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MAJOR ARTICLE

The effect of pertussis vaccination during pregnancy on the binding epitopes and avidity of anti-pertussis toxin igg antibodies in infants and their mothers

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Background: Immunization during pregnancy (IP) against pertussis protects young infants, but the maternally derived antibodies blunt the quantity of infants' antibody responses to their primary vaccination. While the blunting effect has been well studied for antibody quantity, potential blunting that would affect functional characteristics of these antibodies is less studied. This study evaluated the effect of IP on the epitopes and avidity of anti-pertussis toxin (PT) IgG antibodies in infants and their mothers.

Methods: In this prospective open-label controlled clinical trial, 47 pregnant women received diphtheria-tetanus-acellular pertussis (DTaP) vaccine booster, and 22 pregnant women who were not vaccinated served as controls. Sixty-nine infants received hexavalent DTaP vaccine at three

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and five months of age. Anti-PT IgG antibodies' binding strength and their ability to inhibit epitope-specific binding of mouse monoclonal antibodies were measured with ELISA in both maternal and infant samples.

Results: In both study groups antibodies in cord blood showed higher epitope-specific inhibition and avidity than what was induced in infants after two primary vaccine doses, at six months. Higher anti-PT IgG concentrations ($p<0.001$) and epitope-specific inhibition targeting 1B7 ($p=0.049$) and 11E6 ($p=0.024$) were noted at six months in control group infants, suggesting epitope-specific blunting in IP group. No difference was observed in the avidity of anti-PT IgG at six months between two study groups. The increase in avidity after vaccination was the highest in those mothers and infants with lower baseline avidity.

Conclusions: Immunization during pregnancy decreased primary vaccination-induced antibody responses disproportionately against different PT epitopes in infants.

The trial was registered in the EU Clinical Trial database (EudraCT number 2019-001986-34, https://www.clinicaltrialsregister.eu)

Keywords: Immunization in pregnancy; Vaccination; Pertussis; Antibodies; Pertussis toxin; Infants; Epitope; Avidity

BACKGROUND

Acellular pertussis vaccines (aPVs) stimulate antibodies against pertussis toxin (PT), pertactin, filamentous hemagglutinin, and with some formulations to fimbriae. While IgG antibody concentrations against aPV antigens generally correlate with protection against pertussis [1–4], determining the exact protective levels has been challenging. Despite high vaccination coverage and booster doses administered to all age groups, Bordetella pertussis circulates in the population and infects vulnerable infants [5,6]. The transplacental transfer of antibodies and immunization during pregnancy (IP) has been demonstrated to be effective to protect infants less than two/three months of age (before they receive their own vaccines) against severe disease [7]. Maternal antibodies are transferred to the fetus via the placenta, with immunoglobulin G (IgG) being the only class that crosses effectively. IgG transport begins around the 6th week of pregnancy, increases gradually until week 28, and accelerates during the last trimester. Essentially, the earlier the vaccination, the lower the maternal antibody levels before the last trimester, when transfer is most efficient [8,9]. However, studies show that IP decreases the quantity of the antibody response of infants to their primary pertussis vaccination [9–13].

Only limited data exists on the functional characteristics of the IP-induced and correspondingly infants blunted anti-pertussis antibodies, such as their binding strength, binding epitopes, and neutralization efficiency. Antibodies targeting certain epitopes, such as the enzymatically active subunit 1 targeting epitope 1B7 or cell-receptor binding related subunit 2/3 specific epitope

11E6, are preferentially induced after infection over aPVs [14,15] and have been demonstrated to be protective in animal models [16,17]. Whether antibody responses to these potentially protective epitopes are modulated or blunted remains to be studied.

Regarding avidity, the multivalent binding strength between antibodies and the target antigen may demonstrate greater effectiveness at neutralizing the harmful effects of the toxin and can thereafter contribute to longer-lasting immunity against pertussis. Higher-avidity antibodies reflect B-cell maturation and the development of durable immunological memory [18], which in turn contributes to sustained protection against infection [19]. In the case of Hib conjugate vaccines, rising avidity may help explain why strong protection is achieved even when circulating antibody levels are relatively low [18]. A similar pattern is seen with pertussis: elevated antibody titers alone do not reliably predict immunity, and some individuals remain protected despite modest concentrations [20,21]. Earlier studies suggest that newborns of women immunized with tetanus-diphtheria-acellular pertussis (Tdap) during pregnancy have greater avidity of umbilical cord IgG to PT than those of unimmunized women [22]. However, blunting of the avidity development was observed in those infants born from vaccinated mothers at 15 months of age after four primary doses of vaccines [23]. This study aimed to investigate the functional antibody characteristics before infants receive their own vaccines after IP. In addition, the effects of IP and existing immunity on the development of avidity and epitope specificity of anti-PT IgG antibodies were studied both in mothers and their infants first two vaccine doses up to six months post-delivery.

METHODS

Study Design, Participants, and Study Procedures

In total 69 mother-infant pairs completed this prospective, interventional, open-label controlled clinical trial, of whom 47 mothers received Tdap (Boostrix, GSK) vaccination at 30–35 weeks of pregnancy (”IP group”), containing PT, filamentous hemagglutinin, and pertactin [9]. Twenty-two mothers were not vaccinated during pregnancy and served as a control group control group . Serum samples from mothers were collected before vaccination and at 48 hours and six months after delivery. In addition, cord blood samples were collected at delivery. The infants received hexavalent DTaP-IPV-Hib-HepB vaccine (Infanrix hexa, GSK) at three and five months of age. Serum samples were collected before the first pertussis vaccine at three months and one month after two primary doses at six months of age. PBMCs for plasma B cell ELISpot measurement were collected 7 days after the second vaccine dose at 5 months and 7 days of age. Anti-PT IgG and PT-neutralizing antibody concentrations, as well as memory B cell counts against PT, including their methodology, were reported in detail earlier [9]. The trial was registered in the EU Clinical Trial database (EudraCT number 2019-001986-34) and was approved by the Ethics Committee of the Hospital District of Southwest Finland (ETMK Dnro 67 /1800/2019). Written informed consent was obtained from all the participants and the parents of the infant participants.

This exploratory analysis does not represent the whole cohort, since the performed functional antibody assays required considerable quantities of antibodies to be performed, which were used as pre-defined inclusion criteria [13,18]. The baseline characteristics of the study participants were reported previously [11]. Particularly, only a few mother-infant pairs at paired timepoints could be included for longitudinal analysis of epitopes.

Epitope-specific anti-PT IgG antibodies

The binding sites of antibodies on native PT (GSK) were studied based on competition with murine monoclonal antibodies (mAbs) (National Institute for Biological Standards and Control (NIBSC), Potters Bar, UK). Sera from the mothers were tested for 13 different epitopes (Figure 1), whereas the infants' sera, owing to the limited amount of sample, were tested for epitopes 1B7 (S1), 11E6 (S2/3) and 7E10 (S3) [15,16,24].

The blocking of mAb binding to PT by serum antibodies was determined by ELISA as previously described [15] with the following modifications, which aimed to improve the measurement range of this assay to reliably estimate lower antibody levels: The serum samples (with > 10 international units per mL (IU/mL) of anti-PT IgG) were prepared in a twofold dilution series from 1:10–1:80 in 100 µL of 1% BSA-PBS and incubated for two hours at 37°C. PBS was used as a control. However, the serum samples from the mothers were tested only at a dilution of 1:10 in duplicate to epitopes other than 1B7, 11E6, and 7E10. After the wells were washed with 0.9% NaCl-Tween, 100 µL of mAbs in PBS was added according to the NIBSC-recommended dilutions. The duration of P-nitrophenylphosphatase substrate (cat no S0942; Sigma) mediated with anti-mouse alkaline phosphatase (AP124, Merck, Finland) reaction incubation was optimized separately for each epitope by aiming for an absorbance value of 1.5 attained from the sample including only PBS. The absorbance was measured at 405 nm with a Victor Nivo device (Perkin Elmer). The specific inhibition of the maximum signal of mAb binding caused by similar epitope-like antibodies from study sera was determined from background reduced signals as 1 − absorbance (sample)/absorbance (PBS). The serum dilution resulting in a 50% reduction of mouse IgG monoclonal binding, "specific inhibitory value" (IC50), were evaluated from the dilution series of sera, which are later reported as their reciprocal values (1/IC50).

Anti-PT IgG avidity

Serum samples (with > 2 IU/mL anti-PT IgG) were diluted to a concentration of 0.025 anti-PT IgG IU/well and tested as described earlier [25,26]. The sample wells were treated with 100 µl of 6.5 M urea or PBS for 15 minutes, and the avidity-index was calculated from the background (1% BSA-PBS) reduced absorbance values as the absorbance (urea well) / absorbance (PBS well). Any sample with a lower absorbance measured from the PBS well than the in-house anti-PT IgG negative control (0.1 IU/mL) was excluded from further analysis.

Statistics

The analyses were performed with IBM SPSS Statistics for Windows version 28.0 (IBM Corp.). Based on normal distribution assumption by Shapiro-Wilk test, the differences in means between the groups at each timepoint were tested with T-test and Bonferroni correction, and longitudinal analysis was performed with paired t-tests. Three-month time point was used as the baseline for evaluating infants' vaccine responses. To evaluate the effect of the mother's existing memory of vaccination antigens on maternal and infant antibody levels, an analysis by grouping individuals based on high and low baseline antibodies or their affinity was performed. The correlations are reported as Pearson or Spearman's correlation coefficients when applicable. Two-sided statistical significance was set at $p<0.05$.

RESULTS

Epitope-specific anti-PT IgG antibodies in mothers 48 h after delivery

A comprehensive anti-PT IgG epitope profile at 48 hours after delivery was examined in mothers who received the Tdap booster during pregnancy (n=33) (Figure 1). Extensive inhibition of mAb binding by human anti-PT IgG was observed toward S1 epitopes whereas the inhibition of mAbs targeting S2, S3, S4 was notably low (on average <5%), except for 11E6 (S2/3) and 7E10 (S3). At 48 hours post-delivery, mothers with higher pre-vaccination anti-PT IgG concentrations (>10 IU/mL) had more antibodies inhibiting the mabs targeting 6G8 (S4), 3F10 (S1), G9A (S2/3), and 2E12 (S3) epitopes than those mothers with lower anti-PT IgG concentrations pre-vaccination. The magnitude of all the epitope-inhibiting specific responses strongly correlated with the anti-PT IgG concentration (Pearson R=0.842).

Epitope-specific anti-PT immunoglobulins in infants after two primary doses

At six months, sera from infants from the control group had higher inhibition of all three epitope-specific mAb bindings compared to infants born to mothers with Tdap IP IP group (Figure 2), statistical significance was noted only for 11E6 ($p=0.022$). Epitope-specific antibodies correlated moderately, with varying strengths, with avidity, the quantity of PT neutralizing capability of antibodies, PT-specific plasma -, and memory B cells in the control group, but not in the IP group (Figure 3). Thereafter, a proportional analysis model was introduced to take overall anti-PT IgG concentrations into account (Figure 2b). Based on this model, no difference was noted in the relative quantities of epitope-specific antibodies.

To assess whether blunted is due to IP and not just antibodies, epitope responses at six months were compared between infants from IP group and control group who had less than 10 IU/mL anti-PT IgG at three months. Hypothetically, no blunting should occur without maternal antibodies [9]. Infants in the control group had, again, statistically significantly higher epitope-specific inhibition to all three epitopes (Figure 4) and anti-PT IgG at six months ($p<0.05$).

Taking anti-PT IgG concentrations into account, both groups had similar epitope profiles (Figure 4b).

Existing antibodies may influence the readout, as 11% of IP group and 14% of control group mothers had > 50 IU/mL anti-PT IgG before the study. On the contrary, 26% of IP group mothers had, example given, less than a 25 IU/mL increase in anti-PT IgG at delivery compared to baseline. To pinpoint whether epitope responses are blunted due to the presence of maternal antibodies in general, not just IP, the infants were redistributed into groups based on having either lower or higher than 10 IU/mL anti-PT IgG at three months, the criterium that was previously determined to best predict blunting of the infants anti-PT IgG responses [9]. High maternal IgG before vaccination blunted infant anti-PT IgG, 1B7, and 11E6 responses ($p<0.05$), but not 7E10.

Paired sample analysis and epitope-specific transfer to infants

Eleven IP and four control mother-infant pairs were studied for epitope-specific antibody transfer and impact on infants' responses. IP-induced antibodies inhibiting the 1B7, 11E6, and 7E10 epitopes were present in the infants' cord blood and three-month samples (Figure 5). Antibodies against 1B7 were more concentrated in the cord blood sample in both cohorts compared to what was observed in mothers' 48 post-delivery samples but declined faster in comparison to 11E6 and 7E10 epitope-specific antibodies. Unspecific increases of 11E6 (S2/3) ($p=0.343$) and 7E10 (S3) ($p=0.035$) but not S1-targeting antibodies were detected at 48h post-delivery in all unvaccinated mothers (n=4) included in this analysis. These mothers from the control group had very high concentrations of anti-PT IgG (GM of 83 IU/mL) at the time of inclusion in the study but no increase in IgG between their visit during pregnancy and delivery (Figure 5c).

After two primary vaccine doses, there was an increase in the inhibition of epitope-specific mAbs only in IP group infants. However, there was no significant difference in the quantity of epitope-specific antibodies between the study groups at six months of age, despite significantly lower anti-PT IgG concentration in IP group (Figure 5d). The inhibition of epitope-specific mAbs at three months was higher in the control group despite lower anti-PT IgG concentrations, which may reflect these observations. In relative terms, infants in control group induced a lot of anti-PT IgG after their first two vaccination doses, without a comparable increase in the measured inhibition towards the studied epitopes, but hypothetically to other epitopes.

Avidity, Mothers

The avidity of anti-PT IgG in vaccinated mothers did not increase between the vaccination timepoint and the 48 hours after the delivery timepoint, but a significant increase was noted between the 48 hours after delivery timepoint and after six months ((Figure 6, $p=0.013$). A similar increasing trend in avidity was noted among control group. This increase in avidity may be associated with a more than 80% general increase in anti-PT IgG GM concentrations between 48 hours after delivery and six months after in control group. Mothers in IP group with lower

avidity to PT before vaccination had a greater increase in avidity at six months postpartum, whereas a decrease was noted in mothers with higher pre-vaccine avidity (Figure 6c). Nevertheless, those mothers with high avidity pre-vaccination had higher avidity at six months of age (Pearson R between pre-vaccination avidity and avidity at six months =0.566), despite a decrease. There was no difference in the development of avidity noted in mothers based on varying pre-vaccination IgG concentrations (Figure 6b). No correlation was found between the timing of delivery and the avidity of anti-PT IgG at any study timepoint in vaccinated mothers (Spearman R values < ± 0.27). No significant correlation was found either between avidity and anti-PT IgG or PT neutralizing ability at any time point.

Avidity, Infants

One month after receiving two primary vaccine doses, infants had weaker levels of anti-PT IgG avidity compared to those passed from the mother at birth and at three months of age (Table 1). There was no statistically significant difference between the study groups' avidity at six months (p=0.094), or if the classification of infants was based instead of study groups on high or low pre-vaccine anti-PT IgG (p=0.106) or avidity (p=0.185). Similar to the vaccine responses during pregnancy in mothers (Figure 6), infants with the lowest avidity pre-vaccination showed the strongest increase in avidity after vaccination, whereas infants with high pre-vaccination avidity showed a strong decreasing trend, indicating a development trend of blunting for the avidity response (Table 1). The Pearson correlations between the avidity at three months of age and the relationship between the six to three-month avidity levels were -0.770 for IP group and -0.679 for control group. No correlation was found between avidity and anti-PT IgG, PT neutralizing ability or plasma or PT-specific memory B-cells at any time point with either study group.

DISCUSSION

This study supports immunization during pregnancy to ensure sufficient quantity and functionality of PT-specific antibodies for infants before completing their primary vaccination series. Data show that maternally derived antibodies had higher inhibition towards key epitopes, higher affinity, and PT neutralizing ability [9] than antibodies induced by infants. After two doses, infants maintain a high quantity of epitope-specific antibodies at six months.

Maternal antibodies significantly blunted infants' epitope-specific antibody responses, similar as with anti-PT IgG [9, 27]. The lack of correlation between avidity, neutralization ability, plasma and memory B cells to the corresponding epitope-specific inhibition in infants born to vaccinated mothers is concerning, as antibodies targeting these epitopes (1B7, 11E6) are protective in animal models [16,17,28]. PT stimulated fewer B-cells than other vaccine antigens after two doses [9]; future work should asses correlations after the third dose.

Epitope-specific blunting largely disappeared between the study groups if the total anti-PT IgG concentrations were considered (Figure 2b,4b), emphasizing that analysis models must consider multiple factors. Blunting was found not only antibody-mediated; vaccination alone demonstrated reduced anti-PT IgG and epitope-specific responses. Maternal factors such as cytokines [29,30], microbiome [31], and TLR and HLA polymorphisms [32] may influence the outcome.

Maternal antibodies may cause blunting differently depending on PT epitope presentation from infections versus chemically detoxified vaccine PT [14,15,33]. Despite the low transfer of anti-PT IgG from unvaccinated mothers, epitope-specific antibodies appeared in high quantities and blunted the response towards these epitopes effectively in their infants (Figure 5). Hypothetically, antibodies induced by the infants target other epitopes, as these infants had a strong increase in overall anti-PT IgG. High inhibition of 11E6 and 7E10 epitopes in infants of unvaccinated mothers with high pre-study antibody levels suggests of recent infections [15]. These maternal antibodies likely persist at six months.

Epitope-specific inhibitions after Tdap were higher in mothers with higher pre-vaccination anti-PT IgG, indicating stronger responses. Similarly, babies receiving three DTaP doses had higher inhibition of 11E6- and 7E10-specific mAbs than older children after a single booster, indicating benefits of existing memory [15]. Variation in maternal epitope responses may reflect differences in overall anti-PT IgG concentrations or epitope profiles between vaccinated and pre-existing antibodies. E.g., adults given one Tdap dose had less 1B7- and 11E6-specific inhibition than those recently infected [14]. Pertussis exposure may maintain higher epitope-specific antibodies and cell-mediated memory.

Avidity increased most in mothers with low pre-vaccination avidity from baseline to six months postpartum. No correlation was found between delivery timing and avidity development in vaccinated mothers, nor with any of infants' epitope responses. Previous studies report mixed results [34,35]. This study was not designed or powered to evaluate this aspect. Avidity in cord blood exceeded maternal post-delivery samples, consistent with preferential transport of high-avidity maternal antibodies across the placenta [36,37]. Infants showed sharp avidity decline between birth and three months, though PT neutralizing ability remained high [9]. Maternal antibody decay seems differential for avidity and neutralization ability. This remains hypothetical, as the half-life of antibodies is Fc receptor mediated [38]. Subclass degradation kinetics [39] and their selective transport [40–42] may also affect epitope-specific antibody persistence and infant vaccine responses (Figure 5).

Greater avidity in infants born to vaccinated mothers has been observed in cord blood compared to controls [22,43], though we observed no difference in avidity, only quantity. Long term, lower avidity occurs in infants after IP after a fourth vaccine dose at 15 months, correlating with lower anti-PT IgG [23]. Avidity was higher after third vaccine dose in infants of unvaccinated mothers in than after a single booster or infection in older children [25], suggesting more than two

booster doses are needed for avidity maturation. At six months, IP group infants had slightly higher avidity than controls, likely due to residual maternally high-avidity antibodies. Avidity increase after two doses occurred mainly in infants with baseline avidity regardless of concentration, similar to trends in older age groups [26]. Overall, avidity variation among infants was greater at three months than at 6 months.

This study has several limitations. First, strict exclusion criteria were applied to ensure reliable analysis, particularly ruling out samples with low anti-PT IgG. This reduced sample size, statistical power and generalizability; results may not represent the entire cohort or broader population. Second, additional cutoffs were introduced to examine the effect of natural boosting on mothers' and in further blocking of infants' responses [9]. For example, low/high pre-vaccine avidity thresholds were arbitrary and intended for balanced case distribution rather than indication "bad or good" starting points. Previous studies have also included anti-PT IgG concentration as an exclusion criterion for avidity evaluation [43]. However, dilution to matching antibody concentrations demonstrated that existing IgG concentrations alone do not predict avidity or its development (Figure 6b, Table 1) [27]. The lack of strong associations between PT-specific avidity, epitope inhibition, as well as PT neutralizing activity and B-cell counts in emphasize that these are individual characteristics requiring separate study [27]. Finally, this study focused solely on PT-specific responses and extending similar methods to other vaccine antigens would be valuable.

In conclusion, maternal antibodies after IP exhibited greater inhibition to several epitopes and higher affinity than those induced in infants after two primary doses. Having existing memory to PT in mothers positively influenced the magnitude of epitope-specific antibodies and their avidity transferred to the infants. This provides a reassuring rationale for IP, ensuring anti-PT IgG-mediated protection for infants before completing their primary vaccinations. Maternal antibodies caused a blunting particularly toward the 1B7 and 11E6 epitopes. However, the level of avidity remained unaffected up until six months of infant age. The noted blunting and the lack of correlation between plasma and memory B cells to corresponding epitope binding in infants after IP are of potential concern. The results suggest that at least two doses of DTaP are beneficial and necessary for the development of infants' functional antibody responses regarding target epitopes and avidity towards PT.

List of abbreviations:

Acellular pertussis vaccine, aPV; Immunoglobulin G, IgG; Pertussis toxin, PT; Phosphate buffered saline, PBS; Bovine serum albumin, BSA; Subunit 1-5, S1-5; Confidence interval, CI; Geometric mean, GM; Half maximal inhibitory concentration, IC50; Diphtheria-tetanus-acellular pertussis, DTaP, Tdap; Immunization during pregnancy, IP; International units per milliliter, IU/mL

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***Data availability statement**:* The data underlying this article will be shared on reasonable request to the corresponding author.

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***Author Contributions**:* QH and AK were responsible for the conception and design of the study. QH, LI, JM, AK, AMB, PvG, and AB contributed to the data acquisition. AK and QH were responsible for the data analysis and interpretation with contributions from LI, AMB, and JM. AK and QH wrote the first draft of the manuscript, and LI, AMB, JM, PvG, and AB revised it critically. All authors conducted approval for publication of the content and agreed to be available for all aspects of the work to ensure that questions related to the accuracy of any part of the work are appropriately investigated and resolved.

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Figure 1. Overview of anti-PT IgG and epitope-specific inhibition as geometric mean (GM) values of vaccinated mothers (n=33) toward 13 different epitopes (six to S1, three S2/3 (S23), three S3, and one S4 in the respective order) at 48 h after delivery. The cases are differentiated between mothers with lower (orange, n=17) or higher (blue, n=16) than 10 IU/mL anti-PT IgG before vaccination.

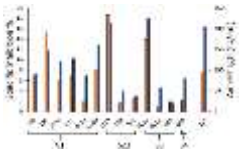

Figure 2. Quantities of epitope-specific mab inhibition of infants at six months of age in the IP group (green, n=27) and the control group (gray, n=18). The box plots show the median, quartile range, and 1.5 times the quartile range of the 1/IC50 (a) and the IC50 values multiplied by the anti-PT IgG concentrations (b) for epitopes 1B7 (S1), 11E(S2/3), and 7E10 (S3) at six months of age. The IC50 indicates the serum dilution at which half of the binding reactions are inhibited. The higher the reciprocal of IC50 (1/IC50, a)) was, the more effective the serum was at blocking the binding of epitope-specific mAbs. The geometric mean of anti-PT IgG concentration (c) is presented for comparison. o = values exceeding 1.5 times the interquartile range. A sample in IP group with an IC50*anti-PT IgG >250 in epitope 7E10 was excluded from the graph. Statistical difference was assessed by T-test.

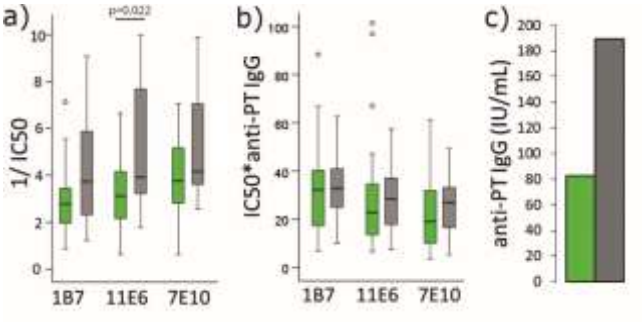

Figure 3. Correlation of epitope-specific mab inhibition and other immune responses in infants, at six months presented as a heatmap. * Measured one week after the second primary dose at five months of age. Figure was created using Biorender. Correlation of ≤ ± 0.35 was considered as weak, between ± 0.35–0.75 as moderate, and ≥ ± 0.75 as strong.

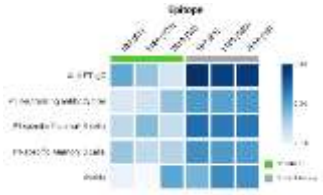

Figure 4. Only samples with < 10 IU/mL anti-PT IgG at three months of age, before primary vaccination, were compared for epitope-specific antibody inhibition between infants at six months of age in the IP group (green, n=12) and the control group (gray, n=14). The box plots show the median, quartile range, and 1.5 times the quartile range of the 1/IC50 (a) and the IC50 values multiplied by the anti-PT IgG concentrations (b). The geometric means of anti-PT IgG concentration (c) at six months of age is presented for comparison. o = values exceeding 1.5 times the interquartile range. A sample in IP group with an IC50*anti-PT IgG >250 in epitope 7E10 was excluded from the graph. * Statistical difference by T-test ($p<0.05$).

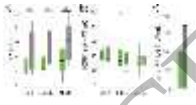

Figure 5. Transfer and development of anti-PT IgG (IU/mL) (d) and PT-specific antibodies inhibiting three epitopes (a-c), defined as geometric mean values of the 1/IC50 (a, b, c), IP group (green, n=11) and the control group (gray, n=4). Only paired samples with at least >10 IU/mL anti-PT IgG at all timepoints were included in the analysis. M = mother, B = baby

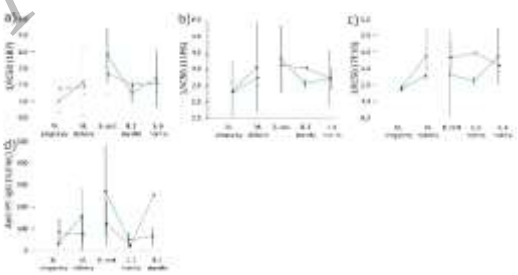

Figure 6. a) Mothers' avidity-index to PT in IP group , (green, n=33) and control group (gray, n=18) before vaccination, at 48 hours after delivery, and six months of age are presented as box plots in their respective order. "X" within the box plot describes the mean value of the avidity-index. In an alternating grouping, the avidity development of mothers in IP group are shown based on having either lower (orange, n= 13) or higher (blue, n=20) anti-PT IgG (10 IU/mL) (b) or mothers with a lower (n=17) or higher (n=16) avidity-index than 35% (c) before vaccination. control group is shown as gray (n=18). A statistically significant difference by paired T-test (p=0.005) was noted at six months between the control group subgroup and the IP group subgroup with high avidity pre-vaccine and between the high and low avidity groups within IP group (p=0.023) (c). M=mother

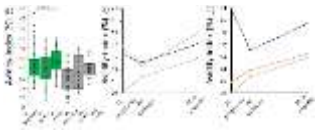

Table 1. Avidity-index and concentration of anti-PT IgG in the study infants.

		Avidity-index % (95% CI)			Anti-PT IgG (IU/mL) (95% CI)		
	N	Cord	3 Mo	6 Mo	Cord	3 Mo	6 Mo
IP group	37	49.8 (46.8-52.7)	38.8 (35.9-41.8)	38.0 (36.0-40.0)	202.8 (141.9-263.7)	32.4 (22.2-42.7)	83.4 (67.0-99.8)
Control group	14	50.5 (47.3-53.7)	40.3 (36.5-44.1)	35.2 (33.2-37.1)	39.6 (9.7-69.6)	7.1 (2.7-11.5)	188.3 (128.0-248.5)
IP group < 10IU/mL anti-PT IgG pre-vaccine	10	48.4 (41.4-55.3)	42.2 (35.4-49.1)	36.8 (32.6-41.0)	28.1 (16.0-40.2)	4.7 (3.1-6.4)	111.9 (78.7-145.1)
IP group ≥ 10IU/mL anti-PT IgG pre-vaccine	27	48.9 (45.3-52.6)	37.4 (34.0-40.9)	38.9 (36.0-41.7)	247.7 (172.1-323.4)	44.0 (30.5-57.5)	64.7 (48.6-80.7)
Control group < 10IU/mL anti-PT	10	48.9	39.4	35.1	18.5	3.8	218.6

IgG pre-vaccine		(44.3-53.5)	(35.0-43.9)	(32.1-38.1)	(10.2-26.9)	(2.1-5.6)	(109.6-327.7)	*
Control group ≥ 10IU/mL anti-PT IgG pre-vaccine	4	51.0 (46.1-55.8)	42.4 (30.1-54.7)	35.4 (28.4-42.3)	146.5 (0.1-300.5)	25.0 (6.1-43.9)	45.3 (14.3-77.8)	
IP group low* avidity pre-vaccine	15	42.1 (37.8-46.4)	30.9 (27.9-33.8)	36.7 (32.3-41.2)	234.3 (135.5-333.2)	44.3 (25.2-63.4)	83.1 (50.9-115.3)	
IP group high* avidity pre-vaccine	22	53.4 (50.1-56.6)	44.1 (41.0-47.1)	39.4 (36.7-42.0)	157.0 (70.1-243.9)	25.9 (11.6-40.3)	73.6 (56.7-90.4)	
Control group low* avidity pre-vaccine	8	48.2 (43.9-52.6)	35.8 (34.8-36.8)	34.8 (31.2-38.4)	49.0 (0.1-119.5)	7.7 (0.1-16.6)	113.4 (43.7-183.2)	
Control group high* avidity pre-vaccine	6	51.2 (44.8-57.6)	46.3 (40.2-52.4)	35.7 (31.4-40.0)	63.3 (0.1-138.3)	12.8 (0.1-26.6)	243.3 (38.3-447.8)	

Arbitrary cutoff for low/high avidity in infants defined as 38% at three months of age

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