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Transplacental transport of IgG antibodies specific for Pertussis, Diphtheria, Tetanus, HiB and MenC is lower in preterm compared to term infants

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Abstract

Background: Maternal antibodies, transported through the placenta during pregnancy, contribute to the protection of infants from infectious diseases during the first months of life. The aim of this study was to measure the concentration of antibodies against several vaccine-preventable diseases in paired maternal and cord blood serum samples in preterm and term infants and to assess placental transfer ratios and infant antibody concentrations against vaccine-preventable diseases.

Methods: Antibody concentrations specific against pertussis proteins (pertussis toxin, filamentous hemagglutinin, pertactin, and fimbriae), diphtheria and tetanus toxins, and antibody concentrations specific against polysaccharides from *Haemophilus influenzae* type b and *Neisseria meningitides* serogroup C were measured in cord blood samples from preterm (<32 weeks and 1500 g) and term infants and maternal serum samples, using afluorescent bead-based multiplex immunoassay.

Results: A total of 96 preterm and 42 term infants and their mothers were included in the study. Placental transfer ratios of antibodies against all vaccine antigens were significantly lower in preterm infants (medians varied from 0.26 to 0.86) compared with term infants (medians, 0.74 –1.89; all antibodies $P < 0.05$). Furthermore, polysaccharide-vaccine–specific antibodies showed lower transplacental transport ratios than protein-vaccine–specific antibodies. Maternal concentrations are the most important determinants of infant antibody concentrations against vaccine-preventable diseases.

Conclusions: Preterm infants benefit to a lesser extent from maternal antibodies against vaccine-preventable diseases than term infants, posing them at higher risk for infectious diseases in the first months of life.

Introduction

Newborn infants, especially preterm infants, have an immature immune system, which is not capable of protecting them actively against vaccine-preventable infections such as diphtheria, tetanus, pertussis, *Haemophilus influenzae* type b (Hib) and *Neizzeria meningitides* serogroup C (MenC).[32-34] Maternal immunoglobulin G (IgG) is transported across the placenta (transplacental transport) by an active, receptor-mediated process during pregnancy thereby protecting term infants against infections.[25] In general, higher IgG concentrations are thought to be associated with longer protection. Previous studies showed that the degree of transplacental transport of IgG is dependent on the duration of the gestation.[26-28] In the first trimester, a small amount of IgG is transported to the fetus.[29,30] The fetal IgG is ~10% of the maternal concentration at 17-22 weeks of gestation, whereas it rises to 50% at 28-32 weeks of gestation as determined by chordocentesis.[25,31] The increase in fetal IgG concentrations between 29 and 41 weeks of gestation is twice that at 17 to 28 weeks of gestation.[30]

Besides gestational age (GA), maternal IgG antibody titer is an important predictor of the neonatal IgG antibody titer, as found in the study of van der Zwet et al.[90] In term infants, the IgG antibody concentration at birth is usually higher than the maternal IgG antibody concentration.[30,115]

In the Netherlands, preterm infants are recommended to be vaccinated according to the same vaccination schedule as term infants, regardless of prematurity. In the Dutch National Immunization Programme, diphtheria, tetanus, pertussis/Hib/inactivaded polio (DTaP-Hib-IPV) vaccines are administered at 2, 3, 4 and 11 months (booster) of life. Extra DTaP-IPV and DT-IPV boosters are administered at 4 and 9 years and Men C vaccine is administered at 14 months of age. Most of the mothers in this study have followed the regular Dutch National Immunization Programme including whole-cell pertussis, but they were not vaccinated with Hib, MenC and acellular pertussis because these vaccinations were only implemented in 1993 (Hib), 2002 (MenC) and 2005 (acellular pertussis). Any antibodies against Hib and MenC found in these mothers must therefore be naturally acquired.

Before acquiring antibodies evoked by vaccination starting at 2 months of life, infants are predominantly protected by maternal IgG obtained during pregnancy. The concentrations of the IgG subclasses in the fetus are not equally distributed.[77] IgG1 and IgG4 are transported more efficiently than IgG3, and IgG2 is the least efficiently transported.[78] Vaccination with protein vaccines elicit mainly IgG1 and IgG3 antibodies whereas polysaccharide vaccines (like MenC and Hib) elicit mainly IgG2 antibodies.[80-83] This would imply that as a result of preferred placental transport of IgG1 over IgG2 the infant would benefit most from proteinvaccine-derived maternal antibodies.

The data about the antibody concentrations at birth of preterm infants with a GA <32 weeks are limited. We hypothesize that IgG antibody concentrations against diphtheria,

tetanus, pertussis, Hib and MenC are lower in preterm infants than in term infants, and that the maternal antibody concentration for these vaccine-preventable infections is a major determinant for the antibody concentrations in infants at birth. Therefore, the aim of this study was to measure the concentration of antibodies against these infectious diseases in mothers and their offsprings with GA <32 weeks or birth weight (BW) <1500 g and in healthy term infants.

Methods

Study population

Preterm infants with a GA <32 weeks and/or BW <1500 g born and their mothers at the VU University Medical Center were eligible for this study. Term infants (GA >37 weeks) born at the VU University Medical Center and their mothers served as controls. The study protocol was approved by the local Medical Ethical Committee. This study was an addendum to the CARROT-study (immune effects of neutral and acidic oligosaccharides in preterm infants), registered as ISRCTN16211826.[116] Written informed consent was obtained from mothers of all infants.

Laboratory analysis

We obtained cord blood from the placenta directly after delivery. If cord blood could not be obtained, blood from the infants was obtained within 48 hours after delivery. Maternal blood was obtained by venous sampling between 2 days before and 2 days after delivery. Serum samples were centrifuged and stored at –80ºC until analysis.

Antibody concentrations in the serum samples were analyzed as previously described by Van Gageldonk et al.[117] In short, serum samples were tested for antibodies to the pertussis vaccine components, pertussis toxin (Ptx, filamentous hemgglutinin (FHA), pertactin (Prn), fimbriae(Fim) and to Dtx and Ttx using a fluorescent bead-based multiplex immunoassay (Luminex xMAP technology). Analysis was performed with a Bio-Plex 200 in combination with Bio-Plex manager software (Bio-Rad Laboratories, Hercules, CA).[117,118] Serum samples were also tested for antibody concentrations against MenC and Hib in a similar multiplex immunoassay as previously described by de Voer et al.[119]

Data analysis

GA, BW and maternal age are described as median and range. For statistical analysis, antibody concentrations below the lower limit of quantitation were assigned as half the lower limit of quantitation (0.001 IU/mL for Dtx and Ttx, 0.01 µg/mL MenC and Hib, and 1 EU/mL for Ptx, FHA, Prn and Fim). All IgG antibody concentrations were expressed as geometric mean concentrations (GMCs) with 95% confidence intervals (CIs). Student *t* test was used

to compare the specific antibody concentrations between preterm and term infant serum samples after natural logarithmic transformation. Placental transfer of IgG antibodies to the vaccine components was defined as the ratio between each individual paired infant and maternal serum sample. In addition, the overall ratio for each antigen was defined as the geometric mean of the individual ratios.

International assigned protective concentrations were used to determine the percentage of mothers and infants with protective IgG concentrations (anti-Dtx and anti-Ttx ≥ 0.01 IU/mL, anti-Hib ≥0.15 µg/mL, MenC ≥ 2 µg/mL). Protective concentrations for anti-Ptx are not internationally assigned, but an arbitrary cut-off value of ≥20 EU/mL was used as previously described.[120,121] A Pearson's correlation was performed to determine the maternal IgG antibody concentration in relation to infant IgG antibody concentration. A multiple linear regression analysis was performed to determine the influence of GA, BW, maternal IgG antibody titer, and maternal age on infant GMCs and transplacental transport ratio. For all statistical analyses, a 2-sided p value of <0.05 was considered significant. SPSS 15.0 (SPSS Inc., Chicago, IL) was used for data analysis.

Results

Study participants

Between April 2007 and December 2008, 96 preterm infants and 42 term infants and their mothers were included in this study.

In the preterm group, median GA was 29.7 weeks (25.0-32.7) and median BW was 1235 g (500-2240). Of these 96 preterm infants, 26 infants had a GA <28 weeks. In the term group, median GA was 38.9 weeks (36.7- 41.3) and median BW was 3421 g (2370-5070). Maternal age was lower in the preterm group than in the term group: 32.0 years (19-41) and 34.2 years (21-43), respectively (P = 0.009). In the preterm group, 57% was born by vaginal delivery and 43% by cesarean section whereas in the term group 29% was born by vaginal delivery and 71% by cesarean section. In the preterm group, 81 cord serum samples, 10 arterial serum samples, 3 capillary serum samples and 2 venous serum samples were collected, and in the term group 42 cord serum samples have been collected. Because type of blood sample did not affect results, all blood samples were included in the analysis.

Seroprevalence of Dtx, Ttx, Ptx, FHA, Prn, and Fim protein-vaccine-specific IgG in maternal and infant serum samples

GMC's 95% CI, and ranges of anti-Dtx, anti-Ttx, anti-Ptx, anti-FHA, anti-Prn and anti-Fim antibodies in maternal and infant serum samples are summarized in Table 4.1. Placental transfer ratio for the antibodies specific against protein vaccines of Dtx, Ttx, Ptx, FHA, Prn and Fim was lower in preterm infants (0.53, 0.83, 0.64, 0.65, 0.66 and 0.69, respectively)

Table 4.1. Number of samples tested, geometric mean concentration (GMCs) and transplacental transport ratios of antibodies to diphtheria (Dtx), tetanus (Ttx), pertussis toxin (Ptx), filamentous hemagglutinin (FHA), pertactin (Prn), fimbriae (Fim), *Neisseria meningitidis* serogroup C (MenC), *Haemophilus influenza type b* (Hib) in preterm and term infants

	GMCs (95% CI) [range] and transplacental transport ratio				
	N	**Cord serum**	**N**	**Maternal serum**	**Ratio**
DTX (IU/ml) preterm	96	0.06 (0.04-0.07) [0.001-1.89]	88	0.11 (0.08-0.14) [0.01-3.66]	0.53[a]
DTX (IU/ml) term	42	0.09 (0.06-014) [0.005-1.55]	39	0.08 (0.05-0.13) [0.01-1.56]	1.18
TTX (IU/ml) preterm	96	0.70 (0.54-0.91) [0.01-8.65]	88	0.82 (0.63-1.06) [0.032-21.46]	0.86 [a]
TTX (IU/ml) term	42	1.23 (0.85-1.79) [0.04-8.14]	39	0.61 (0.39-0.94) [0.02-4.35]	1.89
PTX (EU/ml) preterm	96	5.36 (4.18-6.89) [1-92]	88	7.33 (6.03-9.90)[1-196]	0.64 [a]
PTX (EU/ml) term	42	6.20 (4.10-9.38) [1-136]	39	5.21 (3.48-7.81) [1-105]	1.30
FHA (EU/ml) preterm	96	9.80 (7.88-12.18) [1-140]	88	15.06 (11.95-18.98) [2-171]	0.65 [a]
FHA (EU/ml) term	42	14.14 (10.32-19.37) [1-101]	39	10.16 (7.32-14.13) [1-58]	1.37
Prn (EU/ml) preterm	96	3.18 (2.39-4.25) [1-2.86]	88	4.92 (3.65-6.62) [1-941]	0.66 [a]
Prn (EU/ml) term	42	4.68 (3.22-6.80) [1-71]	39	4.09 (2.77-6.06) [1-37]	1.20
Fim (EU/ml) preterm	96	8.72 (6.52-11.66) [1-462]	88	12.33 (8.97-16.95) [1-649]	0.69 [a]
Fim (EU/ml) term	42	10.05 (6.83-14.78) [1-66]	39	10.39 (6.35-16.99) [1-144]	1.12
Hib (µg/ml) preterm	95	0.15 (0.11-0.22) [0.01-27.56]	88	0.67 (0.47-0.95) [0.01-48.88]	0.26 [b]
Hib (µg/ml) term	40	0.28 (0.17-0.47) [0.01-9.66]	37	0.37 (0.22-0.63) [0.03-7.03]	0.74
MenC (µg/ml) preterm	93	0.08 (0.06-0.11) [0.01-18.88]	85	0.22 (0.16-0.32) [0.01-42.22]	0.38 [b]
MenC (µg/ml) term	42	0.11 (0.06-0.20) [0.01-9.88]	31	0.13 (0.07-0.25) [0.01-9.36]	0.87

NOTE. Maternal serum samples were obtained from mothers between 2 days before and after delivery and cord serum samples were obtained from umbilical cords. CI, confidence intervals; EU, enzyme-linked immunosorbent assay unit; IgG, immunoglobulin G; [a] $p<0.01$, [b] $p<0.05$

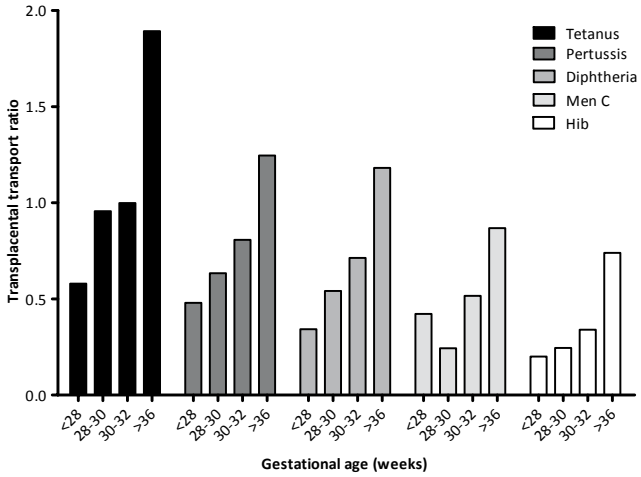

Figure 4.1. The placental transfer ratio of antibodies against the vaccine components plotted against the gestational age. Other Pertussis antigen were similar to Pertussis toxin.

than in term infants (1.18, 1.89, 1.30, 1.37, 1.20, and 1.12, respectively: all P <0.01) (Table 4.1). The median placental transfer ratio of all antibodies against the protein vaccines is 0.67 in preterm infants and 1.34 in term infants. Placental IgG transfer of antibodies against the protein vaccines was lower in preterm infants <28 weeks than in preterm infants >28 weeks (0.48 versus 0.77,P <0.05). The placental transfer ratio of antibodies to vaccine components plotted against the GA is shown in Figure 4.1.

Seroprevalence of MenC and Hib polysaccharide-vaccine-specific IgG in maternal and infant serum samples

GMC's 95% CI, and ranges of anti-MenC and anti-Hib antibodies in maternal and infant serum samples are summarized in Table 4.1. Placental transfer ratios for antibodies against the polysaccharide vaccines of Hib and MenC were lower in preterm infants (0.26 and 0.38, respectively) than in term infants (0.74 and 0.87, respectively, p<0.05) (Table 4.1, Figure 4.1). The median placental transfer ratios of the antibodies against the 2 polysaccharide vaccines is 0.32 in preterm infants and 0.81 in term infants. Placental transfer of antibodies against the 2 polysaccharide vaccines was not different in preterm infants <28 weeks and preterm infants >28 weeks. MenC vaccinated mothers (n=5) showed higher maternal MenC IgG GMCs than non-MenC vaccinated mothers (median 2.34 and 0.13, p<0.001). The infants (n=6, 1 twin) of MenC vaccinated mothers also showed higher MenC IgG GMCs than infants of non-MenC vaccinated mothers (n=90: median 0.54 and 0.05 respectively, P <0.001).

Determinants of infant GMC of the vaccine components

A strong correlation between maternal and infant antibody concentration was found for all measured antibodies against all vaccine components in both preterm infants (R^2: 0.49-0.98) and term infants (R^2: 0.56-0.96). Using a multiple linear regression model, the neonatal GMCs of antibodies against all vaccine components was predominantly determined by the maternal GMC, both in preterm (ß: 0.65-0.95, all P <0.001) and term (ß: 0.70-1.0, all P <0.001) infants. For preterm infants, the influence of GA on infants GMC against antibodies against all protein vaccine components was less strong compared with maternal GMC (ß: 0.09-0.30, P ≤0.05), and similar to the influence of BW on infants GMC (ß: 0.09-0.18, P ≤0.05, except for Dtx vaccine, ß:0.08, p:0.07). In preterm infants, GA and BW did not influence the GMC against the antibodies of the 2 polysaccharide vaccines. In term infants, GA and BW had no influence on the GMCs of antibodies against all vaccine components. Maternal age had no influence on the GMC of all vaccine components in both preterm and term infants.

Determinants of placental transfer ratio

The placental transfer ratio for antibodies specific for protein vaccines is influenced by GA (ß:0.36-0.62, all P ≤0.001) and BW (ß:0.29-0.51, all P ≤0.001). The placental transfer ratio for

antibodies specific for polysaccharide vaccines is less influenced by GA (Hib: ß:0.14, P = 0.12/ MenC ß:0.18, P = 0.04) or BW (Hib: ß:0.15, P = 0.11/MenC: ß:0.20, P = 0.03).

Protective antibody concentrations in maternal and infant serum samples

Protective antibody concentrations at birth are shown in Table 4.2. Low percentages of protective antibody concentrations were found for Ptx and MenC in mothers and infants. Four percent of preterm infants and 5% of term infants were protected against MenC. Mothers also had very low percentages of protection (11%) against MenC. High percentages of protective antibody concentrations for Ttx (100% protected), and Dtx (>95% protected) were found in mothers and infants.

Table 4.2. Percentage of preterm and term infants and their mothers at birth with protective concentrations of IgG to diphtheria toxin (Dtx), tetanus toxin (Ttx), pertussis toxin (Ptx), *Neisseria meningitidis* serogroup C (MenC) and *Haemophilus influenzae* type b (Hib)

Antibody	Protective concentration	Percentage with protective concentrations of IgG			
		Preterm		Term	
		Infant	Mother	Infant	Mother
Dtx	≥*0.01 IU/ml*	97	99	95	95
Ttx	≥*0.01 IU/ml*	100	100	100	100
Ptx	≥*20 EU/ml*	15	25	26	10
Hib	≥*0.15 µg/ml*	46	84	70	76
MenC	≥*2 µg/ml*	4	12	5	10

Discussion

In this study, we found significantly lower transplacental transport of IgG in preterm infants <32 weeks than in term infants for antibodies against diphtheria, tetanus, pertussis, Hib and MenC. Active transport of maternal antibodies is largely restricted to antibodies specific for protein vaccines both for preterm and term infants. This transport was significantly lower in preterm infants than in term infants, reflected by a placental IgG transfer ratio <1 in preterm infants <32 weeks and >1 in term infants.

For polysaccharide vaccines, the transplacental transport ratio was significantly lower in preterm infants than in term infants, and both ratios were lower than that for protein vaccine antibodies. This difference can be explained by the different IgG subclass response after administration of these vaccines. The transplacental transfer of IgG2 subclass antibodies, which is the main subclass induced by polysaccharides in the capsule from bacteria like Hib and MenC, is known to be less effective compared with other IgG subclasses.[77,78] However,

active transport of antibodies against polysaccharides is possible as Munoz et al[122] have shown for pneumococci after maternal vaccination.

The low antibody concentrations of protective IgG against pertussis in infants most certainly predict a susceptibility of the infants for this infection and, therefore, the majority of infants is unprotected against pertussis before the first vaccination.[123] In the Netherlands, epidemic episodes of pertussis occur every 3 years.[124] This is a threat for all infants who are not yet fully vaccinated, especially for the more vulnerable preterm infants with the most immature immune system.

The low percentages of protection against MenC (≥2 μg/mL) in mothers is of special concern, because the MenC vaccination is only administered at 14 months of life, and infants may have an increased risk to develop an infection before vaccination. Most of the mothers in this study are not vaccinated against MenC. The number of vaccinated mothers will increase in coming years. Mothers who already received their anti-MenC vaccination had higher IgG antibody concentrations against MenC, similar to their infants. For diphtheria and tetanus, high percentages of protection were observed. For Hib, 44% of the preterm infants showed protective antibody concentrations.

The increase in transplacental transport of IgG-antibodies elicited by protein vaccines with increasing GA and BW seems to evolve in a linear way. The results of our study are in line with Malek et al, who also showed a linear rise in IgG2 during pregnancy. [25] Although the rise of antibodies with increasing GA seems also linear for the polysaccharide vaccines, it was not significant. This might be partly explained by the different IgG subclass distribution induced by the different vaccines.

Some limitations of the study need to be addressed. First, it was not always possible to obtain cord blood from infants included in our study. In 15 of 96 cases, other blood samples were used for the IgG measurements. However, the difference in the type of blood sampling did not influence the results of the study. Second, the mothers of term infants showed significantly lower concentrations for antibodies directed against all vaccine components than mothers of preterm infants. The GMCs for antibodies against all vaccine components except for Prn of the preterm mothers were in line with the study of de Voer et al, who included a more representative Dutch study group from a general hospital.[120] The antibody concentrations of the term mothers were lower in our study than in the mothers in the general Dutch population.[120] Therefore, the difference in transported antibody concentration found between preterm and term infants in our study is probably an underestimation.

To reduce the risk of preterm infants to vaccine preventable diseases administration of intravenous Ig may be an option. However, recently Ohlsson and Lacy concluded that administration of intravenous Ig for preventing infections in preterm or low BW infants only gives a small reduction (3%-4%) of nosocomial infections, without a reduction in mortality or other important clinical outcomes.[113] Vaccination of mothers during pregnancy may be another option to reach higher antibody concentrations in infants at birth. However, studies

on maternal vaccination [107–111] were only performed in pregnancies ≥30 weeks of gestation and the antibody concentrations were only higher in the infant at birth when the vaccination was given > 2 weeks before delivery. [112]

In conclusion, the transplacental transport of IgG is significantly lower in preterm infants than in term infants. This involves both antibodies elicited by protein vaccines, such as Dtx, Ttx and pertussis, and antibodies elicited by polysaccharide vaccines, such as MenC and Hib. In term infants, low percentages of protective antibody concentrations were found posing these infants at risk for vaccine-preventable diseases in the first months of life. However, preterm infants with an immature immune system have even lower protective antibody concentrations, derived from their mothers, which predisposes them to higher risk for vaccine-preventable diseases.