and stimulation was performed with phorbol myristate acetate (PMA, 50 ng/mL, Sigma-Aldrich) and ionomycin (1 µg/mL, Sigma-Aldrich) for a total of 3.5 h at 37 degrees centigrade. After 30 min, 1.25 µg/mL of monensin (Sigma-Aldrich) was added to stimulated cells for the remaining 3 h. Cells were then washed with phosphate-buffered saline (PBS, Sigma-Aldrich) and re-suspended in MACS buffer. Cell surface staining was performed for 30 min on ice in the dark as follows: BV510-conjugated CD4, PerCP-Cy5.5-conjugated CD8, ECD-conjugated CD14/CD19/CD56, APC-Cy7-conjugated CD3 (all from Biolegend) and Live/Dead (red, 488 nm, Invitrogen, Carlsbad, CA, USA). After washing, cells were fixed with fixation/permeabilization solution (eBioscience, San Diego, CA, USA) for 30 min at room temperature in the dark. Cells were washed with permeabilization buffer (eBioscience). Following centrifugation and re-suspension in MACS buffer, the following intracellular dyes were added for 30 min at room temperature in the dark: PE-Cy7-conjugated IL-6, AF700-conjugated IFN-γ, AF647-conjugated IL-4, BV421-conjugated IL-17A and PE-conjugated IL-8 (all from Biolegend). Cells were then washed and run on an LSRII flow cytometer equipped with blue, red and violet lasers (BD Biosciences, San Jose, CA, USA). The staining procedure was identical for unstimulated cells. At least 50,000 cells were recorded per sample. Unstained and single-stained samples were used as compensation controls in flow cytometry experiments.

Panel 2 assessed the immunophenotype of cells without mitogenic stimulation. Cells were surface stained in MACS buffer with PE-Cy7-conjugated HLA-DR, BV510-conjugated CD4, PerCP-conjugated CD69, ECD-conjugated CD14/CD19/CD56, AF700-conjugated CD45RA, APC-Cy7-conjugated CD3, BV421-conjugated CD25, BV605-conjugated CD31 (all from Biolegend) and Live/Dead (red, 488 nm, Invitrogen). After washing, cells were fixed with fixation/permeabilization solution (eBioscience) for 30 min at room temperature in the dark. Cells were washed with permeabilization buffer (eBioscience). Following centrifugation and re-suspension in MACS buffer, AF647-conjugated FoxP3 (Biolegend) antibodies were added for 30 min in the dark. Cells were then washed and run on an LSRII flow cytometer equipped with blue, red and violet lasers (BD Biosciences). At least 50,000 cells were recorded per sample. Unstained and single-stained samples were used as compensation controls in flow cytometry experiments.

Table 1
Demographics of the neonates investigated in the study.

	Neonatal BCG (n = 8)	Unvaccinated (n = 9)	
	Parents BCG-vaccinated (n = 8)	Parents BCG-vaccinated (n = 3)	Parents unvaccinated (n = 6)
Birthweight, g [IQR]	3495 [3405–3945]	3155 [3125–3420]	3450 [3050–3655]
Gestation, weeks [IQR]	39 [39–39]	39 [39–39]	39 [38–39]
Gender, n (%)	Female 6 (75%) Male 2 (25%)	Female 2 (67%) Male 1 (33%)	Female 2 (33%) Male 4 (67%)
BCG vaccination, DOL [IQR]	1 [1–1]	NA	NA
Ethnicity, n (%)	Caucasian 1 (13%) Non-Caucasian 7 (87%)	Caucasian 1 (33%) Non-Caucasian 2 (67%)	Caucasian 6 (100%) Non-Caucasian 0 (0%)

Abbreviations: DOL; day of life; g, grams; IQR, interquartile range; NA, not applicable.
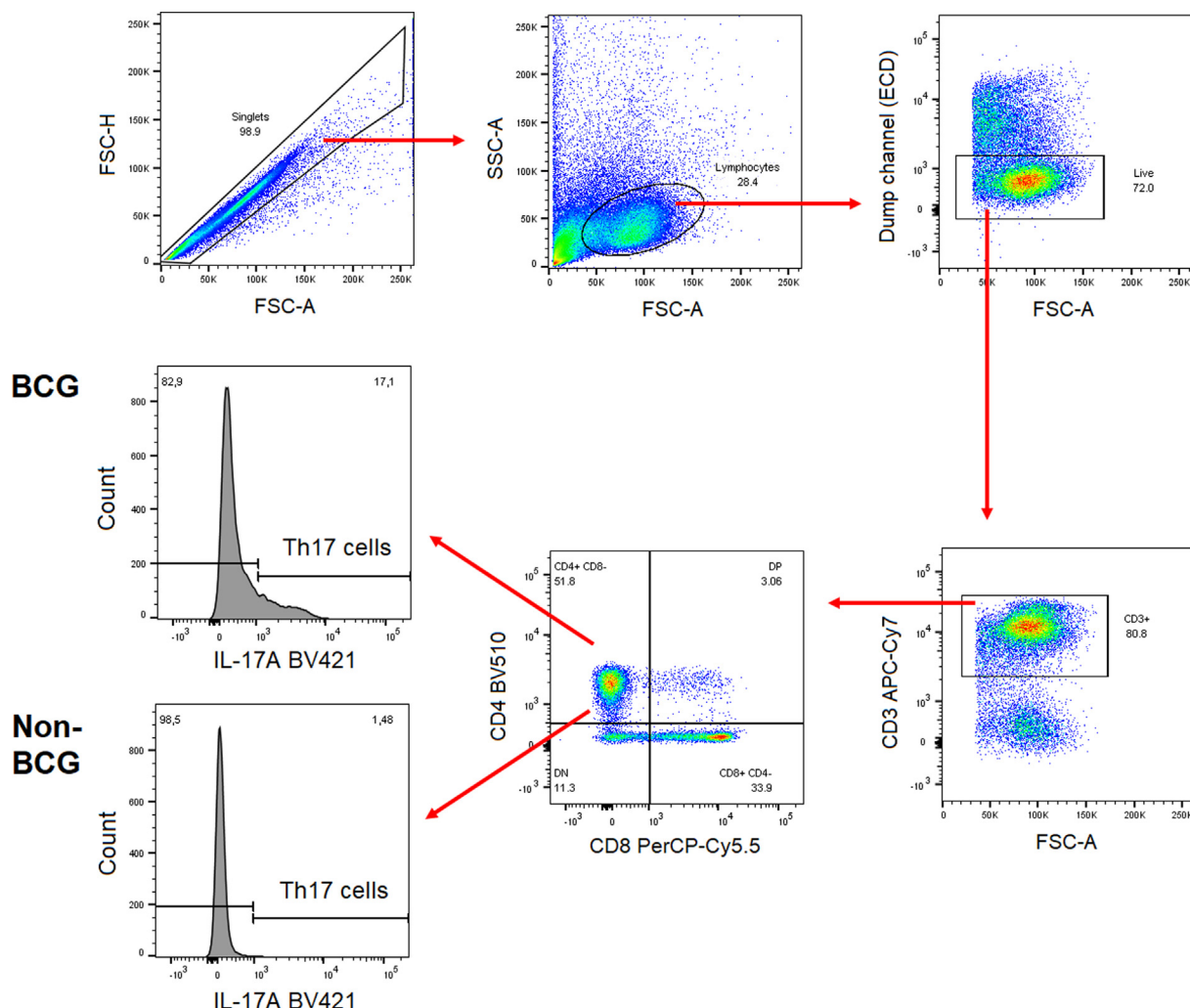
Fig. 1. Gating strategy of immunophenotyping and a representative example of Th17 cell proportions in a BCG vaccinated and a non-vaccinated neonate. Doublets were first excluded based on FSC-A and FSC-H characteristics. Lymphocytes were then identified based on FSC-A and SSC-A characteristics. Dead, as well as CD14+, CD19+ and CD56+ cells were excluded based on positivity in the ECD channel. Further gating was performed within CD3+ cells. FSC – forward scatter characteristics, SSC – side scatter characteristics.

In both panels during the gating process, doublets were first excluded based on FSC-A and FSC-H characteristics. Lymphocytes were then identified based on FSC-A and SSC-A characteristics. Dead, as well as CD14+, CD19 + and CD56 + cells were excluded based on positivity in the ECD channel. Further gating was performed within CD3 + cells (Fig. 1). Flow cytometry data was analysed using the FlowJo software package.

2.3. T cell proliferation

PBMCs from blood samples were isolated and used in mixed lymphocyte reaction (MLR) assays as described earlier.[12] In brief, stimulator maternal PBMCs were irradiated and for neonatal responder cells, T cell enrichment was performed using magnetic bead separation. Proliferation of responder T cells was assessed using the CellTrace Violet dye (Invitrogen). 2×10^5 irradiated stimulator mononuclear cells were added at a 2:1 ratio to each responder sample of 1×10^5 in a 96-well round-bottom plate. Each sample was run in duplicate with appropriate positive and negative controls as described earlier. Samples were incubated for 5 days after which surface staining was performed in MACS buffer using APC-Cy7-conjugated CD3, BV510-conjugated CD4 and PerCP-Cy5.5-conjugated CD8 (all from Biolegend). Samples were washed

and then re-suspended. 1 μL of propidium iodide (Biolegend) was added for live/dead discrimination to each sample immediately before flow cytometry was performed on an LSRII flow cytometer equipped with blue, red and violet lasers (BD Biosciences). At least 20,000 cells were recorded per sample. Unstained and single-stained samples were used as compensation controls in flow cytometry experiments.

During the gating process, doublets were first excluded based on FSC-A and FSC-H characteristics. Lymphocytes were then identified based on FSC-A and SSC-A characteristics. Dead cells were excluded based on positivity in the ECD channel. Further gating was performed within CD3 + cells. Flow cytometry data was analyzed using the FlowJo software package.

2.4. Statistical analysis

Comparisons were made using the Mann-Whitney U test as the distribution of data appeared to be non-normal according to the Shapiro-Wilk test; p values < 0.05 were considered significant. Data are presented as median values [interquartile range]. All analyses were performed overall, by sex, and by parental and neonatal BCG vaccination status using GraphPad Prism 5 software.

3. Results

Among 17 healthy neonates that were bled at 3 weeks of age, 11 were born to BCG-vaccinated parents and 8 of these neonates themselves received BCG at birth; 6 neonates born to unvaccinated parents did not receive BCG. All parent pairs had either both received childhood BCG (n = 11), or none had been vaccinated (n = 6). No episodes of major infections requiring hospitalization was reported in study participants up to one year of age.

We compared intracellular cytokine and cell surface marker expression in T cells at 3 weeks of age in BCG-vaccinated and non-vaccinated neonates. Most notably, the prevalence of T helper 17 (Th17) CD4 + IL-17 + cells in peripheral blood was expanded to 4-fold higher levels in BCG-vaccinated compared to unvaccinated neonates (11.6% [3.6–19.6%] vs 2.8% [1.0–6.6%], p = 0.043), whereas the prevalence of regulatory T cells (Tregs, CD4 + CD25hi FoxP3+) was comparable (Fig. 2, panel A + B). As a consequence, the Th17/Treg ratio was higher in the BCG-vaccinated compared to control neonates (1.84 [0.48–3.42] vs 0.45 [0.18–0.69], p = 0.046). For the prevalence of CD8 + IL-17 + cells, there was also a trend of higher prevalence in BCG-vaccinated neonates (10.1% [4.3–24.6%] vs 3.9% [1.3–6.4%], p = 0.075). The intracellular expression of IL-17 (mean fluorescence intensity) within CD4 or CD8 cells did not differ between the two study groups, however, There was a trend for lower prevalence of Th2 (CD4 + IL-4+) cells in BCG-vaccinated compared to unvaccinated neonates (1.7% [0.8–3.0%] vs 4.3% [1.5–7.6%], p = 0.075), whereas that of Th1 (CD4 + IFN-g +) cells did not differ between the two groups (Fig. 2, panel C + D). No further difference was observed in the studied markers between the two groups. Furthermore, no differences were detected regarding proliferation of CD3, CD4 or CD8 cells at 3 weeks
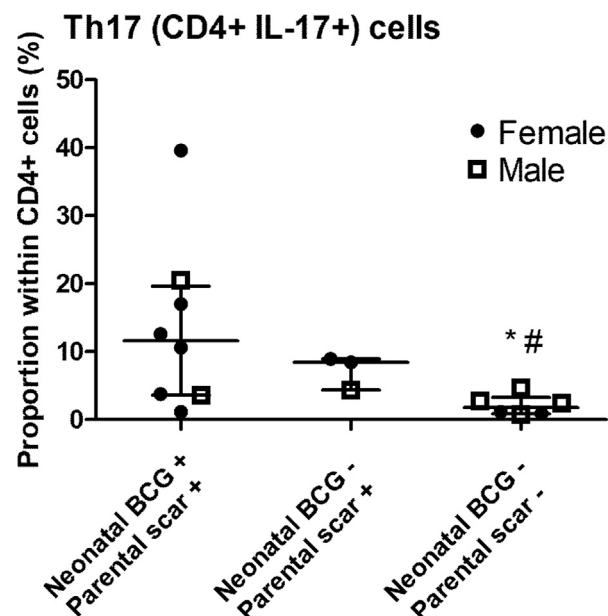

Fig. 3. Th17 cell prevalence in BCG vaccinated neonates, non-vaccinated neonates whose parents (both mothers and fathers) received BCG and developed a BCG scar and non-vaccinated neonates whose parents did not receive BCG. Data are presented as median (horizontal line) and interquartile range (whiskers). * p = 0.028 compared to Neonatal BCG+ and Parental scar + group. # p = 0.048 compared to Neonatal BCG - and Parental scar + group.

of age in response to non-inherited maternal antigen (NIMA) between the two groups.

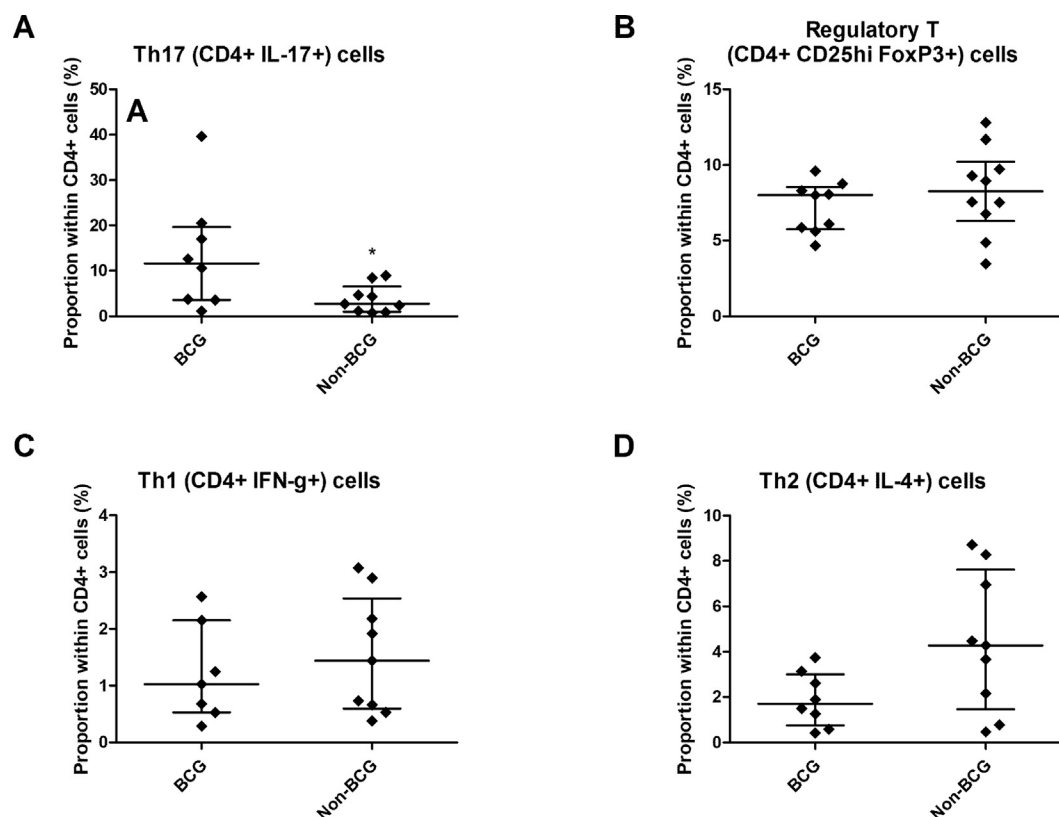

Fig. 2. The prevalence of T helper cell subsets in BCG vaccinated and non-vaccinated neonates. Data are presented as median (horizontal line) and interquartile range (whiskers). * p < 0.05 compared to BCG-vaccinated neonates.
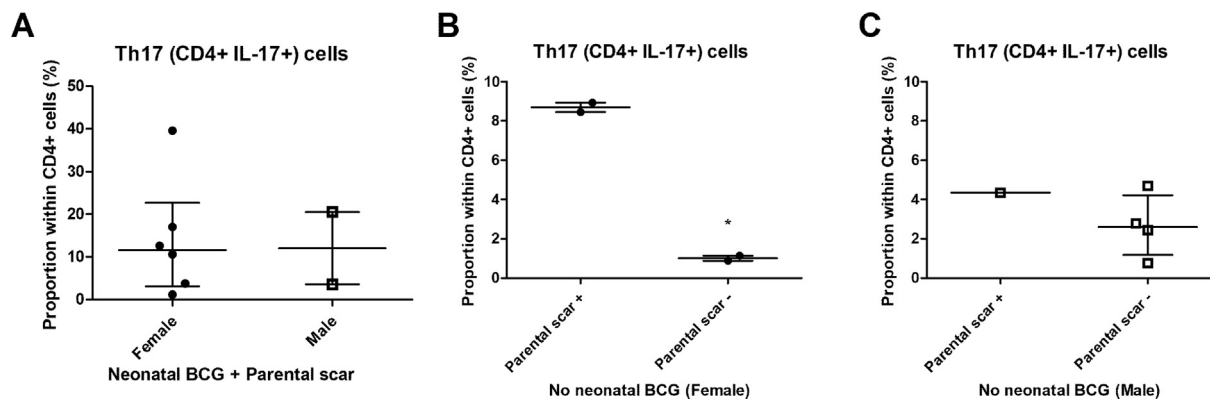
Fig. 4. Th17 cell prevalence in boys and girls amongst BCG vaccinated (A) and non-vaccinated neonates (B + C). Data are presented as median (horizontal line) and interquartile range (whiskers). * $p < 0.05$ compared to Parental scar+.

Interestingly, within the unvaccinated group, the Th17 count of those neonates whose parents were BCG-vaccinated (n = 3) were among the highest and significantly higher when compared with the rest of the neonates (n = 6) within the unvaccinated group (8.5% [4.4–8.9%] vs 1.8% [0.8–3.3%], p = 0.048). Th17 counts among unvaccinated neonates born to vaccinated parents was thus comparable to those of the BCG-vaccinated neonates that were also born to BCG-vaccinated parents (Fig. 3).

When the Th17 data were analyzed by sex, no sex differences were noted for BCG-vaccinated neonates. For unvaccinated females, however, there was an 8-fold higher prevalence of Th17 cells if the parents had been BCG-vaccinated compared to no parental BCG, whereas there was no detectable difference for males (Fig. 4, panel B).

When we compared intracellular cytokine and cell surface marker expression in maternal T cells by maternal BCG scar status, we observed no significant differences. Additionally, there was no correlation between the prevalence of neonatal Th1, Th2, Th17 or Treg cells with the maternal prevalence of the respective cell subset, neither in the whole study population, nor when only BCG-vaccinated neonates or neonates born to vaccinated parents were analyzed.

4. Discussion

We identified a substantial expansion of Th17 (CD4 + IL-17+) cells in peripheral blood of BCG-vaccinated vs unvaccinated neonates and a higher Th17/Treg ratio. Probably as a result of the UK BCG vaccination guidelines, all BCG-vaccinated neonates were born to BCG-vaccinated parents. In the 3 unvaccinated neonates born to BCG-vaccinated parents, Th17 was elevated to levels comparable to the BCG-vaccinated neonates, when compared to unvaccinated neonates born to unvaccinated parents. This pattern was especially evident for females, but subgroup numbers were small.

In our data, the Th17 subset appeared to be the single most important T cell subset influenced by BCG. No functional changes in terms of immunological tolerance towards NIMA was observed. While breastfeeding seems to have an important impact on the active establishment of immunological tolerance and the expansion of Tregs in the first few weeks of life[12], BCG contributes to the development of pro-inflammatory cellular responses that equip the neonate to fight pathogens. Both of these effects are important in the development of an appropriate balance in the evolving adaptive immune system.

Th17 cells play indispensable roles in tissue immunity and Th17 effector cytokines mediate host defense mechanism to a wide array of infections, especially bacterial infections, and are also involved

in the pathogenesis of autoimmune diseases.[13] Interestingly, IL-17A produced by Th17 cells increases G-CSF in human bone marrow stromal cells, expanding granulopoiesis by pushing differentiation of progenitor cells into neutrophil progenitors in vitro. [14] Two recent studies indicate that BCG's immediate NSE might be mediated by emergency granulopoiesis[8,10], an effect induced by G-CSF[10], which might be occurring downstream of a wide expansion in the Th17 cell population. Importantly, a previous study has associated BCG vaccination with sustained non-specific adaptations in immune responses to infections mediated both by induction of innate trained immunity and by induction of long-lasting heterologous Th17 responses.[15]

4.1. Strengths and weaknesses

We present a small but detailed study employing elaborate immunological profiling and functional studies to assess effects of neonatal and parental BCG vaccination on early-life immune responses. The novel finding of a substantial cell expansion restricted to the Th17 subset in a small and rather selected group of neonates, all born by caesarian section, should be interpreted with caution. It is plausible, however, that Th17 could mediate effects of neonatal and/or parental BCG vaccination, given that Th17 plays a crucial role in protection against bacterial infections and is of importance in autoimmune diseases.[13] This is akin to the NSE of both neonatal and maternal BCG, which are especially protects against neonatal sepsis.[7,10,16]

All studied neonates that received BCG were born to couples that had both received BCG, as by parental recall. It is thus not entirely possible to disentangle the effect of neonatal BCG from the effect of parental BCG priming on Th17 cell expansion. Parents that had received BCG were likely different in terms of background factors compared to parents that were unvaccinated, mainly in terms of their ethnicity, since the majority of vaccinated parent pairs were migrants that had received BCG in their native countries. Since the ethnicity of the parents also highly influenced the BCG vaccination status of the offspring due to the UK vaccination guidelines, these factors could have confounded the comparison and our findings should therefore be confirmed in different settings and larger cohorts.

The BCG status was available for both parents for all study participants, and by coincidence, parents were either both vaccinated or both unvaccinated. This is possibly a strength of the study since the relative importance of maternal and paternal priming with BCG is unknown; data from Bissau suggests that both factors might be of importance.[6] If this is the case, then having the data of both parents is important, but our data on the other hand does not pro-

vide information regarding the relative importance of maternal vs paternal priming with BCG. Importantly, we used parental reporting of BCG scar data collected by telephone to establish whether the parent had been vaccinated or not. A physical examination of the scar status had likely been more optimal.

In maternal end pregnancy blood samples, we analyzed intracellular cytokine and cell surface marker expression in maternal T cells by maternal BCG status and identified no differences. Of note, maternal blood samples were sourced before the Caesarean section, and pregnancy is known to alter the prevalence of T helper subsets compared to the non-pregnant state.[17] Therefore, any possible differences might have been masked by pregnancy.

4.2. Perspectives

Harnessing favorable effects of the parent-offspring immune system triad is an important strategy to reduce newborn mortality and morbidity. There are now four studies[4–7] indicating that BCG's NSE are modulated by parental BCG. Our study provides clues to the mechanistic pathway behind such effects, which deserve further analysis in larger cohorts to further shed light on the three-way interaction between neonatal BCG, parental BCG, and sex.

5. Conclusion

A wide array of studies has documented that infant BCG has beneficial NSE, and novel studies indicate that parental priming might potentiate these effects, but the immune pathways have yet to be established. In a small cohort from the UK, receiving neonatal BCG and/or being born to BCG-vaccinated parents was associated with a 4-fold expansion of the Th17 cell subset by 3 weeks of age. It remains to be investigated whether this cell response is implicated in BCG's protective effects against infectious diseases.

CRediT authorship contribution statement

Frederik Schaltz-Buchholzer: Conceptualization, Validation, Writing - original draft. **Gergely Toldi:** Methodology, Investigation, Formal analysis, Writing - reviewing and editing, Visualization.

Declaration of Competing Interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: No funders or companies had any influence on the study design, data collection, analysis, interpretation or writing of the report, nor the decision to submit the paper for publication. None of the authors have any commercial, financial, or personal interests, relationships or other associations that might pose a conflict of interest in relation to this study.

Acknowledgments

References

[1] Higgins Julian PT, Soares-Weiser Karla, López-López José A, Kakourou Artemisia, Chaplin Katherine, Christensen Hannah, et al. Association of BCG, DTP, and measles containing vaccines with childhood mortality: systematic review. *BMJ* 2016;**355**:i5170.

[2] Biering-Sørensen Sofie, Aaby Peter, Lund Najaaraq, Monteiro Ivan, Jensen Kristoffer Jarlov, Eriksen Helle Brander, et al. Early BCG-Denmark and Neonatal Mortality Among Infants Weighing <2500 g: A Randomized Controlled Trial. *Clin Infect Dis* 2017;**65**(7):1183–90. Doi: 10.1093/cid/cix525.

[3] Prentice S, Nassanga B, Webb EL, Akello F, Kiwudhu F, Akurut H, et al. BCG-induced non-specific effects on heterologous infectious disease in Ugandan neonates: an investigator-blind randomised controlled trial. Lancet Infect Dis 2021;21(7):993–1003.

[4] Stensballe Lone Graff, Ravn Henrik, Birk Nina Marie, Kjærgaard Jesper, Nissen Thomas Nørrelykke, Pihl Gitte Thybo, et al. BCG Vaccination at Birth and Rate of Hospitalization for Infection Until 15 Months of Age in Danish Children: A Randomized Clinical Multicenter Trial. *J Pediatr Infect Dis Soc* 2019;**8**(3):213–20. Doi: 10.1093/jpids/piy029.

[5] Berendsen Mike L T, Øland Christian Bjerregård, Bles Pauli, Jensen Aksel Karl Georg, Kofoed Poul-Erik, Whittle Hilton, et al. Maternal Priming: Bacillus Calmette-Guérin (BCG) Vaccine Scarring in Mothers Enhances the Survival of Their Child With a BCG Vaccine Scar. *J Pediatr Infect Dis Soc* 2020;**9**(2):166–72. Doi: 10.1093/jpids/piy142.

[6] Berendsen Mike LT, Schaltz-Buchholzer F., Bles Pauli, Biering-Sørensen S., Jarlov Jensen Kristoffer, Monteiro I., et al. Parental Bacillus Calmette-Guérin vaccine scars decrease infant mortality in the first six weeks of life: A retrospective cohort study. EClinicalMedicine. 2021;39:101049. doi: 10.1016/j.eclinm.2021.101049.

[7] Schaltz-Buchholzer F., Bjerregård Øland Christian, Berendsen Mike L. T., Bjerregaard-Andersen Morten, Stjernholm Elise Brenno, Golding Christian N., et al. Does maternal BCG prime for enhanced beneficial effects of neonatal BCG in the offspring? J Infect. 2021 Dec 24:S0163-4453(21)00646-0. doi: 10.1016/j.jinf.2021.12.028.

[8] Williamson SL, Eleanor Gd, Thillagavathie P, Gergely T. Non-specific effects of BCG vaccination on neutrophil and lymphocyte counts of healthy neonates from a developed country. Vaccine 2021;39(14):1887–91. https://doi.org/10.1016/j.vaccine.2021.02.064.

[9] Benn CS, Netea MG, Selin LK, Peter A. A small jab - a big effect: nonspecific immunomodulation by vaccines. Trends Immunol 2013;34(9):431–9. https://doi.org/10.1016/j.it.2013.04.004.

[10] Brook B, Harbeson DJ, Shannon CP, Cai B, He D, Ben-Othman R, et al. BCG vaccination–induced emergency granulopoiesis provides rapid protection from neonatal sepsis. Sci Transl Med 2020;12(542). https://doi.org/10.1126/scitranslmed.aax4517.

[11] Mawa PA, Webb EL, Filali-Mouhim A, Nkurunungi G, Sekaly R-P, Lule SA, et al. Maternal BCG scar is associated with increased infant proinflammatory immune responses. Vaccine 2017;35(2):273–82.

[12] Wood H, Acharjee A, Pearce H, Quraishi MN, Powell R, Rossiter A, et al. Breastfeeding promotes early neonatal regulatory T-cell expansion and immune tolerance of non-inherited maternal antigens. Allergy 2021;76(8):2447–60.

[13] Wenjun O, Kolls JK, Yan Z. The Biological Functions of T Helper 17 Cell Effector Cytokines in Inflammation. Immunity 2008;28(4):454–67. https://doi.org/10.1016/j.immuni.2008.03.004.

[14] Fossiez F, Djossou O, Chomarat P, Flores-Romo L, Ait-Yahia S, Maat C, et al. T cell interleukin-17 induces stromal cells to produce proinflammatory and hematopoietic cytokines. J Exp Med 1996;183(6):2593–603. https://doi.org/10.1084/jem.183.6.2593.

[15] Kleinnijenhuis J, Quintin J, Preijers F, Benn CS, Joosten LAB, Jacobs C, et al. Long-Lasting Effects of BCG Vaccination on Both Heterologous Th1/Th17 Responses and Innate Trained Immunity. J Innate Immun 2014;6(2):152–8.

[16] Schaltz-Buchholzer F, Biering-Sørensen S, Lund N, Monteiro I, Umbasse P, Fisker AB, et al. Early BCG Vaccination, Hospitalizations, and Hospital Deaths: Analysis of a Secondary Outcome in 3 Randomized Trials from Guinea-Bissau. J Infect Dis 2019;219(4):624–32. https://doi.org/10.1093/infdis/jiy544.

[17] Shigeru S, Akitoshi N, Tomoko S, Mika I. Th1/Th2/Th17 and regulatory T-cell paradigm in pregnancy. Am J Reprod Immunol N Y N 1989 2010;63(6):601–10. https://doi.org/10.1111/j.1600-0897.2010.00852.x.