

Demonstration of Opsonic and Protective Activity of Human Cord Sera against Type III Group B Streptococcus that Are Independent of Type-Specific Antibody¹

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ABSTRACT. In an effort to further understand the host defense against group B streptococcus (GBS), we examined 71 human cord sera for their content of type III GBS IgG antibody by enzyme-linked immunosorbent assay and correlated the results with opsonic and protective activity against type III GBS. Most cord sera (67%) containing $>0.1 \mu\text{g/ml}$ of type III GBS IgG antibody promoted phagocytosis and killing *in vitro* and protection against type III GBS in neonatal rats. However, 26% of cord sera containing $<0.1 \mu\text{g/ml}$ of type III IgG antibody exhibited similar activity *in vitro* and *in vivo* against type III GBS. This opsonic and protective activity was retained in IgG fraction of whole serum, and was not directly associated with complement activity or with fibronectin. Further studies are needed to understand the mechanisms responsible for the opsonic and protective activity of some cord sera against type III GBS that may be independent of antibody to the type-specific polysaccharide antigen. (*Pediatr Res* 24: 628-632, 1988)

Abbreviations

CFU, colony-forming unit
ELISA, enzyme-linked immunosorbent assay
GBS, group B streptococcus
MEM, minimal essential medium with Earle's balanced salt solution
RABA, radioactive antigen-binding assay
PMN, polymorphonuclear leukocytes

Antibody to type-specific polysaccharides of GBS is important in protecting both humans and experimental animals against GBS diseases (1-5) and it has been suggested that a deficiency of type-specific antibody is a major risk factor for neonatal infection with GBS. Nevertheless, several studies from different geographic areas have demonstrated that most adults lack significant type-specific antibody of the IgG class (6-10) which can cross the placenta, and thus most newborns exposed to GBS are not protected by maternal GBS IgG antibody. A deficiency of type-

specific antibody alone therefore cannot explain the relatively low incidence of systemic GBS infection in neonates after intrapartum exposure to GBS. Approximately 50 to 70% of neonates exposed to GBS intrapartum become colonized, but only a small fraction (1-2%) of those neonates develop invasive GBS disease (11).

In studying the host defense against GBS, we observed opsonic and protective activity against type III GBS in the IgG fraction of some cord sera that appeared to be independent of type-specific antibody. This report describes those observations.

MATERIALS AND METHODS

Organism. A type III GBS (strain K79) isolated from the cerebrospinal fluid of a newborn with meningitis (12) was used for *in vitro* and *in vivo* studies.

Human cord sera. Human cord sera were collected aseptically from deliveries of healthy, term infants and only those that were free of maternal blood contamination (defined as IgM $<15 \text{ mg/dl}$) were included in this study. Cord blood was allowed to clot at 0°C (in ice) and serum was stored in aliquots at -70°C within 60 min of collection to preserve endogenous complement activity. At the time of *in vitro* and *in vivo* studies, sera were examined for complement activity by a CH100 assay (Kallestad Laboratories, Austin, TX) and/or CH50 using a standard hemolytic assay (13). Selected sera were also examined for individual complement components by radial immunodiffusion with goat anti-human complement (Nichols Institute, San Juan Capistrano, CA).

Total Ig and type III GBS IgG antibody. Each serum was examined for total IgM concentration by radial immunodiffusion with goat anti-human IgM (Kent Laboratories, Redmond, WA). IgG antibody against the type III polysaccharide was measured by ELISA (6, 14). Selected sera were also examined for total IgG and IgA by radial immunodiffusion with goat anti-human IgG and IgA (Kent) and for fibronectin concentration by radial immunodiffusion with rabbit anti-human fibronectin (Behring Diagnostics, La Jolla, CA).

***In vitro* opsonophagocytic assays.** The opsonic activity of cord sera was determined by the method described previously (15). Neutrophils from normal adults were isolated by dextran sedimentation followed by Ficoll-Hypaque density centrifugation. Washed neutrophils (approximately 1×10^6 PMN/40 μl) were added to $12 \times 75 \text{ mm}$ sterile polystyrene tubes (Falcon Plastics, Oxnard, CA) along with 10 μl of washed logarithmic-phase strain K79 (approximately 2×10^5 CFU), 10 μl of cord sera and 20 μl of MEM (GIBCO Laboratories, Santa Clara, CA) in a total

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volume of 80 μ l. The same MEM was used in washing and resuspending the PMN and bacteria. The phagocytic mixture contained a ratio of PMN to bacteria of approximately 5/1. Control tubes lacking either PMN or serum were included in each experiment. The tubes were incubated at 37°C with tumbling on a multipurpose rotator (Scientific Industries, Inc., Springfield, MA). Aliquots (10 μ l) were taken from each tube at 0 and 1 h, serially diluted 10-fold in sterile distilled water, and plated on blood agar to determine surviving CFU. Results were expressed as the percentage of bacteria killed: $100 - [(CFU \text{ at } 1 \text{ h} / CFU \text{ at } 0 \text{ h}) \times 100]$.

Separation of IgG from whole sera. To determine if the IgG fraction of cord sera was responsible for opsonic activity, IgG was separated from cord sera by the method of Johnson and Libby (16). Briefly, 250 μ l of whole serum diluted 20-fold in 0.05 M EDTA buffer, pH 7.0, was passed through a small column (1 ml bed volume) of quarternary aminoethyl-Sephadex A-50 (Pharmacia Fine Chemical, Piscataway, NJ), which was then eluted with 5 ml of the same buffer. The eluate was concentrated approximately 40-fold with an ultrafiltration membrane (Diaflo XM 50, Amicon Corp, Lexington, MA), dialyzed against phosphate-buffered saline, pH 7.4, overnight at 4°C in cellulose dialysis tubing (mol. wt. cutoff 12,000–14,000, Spectrapor membrane tubing, Spectrum Medical Industries, Inc., Los Angeles, CA) and used for opsonophagocytic and animal experiments. Opsonophagocytic assays were essentially the same as described previously except that cord serum devoid of both type III IgG antibody and opsonic activity was added as a source of complement because complement activity was undetectable in the IgG fractions separated by QAE-Sephadex. Total levels of IgG, IgM, and IgA were measured simultaneously on whole and fractionated serum.

Animal studies. Outbred, pathogen-free Sprague-Dawley pregnant rats (with timed conception) were purchased from Charles River Breeding Laboratories (Wilmington, MA) and gave birth in our vivarium 5–7 days after arrival. Each adult rat and her pups were housed in a separate, solid polypropylene, opaque cage with a filter hood.

GBS bacteremia was induced in 5-day-old rats as described previously (15, 17, 18). At 5 days of age, all members of each litter were divided into two groups to receive intraperitoneally 0.1 ml of cord serum with opsonic activity (>80% killing at 1 h of incubation) or cord serum with less opsonic activity. Two or three rats from each litter served as controls, receiving 0.1 ml of saline. Within 30 min, animals were inoculated subcutaneously with 1×10^5 CFU of type III GBS strain K79. As shown previously (15, 17, 18), this inoculum produces nonlethal bacteremia within 18 h and subsequent death within 5 days in almost 100% of the animals. A total of 18 h after bacterial inoculation, 0.1 ml of blood was obtained from each rat for quantitative cultures. Mortality was recorded for 5 days, and postmortem blood cultures were done to confirm GBS infection.

Statistical methods. The odds ratio was used to determine the association between concentrations of type III GBS of IgG antibody and opsonic activity (19). The χ^2 (with Yates' correction for small samples) was used to compare the incidence of bacteremia and the mortality rates, and the Student's *t* test was used to compare the magnitude of bacteremia among groups of animals (20).

RESULTS

Type III GBS antibody versus opsonic activity. In all, 71 cord sera were examined for type III GBS IgG antibody by ELISA and the results were compared with those of opsonic activity against a type III GBS strain (Fig. 1). Thirty-three cord sera contained ≥ 0.1 μ g/ml of type III GBS IgG antibody and 38 cord sera contained <0.1 μ g/ml of type III IgG antibody. When cord sera were examined for total complement activity by CH100

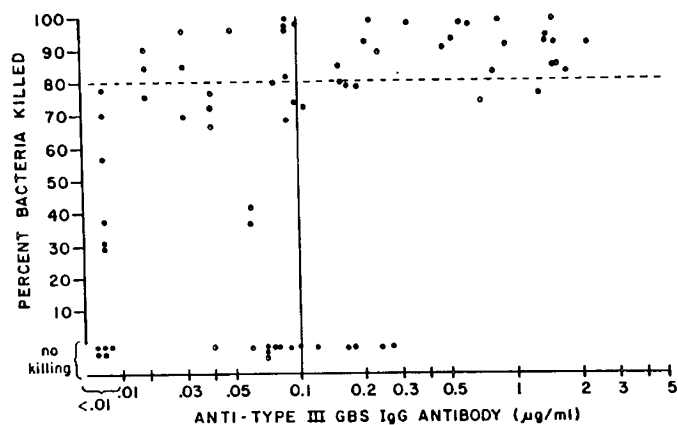


Fig. 1. Comparison of 71 human cord sera for the contents of type III GBS IgG antibody (μ g/ml) and opsonic activity (% bacteria killed after 1 h of incubation).

assay, the majority of cord sera (41/71 or 65%) contained >70 CH100 U/ml (considered normal range). Selected sera with >70 CH100 U/ml were also found to contain 120–180 CH50 U/ml (normal range in our laboratories).

In opsonophagocytic assays, bacterial killing did not occur in control tubes, indicating that phagocytosis and killing of type III GBS organism required both PMN and cord sera. Of the 33 cord sera that contained ≥ 0.1 μ g/ml of type III IgG antibody, most (22/23 or 67%) were, as expected, highly effective in supporting phagocytosis and $\geq 80\%$ killing of the original inoculum of strain K79 after 1 h of incubation. The odds of exhibiting efficient opsonic activity ($\geq 80\%$ killing) were 5.6 times greater for cord sera containing ≥ 0.1 μ g/ml of type III GBS IgG antibody than for cord sera containing lesser amounts of type III IgG antibody. This odds ratio was significant ($p = 0.001$).

Surprisingly, 10/38 (26%) cord sera containing <0.1 μ g/ml of type III GBS IgG antibody were also effective in supporting the killing of $\geq 80\%$ of the original inoculum. The demonstration of efficient opsonic activity was not directly influenced by CH100 values. Six (60%) of the 10 cord sera containing <0.1 μ g/ml of IgG antibody and exhibiting $\geq 80\%$ killing contained >70 CH100 (normal range); whereas 19 (68%) of 28 cord sera with <0.1 μ g/ml of antibody and showing <80% killing contained >70 CH100 (normal range). Similarly, reduced opsonic activity of cord sera containing >0.1 μ g/ml of type III IgG antibody was not due to decreases in CH50 or individual complement components (Table 1).

To examine the significance of these findings further, we studied cord sera with <0.1 μ g/ml of antibody for their protective activity against experimental GBS infection in newborn rats.

Opsonic versus protective activity. First, we evaluated and compared two cord sera (nos. 19 and 23) containing similar amounts of type III GBS IgG antibody but differing in their opsonic activity (96% killing versus no killing, respectively). As summarized in Table 2, these two cord sera contained similar amounts of total IgG, IgM, type III GBS IgG, fibronectin, and CH100. Table 3 summarizes the incidence and magnitude of bacteremia at 18 h after subcutaneous inoculation with strain K79, and overall survival rates in groups of animals receiving cord sera 19 or 23 or saline. After 18 h of infection, all control animals receiving saline (six of six) were bacteremic. The prevalence of bacteremia at this time was significantly lower ($p < 0.01$) in animals receiving cord sera 19 than in those receiving cord sera 23. The magnitude of bacteremia at 18 h was compared among animals with positive blood cultures. Bacterial counts in blood of animals receiving cord sera 19 were significantly lower ($p < 0.01$) than those receiving cord sera 23 or saline. Survival rates over the 5-day period are also summarized in Table 3; the overall rate was significantly higher in animals receiving cord

sera 19 than in those receiving cord sera 23 or saline ($\chi^2 = 6.00$ and 7.65, respectively, $p < 0.02$).

We next attempted to determine if the opsonic and protective activity of cord sera was related to IgG or non-IgG components. For this study, we used two cord sera (nos. 30 and 31) containing low levels of type III GBS IgG antibody (0.02 and 0.03 $\mu\text{g/ml}$), respectively) but exhibiting excellent opsonic activity (90 and 96% bacterial killing, respectively). As shown in Table 4, IgG separated by QAE-Sephadex yielded equivalent amounts of IgG compared with whole serum and contained no detectable IgM and IgA. Inasmuch as the type III GBS IgG antibody concentrations were very low in whole serum, they were not measured in

separated IgG preparations. Although cord serum 31 contained 4.6 mg/dl of fibronectin, fibronectin was not detectable in separated IgG. Opsonophagocytic studies revealed that enhancement of PMN-mediated killing by separated IgG was similar to that by whole serum (Table 4). Similarly, when separated IgG preparations were evaluated for their protective activity against experimental type III GBS infection compared with whole serum, their protective efficacy was similar, as shown by similar prevalence and magnitude of bacteremia at 18 h after subcutaneous inoculation with strain K79 and similar survival rates (Table 5).

DISCUSSION

Our studies revealed that most cord serum containing significant amounts of type III GBS IgG antibody ($\geq 0.1 \mu\text{g/ml}$) were, as expected, highly effective in enhancing phagocytosis and killing of type III GBS ($\geq 80\%$ killing of the original inoculum). This efficient opsonophagocytic activity was also directly correlated with significantly greater protection against bacteremia and death in newborn rats challenged with a type III GBS strain. However, 26% of cord sera containing lesser amounts of type III GBS IgG antibody also exhibited efficient opsonophagocytic and protective activity against type III GBS. This activity was independent of total complement activity, as shown by a lack of correlation between CH100/CH50 values and opsonic activity among cord sera containing similar amounts of type III GBS IgG antibody.

Several studies have shown that IgM monoclonal antibody against type III polysaccharides effectively provides protection against experimental type III GBS infection in neonatal rats and mice (9, 22). Campbell *et al.* (23) have also shown that the opsonophagocytic activities of naturally acquired IgM and IgG antibodies for type III GBS are comparable. A recent report indicated a strikingly high prevalence of IgM antibody to type III GBS compared with IgG antibody in some adult and maternal sera (6). Boyer *et al.* (24) have shown that development of IgM

Table 1. Comparison of CH50 and individual complement components between cord sera possessing similar amounts of type III GBS IgG antibody but differing in their opsonic activity

	Cord serum		Cord serum	
	5S	15S	32	13S
Type III IgG ($\mu\text{g/ml}$)	0.23	0.21	1.77	1.6
CH50 (normal, 120–180 U/ml)	132	156	141	132
Clq (normal, 5–8.6 mg/dl)	3.2	3.9	3.5	3.6
C2 (normal, 2–3.7 mg/dl)	1.8	2.6	2.2	2.0
C5 (normal, 7–16 mg/dl)	8.9	10.9	10.9	7.9
C6 (normal, 3.6–7.5 mg/dl)	2.7	3.1	3.0	2.4
C7 (normal, 3.9–7.8 mg/dl)	5.6	7.2	8.1	6.5
C8 (normal, 2.7–6.0 mg/dl)	<2.4	<2.4	<2.4	<2.4
C9 (normal, 2.4–6.9 mg/dl)	1.7	2.0	<1.7	<1.7
Opsonic activity (% bacteria killed)	No killing	92	60	96

Table 2. Comparison of total IgG, IgM, IgA, type III GBS IgG, fibronectin, CH100, and opsonic activity between cord sera 19 and 23

	Cord serum 19	Cord serum 23
Total IgG (mg/dl)	947	600
Total IgM (mg/dl)	13	10.4
Total IgA (mg/dl)	<5.6	<5.6
Type III GBS IgG ($\mu\text{g/ml}$)	0.09	0.08
Fibronectin (mg/dl)	5.5	6.1
CH100 (U/ml)	71	80
Opsonic activity (% bacteria killed)	96	No killing

Table 4. Total IgG, IgM, IgA, and type III GBS IgG concentrations and opsonic activity in IgG-containing sera separated from cord sera by quaternary aminoethyl-Sephadex in comparison with whole sera

	Cord sera 30		Cord sera 31	
	Whole sera	Separated IgG	Whole sera	Separated IgG
IgG (mg/dl)	950	1050	870	790
IgM (mg/dl)	9.7	<4.9	4.9	<4.9
IgA (mg/dl)	<5.6	<5.6	<5.6	<5.6
Type III GBS IgG ($\mu\text{g/ml}$)	0.02	Not done	0.03	Not done
CH 100 (units/ml)	100	Undetectable	39	Undetectable
Opsonic activity (% bacteria killed)	90	90	99	96

Table 3. Incidence and magnitude of bacteremia and overall survival among animals receiving cord sera 19 or 23 or saline

Cord sera	No. of animals	No. of animals with positive blood cultures (%)*	Magnitude of bacteremia*† (log ₁₀ CFU/ml of blood)	Overall survival (%)
19	13	2 (15%)‡	1.35 ± 0.00	12 (92)§
23	13	10 (77%)	4.91 ± 1.52	6 (46)
Saline	6	6 (100%)	5.06 ± 0.89	1 (17)

* A total of 18 h after infection.

† Expressed as mean ± SD.

‡ The prevalence of bacteremia was significantly less ($\chi^2 = 7.58$, $p < 0.01$) with cord serum 19 than with cord serum 23.

§ The overall survival rate was significantly greater with cord serum 19 than with cord serum 23 or saline ($\chi^2 = 6.00$ and 7.65; $p < 0.02$ and $p < 0.01$, respectively).

Table 5. Incidence and magnitude of bacteremia and overall survival among animals receiving cord sera, separated IgG or saline

Experiment	Sera	No. of animals	No. of positive blood cultures (%) [*]	Magnitude of bacteremia ^{*†} (log ₁₀ CFU/ml of blood)	Survival
1	Cord sera #30	8	2 (25)‡	1.35 ± 0.00	7 (88)
	Separated IgG	8	1 (12)‡	1.35	7 (88)
	Saline	8	7 (88)	3.22 ± 1.82	4 (50)
2	Separated IgG from cord sera #31	9	0	0	9 (100)
	Saline	6	6 (100)§	5.13 ± 0.96	0

* A total of 18 h after infection.

† Expressed as means ± SD.

‡ The prevalence of bacteremia was significantly less with cord sera 30 or separated IgG than with saline ($\chi^2 = 4.06$ and 6.25 , respectively, $p < 0.05$).

§ The prevalence of bacteremia was significantly less ($\chi^2 = 11.12$, $p < 0.001$) with separated IgG than with saline.

specific antibody to type III GBS is age dependent; acquisition of protective levels of IgM antibody to type III GBS ($\geq 0.3 \mu\text{g/ml}$) in infants is observed after 6 months of age (23). These findings suggest that the presence of IgM antibody may in part explain the infrequent occurrence of invasive GBS disease beyond infancy. In the present study, cord sera were selected based on their low total IgM concentrations ($< 15 \text{ mg/dl}$). Type III GBS IgM antibody was not measured because, in our previous studies (6), IgM antibody was undetectable in cord sera free of maternal blood contamination. Moreover, IgG separated from whole serum devoid of IgM showed opsonic and protective activity against type III GBS similar to that of whole serum. Therefore, the excellent functional activity of some cord sera containing $< 0.1 \mu\text{g/ml}$ of type III GBS IgG antibody is not likely due to the presence of IgM antibody.

Other host factors such as fibronectin also did not appear responsible for the functional activity of some cord sera containing insignificant amounts of type III GBS antibody. When two cord sera having similar amounts of type III GBS IgG antibody (0.08 and $0.09 \mu\text{g/ml}$, respectively) as well as fibronectin (6.1 and 5.5 mg/dl , respectively) were examined for their opsonic and protective activity, there was a wide disparity between them. Moreover, separated IgG devoid of fibronectin was found to exhibit similar opsonic and protective activity compared to whole serum.

There are obvious difficulties in comparing measurements of specific antibody in different laboratories. We therefore selected 11 cord sera for study of total type III GBS antibody by the radioactive antigen-binding assay (RABA) (25), which presumably measures all isotypes, in the laboratory of Dr. Morven Edwards of Baylor College of Medicine, Houston, TX. There was a strong correlation between the two assays. All but two cord sera containing $< 0.1 \mu\text{g/ml}$ of type III GBS IgG antibody by ELISA were found to have type III GBS antibody $< 2 \mu\text{g/ml}$ by RABA (considered to be low levels of antibody) (25, 26). Therefore, these sera clearly contained low concentrations of type III IgG antibody. Available data do not indicate that differences in opsonic activity are likely due to differences in antibody affinity for type III polysaccharide. This was suggested by similar slopes of optical density values in ELISA for dilutions of cord sera containing similar amounts of type-specific IgG antibody, but differing in their opsonic activity (data not shown). However, it is possible that some opsonic and protective activity of low concentrations of type III IgG antibody in some sera may be due to greater affinity/avidity.

The type III GBS strain K79 used for this study was found to possess R protein. When cord sera possessing efficient opsonophagocytic activity against strain K79 ($\geq 80\%$ killing of the original inoculum) were reexamined after absorption with type III polysaccharides, opsonophagocytic activity was completely abolished against another type III GBS strain without protein

antigens, but similar opsonic activity ($\sim 60\%$ killing) was present against the R protein-positive strain K79 (data not shown). These two type III strains with or without R protein contained similar amounts of sialic acid (data not shown), indicating that differences in opsonophagocytic activity against two type III GBS strains of sera absorbed with type III polysaccharides are not due to differences in sialic acid content. These findings suggest that additional antibody directed against other surface antigens (e.g. R protein) may possibly be responsible for opsonic activity observed against strain K79 in some cord sera. Linden *et al.* (27) and Christensen *et al.* (28) have suggested that R protein antibody may be important in protecting mice against GBS strains possessing R protein. Additional studies are therefore needed to determine if the opsonic and protective activity that appear to be independent of type-specific GBS antibody may stem from antibodies directed against other surface antigens (e.g. R protein).

We are still far from a complete understanding of the host-microbial factors that determine the development of invasive GBS infection in human neonates. However, our observations suggest that the opsonic and protective activity that appears to be independent of type-specific antibody noted in our report may in part account for the relatively low incidence of systemic GBS infection in neonates subsequent to intrapartum exposure to GBS.

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