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The Protective Value of Maternal Group B *Streptococcus* Antibodies: Quantitative and Functional Analysis of Naturally Acquired Responses to Capsular Polysaccharides and Pilus Proteins in European Maternal Sera

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Background. Group B *Streptococcus* (GBS) is a major cause of neonatal sepsis and meningitis. A vaccine targeting pregnant women could protect infants through placentally transferred antibodies. The association between GBS maternal antibody concentrations and the risk of neonatal infection has been investigated in US and African populations. Here we studied naturally acquired immunoglobulin G (IgG) responses to GBS capsular polysaccharides (CPS) and pilus proteins in European pregnant women.

Methods. Maternal sera were prospectively collected in 8 EU countries from 473 GBS non-colonized and 984 colonized pregnant women who delivered healthy neonates and from 153 mothers of infants with GBS disease. GBS strains from these colonized women and infected infants were obtained in parallel and their capsular and pilus types were identified by serological and molecular methods. Maternal serum concentrations of IgG anti- Ia, -Ib, -III and -V polysaccharides and anti-BP-1, -AP1-2a and -BP-2b pilus proteins were determined by enzyme-linked immunosorbent assay. Antibody functional activity was quantified by Opsonophagocytic Killing Assay.

Results. Antibody levels against CPS and pilus proteins were significantly higher in GBS colonized women delivering healthy babies than in mothers of neonates with GBS disease or non-colonized women. Moreover, maternal anti-capsular IgG concentrations showed a significant correlation with functional titers measured by Opsonophagocytic Killing Assay.

Conclusions. Maternal anti-capsular IgG concentrations above 1 μg/mL mediated GBS killing in vitro and were predicted to respectively reduce by 81% (95% confidence interval, 40%–100%) and 78% (45%–100%) the risk of GBS Ia and III early-onset disease in Europe.

Keywords. group B *Streptococcus*; *Streptococcus agalactiae*; neonatal infection; capsular polysaccharide; pilus island; immune response.

Streptococcus agalactiae (Group B *Streptococcus*, GBS) is a major cause of life-threatening infections in infants and in adults with underlying medical conditions [1, 2]. Maternal colonization is the primary risk factor for early-onset disease (EOD) manifesting within six days from birth with rapid bacterial spreading that can result in pneumonia, sepsis and/or meningitis [1]. Late-onset disease (LOD) in 7–89 day old infants may be acquired perinatally or from community sources and is characterized by meningitis in up to 50% of cases [3].

Intrapartum Antibiotic Prophylaxis (IAP) in women colonized with GBS or having obstetric risk factors, has remarkably reduced EOD incidence [4, 5]. However, IAP has no effect on LOD and concerns have arisen on the emergence of antibiotic resistance and its impact on neonatal intestinal microbiota [6].

A correlation of maternal deficiency in antibodies recognising the capsular polysaccharide (CPS) with increased probability of infant disease [7] suggested that a GBS vaccination program targeting pregnant women could further reduce the incidence of EOD and LOD. CPS antigens are major virulence factors expressed into 10 unique serotypes, of which Ia, Ib, II, III and V account for the majority of disease globally [8–10]. Surface proteins conferring serotype-independent protection in pre-clinical infection models were identified by genomic approaches [11]. Among them, widely represented pilus proteins implicated in adherence and invasion of host cells [12–15].

Vaccines made of CPS conjugated to detoxified Tetanus or Diphteria toxins safely elicited serotype-specific antibodies that were efficiently transferred transplacentally [16, 17]. Yet, GBS vaccine efficacy studies present the challenge of low disease

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incidence and concurrent use of IAP. Lin et al [18, 19] estimated maternal levels of naturally acquired CPS antibodies associated to reduced risks of EOD in a US population. Their studies support a new strategy for assessing vaccine efficacy, based on the quantification of maternal antibodies as surrogates of neonatal protection.

In Europe, GBS national surveillance and prevention guidelines vary among regions. Current disease rates are 0.5–1.4 per 1000 live births, with differences between countries [4, 20–23]. The DEVANI (Design of a Vaccine Against Neonatal Infections) pan-European consortium was established in 2008 to launch a multi-center sero-epidemiological study with three main objectives [24]. First, to standardize diagnosis of maternal colonization and neonatal infection; second, to assess disease burden and sero/genotype distribution; third, to inform vaccine design by investigating naturally acquired antibody responses in pregnant women. Here we report the main results of the last objective, focusing on the analysis of immunoglobulin G (IgG) levels against CPS and pilus protein antigens in European mothers of healthy and GBS infected infants.

MATERIALS AND METHODS

Participants, Specimen Collection and GBS Strain Characterization

DEVANI specimens were collected between 2008 and 2010 with approval by the ethics committee of each participating institution [24]. Informed consent was obtained from all enrolled women and parents of infected neonates. Lower vaginal/rectal swabs and blood samples were prospectively collected from healthy pregnant women using pre-standardized methods [25]. Enrolled cases were identified through daily surveillance according to the following inclusion criteria: GBS isolation from blood, cerebrospinal fluid, or other normally sterile sites in infants aged 1–6 days (EOD) or 7–89 days (LOD). Detailed inclusion criteria and procedures for sample collection/management are in Supplementary Data. Harmonized protocols were used for GBS identification as described [25]. Capsular serotyping was performed by standardized latex agglutination tests (SSI, Denmark) [25, 26]. Capsular and pilus genotypes were determined by polymerase chain reaction [15, 26–28]. Pilus surface expression was assessed by flow cytometry using monoclonal antibodies raised against pilus 1, 2a and 2b proteins [12, 15].

Enzyme-Linked Immunosorbent Assay (ELISA) and Opsono Phagocytic Killing Assay (OPKA)

CPS and pilus-specific IgG concentrations in maternal sera were measured by ELISA using highly purified CPS conjugated to human serum albumin-adipic acid dihydrazide or pilus proteins BP-1 (SAG0645), AP1-2a (SAG1408) or BP-2b (SAN_1518). Anti-CPS reference sera were obtained from Baker et al [29, 30]. Anti-pilus proteins standard sera were prepared by pooling unweighthed high titer sera. Functional activity of anti-GBS antibodies was assessed by OPKA using differentiated HL-60 cells and strains 515-Ia, H36b-Ib and COH1-III [31]. ELISA and OPKA protocols are in Supplementary Data.

Statistical Analysis

Differences between specific IgG levels in maternal sera were assessed using Mann–Whitney and Kolmolgorov test, or Kruskal–Wallis followed by Dunn's test for multiple comparisons; undetectable IgG values were imputed to half of the lower limit of quantification (LLOQ). Demographic differences between cases and controls were assessed by Chi-square test of proportions and Mann–Whitney. Maternal IgG concentrations in case and control sera were analyzed using Kaplan–Meier estimates of their reverse cumulative distributions and by calculating the corresponding non-parametric Absolute Disease Risk (ADR) [32] under an assumed incidence of GBS neonatal infection among colonized mothers of 1% [29]. Interval estimates were calculated using non-parametric bootstrap [33]. For each serotype, serum maternal IgG concentrations were compared to their OPKA titers on a log-log scale using Pearson's correlation coefficient and linear regression.

RESULTS

Collection of European Maternal Sera and GBS Strains

A collection of maternal sera, 984 samples from GBS carriers, 473 from non-colonized women and 153 from mothers of cases (82 EOD and 71 LOD, Supplementary Table 1) was obtained in eight EU countries. Matched GBS strains were collected from colonized women and infected newborns, and the capsular and pilus type frequencies were determined. As shown in Figure 1, serotype III was the most common in all groups. Serotype V accounted for one fifth of carrier and EOD strains and was rare in LOD, type II was more frequent in carrier strains, and types Ia and IV were evenly distributed across groups. Types Ib and IX were less frequent, and 8% of carrier strains were non-typeable.

The distribution and in vitro expression of the three pilus variants was assessed in 146 neonatal and 959 colonizing strains (Supplementary Figure 1). The pilus 1 + 2b combination was the most frequent in neonatal isolates whereas pili 1 + 2a predominated among carriers and one fifth of invasive and colonizing strains carried pilus 2a alone. Pili 2a and 2b were more expressed compared to pilus 1, and most strains from infected neonates (92%) and carrier women (79%) expressed at least one pilus type.

Antibody Responses in GBS Colonized and Non-colonized Pregnant Women

To investigate the impact of carriage on maternal responses to GBS antigens, we compared IgG concentrations against CPS Ia, Ib, III, V and pilus proteins in sera from GBS colonized vs non-colonized women delivering healthy babies. Figure 2 shows that anti-capsular IgG concentrations were significantly higher in colonized mothers against the carried serotype (Ia, Ib, III or

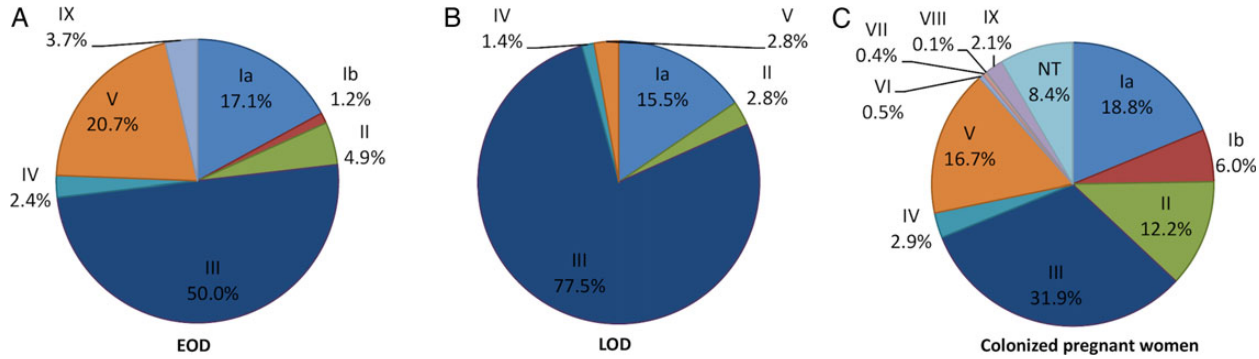

Figure 1. Serotype distribution of group B *Streptococcus* strains isolated from sterile sites in neonates with early-onset disease (EOD) (*A*), late-onset disease (LOD) (*B*) and from vaginal-rectal swabs of colonized pregnant women who delivered healthy babies (*C*). Bacterial strains were collected between 2008 and 2010 in the eight European countries participating to the DEVANI program. Abbreviation: NT, non-typeable.

V) compared to carriers of other serotypes and to non-carriers (*P*-value < .0001). Supplementary Figure 2 reports individual IgG titers against the three pilus proteins BP-1, AP1-2a and BP-2b, in women colonized by strains expressing the corresponding pilus types vs women colonized by GBS isolates not expressing those pilus variants. Similarly to CPS responses, antibody levels against each pilus protein significantly increased during colonization (*P*-values <.0001, <.05, <.01 for BP-1, AP1-2a, BP-2b respectively).

Anti-CPS Ia and III Responses in GBS Colonized Mothers Delivering Healthy Infants and Mothers of Cases

To explore the relationship between maternal CPS-specific antibody concentrations and the risk of neonatal infection, we compared anti-CPS IgG concentrations in sera from mothers of EOD cases with those of women colonized with the same serotype and delivering healthy babies (controls). This analysis was restricted to case and control samples taken within 7 days of delivery from women who gave birth at ≥34 weeks of gestation, to ensure collection close to birth and efficient antibody placental transfer. The complete list of EOD cases including serotype, gestation weeks, time of disease onset and of sera collection, and maternal CPS-specific IgG concentrations, is reported in Supplementary Table 2. Meaningful comparisons between IgG distributions in cases and controls were possible only for serotypes Ia and III, where the number of eligible EOD cases were 8 and 23 out of 14 and 41 respectively. Conversely, only 5 type V cases with ≥34 gestation weeks and collected within 7 days from birth were available.

Demographic characteristics and summary statistics of anti-CPS Ia and III IgG concentrations in the analyzed

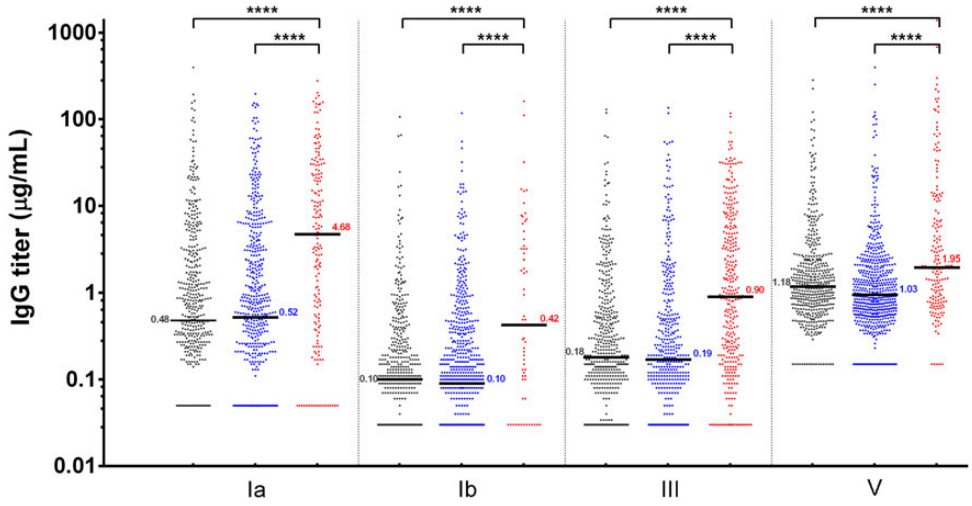

Figure 2. Maternal immunoglobulin G (IgG) concentrations against group B *Streptococcus* (GBS) capsular polysaccharide (CPS) Ia, Ib, III and V measured in individual sera from women colonized by the homologous CPS (red), in sera from mothers colonized by non-homologous CPSs (blue) among serotypes Ia, Ib, III and V and in non GBS colonized women (grey). Horizontal bars indicate the median IgG concentration, the corresponding values are reported next to each bar. Statistical analysis: Kruskal–Wallis followed by Dunn's multiple comparisons test (****P-value < .0001).

Table 1. Demographics of Case-Control DEVANI Samples and Summary Statistics of Anti-Capsular Polysaccharide Immunoglobulin G Concentrations

	GBS Capsular Polysaccharide Type				
	Ia		III		
	Controls (n = 90)	EOD Cases (n = 8)	Controls (n = 190)	EOD Cases (n = 23)	LOD cases (n = 9)
Belgium	17	0	21	0	0
Germany	39	3	84	3	3
Spain	34	3	49	9	0
Italy	0	0	33	6	5
Denmark	0	0	0	2	0
UK	0	1	0	2	1
Bulgaria	0	1	3	1	0
Maternal age[a]	30 (16–34)	31 (30–37)	31 (15–43)	29 (17–40)	30 (22–44)
Gestational age[a]	36 (34–39)	39 (37–42)[b]	36 (34–39)	39 (34–41)[b]	38 (36–41)[b]
Maternal fever >38.5°C	2/82 (2%)	1/8 (13%)	2/168 (1%)	3/21 (14%)	0/9
PROM > 18 h	10/82 (12%)	1/8 (13%)	14/164 (9%)	2/19 (11%)	0/9
IAP	74/90 (82%)	1/8 (13%)	148/190 (78%)	4/23 (17%)	1/9 (11%)
Samples with anti-CPS IgG > LLOQ	82/90 (91%)	7/8 (88%)	169/190 (78%)	18/23 (89%)	8/9 (89%)
Median and range of anti-CPS IgG (μg/mL)	9.3 (<LLOQ, 202.6)	0.63 (<LLOQ, 12.4)	1.1 (<LLOQ, 117)	0.2 (<LLOQ, 52.4)	0.27 (<LLOQ, 3.7)
Median and range of anti-CPS IgG (μg/mL) in no IAP samples	3.7 (n = 16) (<LLOQ 159.9)	0.6 (n = 9) (<LLOQ 0.96)	1.7 (n = 42) (<LLOQ, 46.2)	0.17 (n = 19) (<LLOQ, 4.1)	. . .

Data are no. of subjects (%), unless otherwise indicated.

Abbreviations: CPS, capsular polysaccharide; EOD, early-onset disease; GBS, group B *Streptococcus*; IAP, intrapartum antibiotic prophylaxis; IgG, immonoglobulin G; LLOQ, lower limit of quantitation; LOD, late-onset disease; PROM, prolonged (>18 hours) rupture of membranes.

[a] Median maternal and gestational age (range) in weeks.

[b] Significant difference between cases and controls ($P < .001$). Differences between groups for maternal age and gestational age were assessed by Mann–Whitney test, differences for maternal fever, PROM and IAP by Chi-square test of proportions.

samples are shown in Table 1. Lack of statistically significant differences between cases and controls for intrapartum fever, prolonged rupture of membranes and maternal age, confirmed the suitability of the dataset to assess the role of maternal anti-GBS IgG as an independent variable. Mothers of EOD cases presented a shorter gestational age vs controls ($P < .001$); this difference could not be seen as confounder when assessing the protective value of maternal antibody concentrations.

The proportion of samples with maternal IgG below the LLOQ was not different between cases and controls. However, as shown in Figure 3*A* and 3*B*, the distribution of IgG concentrations among the control samples dominated over that of cases for both serotypes (one-tailed Kolmogoroff-Smirnoff test). The proportion of EOD mothers receiving IAP was significantly smaller than that of controls; for this reason, comparison of antibody concentrations was also undertaken for the subset of cases and controls not receiving antibiotics. The empirical RCD of the 7 Ia and 19 III EOD cases who did not receive IAP lay uniformly below that of their 16 and 19 controls respectively (Figure 3*C* and 3*D*).

We subsequently analysed maternal IgG concentrations in LOD cases. As shown in Supplementary Table 3, none of the 9 type Ia and 38 type III LOD cases with gestational age of 34 weeks and above, had maternal blood collected within one week. Therefore, 9 type III cases identified using the less stringent cut-off of 15 days were selected for analysis, presenting median values of 10 days between birth and disease. Figure 3*E* shows empirical distributions of anti-CPS III IgG in these LOD samples vs the same 190 type III controls used for EOD comparisons. Both Mann–Whitney and Kolmogorrof-Smirnoff tests indicated that maternal IgG concentrations in control sera were significantly higher than those of LOD cases.

To better understand the potential implications of the measured maternal IgG concentrations for prevention of GBS neonatal disease in Europe, we derived the empirical ADR from the RCDs shown in Figure 3, starting from the lowest measureable IgG concentration upwards. The empirical ADR curve estimates are shown in Supplementary Figure 3; the bootstrap prediction intervals of these curves indicate that precision was maximized over the interval LLOQ-3 μg/mL, with more uncertain estimates for higher IgG concentrations due to a lower number of samples. As shown in Supplementary Table 4, the chance of GBS Ia or III EOD in infants born to mothers with anti-CPS IgG at or above 1 μg/mL compared to LLOQ decreased by 81% (40%–100%) and 78% (45%–100%) respectively. The corresponding ADR reductions for type Ia and III EOD cases and controls who did not receive IAP were 100% and 88% (46%–100%). The reduction in type III LOD risk above 1 μg/mL was 76% (21%–100%).

GBS Opsonophagocytic Killing Titers in Sera From Colonized Mothers

The observed protective value of CPS maternal antibodies suggests that these IgG are functional against GBS. This hypothesis

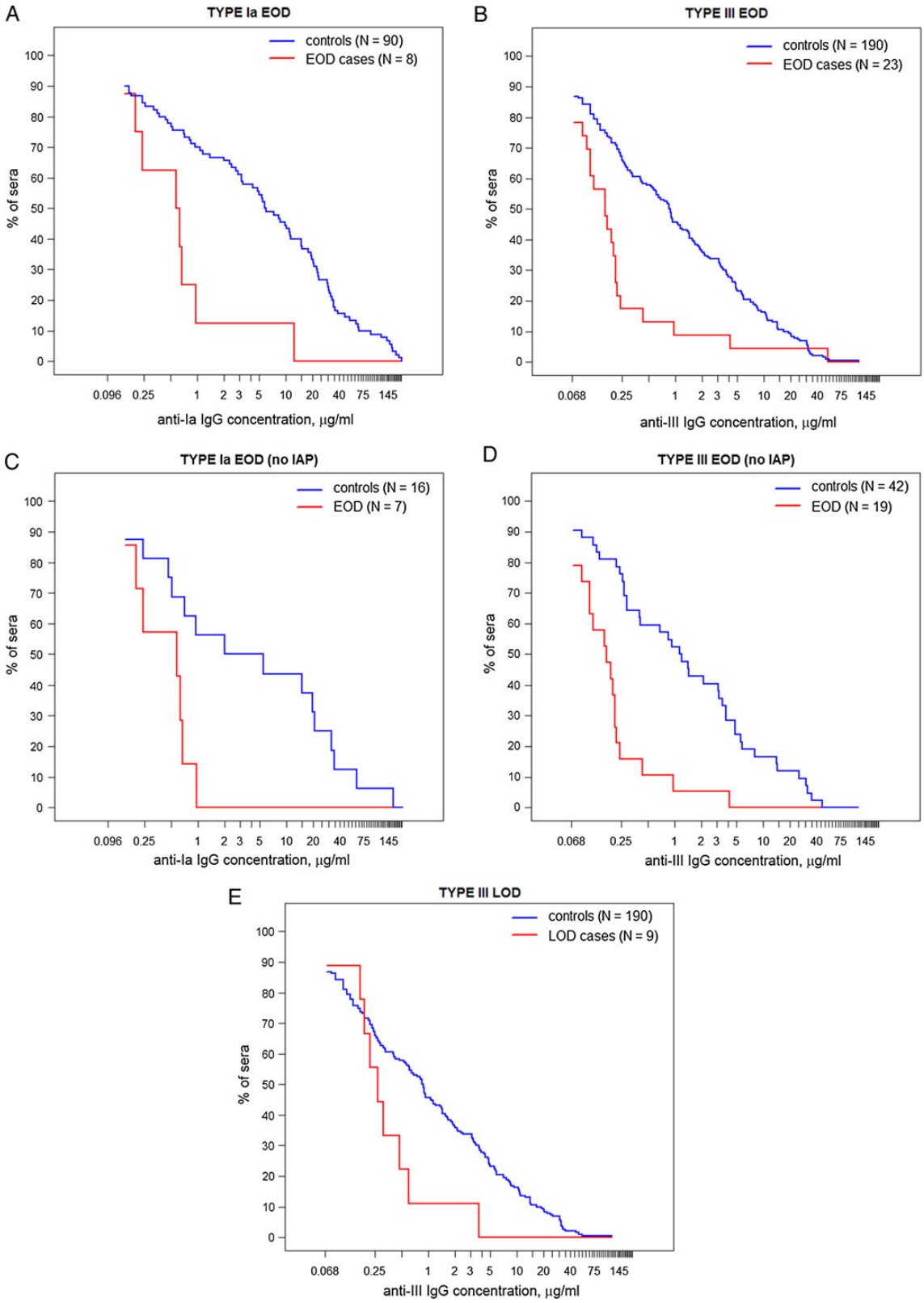

Figure 3. Empirical reverse cumulative distributions (RCD) of immonoglobulin G (IgG) concentrations against polysaccharide type-Ia and III in maternal case and control sera. Numbers on vertical axis represent the percentage of samples with measured anti-capsular polysaccharide IgG concentrations larger or equal to each value shown on the horizontal axis. *A*, Anti-Ia IgG in early-onset disease (EOD) and control samples. *B*, Anti-III IgG in EOD and control samples. *C*, Anti-Ia IgG in EOD and control sera from mothers not treated with antibiotics (no intrapartum antibiotic prophylaxis [IAP]). *D*, Anti-III IgG in EOD and control sera from mothers not treated with antibiotics (no IAP). *E*, Anti-III IgG in late-onset disease (LOD) and control samples. Mann–Whitney and Kolmogorrof-Smirnoff tests indicated that maternal IgG concentrations in control sera were significantly higher than those of EOD and of LOD cases (P-value < .001).

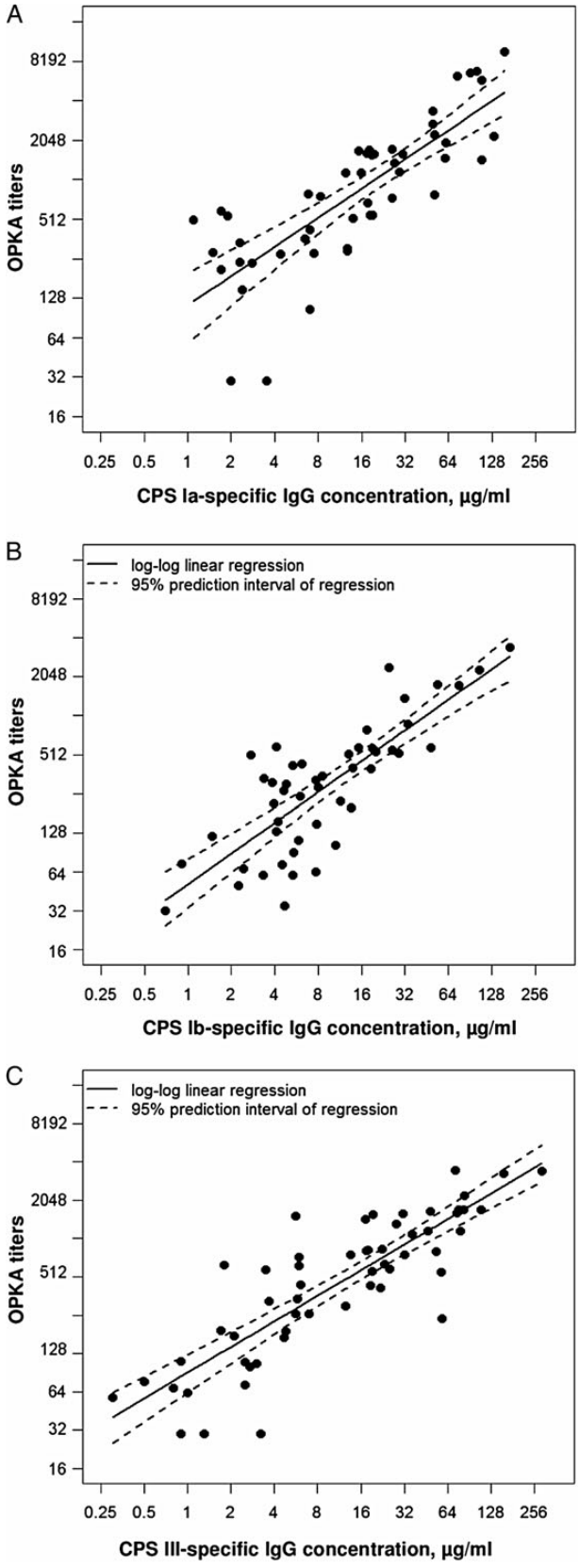

Figure 4. Anti-capsular polysaccharide (CPS) immunoglobulin G (IgG) concentrations against serotypes Ia (*A*), Ib (*B*) and III (*C*) in sera from mothers colonized with the corresponding serotype, plotted vs their opsono phagocytic killing assay (OPKA) titers. Whole lines represent the fitted log-log linear regressions. Dashed curves show the 95% boostrap prediction intervals of the regression lines.

Table 2. Estimated Regression Coefficients and Residuals Standard Error for the Log-Log Linear Regressions of Opsono Phagocytic Killing Assay Titers by Immunoglobulin G Concentrations of the Three Group B *Streptococcus* Serotypes

	Type-Ia	Type- Ib	Type-III
Pearson correlation coefficient (*r*)	0.8	0.8	0.85
Intercept	6.8 (5.9, 7.6)	5.7 (5.1, 6.2)	6.5 (6.0, 7.0)
Slope	0.7 (0.6, 0.9)	0.8 (0.6, 0.9)	0.7 (0.6, 0.8)
residuals SE	1.1	1.0	1.0

95% prediction intervals are shown in brackets.

Abbreviation: SE, standard error.

was investigated by testing in OPKA a subpanel of sera from colonized mothers of healthy babies with IgG levels covering the full range of measurable antibody responses against CPS Ia, Ib or III (49, 48 and 56 sera respectively). Statistically significant Pearson correlations between the logarithm of the CPS IgG concentrations and of their OPKA titers were estimated for anti-Ia (80%), Ib (80%) and III (85%) (*P*-values <.0001). All correlations considering heterologous serotypes were not statistically significant, having a maximum of 30%. ELISA-OPKA correlations were also investigated using log-log linear regression (Figure 4). Table 2 reports the Pearson correlations and the fitted regression coefficients for the three serotypes. These estimates show that a doubling in IgG concentration corresponds to expected OPKA titer increases between 70% and 80%, and that IgG concentrations at the ELISA LLOQ correspond to OPKA titers below the detection limit. Also, OPKA titers for 1 µg/mL CPS IgG are expected to range between 64 and 128 for the three serotypes. To assess whether these titers may be considered as reference values for neonatal EOD protection, we tested by OPKA all sera from mothers of Ia and III EOD cases (8 and 23 respectively). None of these sera had measurable OPKA titers except 1 Ia and 2 III cases exhibiting serotype specific IgG concentrations above 4 µg/mL.

Anti-pilus Protein Responses in GBS Colonized Mothers of Healthy Neonates or EOD Cases

Antibody levels against BP-1, AP1-2a and BP-2b were investigated in sera from mothers carrying strains expressing either of the three pilus types and delivering healthy babies or EOD cases. Consistently with our analysis of anti-CPS maternal antibody concentrations, this analysis was restricted to sera collected within 7 days from delivery from mothers delivering after 34 gestation weeks. Table 3 shows that maternal titers against pili 1 and 2a were significantly lower among EOD samples compared to their controls, while no statistically significant differences were detected between maternal titers against pilus 2b (two-samples Kolmogoroff-Smirnoff test). The RCDs of anti-pilus titers depicted in Supplementary Figure 4 confirmed that the distribution of control samples dominates that of the EOD cases for pili 1 and 2a, whereas the RCDs of cases and controls are

Table 3. Summary Statistics of Group B *Streptococcus* Anti-pilus Immunoglobulin G Concentrations for the Analyzed DEVANI Samples

	Pilus 1		Pilus 2a		Pilus 2b	
	Controls (n = 428)	EOD Cases (n = 35)	Controls (n = 568)	EOD Cases (n = 25)	Controls (n = 107)	EOD cases (n = 18)
Samples with anti-pilus IgG > LLOQ	411/428 (96%)	32/35 (91%)	501/568 (88%)	21/25 (84%)	99/107 (93%)	14/18 (78%)
Median and range anti-pilus IgG (EU/mL)	21.3 (<LLOQ, 1083)	13.5 (<LLOQ, 554.5)	37.3 (<LLOQ, 532)	28.2 (<LLOQ, 160.4)	83 (<LLOQ, 1873)	85.3 (<LLOQ, 367)

Abbreviations: EOD, early-onset disease; IgG, immunoglobulin G; LLOQ, lower limit of quantitation.

interweaved for pilus 2b EOD throughout the range of measured titers.

DISCUSSION

Despite remarkable accomplishments in the prevention of neonatal infection after the introduction of IAP, GBS remains a global leading cause of infant invasive disease. Accurate surveillance studies are required to evaluate disease incidence, colonization, and epidemiology. One main aim of the DEVANI network was to lay the foundations for standardized surveillance in Europe. Among the 25 000 pregnancies followed, 153 neonatal cases were documented, and the corresponding invasive strains and maternal sera collected. The serotype distribution of colonizing and invasive strains was in line with other recent EU reports [4, 20, 22, 34–36]. Serotypes Ia-Ib-II-III-V, accounted for 88%, 94% and 99% of carrier, EOD and LOD isolates respectively. The abundance of pilus 1 + 2b LOD strains reflects the high frequency of the hypervirulent type III ST-17 lineage [37]. Conversely, the prevalence pilus 1 + 2a colonizing isolates matches the higher frequencies of serotypes V, II and Ib in this group.

A systematic investigation on the relationship between serum specific IgG levels in European pregnant women, their colonization status and their probability of delivering an infected baby had not been conducted prior to DEVANI. Several observations were made during our analysis. First, GBS vagino/rectal colonization was associated with increased CPS and pilus specific antibodies. Second, anti-Ia or-III IgG concentrations in control samples were at least four-fold greater than those of EOD cases for the corresponding serotypes. A similar difference was observed for type III LOD samples. Third, anti-pilus 1 and 2a IgG were lower in mothers of cases compared to controls, suggesting a possible additional contribution of these antibodies to neonatal protection. Multiple regression would allow estimating simultaneously the protective value of antibodies directed against CPS and the pilus proteins; however, this analysis would require a larger number of samples with measurable antibody responses against all antigens compared to those available in the DEVANI study.

The low rates of GBS neonatal infection together with IAP use highlight the difficulty of conducting adequately powered vaccine efficacy studies. Prediction of efficacy by serological correlates of protection [38] could provide a path forward for vaccine development. Pioneering US investigations by Lin et al indicated that the relative risk of developing Ia or III EOD was reduced by about 90% if maternal IgG concentrations were higher than 5 or 10 μg/mL respectively [18, 19]. In a similar US study, Baker et al predicted that the absolute risk of contracting Ia, III and V EOD would decrease by 70% if maternal CPS-specific antibody concentrations were equal or higher than 1 μg/mL [29]. Differences in antibody concentrations predictive of protection in these two studies could be due to the different assays, standard sera, statistical analyses or sampling variation. Using the same standard serum and a similar ELISA as in Baker et al [29], we estimated that over 75% of European Ia and III EOD cases could be prevented by raising maternal anti-CPS IgG above 1 μg/mL, consistently with what reported by those authors. Notably, these estimates are more conservative than the observed proportions of cases with maternal IgG concentrations below 1 μg/mL (87% and 91% for Ia and III EOD). A recent South African study using the same standard sera estimated a 90% ADR reduction by 6 and 3 μg/mL Ia and III–specific IgG respectively [39]. One driver of these higher thresholds could be the lower IgG concentrations measured in the African control group (median 0.29 μg/mL for both serotypes) compared to Baker et al (1.8 and 1.6 μg/mL for Ia and III respectively) and this EU study (4.7 and 0.9 μg/mL).

One limitation of our study is that not all maternal blood samples were collected at the time of delivery, with most cases being identified retrospectively. To ensure that the compared maternal case-control IgG concentrations reflected those transferred to infants, only samples taken within 7 days (EOD) and 15 days (LOD) were used to derive non-parametric ADR estimates. By deriving the ADR from the Kaplan–Meier distributions of the case and control samples and by using the nonparametric bootstrap to derive its prediction intervals, these results do not rely on a priori assumptions about the ADR shape. More precise estimation, possibly based on parametric ADR models, will require prospective collection of a larger number of case samples.

Finally, the measured correlation between anti-CPS IgG concentrations and OPKA functional titers in European maternal sera reinforces the protective value of these antibodies and further supports the use of IgG concentrations estimated from

case-control studies to assess the protective efficacy of CPS based vaccines against this relevant human pathogen.

Supplementary Data

Supplementary materials are available at http://cid.oxfordjournals.org. Consisting of data provided by the author to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the author, so questions or comments should be addressed to the author.

Notes

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