Journal of Infection 76 (2018) 449–456

Contents lists available at ScienceDirect

Journal of Infection

journal homepage: www.elsevierhealth.com/journals/jinf

Functional activity of maternal and cord antibodies elicited by an investigational group B *Streptococcus* trivalent glycoconjugate vaccine in pregnant women

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ARTICLE INFO

Article history:
Accepted 6 January 2018
Available online 31 January 2018

Keywords:
GBS
Neonatal infection
Capsular polysaccharide
Immune responses

ABSTRACT

Objectives: The main aim of this exploratory study was to evaluate functional activity of antibodies elicited by a maternal Group B *Streptococcus* (GBS) investigational vaccine composed of capsular polysaccharides Ia, Ib, and III conjugated to genetically detoxified Diphtheria toxin CRM_{197}. The second objective was to investigate the relationship between serotype-specific IgG concentrations and functional activity in maternal and cord sera.
Methods: Maternal and cord sera collected at baseline and at delivery from vaccine and placebo recipients during a double-blind placebo-controlled Phase II study (www.clinicaltrials.gov, NCT01446289) were tested in an opsono-phagocytic bacterial killing assay. Cord sera from vaccine recipients were also passively transferred to newborn mice to investigate conferred protection against bacterial challenge.
Results: Antibody-mediated GBS phagocytic killing was significantly increased in maternal serum at delivery and in cord sera from the investigational vaccine group as compared to the placebo group. Anti-capsular IgG concentrations above 1 μg/mL mediated *in vitro* killing against GBS strains belonging to all three serotypes and IgG levels correlated with functional titers. Passively administered cord sera elicited a dose-dependent protective response against all GBS serotypes in the *in vivo* model.
Conclusions: The maternal vaccine elicited functional antibodies that were placentally transferred. Anti-capsular IgG concentrations in maternal and cord sera were predictive of functional activity and *in vivo* protection in the mouse model.

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https://doi.org/10.1016/j.jinf.2018.01.006
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Introduction

Group B *Streptococcus* (GBS) is a leading cause of invasive disease in early infancy. Up to 30% of pregnant women carry GBS bacteria in their lower gastrointestinal or genitourinary tract and up to 1% of neonates born to colonized mothers become infected *in utero* or during delivery.[1,2] GBS neonatal disease is classified into two types based on onset of disease. Early onset disease (EOD) mainly presents during the first week after birth while late onset disease (LOD) occurs between >7 days and 90 days after birth. The most common disease manifestations are sepsis, pneumonia, and meningitis, with frequent sequelae and possible death. EOD incidence has declined after implementation of intrapartum antibiotic prophylaxis[3–5] although this practice does not impact LOD and widespread use of antibiotics has raised concerns over allergic reactions and potential emergence of resistant strains. A safe and effective GBS vaccine is therefore needed to further reduce the mortality and severe morbidity associated with both EOD and LOD and decrease the risk of antibiotic resistance.

Placental transfer of naturally acquired maternal antibodies against GBS that protect infants from invasive infection was first reported in a small case study in 1976.[6] A direct relationship between maternal immunoglobulin G (IgG) levels against three of the ten known GBS capsular polysaccharides (CPS) and reduced risk of neonatal infection has since been established.[7–11] Investigational glycoconjugate vaccines targeting the most frequent CPS are under development for maternal administration with the aim of eliciting and enhancing specific antibodies for placental transfer and neonatal protection.[12–14]

A recent Phase II randomized placebo-controlled, observer-blind, multicenter study conducted in Belgium and Canada investigated the safety and immunogenicity of an investigational glycoconjugate formulation of three major CPS serotypes (Ia, Ib, III) among women receiving one dose of vaccine during the third trimester of pregnancy, and antibody placental transfer. Study results indicated an acceptable safety profile and 16-, 23-, and 20-fold increase in IgG geometric mean concentrations against CPS Ia, Ib, and III respectively in vaccinated women from baseline to delivery, with placental transfer rates between 68% and 81%.[15]

Protection mediated by CPS-specific antibodies relies on phagocytic killing of opsonized bacteria by host effector cells.[16] Here we used an opsono-phagocytic bacterial killing assay (OPKA) to assess killing of GBS serotypes Ia, Ib, and III by differentiated human HL-60 effector cells, in the presence of maternal or cord sera from subjects who participated in the trivalent Phase II study.[15] The relationship between CPS-specific IgG concentrations and OPKA titers against serotypes Ia, Ib, and III was investigated both in maternal and cord samples. Functional activity of placentally transferred IgG was also assessed *in vivo* by testing the capacity of cord sera to passively protect newborn mice from GBS infection.

Materials and methods

Study design and participants

Study NCT01446289 (Clinical Trial Registration: ClinicalTrials.gov, www.clinicaltrials.gov)[15] enrolled 86 pregnant women from Belgium and Canada aged 18–40 years at 24–35 gestation weeks and randomized 3:2 to receive one dose of an investigational GBS vaccine containing CPS Ia, Ib, and III conjugated to CRM_{197} detoxified Diphtheria toxin, or placebo, by intramuscular injection. A 0.5-mL dose of vaccine reconstituted with 0.9% sodium chloride contained 5 micrograms of each capsular polysaccharide individually conjugated to CRM_{197}.

The study was conducted in accordance with the Declaration of Helsinki and the principles of Good Clinical Practice. The protocol was reviewed and approved by the appropriate ethics review committees and institutional review boards, and informed written consent was obtained from all women on behalf of themselves and their infants before enrollment in the trial.

Blood samples were collected from women before (baseline) and 30 days after vaccination, at delivery (+72 hours collection window) and 91 days postpartum. Cord blood was taken at birth and when cord blood was not available, infant peripheral blood was collected within 72 hours from birth. All sera were stored at –20 °C.

The analysis of antibody functional activity was conducted in the Belgian subset of 55 maternal serum pairs collected at baseline and at delivery, and 53 cord/infant samples collected at delivery with sufficient volumes for OPKA (0.5 mL) and for mouse passive protection assays (1.5–2.0 mL). A total of 33 maternal and 31 cord samples from the vaccine group and 22 maternal and cord samples from the placebo group were available and used for OPKA analysis. Demographic characteristics were consistent across vaccine and placebo groups. The overall mean age at vaccination was 30.2 years (standard deviation [SD] ± 4.7), and 98% of women were Caucasian. The mean gestational age at enrollment was 29.7 weeks (SD ± 3.2) and 38.9 weeks (SD ± 1.1) at delivery. All women delivered after at least 37 weeks of gestation. The number of weeks between vaccination and delivery ranged between 3 and 16, with a mean of 9.1 (SD ± 3.5).

Mouse protection experiments were conducted by passive administration of available cord sera. These included samples from the vaccine group having quantifiable IgG anti-Ia (n = 9), Ib (n = 8), and III (n = 10) concentrations, samples from the placebo group with quantifiable IgG anti-Ia (n = 5) concentrations, and negative samples showing no quantifiable IgG anti-Ia (n = 2), Ib (n = 5), and III (n = 4) (see Supplementary Table S1).

Determination of IgG concentrations against CPS Ia, Ib and III

The enzyme-linked immunosorbent assay (ELISA) protocol used to measure maternal and cord CPS-specific IgG concentrations has been previously reported.[15] Briefly, 96-well plates were coated with 100 ng of GBS polysaccharides (Ia, Ib, or III) conjugated to human serum albumin *via* an adipic acid dihydrazide linker. The plates were incubated with serially diluted serum samples for 1 hour at 37 °C, washed and further incubated for 90 minutes at 37 °C with an alkaline phosphatase-conjugated goat anti-human IgG. After further washing, SeramunGelb pNPP was added to the plates and incubated for 30 minutes at room temperature. The reaction was stopped with SeramunGelb stop. Optical density values were measured at 405 nm using a BEP III ELISA processor. Antibody concentrations were assigned by Mikrowin 2000 software analysis using standard curves from weighed standard sera for serotypes Ia,[8] Ib,[17] and III.[9] The assay lower limits of quantification (LLQ) were 0.326, 0.083, and 0.080 μg/mL for serotypes Ia, Ib, and III respectively. Individual IgG concentrations below the LLQ were assigned an arbitrary value of half the LLQ for calculation of geometric mean concentrations.

OPKA for determination of antibody functional activity

OPKA were performed with GBS strains 515, H36b, and COH1 representing serotypes Ia, Ib, and III respectively and differentiated HL-60 cells as previously described.[11,18] The assays were conducted in 96-well microtiter plates, in a total volume of 125 μL/well. Each reaction contained heat inactivated test serum (12.5 μL), GBS (6×10^4 colony forming units [CFU]), differentiated HL-60 cells ($1.5–2.5 \times 10^6$ cells) and either 10%, 5%, or 2% baby rabbit complement (Cederlane) in Hank's balanced salt solution (Gibco) for serotypes Ia, Ib, and III respectively. For each serum sample, four

serial dilutions were tested. Negative controls lacked effector cells, or contained either negative sera or heat inactivated complement.

After reaction assembly, plates were incubated for 1 h at 37 °C. Prior to incubation [T0] and after 1 h incubation [T60] reactions were diluted in sterile water, plated onto trypticase soy agar plates with 5% sheep blood (Becton Dickinson) and incubated over night at 37 °C. Final bacterial counts were performed manually. GBS killing was calculated for each tested serum dilution as (CFU at T0–CFU at T60)/(CFU at T0). OPKA titers were expressed as the reciprocal serum dilution mediating 50% bacterial killing estimated through piecewise linear interpolation of killing measured at each serum dilution. The LLQ of the assay was 1:30 based on the minimum dilution of the test sample. The assay coefficient of variation (based on analysis of 10 sera by two different operators on 3 different days) was 30% for all serotypes. Individual OPKA titers below the LLQ (<30) were assigned an arbitrary value of half the LLQ for the determination of geometric mean titers (GMTs), graphical representation and non-parametric hypothesis testing.

In vivo *passive protection model*

Groups of 8–10 newborn CD1 mice (Charles River Laboratories, Calco, Italy) received one intraperitoneal injection of cord sera containing different concentrations of anti-Ia, Ib, or III IgG, from 0 to 500 ng, diluted in phosphate buffered saline (20 μL/mouse) within 24 hours from delivery. Supplementary Table S1 reports the total number of tested cord samples for the three investigated anti-Ia, Ib, or III IgG (ranges 15–30 ng, 100–150 ng, or 250–500 ng) and the number of passively immunized pups in each group, including negative sera recipient groups. Twenty-four hours after passive transfer with cord sera, pups were injected intraperitoneally with a 70%–100% lethal dose of GBS strains 090 (Ia, $1.5–3 \times 10^2$ CFU/mouse), H36B (Ib, $1.1–1.7 \times 10^6$ CFU/mouse), or M781 (III, $1.1–2.8 \times 10^5$ CFU/mouse) in Todd-Hewitt broth.[19] After bacterial challenge, mice were monitored every 12 hours for 4 days and euthanized for humane reasons when they exhibited pre-established endpoints. The number of surviving pups after 4 days of infection was used to evaluate protection by the passively transferred sera. All animal experiments were approved by and conducted according to the guidelines of Animal Welfare from GSK and the Italian Istituto Superiore di Sanità.

Statistical analysis

The numbers of sera presenting detectable OPKA titers (>30) at baseline and at delivery within each treatment group (vaccinated or placebo) were compared using the McNemar's test of hypothesis and the Bayesian analysis described in detail in the Supplementary materials. The proportions of sera presenting detectable OPKA titers either at baseline or at delivery were compared across treatment groups using the chi-square test for proportions. OPKA titers measured in maternal sera and their corresponding cord sera were compared using Spearman's rank correlation. Geometric mean OPKA titers between vaccine and placebo groups were compared using the Mann–Whitney test. Anti-capsular IgG concentrations and OPKA titers in samples presenting detectable values in both assays were compared using the chi-square test for proportions and orthogonal Deming regression. Analyses were conducted using the statistical software *R*, version 3.3.1[20] and Graph Pad Prism version 6.0.

Results

Antibody-mediated GBS opsono-phagocytic killing in maternal and cord sera

Antibody functional activity in samples collected prior to vaccination and at delivery was assessed by OPKA. A total of 55 maternal serum pairs and 53 cord samples were available for analysis. A summary of the obtained data is presented in Table 1 and in Fig. 1. For all serotypes, the rate of OPKA-positive (titers >30) maternal samples at delivery was higher in the investigational vaccine recipients (herein "vaccine group") compared to the placebo group (p-values <.0001). As shown in Table 1, among the 33 women belonging to the vaccine group, the rate of OPKA-positive maternal sera increased from 33%, 18%, and 42% at baseline to 97%, 61%, and 88% at delivery for serotypes Ia, Ib, and III, respectively (p-values <.001, posterior probabilities above 95%). Among the 22 placebo maternal samples, 55%, 5%, and 36% were OPKA-positive before treatment and 55%, 14%, and 41% at delivery for serotypes Ia, Ib, and III, respectively, with no statistically significant change (p-values >.05, posterior probabilities ≤16%).

Sixteen out of 33 maternal sera from the vaccine group were OPKA-positive at delivery against all three serotypes compared to 0 out of 22 in the placebo group (p-values <.001). Further, 28 out of 33 of the women receiving the investigational vaccine were OPKA positive at delivery against both the major serotypes Ia and III, (p-values <.001) compared to 4 out of 22 placebo recipients.

The OPKA GMTs at delivery against serotypes Ia, Ib, and III were higher in the vaccine group compared with placebo (Table 1, all p-values <.001). In the vaccine group, GMTs at delivery were higher in women presenting detectable OPKA activity prior to vaccination compared to OPKA negative women at baseline (2244, 5100,

Table 1
ELISA and OPKA analysis of functional activity in maternal and infant cord sera from the vaccine and placebo groups.

		GBS Serotype Ia			GBS Serotype Ib			GBS Serotype III		
		Maternal Baseline	Maternal Delivery	Infant Cord	Maternal Baseline	Maternal Delivery	Infant Cord	Maternal Baseline	Maternal Delivery	Infant Cord
Vaccine	Total no. of sera	33	33	31	33	33	31	33	33	31
	no. of OPKA ≥30	11 (33%)	32 (97%)	29 (94%)	6 (18%)	20 (61%)	14 (45%)	14 (42%)	29 (88%)	22 (71%)
	OPKA GMT[a]	36	555	294	23	234	102	56	890	326
	Max OPKA titer	732	15,900	33,572	287	16,115	26,523	1196	26,138	20,917
	ELISA GMC (μg/mL)[a]	0.2	3.3	2.2	0.1	3.6	3.8	0.1	2.9	2.1
Placebo	Total no. of sera	22	22	22	22	22	22	22	22	22
	no. of OPKA ≥30	12 (55%)	12 (55%)	15 (68%)	1 (5%)	3 (14%)	1 (5%)	8 (36%)	9 (41%)	2 (9%)
	OPKA GMT [a]	79	70	61	17	20	16	46	55	20
	Max OPKA titer	1524	1517	1553	189	144	49	1640	933	579
	ELISA GMC (μg/mL) [a]	0.5	0.5	0.4	0.1	0.1	0.1	0.1	0.1	0.1

[a] For GMC and GMT estimation, antibody concentrations and OPKA titers below the LLQ were assigned an arbitrary value of half the LLQ.
Note: In brackets are reported the % of OPKA positive sera.
Abbreviations: ELISA, enzyme-linked immunosorbent assay; GBS, Group B *Streptococcus*; GMC, geometric mean concentration; GMT, geometric mean titer; LLQ: lower limit of quantification; OPKA, opsono-phagocytic bacterial killing assay.

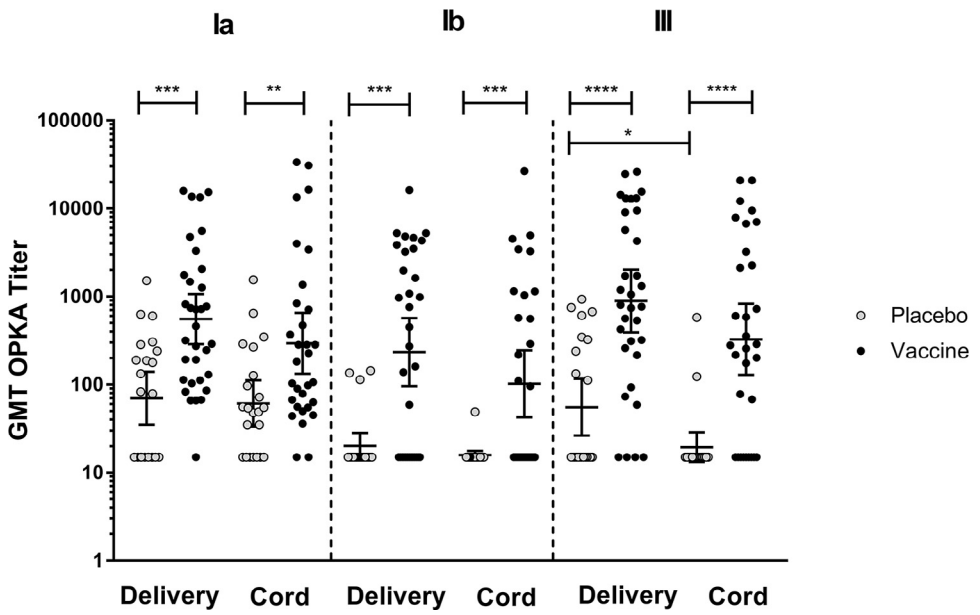

Fig. 1. Opsono-phagocytic bacterial killing titers in maternal and cord sera. OPKA titers against GBS serotypes Ia, Ib and III are presented at delivery in maternal sera and in paired infant cord sera from the placebo and vaccine groups. Each point represents one individual serum; individual titers below the LLQ (<30) were assigned an arbitrary value of half the LLQ; geometric mean titers with 95% confidence intervals are indicated by horizontal bars. Mann–Whitney test *p*-values: * $p < .05$; ** $p < .01$; *** $p < .001$; **** $p < .0001$.

and 5671 versus 317, 797, and 469 for serotypes Ia, Ib, and III, respectively, all *p*-values <.001).

The rate of OPKA-positive cord sera was again higher in the vaccine group compared to placebo for all serotypes (94% [Ia], 45% [Ib], 71% [III] versus 68%, 5%, and 9%; *p*-values <.001). All 31 cord sera from the vaccine group were positive against at least one serotype and 26 were positive against at least two serotypes, while 16 out of 22 placebo cord sera were OPKA positive against at least one serotype and 2 against two serotypes.

The proportions of OPKA-positive maternal sera at delivery that also presented OPKA-positive cord sera were 95% (41/43) for serotype Ia and 67% for serotypes Ib and III (14/21 and 24/36 respectively). The correlations between the quantifiable maternal OPKA titers at delivery and their corresponding cord titers (41, 14, and 24 GBS Ia, Ib, and III sample pairs) were 76% (Ia and Ib) and 81% (III) (all *p*-values <.001).

Association between OPKA functional activity and anti-capsular IgG

The relationship between opsono-phagocytic activity against GBS Ia, Ib, and III in maternal and cord sera and the corresponding CPS-specific IgG concentrations was investigated.[15] Table 2 shows the number of samples presenting ELISA IgG concentrations and OPKA titers within the quantifiable range of the assays, for each serotype and serum source (maternal at baseline, maternal at delivery, paired infant cord) across the vaccine and placebo groups. Table 2 also shows that IgG concentrations and OPKA titers were measured from sera presenting respectively OPKA titers or IgG concentrations below their LLQs. It was not possible to include these data in ELISA-OPKA rank correlations and regression analyses.

The association between ELISA and OPKA positivity was significant for all serotypes and serum sources (*p*-value <.05) except for serotype Ib cord sera (*p*-value = .3). This discrepancy was due to 21 cord sera with IgG anti-Ib above the LLQ (0.083 μg/mL) but undetectable OPKA activity. The concentrations in maternal sera and cord sera presenting no quantifiable OPKA titers in the vaccine and placebo groups are shown in Supplementary Table S2. Anti-Ia IgG concentrations above the LLQ (0.326 μg/mL) accurately predicted positive OPKA titers in all serum sources. For the other serotypes, the minimum IgG concentration predicting an OPKA-positivity was 0.7 μg/mL for all III sera, 0.9 μg/mL for Ib maternal sera, and 1.2 μg/mL in Ib cord sera.

Correlations between the IgG concentrations and their corresponding OPKA titers were estimated for all three serotypes and

Table 2
Number of vaccine or placebo sera with detectable ELISA IgG concentrations and OPKA titers by serum source for each Group B *Streptococcus* serotype.

			Maternal Baseline		Maternal Delivery		Infant Cord	
			OPKA titer					
			<LLQ	≥LLQ	<LLQ	≥LLQ	<LLQ	≥LLQ
Serotype Ia	ELISA IgG titer	<LLQ	32	10	11	6	9	9
		≥LLQ	0	13	0	38	0	31
Serotype Ib		<LLQ	29	0	16	0	5	0
		≥LLQ	17	7	15	22	21	11
Serotype III		<LLQ	25	10	13	5	16	0
		≥LLQ	8	12	4	33	6	18

Note: Lower limit of quantification (LLQ) of ELISA: 0.326 μg/mL (Ia), 0.083 μg/mL (Ib) and 0.080 μg/mL (III); LLQ of OPKA: 1:30 for all serotypes.
Abbreviations: ELISA, enzyme-linked immunosorbent assay; OPKA, opsono-phagocytic bacterial killing assay.

Table 3
Rank correlations between measured ELISA IgG concentrations and corresponding OPKA titers at delivery.

	GBS Serotype		
	Ia	Ib	III
Maternal ELISA vs OPKA	78	86	78
Cord ELISA vs OPKA	90	96	93
Maternal ELISA vs cord OPKA	91	94	92

Notes: All correlations were significant for each serotype (Spearman correlation *p*-values <.001). No significant difference was detected between correlations.
Abbreviations: ELISA, enzyme-linked immunosorbent assay; GBS, Group B *Streptococcus*; OPKA, opsono-phagocytic bacterial killing assay.

serum sources (Table 3). These results indicated a strong association between the IgG measured in maternal and cord sera and the corresponding OPKA titers for all three serotypes (*p*-values <.001) when both measurements were within the quantifiable ranges.

This relationship was further investigated using regression analysis. Fig. 2 reports the fitted log2-log2 orthogonal regression lines between maternal IgG concentrations and their corresponding OPKA titers at delivery (panels A–C), cord sera IgG and their OPKA titers (D–F), and maternal IgG at delivery versus cord OPKA titers (G–I); all quantifiable measurements for both the vaccine and placebo groups were included in each analysis. All regression slopes were positive and statistically significant, ranging from 0.5 for serotype Ib maternal delivery sera up to 1.1 for serotype Ia maternal ELISA at delivery versus cord OPKA (Supplementary Table S3). These estimates showed that, when IgG concentrations are doubled, the corresponding OPKA titer is predicted to increase between 50% and 110%. Of note, measurements from both the vaccine and the placebo groups fell within the same regression line (Fig. 2), although the limited number of placebo OPKA-positive samples did not allow for a statistical comparison of ELISA-OPKA data between the vaccine and placebo groups.

Prediction of mouse neonatal protection by anti-capsular IgG concentrations

Functional activity in cord sera was also investigated in a preclinical *in vivo* model that mimics human neonatal sepsis where newborn mice were passively transferred with cord sera from the vaccine group containing different anti-CPS Ia, Ib, or III IgG concentrations, or no specific IgG (negative controls), followed by GBS intraperitoneal challenge (Supplementary Table S1). Fig. 3 shows the mean protection levels against GBS Ia, Ib or III challenge in animals who received anti-CPS IgG in the ranges of 15–30 ng, 100–150 ng, or 250–500 ng. For all serotypes, statistically significant protection (*p*-values <.001) was observed in mice receiving at least 100 ng of specific IgG compared to negative controls. Protection levels were dose-dependent and highest protection rates were achieved with 250–500 ng dosages.

For serotype Ia passive administration of cord serum from subjects belonging to the placebo group versus the vaccine group for each equivalent specific IgG range was also compared. Significant protection was achieved in pups administered cord serum from the vaccine or the placebo groups for concentrations above 100 ng compared to pups receiving 15–30 ng of IgG or the negative sera (Supplementary Fig. S1), confirming similar levels of functional activity of CPS-specific IgG elicited either by natural exposure or by vaccination.

Discussion

This exploratory analysis demonstrated that administration of a single dose of an investigational GBS glycoconjugate trivalent vaccine during pregnancy resulted in statistically significant increases in antibody mediated GBS opsono-phagocytic killing in both maternal and cord blood at delivery compared with placebo. Cord sera passively transferred to newborn mice conferred a dose-dependent protective response against all three GBS serotypes, with significantly higher survival rates in pups receiving doses of specific antibody above 100 ng (approximately equivalent to 1 μg/mL of specific IgG in the mouse blood) compared to negative controls. Consistently, maternal and cord IgG levels above 1 μg/mL were predictive of OPKA functional activity for all three serotypes. These results confirmed that CPS-specific IgG elicited by the trivalent vaccine plays a major role in the defense against GBS.

Higher OPKA titers post-vaccination were observed in the population of women presenting detectable functional activity at baseline compared with those having negative OPKA titers prior to vaccination. These data are consistent with the previously reported ELISA results from the same study indicating higher immune responses in the primed population.[15]

The rate of OPKA-positive sera was higher against Ia and III compared to Ib both at baseline and post-vaccination, despite similar IgG concentration distributions between the three capsular types. This suggests that marginally higher anti-CPS IgG may be necessary to mediate phagocytic killing of GBS Ib compared to the other serotypes; whether this difference applies only to the H36B strain used here for OPKA or also to other Ib isolates will require further investigations. The higher number of Ia and III OPKA positive sera compared to Ib could also be associated with the higher frequency of these two serotypes among colonized women, and therefore a stronger priming/boosting effect that could result in higher affinity and more functional antibodies.

From a clinical development perspective, the low incidence of GBS invasive disease in neonates indicates that an extremely large Phase III study involving pregnant women would be necessary to demonstrate efficacy of an investigational vaccine; moreover, these assessments would be particularly difficult if conducted in regions currently using intrapartum antimicrobial prophylaxis. Based on the inverse relationship between maternal anti-CPS antibody and the occurrence of neonatal infection,[6] putative maternal CPS-specific IgG concentrations predictive of infant protection have been developed in case-control studies where sera from mothers delivering neonates with invasive GBS disease were matched with GBS-colonized mothers delivering non-infected infants.[7,10,11] To support IgG-based GBS serocorrelates, it is important to investigate how IgG concentrations in sera can predict functional activity levels. A positive correlation between naturally acquired maternal IgG concentrations anti-CPS Ia, Ib, and III and OPKA functional titers was reported in the European DEVANI study.[11] Here we have extended this observation by showing that capsular-specific IgG concentrations in maternal sera from vaccinated women are predictive of OPKA titers against each GBS serotype. The analysis conducted on cord sera showed the strongest correlation between ELISA IgG levels and OPKA titers, which could be related to placental transfer of a high affinity IgG subpopulation. Quantifiable measurements from both the vaccine and the placebo groups fell within the same regression line, suggesting comparable functional activity of naturally acquired and vaccine-induced GBS antibodies. In the mouse passive immunization model, equivalent amounts of cord anti-Ia IgG from either the placebo or the vaccine group provided comparable neonatal protection against GBS Ia challenge, supporting similar functional activity of antibodies originating from natural exposure or from vaccination. If confirmed, this relationship could further support the use of IgG concentrations derived from case-control epidemiological studies as surrogates of protection to predict the efficacy of maternal vaccines targeting GBS neonatal disease.

Our study has some limitations. Firstly, a relatively small number of maternal serum pairs and cord samples was available for

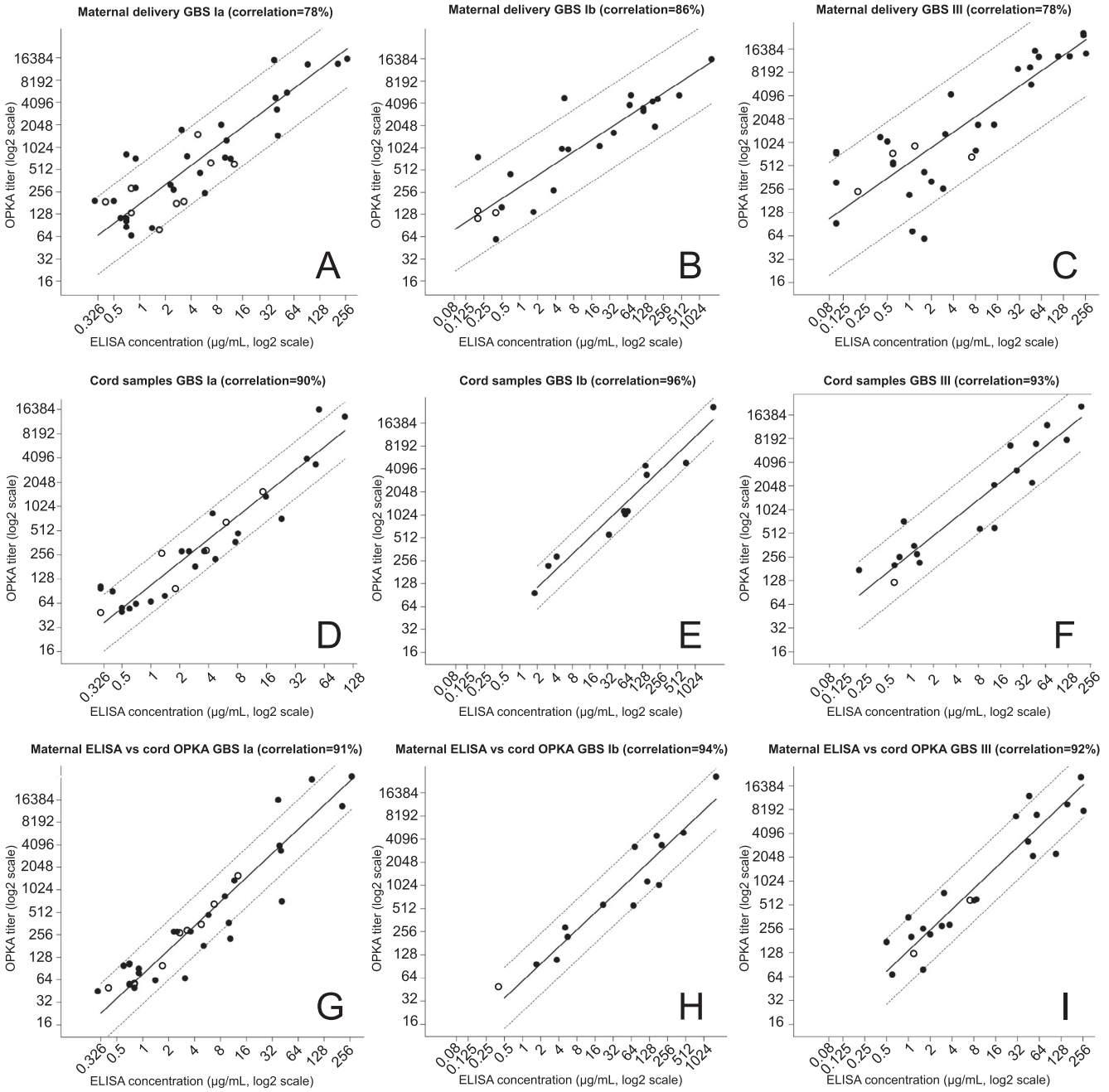

Fig. 2. Correlation between functional activity of anti-GBS antibodies and CPS-specific IgG concentrations in maternal and cord sera. Log2-log2 linear orthogonal regression analysis of quantifiable anti-CPS Ia, Ib, and III IgG concentrations and corresponding measurable OPKA titers are presented for individual maternal sera at delivery and paired infant cord sera. In each panel, vaccinated and placebo subjects are represented by full and empty dots respectively. Dashed lines represent the 95% probability bands of the regression lines. Spearman correlations between IgG concentrations and OPKA titers are reported in the panel titles.

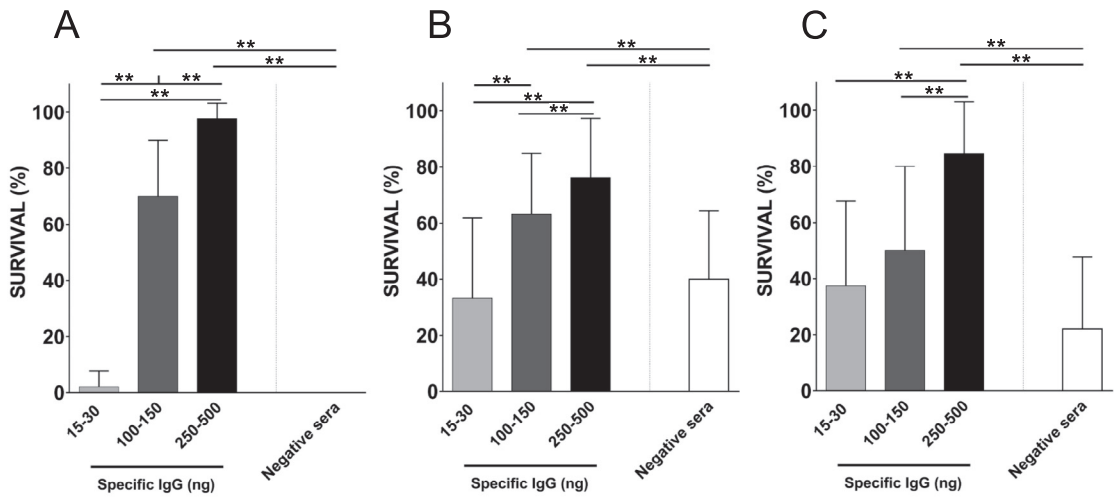

Fig. 3. Passive protection of newborn mice by cord sera. Within 24 hours from delivery, mice received 20 μL of cord sera containing different concentrations of IgG anti-CPS Ia (A), Ib (B) or III (C) in the ranges indicated in the x-axis; animals treated with negative sera were used as controls. Pups were infected with GBS 090 (Ia, A), H36b (Ib, B) or M781 (III, C) 24 hours later. Histograms report the estimated protection average for each serotype/IgG range and lines indicate the standard deviation. ** *p*-values ≤.001.

assessment; more precise predictions on the relationship between maternal and cord IgG titers and antibody functional activity will be possible with future analysis of higher numbers of sera. Secondly, the current lack of standardized assays across laboratories limits the possibility to extrapolate the results of this analysis to data measured in other studies. Assay standardization will also facilitate the definition of global serocorrelates of protection against GBS neonatal invasive disease.

Overall, this study provides further evidence that CPS-specific IgG play a major role in neonatal defense against GBS and that elicitation of functional antibodies by glycoconjugate vaccine combinations in pregnant women may constitute a suitable approach to the prevention of perinatal infections caused by GBS strains belonging to different serotypes.

Funding

This Phase II study was initially supported by Novartis Vaccines and Diagnostics, Inc., prior to the divestiture of its non-influenza vaccine business, which was acquired by GlaxoSmithKline Biologicals SA in March 2015. Since March 2015, GlaxoSmithKline Biologicals SA supported this Phase II study and also took responsibility for all costs associated with the development and publishing of this manuscript.

Authors contributions

MF, IM, GG, KS, SH, GD, RD, FW and PF designed the study. MF, IM, EF, GT, RD, SF, SH, EC, GD, FW and PF acquired the data. MF, IM, EF, GT, GG, RD, KS, FR, SF, SH, EC, GD and FW analyzed the data. MF, IM, EF, GT, RD, FR, SF, SH, EC, GD and FW contributed to the conduct of the study. All reviewed and revised the manuscript, and approved the final manuscript as submitted.

Conflicts of interest

KS, SF, FW and GG were employees of the GSK group of companies, and were also former employees of the Novartis Vaccines and Diagnostics Division. MF, GT, FR, EC, EF, PF and IM are employees of the GSK group of companies and were employees of the Novartis Vaccines and Diagnostics Division. FR and IM own shares of the GSK group of companies. SH declares having received grant funds paid to his institution by the Novartis Vaccines and Diagnostics Division for performance of the GBS clinical trial. Outside of the submitted work, SH declares having received consulting fees from the GSK group of companies and Sanofi Pasteur for serving on ad hoc advisory boards. All other authors have no conflicts of interest to declare. On 2 March 2015 Novartis non-influenza vaccines business was acquired by the GSK group of companies.

Acknowledgments

The authors thank the staff at all participating study centers for their careful attention to detail and the volunteers and their infants who participated in the study, as well as investigator Peter Dull. We also thank Marzia Giuliani, Elena Mori and Sherryl Baker for their support and useful discussions. Writing support was provided by Annick Moon (freelance for XPE Pharma and Sciences) and manuscript coordination was provided by Stéphanie Deroo (XPE Pharma & Sciences c/o GSK).

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.jinf.2018.01.006.

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