Influence of maternal vaccination against diphtheria, tetanus, and pertussis on the avidity of infant antibody responses to a pertussis containing vaccine in Belgium

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INFLUENCE OF MATERNAL VACCINATION AGAINST DIPHTHERIA, TETANUS AND PERTUSSIS ON THE AVIDITY OF INFANT ANTIBODY RESPONSES TO A PERTUSSIS CONTAINING VACCINE IN BELGIUM

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List of abbreviations: RAI: relative avidity index; PT: pertussis toxin; FHA: filamentous hemagglutinin; Prn: pertactin; DT: diphtheria toxin; TT: tetanus toxin; IgG: immunoglobulin G; M: molar; Tdap vaccine: trivalent Diphtheria and Tetanus Toxoids and Acellular Pertussis Vaccine

Abstract

Maternal antibodies induced by vaccination during pregnancy cross the placental barrier and can close the susceptibility gap to pertussis in young infants up to the start of primary immunization. As not only the quantity but also the quality of circulating antibodies is important for protection, we assessed whether maternal immunization affects the avidity of infant vaccine-induced IgG antibodies, in the frame of a prospective clinical trial on pregnancy vaccination in Belgium. Infants born from Tdap (Boostrix®) vaccinated (N=55) and unvaccinated (N=26) mothers were immunized with a hexavalent pertussis containing vaccine (Infanrix Hexa®) at 8, 12 and 16 weeks, followed by a fourth dose at 15 months of age. Right before and one month after this fourth vaccine dose, the avidity of IgG antibodies against diphtheria toxin (DT), tetanus toxin (TT), pertussis toxin (PT), filamentous hemagglutinin (FHA) and pertactin (Prn) was determined using 1.5M ammonium thiocyanate as dissociating agent. In both groups, antibody avidity was moderate for TT, PT, FHA and Prn and low for DT after priming. After a fourth dose, antibody avidity increased significantly to high avidity for TT and PT, whereas it remained moderate for FHA and Prn and low for DT. The avidity correlated positively with antibody level in both study groups, yet not significantly for PT. When comparing both study groups, only PT-specific antibodies showed significantly lower avidity in infants born from vaccinated than from unvaccinated mothers after the fourth vaccine dose. The clinical significance of lower avidity of vaccine induced infant antibodies after maternal vaccination, if any, needs further investigation.

Introduction

Pertussis, caused by *Bordetella pertussis*, is a highly contagious respiratory disease, which can be mortal for newborns and young infants [1,2]. In Belgium, the hexavalent vaccine containing diphtheria, tetanus and acellular pertussis is used for primary immunization at 8, 12 and 16 weeks of age with a fourth dose at 15 months of life. To protect these highly susceptible infants from pertussis disease during the first months of life, the National Immunization Technical Advisory Group in Belgium has recommended a trivalent tetanus toxoid, reduced diphtheria toxoid and acellular pertussis (Tdap) vaccine for every pregnant woman between 24 and 32 weeks of gestation since July 2013 [3].

We previously reported that Tdap vaccination during pregnancy induces high titers of maternal antibodies, passing the placental barrier and closing the susceptibility gap to pertussis, diphtheria and tetanus in young infants up to the start of the primary immunization [4]. However, blunting was noticed post-primary vaccination for DT and PT specific responses in infants from vaccinated women [4]. At month 15, before the fourth vaccine dose, we observed a steep decay in antibody titers. Antibody levels rose again significantly for all five antigens one month after the fourth dose, albeit with lower PT-specific IgG in infants from the maternal vaccine group than in those from the control group [5]. The clinical relevance of this blunting effect is not clear, since there are unfortunately no quantifiable correlates of protection for pertussis [6], although antibodies to PT, Prn and fimbriae are thought to be of importance [1].

However, other factors such as antibody isotype, affinity, avidity, immunoglobulin gene sequence, and biological activity (i.e. bactericidal or neutralizing activity) are also important for antibody mediated protection, as extensively documented for *Haemophilus influenza*e Type b antibodies induced by DTaP/Hib combination vaccines [7,8]. Avidity or functional

affinity is an important parameter that determines the strength of the antigen-antibody binding [9]. Following a T-cell dependent response, somatic hypermutation combined to the selection of high affinity B-cells clones in the germinal center result in the maturation of avidity [10–12]. Therefore, measuring antibody avidity can be used to assess a good priming of immunological memory [13]. The immune system of neonates is different from that of adults, including weak germinal centre B cell responses [14] and rare plasma cells [15]. This is illustrated by lower antibody titers induced by vaccination in infants than those observed later in life [10,16,17]. While somatic hypermutation is already functional at birth [18], the selection of B cell producing antibody with high avidity is acquired gradually with age [10,12,15,20].

Many studies have reported on antibody avidity for pertussis antigens [21–25] and tetanus toxoid [10,26], before and after infant immunization, and showed that avidity rises with the number of vaccine doses [21,22,24] and with increasing age [25]. In the context of pregnancy vaccination, Abu Raya *et al.* previously reported a higher antibody avidity to PT in cord samples of newborns of Tdap immunized women during pregnancy, than in newborns of unimmunized women (with very low anti-PT IgG titers) [23]. In addition, they found significantly higher anti-PT avidity in newborns when maternal vaccination was given at 27-30 weeks rather than beyond 30 weeks of gestation [23]. The latter results were not in accordance with our own findings [27] as we found no correlation between avidity and gestational age at vaccination, neither in maternal nor in cord samples.

As far as we know, this is the first report analysing the impact of maternal immunization on avidity of infant antibodies in response to a booster hexavalent acellular pertussis containing vaccine. We have used a convenience number of samples from our previously published studies [4,5] to assess the avidity of vaccine-induced IgG antibodies to diphtheria toxin (DT), tetanus toxin (TT), pertussis toxin (PT), filamentous hemagglutinin (FHA) and pertactin (Prn)

96 before and one month after the fourth vaccine dose at 15 months of age, in infants born to
97 women immunized with a Tdap vaccine during pregnancy or to unimmunized women.

Materials and methods

Study population and serum samples

The present study is a spin-off of our previously reported clinical trial in Belgium on Tdap vaccination during pregnancy (Clinicaltrials.gov identifier: NCT01698346) [4,5]. Healthy pregnant women were either vaccinated with the Tdap vaccine (Boostrix®, GSK Biologicals, Rixensart, Belgium) between 22 and 33 (mean gestational age 28.6) weeks of gestation (vaccine group) or received no vaccine (control group) and were followed until vaginal delivery (80% of women). Their infants, born between April 2012 and April 2014, were vaccinated with the hexavalent vaccine (Infanrix hexa®, GSK Biologicals, Rixensart, Belgium) according to the national 8, 12, 16 weeks priming schedule and the fourth dose at 15 months. The original study included 55 infants in the vaccine group and 26 infants in the control group. The remaining available serum samples (46 and 24 at pre-dose 4; 46 and 23 at post-dose 4 in the vaccinated and control group respectively) collected in infants before and one month after the fourth vaccine dose were used to answer the present study question. Extended clinical information can be found in our previous publications [4,5]. No significant differences in demographic characteristics between both study groups were encountered.

Avidity assay

Antibody avidity was measured with the same commercial IgG ELISAs as in our previous studies [4,5] i.e. (Virion/Serion® for anti-PT, Euroimmun® for anti-FHA and anti-Prn, Virotech/Sekisui® for anti-DT and anti-TT) using 1.5M ammonium thiocyanate (NH_4SCN, Sigma-Aldrich 221988, St Louis, MO) as dissociating agent according to a well-established method [21]. Briefly, the ELISA was performed in duplicate wells for each antigen, using the same diluted serum sample either left untreated or treated with NH_4SCN. For each antigen,

100µl of serum sample dilution (1/101), standards and control sera were incubated in a pre-coated well according to manufacturers' instructions. After incubation and subsequent washing, 100µL of a 1.5M NH_4SCN solution or PBS was added to the appropriate wells. The plate was incubated for 20 min at 37°C, prior to washing and adding the conjugate solution, followed by the substrate and the stop solution.

IgG concentrations with or without NH_4SCN treatment were expressed in international unit IU/mL for all antibodies. The relative avidity index (RAI) was expressed as the percentage between the IgG concentration of the sample treated with NH_4SCN and the untreated sample. Using the same criteria as Almanzar *et al,* a RAI < 40% was considered as low, a RAI between 40% and 60% considered as moderate and a RAI > 60% as high [21].

The analytical performance of the avidity test was optimized using different concentrations of NH_4SCN ranging from 0 M to 3 M. Five serum samples (diluted 1:101) collected at delivery from women immunized with Tdap were treated for 20 min with NH_4SCN at 37°C, the concentration reducing the anti-PT IgG level by 50% was determined to be 1.5M and this concentration was subsequently used for all avidity analyses. Hendrikx *et al* previously used NH_4SCN concentrations in the same range to test the avidity of anti-PT (1M) and anti-Prn (1.5M) antibodies in Dutch children [28].

Laboratory personnel was not blinded to the sample allocation but an external validation for the ELISA was performed by the Canadian Center for Vaccinology in Halifax, Canada and a positive correlation ($p<0.001$) between both labs was found [4].

Statistical analyses

Geometric means RAI were calculated with their 95% confidence intervals. Data distribution was assessed by One-Sample Kolmogorov-Smirnov test for normality. The differences

between pre- and post-fourth vaccine dose were tested by paired t-test or alternatively by Wilcoxon matched-pair signed-rank test. The two infants' groups were compared by parametric unpaired t-test or its nonparametric alternative Mann-Whitney U test. Pearson correlations were used to analyse the relationship between antibody titers and RAI. All tests were two-sided. A statistically significant difference was considered with a p-value < 0.05 and GraphPad Prism version 6 was used to analyse data.

As described before [5], multiple linear regression models were used to identify mother and child variables that could potentially influence the RAI at the different time points. Only significant results were reported.

Results

Antibody avidity in infants in pre- and post-fourth vaccine dose

In order to find out whether the fourth vaccine dose affected antibody avidity, we tested this parameter in an adapted ELISA with 1.5 M NH_4SCN as dissociating agent. Prior to the fourth vaccine dose, mean antibody avidity in both groups was moderate for PT, FHA, Prn and TT (RAI between 40% and 60%) and low for DT (less than 40%), with no significant difference between the two groups (Table 1 and Figure 1). At post-dose 4, antibody avidity increased significantly for all antibodies in the control group **(Figure 1**), albeit the increase in RAI of DT-specific antibodies was very modest (25.8% pre-dose 4 cfr. 31.8% post-dose 4). In the vaccine group, avidity of antibodies increased significantly for TT, PT and Prn ($p<0.001$) but not for DT ($p=0.31$) and FHA ($p=0.11$) (Figure 1). After the fourth dose, antibody avidity to TT, PT, FHA and Prn was comparable in both groups, but the avidity to PT was significantly lower in the vaccine than in the control group ($p<0.003$) (**Table 1**). Overall antibody avidity was high against TT, PT and Prn, moderate against FHA and low against DT (Table 1 and Figure 1).

Parameters influencing antibody avidity

There was no influence of gestational age on the antibody avidity of infant antibodies measured right before or one month after the fourth vaccine dose. However, a detailed regression analysis of antibody avidity and several other parameters showed for the infants of the vaccine group, a significant decrease in RAI of anti-TT antibodies at pre-booster (month 15) with increasing interval between dose 3 (at 16 weeks of age) and pre-dose 4 blood sampling ($p=0.025$). In the vaccine group, avidity of infant anti-Prn antibodies at post-dose 4

(month 16) was influenced significantly by the age of the mother at the time of delivery (p=0.011), avidity increasing with increasing age of the mother.

In the infants of the control group **(but not in the infants of the vaccine group)**, avidity of anti-DT antibodies at month 15 (pre-booster) increased significantly (p=0.009) with increasing interval between vaccine dose 3 and the month 15 blood sampling. In the infants of the control group, age of the mother at the moment of the delivery also influenced significantly the antibody avidity at month 16 against Prn (p=0.032) and FHA (p=0.029), avidity increasing with increasing age of the mother. Finally, a significant influence of the interval between vaccine dose 4 and the post-dose 4 blood sampling (p=0.019) was found, avidity against TT increasing with increased time interval.

Correlation between IgG titer and avidity

We have previously reported vaccine-specific IgG antibody titers in this cohort, in the form of geometric mean concentrations [5]. Comparing the individual antibody (Supplementary Figure S1) and RAI results, Pearson correlation coefficients were calculated. Before the fourth vaccine dose, negative correlation coefficients were found between RAI and antibody levels in the vaccine group for FHA (-0.165, NS), Prn (-0.424, p=0.003) and TT (-0.332, p=0.024) and positive coefficients for PT (0.497, NS) and DT (0.228, NS) respectively (Table 2). In the control group, a negative correlation was found for TT (-0.125, not significant NS) and a positive for DT (0.930 NS), PT (0.343, NS), FHA (0.412, p=0.045) and Prn (0.433, p=0.034). At post-dose4, RAI correlated positively with antibody level in both study groups for all five antigens (Table 2). In the vaccine group, the following Pearson correlation coefficients were found: PT (0.157, NS), Prn (0.496, p=0.001), FHA (0.738, p<0.001), DT (0.581, p<0.001) and TT (0.395, p=0.007). In

the control group, the positive correlation coefficients were for PT (0.287, NS), Prn (0.864, p<0.001), FHA (0.850, p<0.001), DT (0.447, p=0.032) and TT (0.809, p<0.001).

Evolution of avidity and antibody titer to PT from delivery up to 1 month after the fourth vaccine dose in the vaccine group.

In a pilot study, we previously analysed in the vaccine group, the PT specific antibody titer and avidity in serum from women at delivery and in the umbilical cord [27]. Figure 2 shows these results (obtained using the same ELISA and avidity testing protocol) together with the avidity and IgG levels in infants before and after the fourth vaccine dose. At delivery, avidity was moderate in women (serum) and newborns (cord blood) (geometric mean of RAI of 51.9% and 46.9% respectively) and was significantly higher in women than in their infants (paired t-test, p<0.0001), while geometric mean concentration was higher in infants' cord blood (101 IU/mL) than in mothers' serum at delivery (31.4 IU/mL). The reason why avidity of anti-PT antibodies was higher in mothers than in infants at time of birth is not clear, but may be related to the fact that antibody avidity of cord blood results from *continuous* transplacental transfer during the second and third trimester of pregnancy, whereas avidity in serum of mothers at delivery measures only the *endpoint* of a progressive maturation after the maternal booster vaccination.

In infants, anti-PT antibody avidity was moderate (55.4%) prior to the fourth vaccine dose (significantly higher (p< 0.0001) than in cord but not significantly different from maternal antibody avidity (p= 0.1379), and increased to RAI of 68 % after the fourth vaccine dose. **Avidity of infant anti-PT antibodies post fourth vaccine dose was significantly higher than avidity of maternal and cord anti-PT antibodies (p<0,0001). In infants, anti-PT IgG level at month 15 was low (5.4 IU/mL) and one month later mean anti-PT IgG level had increased to**

36.3 IU/mL. This infant anti-PT level at month 16 was not statistically different from maternal anti-PT IgG level (p=0.9315).

Discussion

To evaluate the efficacy of a vaccine, it is important to consider antibody levels as well as antibody avidity [29]. In this study, we used an elution ELISA modified by a chaotropic agent to assess the avidity of specific antibodies to PT, FHA, Prn, DT and TT in infants born to vaccinated and unvaccinated mothers. The choice of the chaotropic agent is a critical issue when evaluating antibody avidity [24,24]. A number of reports have measured avidity with different chaotropic agents [21–24,30] and NH_4SCN proved to be easy to use in a modified ELISA assay and convenient to measure the strength of antigen-antibody complex [21]. In our study we used a 1.5 M concentration of NH_4SCN to determine the relative avidity index RAI for all five vaccine antigens and we used criteria defined by Almanzar *et al.* for interpretation [21]. Although these cut-off values defining low (RAI < 40%) and high (RAI > 60%) avidity are arbitrary to some extent, as the results depend on the assay conditions [21], within a defined setting these criteria enable a monitoring of the avidity of the same antibody specificity over time. Also, use of 1.5M NH_4SCN allowed us to discriminate between the different vaccine antigens, and in particular diphtheria-specific antibodies were found to remain of low avidity in both infant groups, despite the fact that antibody concentrations increased dramatically after the fourth vaccine dose. Interestingly, avidity of anti-DT antibodies increased significantly (p=0.009) with increasing interval between vaccine dose 3 and the pre-dose 4 blood sampling in the control group, indicating that in this infant population, age is an important factor for antibody maturation (independent of vaccine dose).

In this spin-off study of a previously reported clinical trial in Belgium on Tdap vaccination during pregnancy, we assessed antibody avidity in infants born from Tdap immunized women during pregnancy (vaccine group) before and 1 month after a fourth hexavalent vaccine dose as compared to infants from unimmunized women during pregnancy (control group). In a previous pilot study limited to pertussis toxin only, we found that antibodies in cord blood of infants from the vaccine group had a moderate avidity i.e. 46.9% [27], whereas Abu Raya *et al*. reported a higher avidity (RAI= 73.8%) in cord blood samples after maternal vaccination [23,31]. The higher RAI values of the Israeli study may have been the result of differences between the maternal cohorts in terms of priming and natural exposure to *Bordetella pertussis*, or in the time of vaccination during pregnancy [32,33]. More likely, differences in laboratory technique used to determine the relative avidity may explain the discrepancy. Indeed, in contrast to Abu Raya *et al*. who used 0.25M of NH_4SCN [23], we used a 6-fold higher concentration of 1.5 M NH_4SCN, concentration also used in previous studies by Almanzar *et al.* and Prelog *et al.* [21,22]. As the avidity index is indeed a relative value, depending on the NH_4SCN concentration used, RAI values will vary in function of the precise assay conditions and can only be compared with other values generated under the same conditions.

All currently used infant subunit vaccines delivered before six months of age need a series of vaccine doses to elicit protection [34]. In the present study, a significant increase in antibody avidity was found in the control group for all five vaccine-induced antibodies after the fourth vaccine dose, albeit that the increase in avidity of DT specific antibodies was very modest and resulting avidity remained low (RAI 31.8%). Moreover, as expected after re-exposure of the same antigen to a primed immune system [21], no increase of RAI was observed for FHA and DT in the vaccine group, with avidity remaining moderate for FHA (50.5%) and low (28.6%) for DT specific antibodies. Furthermore, although avidity of anti-PT antibodies increased to high

after the fourth vaccine dose in all study groups, it was significantly lower (p=0.003) in infants from the maternal vaccine group compared to those from the control group. The clinical relevance of this blunting effect is not clear since there are unfortunately no protective antibody levels known for pertussis, i.e. no threshold antibody (nor avidity) levels [6].

Blunting of the antibody response in infants to primary infant vaccination has been described by others, and by our group, after maternal Tdap vaccination during pregnancy [4,5,35–37]. This blunting of pertussis immune responses was reported to resolve with a fourth infant vaccine dose [35,36], although we have still observed interference for PT-specific antibodies after the fourth vaccine dose [5] as well as for their avidity. Diphtheria-specific IgG antibody response was also found to be lower in the vaccine group than in the control group at week 20 after the first three immunizations (p=0.002) [4] and at month 16 after the fourth vaccination dose (p=0.023) [5]. Ladhani *et al* have described a similar interference of maternal vaccination on DT-specific antibodies and on some of the serotype specific responses to the pneumococcal CRM (a naturally occurring diphtheria toxin variant) - conjugated vaccines [36] and suggested that the administration of a diphtheria containing vaccine in pregnancy may influence the CRM-197 conjugated vaccine responses in infants. In that view, the present results showing adequate IgG antibody responses to infant diphtheria toxoid, yet low avidity of the induced antibodies could be explained by an interference by the maternal Tdap vaccination as well.

Full protection against diphtheria is achieved when IgG anti-DT titers are above 0.1 IU/ml [38]. All infants in our study had anti-DT IgG titers > 0.1 IU/ml and were sufficiently protected at month 15 prior to the fourth vaccine dose. However, RAI of anti-DT antibodies was low (in both groups) and remained so after the fourth vaccine dose. Booy *et al.* [39] and Tiru *et al.*

[40] have reported on widening of the 3 DTP primary dose intervals and showed that antibody responses to three doses were lower following a shorter 8-12-16 week schedule than following a 8–16–24 week or a 3–5–12 months schedule respectively. With the rapid vaccination schedule, most infants failed to have good response to DT, which is a relatively weak vaccine antigen [39,40]. To our knowledge, avidity of the anti-DT antibodies was not tested in these previous studies, but a comparison of anti-DT avidity in large cohorts of infants vaccinated with these different vaccination schedules would certainly be interesting.

Paediatric vaccination provides adequate protection against diphtheria in infants and adolescents, but a significant susceptible population with low anti-DT levels was reported in Europe among adults and the elderly [41]. With a good vaccination coverage, diphtheria infections could be asymptomatic or less severe, but, as for pertussis, asymptomatic carriers of toxin-producing strains may contaminate unvaccinated or incompletely vaccinated infants [42]. A sad example was the recent death of an unvaccinated child this year in Belgium [43].

After the fourth vaccine dose, antibody levels increased significantly in both groups and this increase correlated positively with increases in RAI in both study groups ($p < 0.05$), even if the correlation was not significant for PT. Before the fourth vaccine dose however, RAI in the vaccine group correlated negatively with the antibody levels for Prn and TT, showing that high antibody levels do not necessarily mean high avidity and *vice versa*. In the vaccine group, regression analysis showed a significant influence of the interval between the third vaccine dose and the pre-fourth dose blood sampling on the avidity of tetanus-specific antibodies ($p=0.025$) at month 15, the TT RAI decreasing with increasing interval. In contrast, in the control group, avidity of DT-specific antibodies increased significantly ($p=0.009$) with increasing interval between the third vaccine dose and the pre-fourth dose blood sampling. It

317 is not clear for the moment why these correlations were different between the two study
318 groups. Another interesting observation was the fact that the age of the mother at delivery
319 influenced antibody avidity of Prn-specific antibodies measured at month 16 in infants of both
320 groups. Obviously, analysis of larger cohorts of infants would be needed to confirm these
321 observations.

322 Throughout the first 2 years of life, there is a gradual acquisition of immune competence and
323 a progressive affinity maturation of IgG antibodies after immunization [44]. For pertussis
324 toxin, Prelog *et al.* have reported on moderate and high avidity of anti-PT antibodies
325 respectively before and after the adolescent booster vaccine dose [22]. The infants in our two
326 study groups also had moderate avidity antibodies against PT before the fourth dose (55.4%
327 and 59.6 % respectively in the vaccine and control group) which increased to high avidity
328 (68.1% and 78.6%) after the fourth vaccine dose, indicating a good priming of B-cell memory
329 [22] to PT in both groups and providing evidence of additional avidity maturation with the
330 number of vaccine doses. **For technical reasons, serum samples could not be tested for**
331 **antibody avidity after the completed primary vaccination at month 5, and therefore**
332 **comparison with antibody avidity at month 15, prior to the fourth vaccine dose, and analysis**
333 **of the impact of increasing age on avidity maturation, as reported by Ibrahim *et a*l [25] could**
334 **not be performed.**

335 In conclusion, among the five vaccine antigens, PT and TT specific antibodies increased to the
336 highest avidity, although there was a minor, but significant, negative effect of maternal
337 vaccination on the PT-specific avidity as observed with antibody level [4,5]. Unfortunately, no
338 correlates of protection exist for pertussis. More specifically, the relative importance of anti-
339 PT IgG antibodies for protection against pertussis is not clear and could be questioned on the

basis of the low and rapidly decaying antibody responses, induced after vaccination [45] and the high anti-PT titers detected in pertussis patients. On the other hand, higher pertussis antibodies in general have been associated with enhanced protection against disease [45–47]. Further analysis of the bactericidal properties and opsonizing potential of pertussis-specific antibodies induced in the context of maternal immunization is needed to clarify whether the minor blunting of PT-specific infant immune responses and of avidity of induced antibodies, related to maternal vaccination has any clinical relevance.

Limitations of the study

This study was limited by the reduced number of infants in the control group, making strict randomization impossible. Another limitation is the fact that serum samples could for practical reasons not be tested for avidity at month 5 after the first three vaccine doses. Laboratory personnel was not blinded to the sample allocation.

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Conflict of interest

Authors do not have any conflict of interest.

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Legend to the Figures and Tables

Figure 1: Distribution of Relative Avidity Index (RAI %) in infant samples before (pre, black symbols) and 1 month after (post, blue symbols) the fourth vaccine dose. Vaccine group = infants born from mothers immunized with Tdap vaccine during pregnancy. Control group = infants born from non-immunized mothers during pregnancy. Black lines indicate the geometric mean of RAI and blue dotted lines represent the avidity classification areas (RAI < 40% was considered as low, a RAI between 40% and 60% considered as moderate and a RAI > 60% as high). P-values show the statistical significance comparing the pre- and the post-dose 4 responses for all 5 antigens in both groups.

Figure 2: Geometric mean concentration of IgG (IU/mL) and geometric mean of relative avidity index (%) of PT-specific antibodies, at delivery in women immunized with Tdap vaccine during pregnancy (Maternal) and in their infants at birth (Cord), prior to the fourth vaccine dose (Vaccine pre) and one month after the fourth vaccine dose (Vaccine post) at 15 months of age.

Supplementary Figure S1: Distribution of the five vaccine-induced IgG antibodies (IU/ml) responses in infants before (pre, black symbols) and 1 month after (post, blue symbols) the fourth vaccine dose. Vaccine group = infants born from mothers immunized with Tdap vaccine during pregnancy. Control group = infants born from non-immunized mothers during pregnancy. Black line indicates the geometric mean concentration. P-values show the statistical differences between groups.

Table 1: Geometric Mean (GM) of Relative Avidity Index (RA%I) with 95% confidence interval (CI) expressed in percentage (%) for IgG antibodies against PT, FHA, Prn, DT and TT and p-values comparing the two study groups of infants either before or one month after the fourth vaccine dose. Differences between both groups were not statistically different except for RAI of anti-PT antibodies after the fourth vaccine dose (p = 0.003).

Table 2: Correlations between IgG titer (IU/ml) and Relative Avidity Index (% RAI) before (pre-dose 4) and 1 month after (post-dose 4) the fourth vaccine dose. Vaccine group = infants born from mothers immunized with Tdap vaccine during pregnancy. Control group = infants born from non-immunized mothers during pregnancy. R^2: Pearson correlation coefficient.

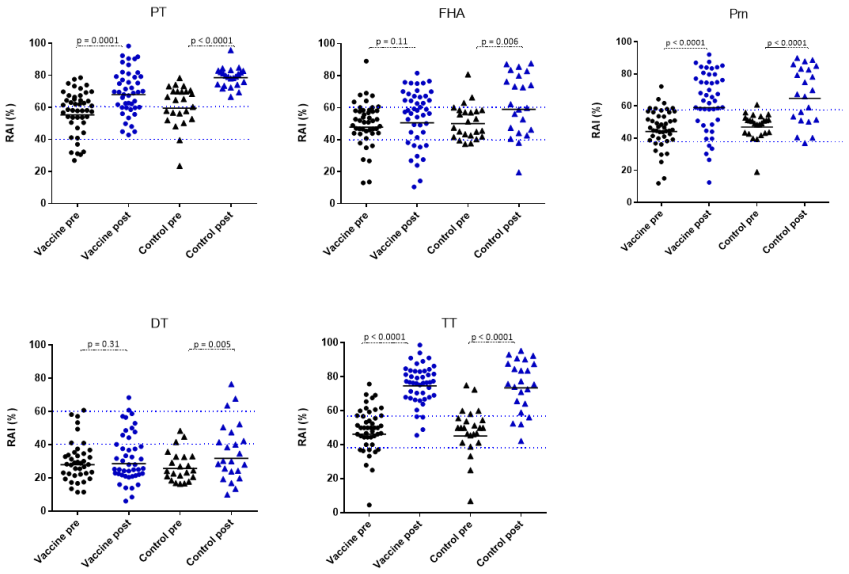

Figure 1: Distribution of Relative Avidity Index (RAI %) in infant samples before (pre, black symbols) and 1 month after (post, blue symbols) the fourth vaccine dose. Vaccine group = infants born from mothers immunized with Tdap vaccine during pregnancy. Control group = infants born from non-immunized mothers during pregnancy. Black lines indicate the geometric mean of RAI and blue dotted lines represent the avidity classification areas (RAI < 40% was considered as low, a RAI between 40% and 60% considered as moderate and a RAI > 60% as high). P-values show the statistical significance comparing the pre- and the post-dose 4 responses for all 5 antigens in both groups.

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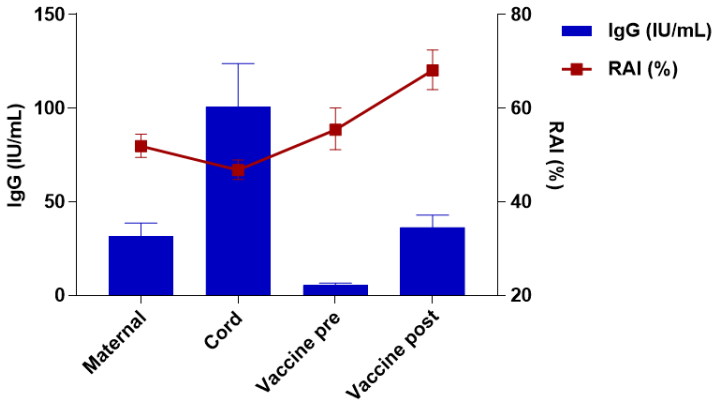

Figure 2: Geometric mean concentration of IgG (IU/mL) and geometric mean of relative avidity index (%) of PT-specific antibodies, at delivery in women immunized with Tdap vaccine during pregnancy (Maternal) and in their infants at birth (Cord), prior to the fourth vaccine dose (Vaccine pre) and one month after the fourth vaccine dose (Vaccine post) at 15 months of age.

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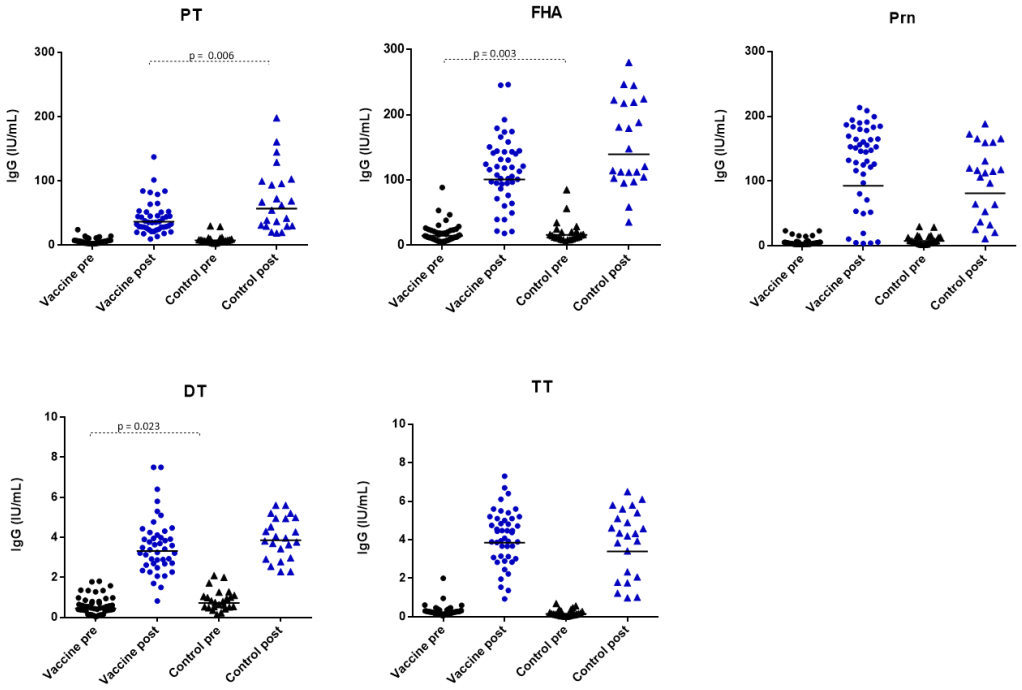

Supplementary Figure S1: Distribution of the five vaccine-induced IgG antibodies (IU/ml) responses in infants before (pre, black symbols) and 1 month after (post, blue symbols) the fourth vaccine dose. Vaccine group = infants born from mothers immunized with Tdap vaccine during pregnancy. Control group = infants born from non-immunized mothers during pregnancy. Black line indicates the geometric mean concentration. P-values show the statistical differences between groups .

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RAI (%) GM (95% CI)	Before the fourth vaccine dose		1 month after the fourth vaccine dose	
	Vaccine group	Control group	Vaccine group	Control group
N	46	24	45	23 (22 for FHA and Prn)
Pertussis toxin (PT)	55.40 (51.14-60.01)	59.64 (53.48-66.52)	68.06 (63.98-72.41)	78.65 (76.04-81.36)
p-value	0.201		0.003	
Filamentous hemagglutinin (FHA)	47.82 (43.04-53.13)	50.13 (46.05-54.57)	50.51 (44.32-57.57)	58.94 (50.06-69.39)
p-value	0.761		0.092	
Pertactin (Prn)	44.13 (39.94-48.76)	46.89 (42.68-51.52)	59.05 (52.56-66.34)	64.82 (57.18-73.49)
p-value	0.582		0.347	
Diphtheria toxoid (DT)	27.97 (24.54-31.86)	25.76 (22.30-29.75)	28.59 (24.54-33.30)	31.82 (25.44-39.80)
p-value	0.301		0.426	
Tetanus toxoid (TT)	46.19 (40.75-52.24)	45.12 (37.09-54.88)	74.68 (71.14-78.39)	73.46 (66.76-80.83)
p-value	0.880		0.880	

Table 1: Geometric Mean (GM) of Relative Avidity Index (RA%I) with 95% confidence interval (CI) expressed in percentage (%) for IgG antibodies against PT, FHA, Prn, DT and TT and p-values comparing the two study groups of infants either before or one month after the fourth vaccine dose. Differences between both groups were not statistically different except for RAI of anti-PT antibodies after the fourth vaccine dose (p = 0.003).

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Antibody	Vaccine pre-dose4		Vaccine post-dose4		Control pre-dose4		Control post-dose4	
	R^2	p-value	R^2	p-value	R^2	p-value	R^2	p-value
PT	0.497	0.000 **	0.157	0.30 NS	0.343	0.10 NS	0.287	0.18 NS
Prn	-0.424	0.003 **	0.496	0.001 **	0.433	0.034 *	0.864	0.000 **
FHA	-0.165	0.27 NS	0.738	0.000 **	0.412	0.045 *	0.850	0.000 **
DT	0.228	0.16 NS	0.581	0.000 **	0.93	0.68 NS	0.447	0.032 *
TT	-0.332	0.024 *	0.395	0.007 **	-0.125	0.56 NS	0.809	0.000 **

Table 2: Correlations between IgG titer (IU/ml) and Relative Avidity Index (% RAI) before (pre-dose 4) and 1 month after (post-dose 4) the fourth vaccine dose. Vaccine group = infants born from mothers immunized with Tdap vaccine during pregnancy. Control group = infants born from non-immunized mothers during pregnancy. R^2: Pearson correlation coefficient.

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