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MAJOR ARTICLE

Impact of Maternal Pertussis Antibodies on the Infants' Cellular Immune Responses

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Introduction. Maternal antibody interference of the infant's humoral immune responses raises some concern to the strategy of maternal Tdap (tetanus, diphtheria, acellular pertussis [aP]) vaccination. This study assessed the impact of maternal Tdap antibodies on the infant's pertussis-specific T lymphocyte responses following infant vaccination with an aP containing vaccine, in a term and preterm born cohort.

Methods. Heparin samples (±0.5 mL) were conveniently drawn from infants of a Belgian prospective cohort study (N = 79, NCT02511327), including Tdap vaccinated (Boostrix®) and nonvaccinated women (no Tdap vaccine in the last 5 years) that delivered at term or prematurely. Sampling was performed before and 1 month after primary (8-12-16 weeks) and booster vaccination (13 or 15 months) with DTaP-IPV-HB-PRP~T vaccine (Hexyon®). Pertussis toxin (PT)-specific $CD3^+$, $CD3^+$ $CD4^+$ and $CD3^+$ $CD8^+$ lymphoblasts and their cytokine secretions were measured using a flow cytometric assay on whole blood (FASCIA) and multiplex technology (Meso Scale Discovery), respectively.

Results. In total, 57% of all infants were considered PT-specific $CD3^+$ $CD4^+$ lymphoblasts responders after primary and booster vaccination, whereas 17% were $CD3^+$ $CD8^+$ lymphoblast responders. Interferon (IFN)-γ, interleukin (IL)-13, IL-17A, and IL-5 cytokine secretions after primary and booster vaccination were indicative of a mixed T helper (Th) 1/Th2/Th17 cell profile. Lymphoblast and cytokine levels were comparable between term and preterm infants. Nonresponders for IL-13 after booster vaccination had higher maternal PT immunoglobulin G (IgG) levels at birth when compared to responders.

Conclusions. Term and preterm born infants are capable of inducing Th1, Th2, and Th17 responses after aP vaccination, yet maternal vaccination modulate these responses. Evaluation of this effect in larger trials is needed.

Keywords. cell-mediated immune response; maternal antibodies; maternal immunization; preterm born infants; Tdap.

Pertussis vaccination during pregnancy ensures efficient and timely transplacental transfer of protective pertussis antibodies from the mother to the fetus, conferring additional protection against *Bordetella pertussis* until the start of the infants' primary vaccinations or until the most vulnerable period passes [1, 2]. Next to the protective properties of these actively acquired tetanus, diphtheria, acellular pertussis (Tdap) maternal antibodies (Mabs), they are known to interfere with the infants' humoral immune response after their own primary [3, 4] and booster vaccinations [5–7]. Here significantly lower vaccine-antibody levels are observed in infants born to vaccinated when compared to unvaccinated women. Although, one surveillance study from England has demonstrated no evidence of an increased burden of disease [8], it remains challenging to determine the clinical significance of Mabs interference because there is no serological correlate of protection for pertussis.

T cells also play a prominent role in the protection against *B. pertussis* infection, as they confer important functions like bacterial clearance and neutrophil recruitment [9]. Unfortunately, the influences of Mabs on the T-cell repertoire have been studied less extensively compared to the humoral responses. Current research indicates that the T-cell compartment remains largely unaffected by Mabs [10]. Although, inhibition of the T follicular helper (Tfh) cell expansion in mice [11] and alterations of the cytokines secretions in human [12, 13] in the presence of Mabs (against influenza, tetanus, and pertussis, respectively) have been observed. These observations demonstrate that determining the impact of Mabs on all aspects of the infant's humoral and cellular immune system are essential. This Belgian clinical study investigated the effect of pertussis-specific Mabs on the humoral and cellular immune response after primary and

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booster DTaP-IPV-HB-PRP~T (Hexyon®) vaccination in term and preterm infants. In a first report, comparable humoral responses in term and preterm infants of vaccinated women after primary vaccination were demonstrated. Yet when comparing term and preterm infants from vaccinated and non-vaccinated women, Mabs interference was observed for some of the Tdap antigens [14]. This paper covers the parallel evaluation of the infants' pertussis-specific T-cell responses before and after primary and booster DTaP-IPV-HB-PRP~T vaccination in the presence of vaccine-induced Mabs.

MATERIALS

Study Design

A prospective controlled cohort study (N = 234, NCT02511327) conducted in Flanders (Belgium) recruited mother-infant pairs, assigning them to 4 different cohorts based on the women's vaccination status (did or did not receive Tdap vaccine [Boostrix®, GSK Biologicals] during pregnancy) and their gestational age (GA) at delivery (Supplementary Figure 1*A*):

- VT Cohort: Vaccinated women and their Term (GA ≥ 37 weeks) infant.
- VP Cohort: Vaccinated women and their Preterm (GA < 37 weeks) infant.
- UnVT Cohort: UnVaccinated women (no pertussis containing vaccine in the last 5 years) and their Term (GA ≥ 37 weeks GA) infant,
- UnVP Cohort: UnVaccinated women (no pertussis containing vaccine in the last 5 years) and their Preterm (GA < 37 weeks) infant.

From this cohort, infants were conveniently selected to participate in a sub-study where the cell-mediated immune (CMI) responses were evaluated.

Study Procedures

Maternal vaccination was planned within the regular healthcare system following Belgian recommendation, that is, between 24 and 32 GA. Women were excluded when suffering from mental illness, immunological disorders, or when receiving experimental medication during pregnancy. Infants with serious medical condition or immune disorders were excluded from the study (full list of inclusion/exclusion criteria available in Maertens et al [14]). Informed consent was obtained. Within the regular planned well-baby clinic visits, newborns received the hexavalent DTaP-IPV-HB-PRP~T vaccine (Hexyon®, Sanofi Pasteur) at the age of 8, 12, and 16 weeks (primary vaccination). A fourth dose (booster vaccination) was administered at 13 months for preterm born infants or at 15 months for term born infants. Hexyon® contains diphtheria toxoid (≥ 20 international units [IU]), tetanus toxoid (≥ 40 IU), pertussis toxoid (PT; 25 µg) and filamentous hemagglutinin (FHA; 25 µg), inactivated poliovirus type 1, 2, and 3 (40, 8, and 32 D-antigen units, respectively), hepatitis B surface antigen (10 µg) and Hemophilus influenzae type b polysaccharide (12 µg) conjugated to tetanus protein (22–36 µg). Cord and maternal blood samples (8mL) were collected 72 hours after delivery. Infants were sampled (serum tube, ±5 mL) before and 28–35 days after their primary and booster vaccination. This report includes infants of whom an additional heparin tube (±0.5 mL) at each timepoint was collected (convenience sample; Supplementary Figure 1B). This study was approved by the Ethical Committee at the University Hospital Antwerp (B300201422982).

Serological Assessment

Serum samples were evaluated for PT-specific immunoglobulins G (IgG) at the Global Clinical Immunology laboratory of Sanofi Pasteur in Swiftwater, Pennsylvania, using an in-house electrochemiluminescent assay (Meso Scale Discovery technology). Samples below the lower limit of quantification (LLOQ) for PT (2 EU/mL) were assigned LLOQ/2 [15]. A full report on maternal and infant antibody titers is available in Maertens et al [14].

Flowcytometric Acquisition of PT-Specific lymphocytes

PT-specific lymphocytes ($CD3^+$, $CD3^+$ $CD4^+$, and $CD3^+$ $CD8^+$ blasts) were measured on heparin blood samples using a specialized Flowcytometric Assay that detects the Specific Cell-mediated Immune response in Activated whole blood (FASCIA). Based on previous protocols [16–18] whole blood was diluted 1/10 in Roswell Park Memorial Institute (RPMI) 1640 (Life technologies) supplemented with 50 µg/mL gentamycine, 2 mM L-glutamine, MEM non-essential AA, Na pyruvate, 2B-mercapthoethanol and fetal bovine serum. Diluted blood was either left unstimulated (negative control) or stimulated with 5 µg/mL PT antigen (heat-inactivated PT, *Bordetella pertussis* strain 165, List Biological) or 1 µg/mL Staphylococcal enterotoxin B (SEB, positive control). Per condition, 2 × 200µL diluted blood was incubated for 6–7 days (37°C, 5% CO^2) on a 96-well plate in duplicate. After incubation, cell culture supernatant was removed and stored at −80°C for the cytokine analysis (see below). Cell pellets from duplicates were pooled and stained with APC-CD3 (BD Biosciences), PE-Cy7-CD4 (BD Biosciences) and Pacific Blue CD8 (Life technologies) at room temperature for 15 minutes. Red blood cells and debris were removed after a 10-minute incubation with IOTest lysis solution (Beckman Coulter), followed by centrifugation at 350G for 5 minutes. Supernatant was removed and the pellet was washed with 2 mL FACS buffer (BD FACS sheath supplemented with 0.1% bovine serum albumin and 0.05% azide). After centrifugation, samples were acquired on CyFlowML (Sysmex) in 1 mL FACS buffer. Proliferation was identified using FlowJo software,

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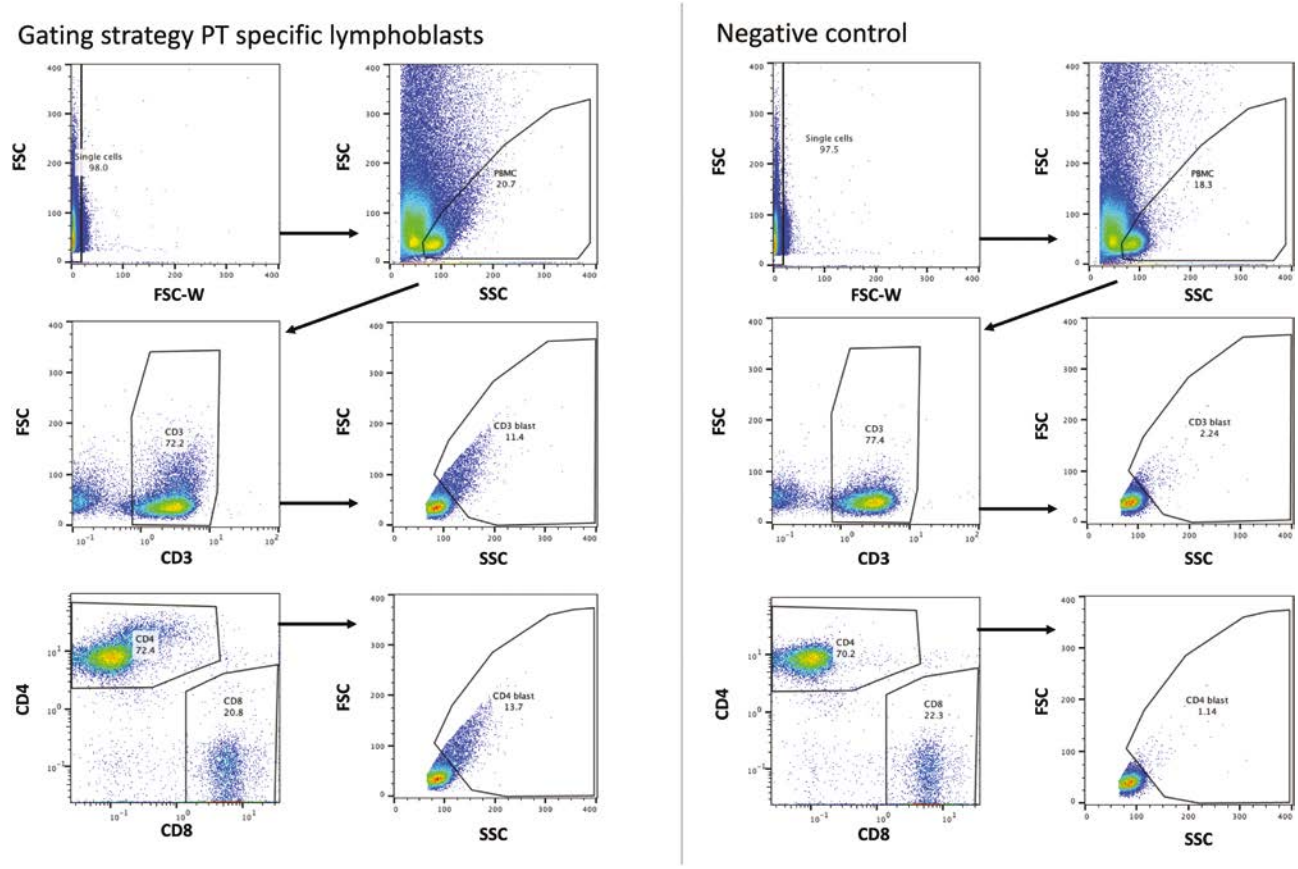

Figure 1. Flow cytometry gating strategy of the PT-specific lymphoblast populations (*left*), which were compared against the negative control (*right*). Single cells were selected using a narrow gate on the FSC/FSC-W plot, after which the total lymphocytes were gated on an FSC/ SSC plot. Next, $CD3^+$, $CD3^+$ $CD4^+$, and $CD3^+$ $CD8^+$ populations were selected, and the percentage of lymphoblasts were identified based on their morphologic characteristics using the FSC/SSC plot (FSC/SSC lymphoblast plot not shown for $CD8^+$). Abbreviations: FSC, forward scatter; FSC-W, forward scatter-width; PT, pertussis toxin; SSC, side scatter.

measuring blast cells based on forward and side scatter (Figure 1). Similar to Palazzo et al [19], data were expressed as percentage of PT-stimulated blasts subtracted by the percentage of blasts in the unstimulated culture. FASCIA protocol and PT concentrations were validated and optimized on blood samples from healthy adults before and after TdaP (Boostrix®) vaccination.

Cytokine Detection

Cytokine secretions of interferon-γ (IFN-γ), interleukin (IL)-10, IL-13, IL-2, IL-4, tumor necrosis factor (TNF)-α, IL-17A, and IL-5 were measured on stored culture medium using the V-PLEX Cytokine and Proinflammatory Panel 1 Human Kit (Meso Scale Discovery), following the manufacturers recommendations. Lower limits of detection (LLOD) for IFN-γ, IL-10, IL-13, IL-2, IL-4, TNF-α, IL-17A, and IL-5 were 0.37 pg/mL, 0.04 pg/mL, 0.24 pg/mL, 0.09 pg/mL, 0.02 pg/mL, 0.04 pg/mL, 0.31 pg/mL, and 0.14 pg/mL, respectively. Similar to the flowcytometric data, cytokine secretions were controlled with the negative controls. Values below the LLOD were assigned the adapted LLOD/2 [15].

Statistics

Sample size calculation was performed for the for the assessment of the humoral responses (primary aim) in the study [14], secondary aims were based on convenience samples. JMPpro version 14, R studio (version 1.3.1093) and GraphPad Prism version 7 statistical software was used. Demographic and descriptive analyses were carried out using χ^2, (paired) Student *t* test, Mann-Whitney *U* or Wilcoxon tests, where appropriate. Statistical significance was defined by P value < .05. Group comparisons were analyzed with non-parametric Wilcoxon test. Here, cutoff for statistical significance was set at 1%, which accounted for multiple testing. Infants were considered positive responders when the in vitro CMI response (lymphoblast or cytokine response) after vaccination was higher than the 95% confidence interval (CI) of the responses before vaccination. Principal component analysis (PCA) was performed on lymphoblast calculations ($CD3^+$, $CD3^+$ $CD4^+$and $CD3^+$ $CD8^+$), normalized serum PT IgG and cytokine data (IFN-γ, IL-10, IL-13, IL-2, IL-4, TNF-α, IL-17A, and IL-5; natural logarithm) to examine the variance within the data. Correlations between symmetrized serum PT IgG and each immune component

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separately were examined using a simple linear regression (LR). Next, linear mixed effect model (LMM) was made to illustrate the different immune components over the course of the infant's vaccination schedule and accounted for more complexity. Model selection was performed based on Akaike information criterion (AIC). Age, cohort, subject, and interaction between serum PT IgG levels and the sampling timepoints were included in the model (Model specifications in the Supplement) [20]. Model analysis was followed by Tukey pairwise comparisons to analyze possible differences between the cohorts.

RESULTS

Demographics of the CMI Subpopulation

Heparin blood samples were conveniently drawn from 79 infants (8 twins; N = 28 VT; N = 38 VP; N = 6 UnVT; N = 7 UnVP cohort). Baseline characteristics of infants and their mothers (N = 71) did not significantly differ from the total study population [14]. Most differences between cohorts were related to preterm delivery (GA at delivery, interval between maternal vaccination and delivery, stay at neonatal care unit, birth weight, and weights at the sampling timepoints, age of vaccination; Table 1).

PT-Specific Lymphoblast Proliferation and Cytokine Secretion

T-cell proliferation after in vitro stimulation with PT showed that primary vaccination significantly increased the infants' PT-specific $CD3^+$ and $CD3^+$ $CD4^+$ lymphoblasts, although PT-specific $CD3^+$ $CD8^+$ lymphoblasts did not increase at any timepoint. Over time slight waning of $CD3^+$and $CD3^+CD4^+$ lymphoblasts populations was observed, which rose again after booster vaccination (Figure 2). Overall, this corresponded to 57% of infants being $CD3^+$ CD4+ lymphoblasts responders, while only 17% $CD3^+$ $CD8^+$ lymphoblasts responders were reported (Table 2). Analysis of the infant's cytokine secretions revealed that lymphoblasts non-responders were still capable of producing cytokines. Primary vaccination resulted in significantly increased IFN-γ, IL-2, IL-13, IL-5, and IL-17A cytokine secretions after PT stimulation in infants (Figure 2). IFN-γ, IL-13, IL-5, and IL-17A secretions remained stable at the different points in time, with 60-70% of the infants being responders (Table 2). A gradual increase of IL-2 secretions was observed (from 56% to 72%, and 80% responders after booster vaccination). Primary vaccination did not induce IL-10, TNF-α, and IL-4 cytokine secretions. Yet booster vaccination significantly increased IL-4 secretions in all cohorts (92% responders). Lymphoblast populations and cytokines secretions were similar between all cohorts.

PT IgG Levels in Cord Blood of Responders Versus Non-Responders

Infants born to vaccinated or unvaccinated women, who were non-responders for IL-13 1 month after booster vaccination (±36% of the infants), had significantly higher PT IgG levels in their cord blood when compared to the levels of responders (*P* value = .002; Figure 3). A more detailed analysis showed that mainly preterm not term infants who were non-responders for $CD3^+$, $CD3^+$ $CD4^+$, $CD3^+$ $CD8^+$, IFN-γ, IL-13, and/or IL-5 after booster vaccination had significantly higher PT IgG levels at birth (Supplementary Figure 2).

Principal Component Analysis of the Immune Components

Most of the variability between the different immune factors was explained by PCA1 and PCA2 (41.8% and 14.1%, respectively). Primary and booster vaccination induced a cluster shift (Supplementary Figure 3), which can be explained by a change in immune components correlated to PCA1 ($CD3^+$, $CD3^+$ $CD4^+$, IFN-γ, IL-13, IL-17A, and IL-5, 60% positively correlated), and to a lesser extent to PCA2 (IL-10 and TNF-α, 70% positively correlated). A separate PCA for the vaccinated cohorts (VT and VP) demonstrated similar findings. No PCA was performed for the unvaccinated cohorts due to sample size limitations.

Correlation Between the Infant's Immune Components and Serum PT IgG

Positive correlation slopes were detected between the infants' PT IgG levels and several immune components after primary ($CD3^+$, $CD3^+$ $CD4^+$, IL-13, IL-4, IL-17A, and IL-5) and booster vaccination ($CD3^+$, $CD3^+$ $CD4^+$, $CD3^+$ $CD8^+$, IL-13, IL-17A, IL-5, and IFN-γ; Figure 4). At all other time points no correlations were observed. Although goodness of fit was achieved by testing normality of the residuals, a lot of the variance remained unexplained with the LR model.

Linear Mixed Models of the Different Immune Components

The LMM was used to study the overall differences of the immune components over time between and within the cohorts, estimating approximately 18–73% of the variability (depending on the immune component) within the data (Figure 5). The best model fit was observed when accounting for the individual subject's variability with a random intercept, and when the respective age of the infants and serum PT IgG levels at each sampling point were added as model predictors. The model predicted that when serum PT IgG levels were high after primary or booster vaccination, also high $CD3^+$ $CD4^+$, IFN-γ, IL-13, IL-17A, and IL-5 levels were observed. Tukey pairwise comparison showed no differences between the cohorts. Parameter estimates for each modeled immune component can be found in Supplementary Table 1.

DISCUSSION

Previously we showed that Tdap vaccination during pregnancy increased the Tdap antibody titers in cord blood of term and preterm born infants, which persisted before primary vaccination [14]. The presence of these persisting Mabs

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Table 1. Demographic Characteristics of Mothers and Infants Included for the Assessment of the CMI Response

		VT Cohort	VP Cohort	UnVT Cohort	UnVP Cohort
Women	N (women)	27	31	6	7
	Mean age at delivery in years (SD)	30.8 (3.7)	31.0 (3.7)	31.4 (4.2)	34.8 (6.7)
	Ethnicity, N (%)				
	White	25 (92.6)	30 (96.8)	6 (100.0)	6 (85.7)
	Non-White	2 (7.4)	1 (3.2)	0 (0.0)	1 (14.3)
	Median GA at delivery (Min-Max)	39.6 (37.1–41.1)	34.9 (28.6–36.7)	39.5 (37.0–40.4)	34.3 (30.0–36.7)
	Median GA at vaccination (Min-Max)	29.4 (24.3–34.3)	29.0 (23.4–34.0)	NA	NA
	Median interval between maternal vaccination and delivery in weeks (Min-Max)	9.6 (3.1–16.4)	5.6 (0.7–13.0)	NA	NA
	Mode of delivery, N (%)				
	Vaginal	18 (66.7)	15 (48.4)	4 (66.7)	5 (71.4)
	C-section	9 (33.3)	16 (51.6)	2 (33.3)	2 (28.6)
Infants	N (included infants)	28 (1 twin)	38 (7 twins)	6	7
	Gender, N (%)				
	Male	12 (42.9)	18 (47.4)	4 (66.7)	6 (85.7)
	Female	16 (57.1)	20 (52.6)	2 (33.3)	1 (14.3)
	Breastfeeding at birth, N (%)				
	Yes	25 (89.3)	31 (81.6)	5 (83.3)	6 (85.7)
	No	3 (10.7)	7 (18.4)	1 (16.7)	1 (14.3)
	Median duration of breastfeeding in weeks (Min-Max)	18.3 (0.1–70.3)	12.0 (0.4–48.9)	15.0 (4.4–69.3)	7.3 (0.3–61.3)
	Stayed in the neonatal care unit, N (%)				
	Yes	2 (7.1)	33 (86.8)	0 (0.0)	4 (57.1)
	No	26 (92.9)	5 (13.2)	6 (100.0)	3 (42.9)
	Median duration of stay at neonatal care unit in days (Min-Max)	2.0 (1.0–3.0)	17.0 (1.0–71.0)	NA	33.5 (16.0–45.0)
	Mean birth weight in grams (SD)	3395.1 (398.7)	2253.2 (564.8)	3565.8 (403.5)	2165.7 (492.5)
	Mean weight before primary vaccination in grams (SD)	5033.5 (503.9)	3958.3 (737.5)	5235.0 (862.5)	3924.3 (828.8)
	Mean weight 1 month after primary vaccination in grams (SD)	7275.2 (801.5)	6547.5 (928.0)	7525.0 (978.4)	7072.5 (827.7)
	Mean weight before booster vaccination in grams (SD)	10367.1 (831.0)	9540.4 (945.8)	10262.0 (986.0)	9875.0 (1234.3)
	Mean weight 1 month after booster vaccination in grams (SD)	10478.5 (815.7)	9779.6 (1035.9)	10050.0 (1060.7)	10300.0 (1246.3)
	Mean age at blood sampling before primary vaccination in weeks (SD)	7.9 (0.3)	7.9 (0.3)	8.0 (0.5)	8.0 (0.6)
	Mean age at blood sampling 1 month after primary vaccination in months (SD)	5.2 (0.4)	5.3 (0.3)	5.4 (0.4)	5.6 (0.7)
	Mean age at blood sampling before booster vaccination in months (SD)	14.8 (0.3)	13.5 (0.8)	15.3 (0.7)	13.5 (0.8)
	Mean age at blood sampling 1 month after booster vaccination in months (SD)	16.2 (0.7)	15.1 (1.0)	16.2 (0.9)	14.7 (0.9)
	Mean age at hexavalent vaccine dose 1 in weeks (SD)	9.0 (0.8)	8.8 (0.7)	9.1 (0.8)	9.7 (1.1)
	Mean age at hexavalent vaccine dose 2 in weeks (SD)	13.4 (1.1)	14.0 (1.1)	13.8 (0.9)	14.5 (1.5)
	Mean age at hexavalent vaccine dose 3 in weeks (SD)	18.4 (1.8)	18.8 (1.4)	18.5 (0.8)	19.8 (2.7)
	Mean age at hexavalent vaccine dose 4 in months (SD)	15.15 (0.7)	14.0 (1.0)	15.2 (0.8)	13.7 (0.9)

Abbreviations: CMI, cell-mediated immunity; GA, gestational age; NA, not applicable; UnVP, *unv*accinated women and their *p*reterm infants; UnVT, *unv*accinated women and their *t*erm infants; VP, *v*accinated women and their *p*reterm infants; VT, *v*accinated women and their *t*erm infants.

were shown to interfere with the infant's humoral immune responses at primary vaccination, as significantly lower antibody titers for FHA and DT in infants of in-pregnancy vaccinated women were observed when compared with infants of unvaccinated women (in preterm infants only interference for DT was observed). Here we provide evidence that pertussis-specific Mabs do not significantly interfere with the infant's CMI responses after primary vaccination, as no major differences were detected between the cohorts for any of the cellular immune components. However, after booster vaccination

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infants who were considered IL-13 non-responders had significantly higher concentrations of Mabs at birth (PT IgG). Specifically, preterm infants with high PT IgG Mabs in their cord blood were observed to be non-responders for $CD3^+$, $CD3^+$ $CD4^+$, $CD3^+$ $CD8^+$, IFN-γ, IL-13, and IL-5 after booster vaccination. It is possible that this effect is caused by earlier booster vaccination in preterm infants. However, Rowe et al. described similar kinetics, as tetanus Mabs at 2 months of age were positively correlated with IL-4, IL-13, and IL-5 cytokine responses in the infant at 18 months but not at 6 months of age [12]. This implies that Mabs do allow priming of the infant's CMI responses after primary vaccination, yet modulate the infant's T helper (Th) 1/Th2 cytokine balance later in life. The communication between the maternal-antibody: antigen complexes and the Fc receptors on different infant immune cells might promote this mechanism, as was suggested by Rice et al [13]. They reported the impact of Tdap-IPV vaccination during pregnancy on the infants' cytokine and innate immune responses, demonstrating lower IL-10 and IL-4 responses after infant primary vaccination when born to vaccinated women. In accordance, our results show that the infants' serum PT IgG positively correlated with $CD3^+$, $CD3^+$ $CD4^+$, and $CD3^+$ $CD8^+$ lymphoblast proliferation, and IFN-γ, IL-13, IL-4, IL-17A, and IL-5 cytokine secretions after primary and/

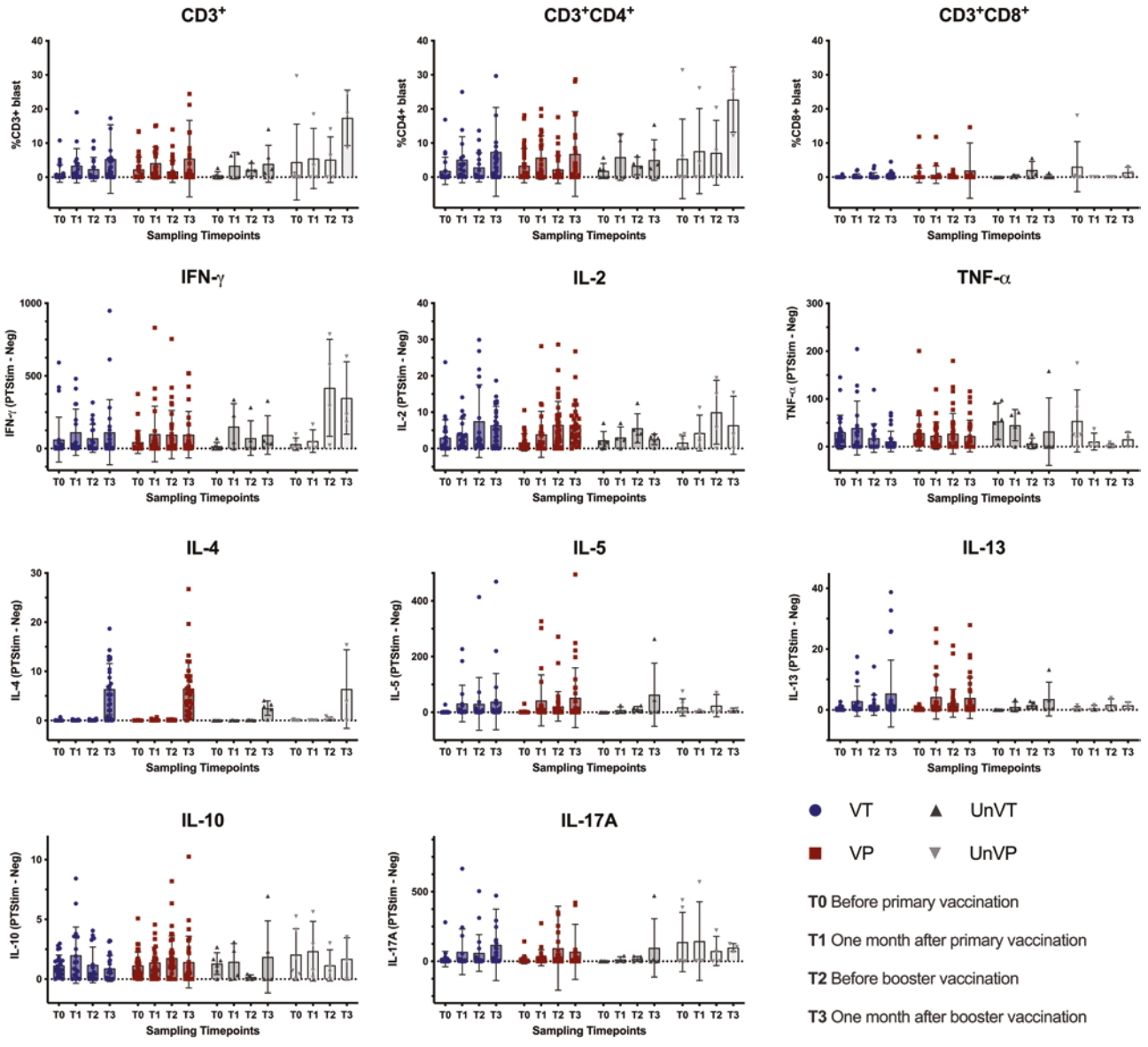

Figure 2. General overview of the $CD3^+$, $CD3^+$ $CD4^+$, and $CD3^+$ $CD8^+$ lymphoblasts populations (in percentages) and the IFN-γ, IL-10, IL-13, IL-2, IL-4, TNF-α, IL-17A, and IL-5 cytokine secretions (pg/mL) over time. Outliers were excluded from the graphs. No significant differences were observed between the different cohorts. Abbreviations: IFN, interferon; IL, interleukin; TNF, tumor necrosis factor.

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Table 2. Percentages of Infants Responding to Pertussis Toxin (PT) Antigen Stimulation (Number of Responders/Total Tested) 1 Month After Primary Vaccination (T1), Before Booster Vaccination (T2), and 1 Month After Booster Vaccination (T3)

% Responders	Cohort	T1 Post Primary Vaccination	T2 Before Booster Vaccination	T3 Post Booster Vaccination
$CD3^+$ lymphoblast	Total	55.32 (26/47)	35.48 (22/62)	56.06 (37/66)
	VT	56.25 (9/16)	36.84 (7/19)	61.54 (16/26)
	VP	56.52 (13/23)	28.57 (10/35)	45.16 (14/31)
	UnVT	50.00 (2/4)	75.00 (3/4)	66.67 (4/6)
	UnVP	50.00 (2/4)	50.00 (2/4)	100.00 (3/3)
$CD3^+$ $CD4^+$ lymphoblast	Total	57.45 (27/47)	37.10 (23/62)	57.58 (38/66)
	VT	68.75 (11/16)	42.11 (8/19)	65.38 (17/26)
	VP	52.17 (12/23)	28.57 (10/35)	45.16 (14/31)
	UnVT	50.00 (2/4)	75.00 (3/4)	66.67 (4/6)
	UnVP	50.00 (2/4)	50.00 (2/4)	100.00 (3/3)
$CD3^+$ $CD8^+$ lymphoblast	Total	17.02 (8/47)	16.13 (10/62)	18.18 (12/66)
	VT	18.75 (3/16)	15.79 (3/19)	19.36 (5/26)
	VP	13.04 (3/23)	11.43 (4/35)	12.90 (4/31)
	UnVT	50.00 (2/4)	75.00 (3/4)	16.67 (1/6)
	UnVP	00.00 (0/4)	00.00 (0/4)	66.67 (2/3)
IFN-γ	Total	64.00 (32/50)	57.81 (37/64)	60.60 (40/66)
	VT	66.67 (12/18)	57.89 (11/19)	59.26 (16/27)
	VP	62.50 (15/24)	52.78 (19/36)	54.84 (17/31)
	UnVT	75.00 (3/4)	60.00 (3/5)	80.00 (4/5)
	UnVP	50.00 (2/4)	100.00 (4/4)	100.00 (3/3)
IL-10	Total	48.00 (24/50)	37.50 (24/64)	34.85 (23/66)
	VT	50.00 (9/18)	26.32 (5/19)	33.33 (9/27)
	VP	45.83 (11/24)	50.00 (18/36)	32.26 (10/31)
	UnVT	50.00 (2/4)	00.00 (0/5)	40.00 (2/5)
	UnVP	50.00 (2/4)	25.00 (1/4)	66.67 (2/3)
IL-13	Total	62.00 (31/50)	56.25 (36/64)	63.64 (42/66)
	VT	61.11 (11/18)	42.11 (8/19)	66.67 (18/27)
	VP	70.83 (17/24)	55.56 (20/36)	54.84 (17/31)
	UnVT	25.00 (1/4)	100.00 (5/5)	80.00 (4/5)
	UnVP	50.00 (2/4)	75.00 (3/4)	100.00 (3/3)
IL-2	Total	56.00 (28/50)	71.87 (46/64)	80.30 (53/66)
	VT	66.67 (12/18)	57.89 (11/19)	77.78 (21/27)
	VP	41.67 (10/24)	75.00 (27/36)	83.87 (26/31)
	UnVT	75.00 (3/4)	100.00 (5/5)	80.00 (4/5)
	UnVP	75.00 (3/4)	75.00 (3/4)	66.67 (2/3)
IL-4	Total	46.00 (23/50)	62.50 (40/64)	92.42 (61/66)
	VT	55.56 (10/18)	52.63 (10/19)	88.89 (24/27)
	VP	45.83 (11/24)	63.89 (23/36)	96.77 (30/31)
	UnVT	25.00 (1/4)	80.00 (4/5)	100.00 (5/5)
	UnVP	25.00 (1/4)	75.00 (3/4)	66.67 (2/3)
TNF-α	Total	42.00 (21/50)	31.50 (20/64)	22.73 (15/66)
	VT	44.44 (8/18)	31.58 (6/19)	14.81 (4/27)
	VP	37.50 (9/24)	36.11 (13/36)	29.03 (9/31)
	UnVT	75.00 (3/4)	20.00 (1/5)	20.00 (1/5)
	UnVP	25.00 (1/4)	00.00 (0/4)	33.33 (1/3)
IL-17A	Total	68.00 (34/50)	51.56 (33/64)	60.61 (40/66)
	VT	72.22 (13/18)	52.63 (10/19)	77.78 (21/27)
	VP	66.67 (16/24)	50.00 (18/36)	45.16 (14/31)
	UnVT	50.00 (2/4)	60.00 (3/5)	40.00 (2/5)
	UnVP	75.00 (3/4)	50.00 (2/4)	100.00 (3/3)
IL-5	Total	78.00 (39/50)	70.31 (45/64)	74.24 (49/66)
	VT	83.33 (15/18)	68.42 (13/19)	81.48 (22/27)
	VP	79.17 (19/24)	66.67 (24/36)	64.52 (20/31)
	UnVT	75.00 (3/4)	1000.00 (5/5)	80.00 (4/5)
	UnVP	50.00 (2/4)	75.00 (3/4)	100.00 (3/3)

An infant was considered to be a positive responder when their response to the PT antigen after vaccination was higher than the 95% confidence interval (CI) of the responses before vaccination.

Abbreviations: IFN, interferon; IL, interleukin; PT, pertussis toxin; TNF, tumor necrosis factor. UnVP, *unv*accinated women and their *p*reterm infants; UnVT, *unv*accinated women and their *t*erm infants; VP, *v*accinated women and their *p*reterm infants; VT, *v*accinated women and their *t*erm infants.

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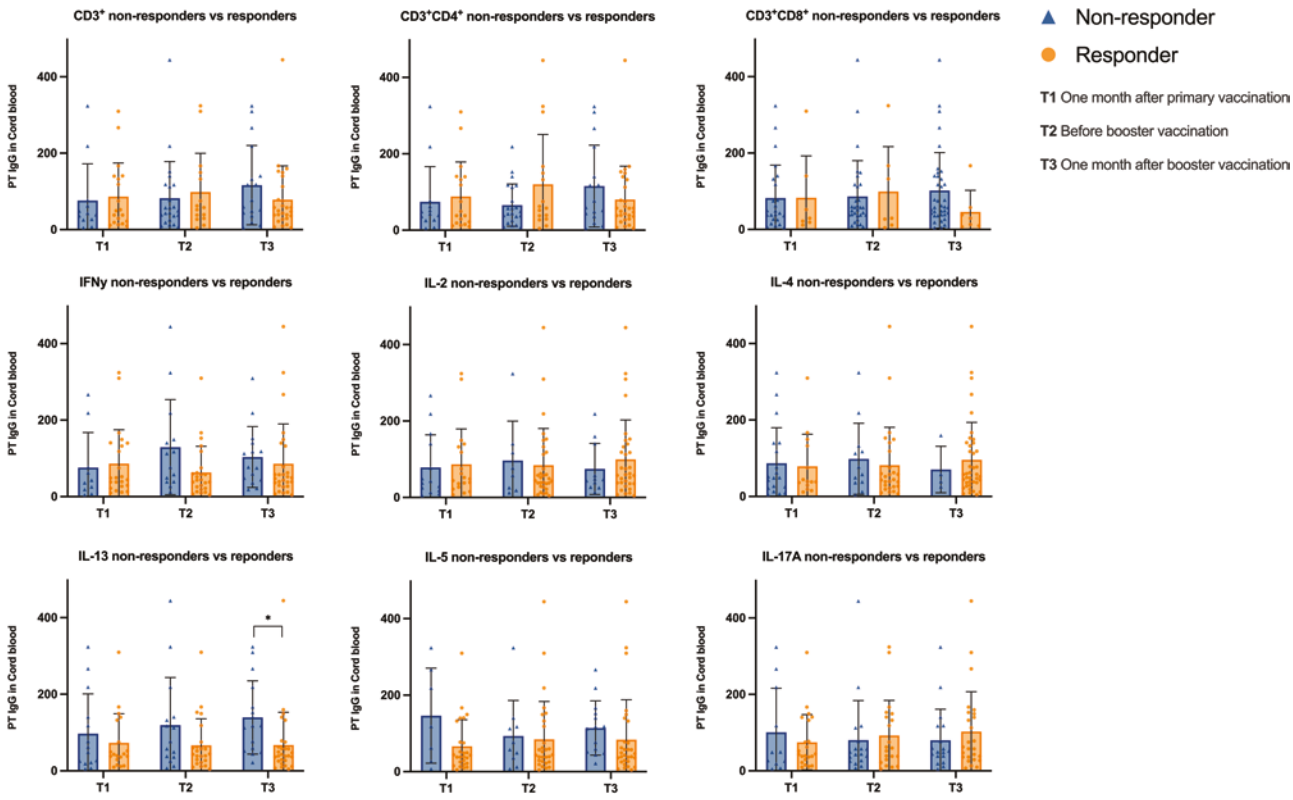

Figure 3. Comparison of the PT IgG levels (EU/mL) in cord blood between responders and non-responders for the different immune components ($CD3^+$, $CD3^+$ $CD4^+$, and $CD3^+$ $CD8^+$ lymphoblasts and the IFN-γ, IL-13, IL-2, IL-4, IL-17A, and IL-5 cytokines; IL-10, TNF-α not shown) 1 month after primary vaccination (T1), before booster vaccination (T2) and 1 month after booster vaccination (T3). * indicating significant differences after correction for multiple testing (*P* value < .01). Abbreviations: IFN, interferon; IgG, immunoglobulin G; IL, interleukin; PT, pertussis toxin; TNF, tumor necrosis factor.

or booster vaccination (LR model and LMM model). These findings again strengthened the evidence that interference by Mabs could modulate the infant's CMI responses, especially after booster vaccination.

Next to these possible influences of the women's vaccination status, we demonstrate that prematurity did not influence the CMI responses, as preterm born infants had comparable responses to those of term born infants. This complements our previous serological findings, showing that primary and booster vaccination with Hexyon® vaccine is immunogenic in both preterm and term born infants [14]. These results confirm previous literature describing the immune competence of term and preterm infants after acellular pertussis (aP) vaccination [21, 22].

The aP vaccines are known to induce a mixed Th1/Th2 profile after vaccination [23, 24]. A similar profile was observed in this study, as more than 57% of all infants responded to primary and/or booster vaccination with $CD3^+$ $CD4^+$ lymphoblast proliferation, IFN-γ, IL-2, IL-13, and IL-5 cytokine secretions. Booster, not primary vaccination elevated the levels of IL-4 in nearly all infants, confirming more Th2 responses after aP booster vaccination shown by previous studies [22, 23, 25]. IL-2 cytokine secretions significantly increased over time, indicating the induction and persistence of Th1 responses. The lack of anti-inflammatory IL-10 cytokines after vaccination, suggest that no suppressive regulatory T cells are induced, which in turn allows for more protective Th1 responses to take place [24]. However, these lower levels might be caused by our experimental set-up, as IL-10 is mainly induced by FHA not PT antigens [26]. On the other hand, the low TNF-α levels (primarily induced by PT antigens) imply poorer pro-inflammatory responses [27]. Next to these Th1 and Th2 responses, also the importance of Th17 cells in the protection against *B. pertussis* at the lung and nasal mucosae, has been recognized over the last years [24, 25]. More than 60% of all infants produced IL-17A, suggesting that Th17 cells are systemically induced after vaccination. However, whether Hexyon® also provides protective mucosal Th17 cells, remains to be investigated. Furthermore, it should be noted that cytokine secretions dependent on the administered vaccine type [28, 29] and the duration of in vitro cell-culture. Moreover, they could also be the secretion products of other immune cells within the culture. Other weaknesses of the study, like the small sample size of the unvaccinated cohorts and the different immunization schedule for the preterm infants, were recognized. However, these limitations were inherent to real-life situations of the Belgian population, as in Flanders vaccinating pregnant women with Tdap reached a coverage of ± 70% in 2016 and

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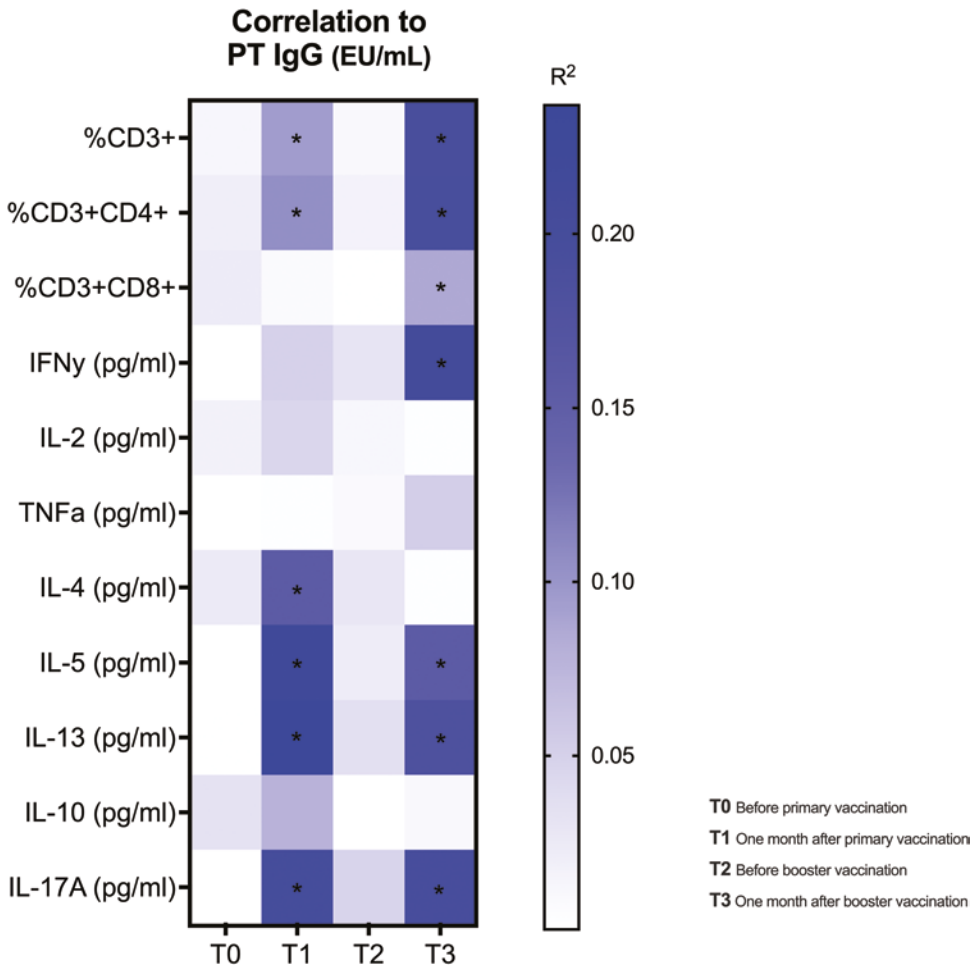

Figure 4. Pearson correlation of the different immune components ($CD3^+$, $CD3^+$ $CD4^+$, and $CD3^+$ $CD8^+$ lymphoblasts and the IFN-γ, IL-10, IL-13, IL-2, IL-4, TNF-α, IL-17A, and IL-5 cytokines) with PT IgG in the infant's serum over time. Serum PT IgG and cytokine levels were normalized (natural logarithm). *Indicating a significant positive Pearson correlation ($P < .05$), R^2 represents the variability explained by the PT IgG predictor. Abbreviations: IFN, interferon; IgG, immunoglobulin G; IL, interleukin; PT, pertussis toxin; TNF, tumor necrosis factor.

earlier booster vaccination for preterm infants is nationally recommended. Limitations within our experimental setup, like the limited amount of T-cell markers, the mitogenic characteristics of the PT-stimulating antigen, the absence of other stimulating vaccine-antigens, and the strict responder definitions, might have resulted in a deviation from the in vivo situation. Regardless, this study provides a unique insight into the CMI responses of term and preterm born infants, which was made possible through assessing small volume samples with FASCIA.

CONCLUSION

This study identified that primary and booster vaccination with aP vaccine induced a mixed Th1, Th2, and Th17 CMI response, which was comparable in term and preterm infants. The induction and persistence of IFN-γ, IL-2, and IL-17A cytokines after vaccination provides indirect evidence that bacterial clearance functions can be established by Th1 and Th17 cells. Moreover, we demonstrate that Mabs might modulate the infants' booster CMI responses. Even though the proven benefit of vaccination during pregnancy should not be discarded, these observations raise questions on Mab interference and its possible long-term effects. Proliferation assays like FASCIA combined with cytokine assessment can be an advantage to future large-scale and long-term surveillance studies involving the evaluation of both humoral and CMI responses.

Supplementary Data

Supplementary materials are available at *Clinical Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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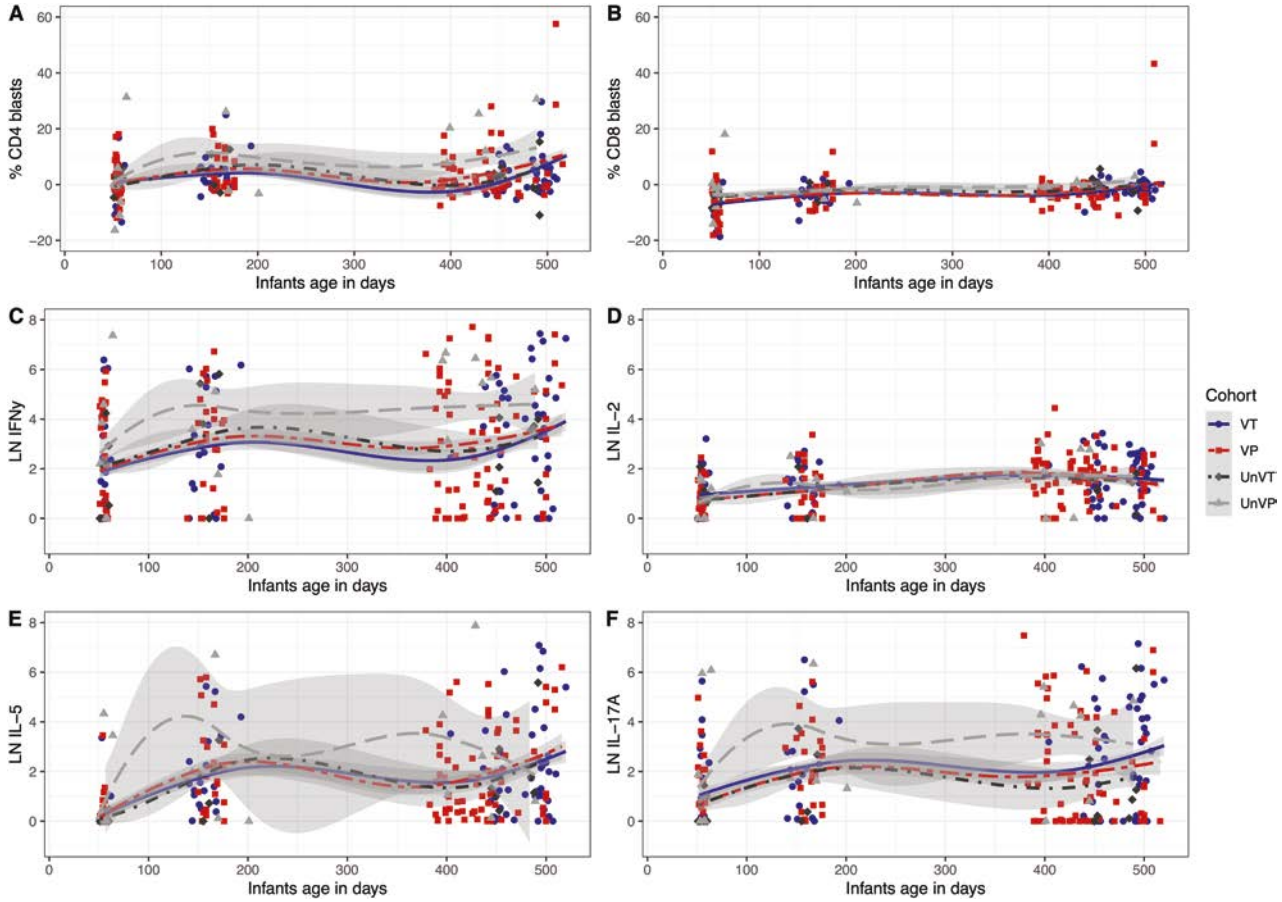

Figure 5. Representation of the linear mixed effect model for the different immune components: $CD3^+$ $CD4^+$ (*A*) and $CD3^+$ $CD8^+$ lymphoblasts (*B*) in percentages and the IFN-γ (*C*), IL-2 (*D*), IL-5 (*E*), and IL-17A (*F*) cytokines (pg/mL) expressed in natural logarithm. Abbreviations: IFN, interferon; IL, interleukin.

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References

1. Baxter R, Bartlett J, Fireman B, Lewis E, Klein NP. Effectiveness of vaccination during pregnancy to prevent infant pertussis. Pediatrics **2017**; 139:e20164091.
2. Dabrera G, Amirthalingam G, Andrews N, et al. A case-control study to estimate the effectiveness of maternal pertussis vaccination in protecting newborn infants in England and Wales, 2012–2013. Clin Infect Dis **2015**; 60:333–7.
3. Maertens K, Cabore RN, Huygen K, Hens N, Van Damme P, Leuridan E. Pertussis vaccination during pregnancy in Belgium: results of a prospective controlled cohort study. Vaccine **2016**; 34:142–50.
4. Ladhani SN, Andrews NJ, Southern J, et al. Antibody responses after primary immunization in infants born to women receiving a pertussis-containing vaccine during pregnancy: single arm observational study with a historical comparator. Clin Infect Dis **2015**; 61:1637–44.
5. Maertens K, Cabore RN, Huygen K, et al. Pertussis vaccination during pregnancy in Belgium: follow-up of infants until 1 month after the fourth infant pertussis vaccination at 15 months of age. Vaccine **2016**; 34:3613–9.
6. Halperin SA, Langley JM, Ye L, et al. A randomized controlled trial of the safety and immunogenicity of tetanus, diphtheria, and acellular pertussis vaccine immunization during pregnancy and subsequent infant immune response. Clin Infect Dis **2018**; 67:1063–71.
7. Wanlapakorn N, Maertens K, Vongpunsawad S, et al. Quantity and quality of antibodies after acellular versus whole-cell pertussis vaccines in infants born to mothers who received tetanus, diphtheria, and acellular pertussis vaccine during pregnancy: a randomized trial. Clin Infect Dis **2020**; 71:72–80.
8. Amirthalingam G, Campbell H, Ribeiro S, et al. Sustained effectiveness of the maternal pertussis immunization program in England 3 years following introduction. Clin Infect Dis **2016**; 63:S236–43.
9. Ross PJ, Sutton CE, Higgins S, et al. Relative contribution of Th1 and Th17 cells in adaptive immunity to *Bordetella pertussis*: towards the rational design of an improved acellular pertussis vaccine. PLoS Pathog **2013**; 9:e1003264.
10. Orije MRP, Maertens K, Corbiere V, et al. The effect of maternal antibodies on the cellular immune response after infant vaccination: a review. Vaccine **2020**; 38:20–8.
11. Vono M, Eberhardt CS, Auderset F, et al. Maternal antibodies inhibit neonatal and infant responses to vaccination by shaping the early-life B cell repertoire within germinal centers. Cell Rep **2019**; 28:1773–84 e5.

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12. Rowe J, Poolman JT, Macaubas C, Sly PD, Loh R, Holt PG. Enhancement of vaccine-specific cellular immunity in infants by passively acquired maternal antibody. Vaccine **2004**; 22:3986–92.
13. Rice TF, Diavatopoulos DA, Guo Y, et al. Modification of innate immune responses to *Bordetella pertussis* in babies from pertussis vaccinated pregnancies. EBioMedicine **2021**; 72:103612.
14. Maertens K, Orije MRP, Herzog SA, et al. Pertussis immunization during pregnancy: assessment of the role of maternal antibodies on immune responses in term and preterm born infants. Clin Infect Dis **2021**.
15. Tran TMP, Abrams S, Aerts M, Maertens K, Hens N. Measuring association among censored antibody titer data. Stat Med 2021;40:3740–61. doi:10.1002/sim.8995
16. Svahn A, Linde A, Thorstensson R, Karlen K, Andersson L, Gaines H. Development and evaluation of a flow-cytometric assay of specific cell-mediated immune response in activated whole blood for the detection of cell-mediated immunity against varicella-zoster virus. J Immunol Methods **2003**; 277:17–25.
17. Dreesman A, Dirix V, Smits K, et al. Identification of mycobacterium tuberculosis infection in infants and children with partial discrimination between active disease and asymptomatic infection. Front Pediatr **2019**; 7:311.
18. Carollo M, Palazzo R, Bianco M, Smits K, Mascart F, Ausiello CM. Antigen-specific responses assessment for the evaluation of *Bordetella pertussis* T cell immunity in humans. Vaccine **2012**; 30:1667–74.
19. Palazzo R, Carollo M, Bianco M, et al. Persistence of T-cell immune response induced by two acellular pertussis vaccines in children five years after primary vaccination. New Microbiol **2016**; 39:35–47.
20. Molenberghs G, Verbeke G. Linear Mixed Models for Longitudinal Data. New York, NY: Springer, **2000**.
21. Mascart F, Verscheure V, Malfroot A, et al. *Bordetella pertussis* infection in 2-month-old infants promotes type 1 T cell responses. J Immunol **2003**; 170:1504–9.
22. Vermeulen F, Verscheure V, Damis E, et al. Cellular immune responses of preterm infants after vaccination with whole-cell or acellular pertussis vaccines. Clin Vaccine Immunol **2010**; 17:258–62.
23. Ryan M, Murphy G, Ryan E, et al. Distinct T-cell subtypes induced with whole cell and acellular pertussis vaccines in children. Immunology **1998**; 93:1–10.
24. Chasaide CN, Mills KHG. Next-generation pertussis vaccines based on the induction of protective T cells in the respiratory tract. Vaccines **2020**; 8:621.
25. Higgs R, Higgins SC, Ross PJ, Mills KH. Immunity to the respiratory pathogen *Bordetella pertussis*. Mucosal Immunol **2012**; 5:485–500.
26. McGuirk P, McCann C, Mills KH. Pathogen-specific T regulatory 1 cells induced in the respiratory tract by a bacterial molecule that stimulates interleukin 10 production by dendritic cells: a novel strategy for evasion of protective T helper type 1 responses by *Bordetella pertussis*. J Exp Med **2002**; 195:221–31.
27. Wolfe DN, Mann PB, Buboltz AM, Harvill ET. Delayed role of tumor necrosis factor- alpha in overcoming the effects of pertussis toxin. J Infect Dis **2007**; 196:1228–36.
28. Vermeulen F, Dirix V, Verscheure V, et al. Persistence at one year of age of antigen-induced cellular immune responses in preterm infants vaccinated against whooping cough: comparison of three different vaccines and effect of a booster dose. Vaccine **2013**; 31:1981–6.
29. Mascart F, Hainaut M, Peltier A, Verscheure V, Levy J, Locht C. Modulation of the infant immune responses by the first pertussis vaccine administrations. Vaccine **2007**; 25:391–8.

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