

Impaired Chemotaxis by Type III Group B Streptococci in Neonatal Sera: Relationship to Diminished Concentration of Specific Anticapsular Antibody and Abnormalities of Serum Complement

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Summary

A chemotaxis (CTG) assay employing adult or neonatal sera, type III group B streptococci (GBS) and polymorphonuclear leukocytes (PMNs) was designed to evaluate the role of PMN mobilization in the pathogenesis of type III GBS infection in neonates. Generation of C5a in healthy adult sera with moderate-high (3-40 µg/ml) or low (≤ 2 µg/ml) levels of specific anticapsular antibody was confirmed by PMN aggregometry and by the neutralization of CTG by goat anti-human C5. CTG was significantly ($P < 0.001$) greater in high as compared to low specific antibody-containing adult sera; stepwise increases in CTG occurred when specific IgG was added to untreated, but not heat-inactivated, hypogammaglobulinemic serum. Immunospecificity of CTG was shown by a failure of type III GBS to generate C5a in heterologous (type Ia) high antibody sera. Mean CTG values in three high and 16 low antibody-containing sera from healthy term neonates were 24% and 62% of high ($P < 0.001$) and low ($P < 0.01$) antibody adult sera, respectively. The addition of both complement and specific IgG to low antibody-containing neonatal sera was required to enhance their CTG activity to high antibody adult values. CTG by type III GBS in neonatal sera-neonatal PMN mixtures was only 25% (high antibody sera) and 14% (low antibody sera) of values for paired maternal sera mixtures reacted with adult PMNs ($P < 0.001$). These studies demonstrate that CTG by type III GBS in neonatal sera is markedly diminished and that low concentrations of specific anticapsular antibody and abnormalities of complement function contribute to impaired PMN mobilization in human neonates.

Abbreviations

Ab, antibody
ACP, alternative complement pathway
C4D, C4 deficiency
CH50, hemolytic complement activity
CL, chemoluminescence
CTG, chemotaxis
DPBS, Dulbecco's Phosphate Buffered Saline
GBS, group B streptococci
GPS, guinea pig sera
PMN, polymorphonuclear leukocyte
THB, Todd-Hewett broth
ZAP, zymosan-activated plasma

Among inflammatory functions, the localized recruitment of phagocytic cells to foci of microbial invasion represents a critical and first line host defense mechanism (5, 32, 49). Impaired leukocyte tissue mobilization has been demonstrated in human neo-

nates and other individuals with disorders characterized by a high risk for the development of severe pyogenic infections (5, 19, 37). Previous investigations in human neonates have demonstrated abnormalities in both the humoral and cellular contributions to leukocyte motility *in vitro* (5, 22, 25, 27, 31, 33, 36, 46), but specific serum requirements for generation of chemotactic factors (chemotaxis) by GBS have not been determined. The experiments described in this report were designed to study the chemotactic properties of type III GBS and to define chemotaxis requirements in human sera for this serotype. Methods were developed to evaluate selectively the functional importance of serum antibody (IgG) specific for native III capsular polysaccharide and complement in the generation of C5a by GBS in sera from neonates and healthy adults. Markedly diminished CTG by type III GBS in neonatal sera was demonstrated and was related both to low concentrations of specific antibody and abnormalities of complement function.

MATERIALS AND METHODS

Isolation of PMN. Venous blood samples were collected from a single adult volunteer and the placentas of healthy neonates in a syringe containing 10 units of sodium heparin per ml of blood. After sedimentation of erythrocytes in dextran, leukocyte-rich plasma was layered over a Ficoll-Hypaque solution consisting of 10 parts of 33.9% Hypaque (Winthrop Labs, New York, NY) and 24 parts of 9% Ficoll (Sigma Chemical Co., St. Louis, MO) and centrifuged at $800 \times g$ for 30 min. For use in CL and aggregometry studies, remaining erythrocytes were lysed in distilled water, and isotonicity was restored with 1.8% NaCl. Purified suspensions of PMNs were washed thoroughly and resuspended in DPBS, pH 7.3, which contained 0.2% dextrose (Grand Island Biological Co., Grand Island, NY). The final cell concentration was adjusted to a 1×10^7 PMN/ml by addition of DPBS.

Preparation of organisms. The strains of GBS used in these investigations were type III (M732, M781, and M803) and type Ia (R515) clinical isolates from infants with neonatal meningitis and Lancefield prototype Ia strain 090. Frozen aliquots (-20°C) of bacteria were inoculated into 10 ml of THB and incubated at 37°C overnight. The suspensions were adjusted to an appropriate optical density for each strain (0.85-0.95 at 540 nm) (Spectronic 20, Bausch & Lomb, Rochester, NY). These suspensions were centrifuged at $1000 \times g$ for 10 min at 25°C , washed, and resuspended in an equal volume of DPBS. These preparations consistently contained 5×10^8 - 10^9 colony forming unit/ml.

Sera. Samples of whole blood were obtained from 13 healthy adult volunteers, the placentas of 21 healthy full-term (gestational age, 37-40 wk) infants, and from the mothers of five of these infants at the time of delivery. Informed consent to perform

phlebotomy was obtained from the mothers of study infants and adult control donors. Placental samples were processed immediately. Samples were allowed to clot at room temperature (about 25°C for 30 min) and sera were separated by centrifugation and frozen in aliquots at -70°C before testing. Aliquots of these sera were heated at 56°C for 30 min to inactivate complement or were chelated at 25°C for 10 min with 4 mM MgCl and 8 mM EGTA (Sigma Chemical Co.) (15). Sera from GPS with homozygous C4D and normal guinea pigs were processed in a similar manner following phlebotomy.

Sera were selected for use in these investigations on the basis of their concentrations of specific antibody to capsular antigens of type III GBS (4). Adult and neonatal samples were categorized into groups containing moderate-high (3–42 µg/ml) or low (<2 µg/ml) levels of specific antibody. In functional assays, comparisons between test sera containing >2 µg Ab/ml in contrast to <2 µg Ab/ml were based on previous studies demonstrating susceptibility to type III GBS disease only among infants whose serum contains <2 µg Ab/ml (7). Serum from an adult with common variable immune deficiency disease [IgG, 143 mg/dl; IgA, 9 mg/dl; IgM, 21 mg/dl; and specific antibody, <0.3 µg/ml (type III, <1.8 µg/ml (type Ia))] was employed in reconstitution experiments requiring exogenous complement.

Serum complement determinations. Quantitation of CH50 in test sera was determined as previously described (30), and concentrations of complement components C3, C4, and Factor B were determined with an immunonephelometric technique (Atlantic Antibody, Scarborough, MN) (11). Concentration of C5 was determined by radial immunodiffusion with C5 antisera (Miles Labs, Elkhart, IN) (29). Partially purified C5 was prepared by the method of Hammer *et al.* (20) with only minor modifications. The material was not passed over an immunoabsorbent for IgG and IgA, the only contaminants demonstrated by SDS-PAGE. The concentration of C5 determined by electroimmunoassay was 27 µg/ml. Hemolytic activity was determined using cellular intermediates and complement component from Cordis Laboratories (Miami, FL). The hemolytic activity was 569 units/µg/ml as compared to a normal serum with an activity of 994 unit/µg/ml.

IgG preparation. Purified IgG was prepared from adult donor serum known to contain a high concentration (20 µg/ml) of specific antibody to native type III GBS polysaccharide. Serum was treated with octanoic acid followed by DEAE chromatography (43). Contaminating IgG or IgA were removed by passage over the appropriate antibody coated sepharose 4B column.

Radioactive antigen-binding assay. The concentration of antibody to native type Ia or III GBS polysaccharides in adult or neonatal human sera and in GPS was determined by a radioactive antigen-binding assay described previously (8, 9). Antigens used in the present studies were purified native type III and type III capsular polysaccharides intrinsically labeled with [³H]. Antigens were provided by Dr. Dennis Kasper, Channing Laboratory, Harvard Medical School, Boston, MA.

Luminol-enhanced phagocytosis-CL assay. Opsonic mixtures containing bacterial suspensions (0.5 ml; 5×10^8 colony forming units in DPBS), serum (0.25 ml), and DPBS (0.25 ml) were reacted for 45 min at 37°C in a shaking water bath. Bacteria were then centrifuged at 4°C, washed twice and resuspended in DPBS. Final phagocytic reaction mixtures contained 2.5×10^6 PMNs, 4×10^8 colony forming units of opsonized or unopsonized bacteria, and 10^{-7} M luminol (5-amino-2,3-dihydroxy-1,4-phthalazine dione) (Sigma Chemical Co.). Quantitation of CL was performed on duplicate samples in a liquid scintillation counter (model #C-2425 Tri-Carb, Packard Instruments, Houston, TX) with the coincidence circuit disengaged. CL data was presented as the integral (area under the curve) of the CL response between 0–75 min after initiation of phagocytosis (4).

CTG assays. Opsonic mixtures containing type III or type IA Group B S and test sera as prepared for CL experiments were incubated at 37°C for 45 min before CG determinations. Opsonized organisms were removed from test sera by centrifugation at 10,000 × g (4°C) for 15 min. The supernatant of each opsonic

mixture was transferred to sterile vials and heated for 30 min at 56°C to avoid nonspecific complement activation (23). Aliquots were then diluted to a final concentration of 12½% (v/v) in DPBS for use in chemotaxis assays. Control opsonic mixtures containing type Ia or type III GBS without serum and serum alone were included in each experiment.

Directed migration of healthy adult or neonatal PMN leukocytes was assessed by a modified Boyden technique (5). Supernatants of opsonic reaction mixtures were placed in the stimulant compartments of blindwell chemotaxis chambers together with 3-µm micropore filters (Millipore Corp., Bedford, MA). PMNs (0.2 ml, 2×10^6 PMN/ml) were then added to the cell compartment of chambers, which were incubated at 37°C in a CO₂ environment for varying time intervals. The behavior of PMN migration in this system was evaluated by determining the depths into filters at which only two cells were in focus in one high power field ("leading front") after 80 min incubation (14). Directed migration values were determined by subtracting random motility values (phosphate buffered saline alone in the stimulant compartment) from total migration "leading front" values.

Characterization of serum-derived chemotactic factor(s). To determine the nature of the chemotactic factor(s) produced in serum by type III GBS, test mixtures were employed in PMN aggregometry and adherence assays. PMN aggregometry was performed as previously described (14, 21, 35) using test serum incubated with zymosan, bacteria, or DPBS to effect aggregation of cytochalasin B pretreated (50 µg/ml, 37°C, 15 min) PMNs obtained from a single healthy adult. In preliminary experiments, an arbitrary scale was established in which ZAP yielded 100 ZAP deflection units; a 1:2 dilution of ZAP in DPBS yielded 50 ZAP units, etc. The aggregating activities of GBS-serum reactants were measured at 4-min intervals and were expressed in ZAP units (39). In parallel experiments, selected GBS-serum reactants were also assessed for enhancement of PMN adherence for protein-coated substrates as previously described (40).

Anti-human C5 (goat) antiserum (Behring Diagnostics, Somerville, NJ) was heated (56°C, 30 min) and centrifuged at 10,000 × g for 10 min. A 100 µl of antiserum was then incubated for 30 min at 37°C with 0.5 ml of the supernatant of selected opsonic mixtures. The resulting preparations were then assayed for PMN chemotactic, aggregating, and adherence promoting activities (39, 40).

RESULTS

Chemotactic and chemotaxigenic activity generated by Type III or Ia GBS. To determine the relative contributions of chemotaxigenic activity derived from bacteria-serum interactions compared with that due to bacteria alone, dose response experiments were performed (Fig. 1). Chemotactic activity was observed in the supernatants of both type III and Ia GBS suspensions incubated in the absence of serum; however, this activity was not detectable when these reactants were diluted to a 10% (v/v) concentration in DPBS. Chemotactic activity generated by both GBS serotypes in the presence of 25% (v/v) serum was significantly greater than that generated without serum. Anti-C5 pre-incubation neutralized activity generated in GBS-serum mixtures but not that of GBS organisms without serum. As shown, maximal activity in this experimental system was demonstrated when 15% (v/v) concentrations of opsonic supernatants were employed; lower values occurred at higher or lower concentrations. A 12.5% (v/v) final concentration was selected for all subsequent experiments to exclude the influence of serum independent (bacterial) chemotactic factors and thus, to allow selective evaluations of CTG by GBS in test sera.

CTG in Serum by Type III GBS: Role of Specific Antibody and Complement

Kinetics of CTG. The kinetics of chemotaxigenic activity generated by type III GBS in untreated, chelated, or heated serum is illustrated in Figure 2. Test sera included normal adult serum containing a high level of type III-specific anticapsular antibody

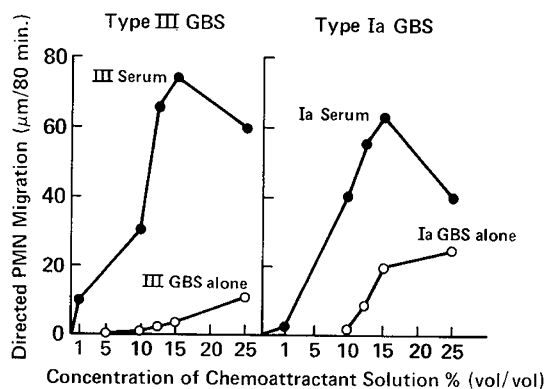


Fig. 1. Chemotaxis by type III (M732) or type Ia (515 group B streptococcus (GBS) (●—●) in healthy adult serum containing high concentrations of type III or Ia specific antibody and cytotoxic activities of supernatants of GBS suspensions without serum (○—○). Serial dilutions of the supernatants of GBS-serum/PBS mixtures were incorporated in the stimulant compartment of Boyden Chambers. Values shown represent the mean of three experiments.

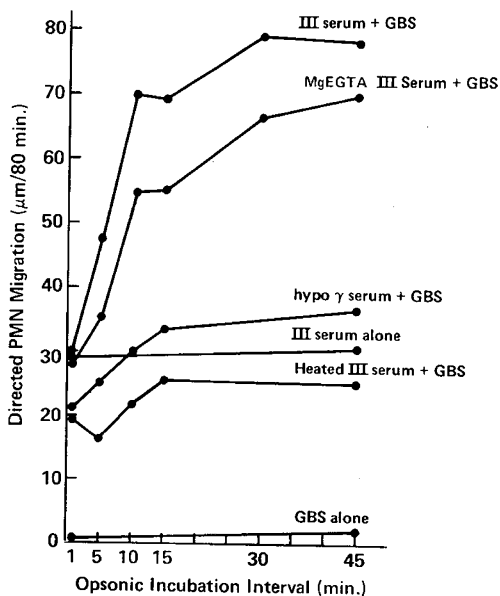


Fig. 2. The kinetics of chemotaxis by type III GBS in (1) adult serum containing high concentrations of specific antibody (III serum), (2) the same serum following MgEGTA-chelation (MgEGTA) or heat-inactivation (●) and (3) hypogammaglobulinemic serum (hypo γ). These data represent the mean of three separate experiments performed in triplicate. Values for III serum at 5, 10, 15, and 30 min time intervals are significantly ($P < 0.05$) increased compared to MgEGTA-chelated serum.

(29.1 $\mu\text{g/ml}$) and hypogammaglobulinemic serum ($<0.3 \mu\text{g/ml}$). These studies were performed by incubating 5×10^8 organisms in 25% (v/v) test sera for 1, 5, 10, 15, 30, or 45 min. Maximal activity generated in untreated high antibody-containing sera was observed after 10–15 min incubation intervals. In contrast, the rate of CTG in MgEGTA-treated sera was delayed, but maximal values achieved were $\approx 88\%$ of those in untreated sera. After 45-min incubation intervals, only minimal increments were observed in hypogammaglobulinemic serum, and determinations in heated serum or serum incubated without GBS were essentially comparable to respective 1-min interval samples.

CTG by type III GBS in untreated, MgEGTA-chelated and heated adult sera. The chemotaxigenic contributions of specific antibody and/or complement are summarized in Figure 3. Data shown represent experiments performed with strain M732 although similar results were observed when these sera were tested

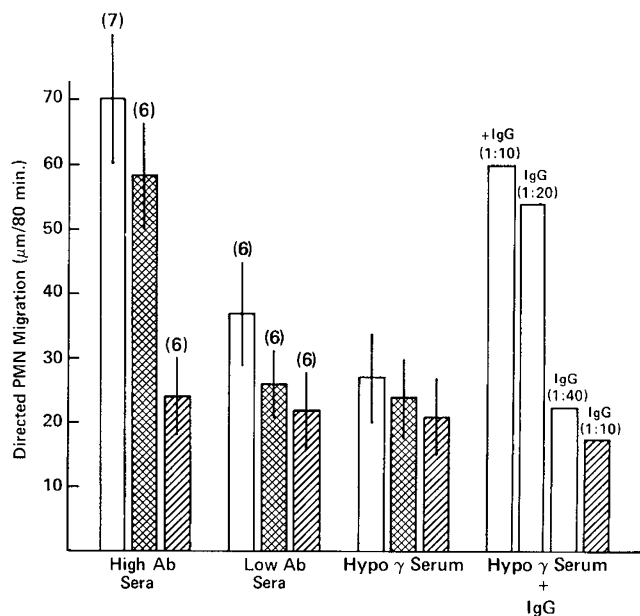


Fig. 3. Chemotaxis by type III GBS in untreated (□), MgEGTA-chelated (▨) or heated (▧) sera containing moderate-to-high (4.8–41.8 $\mu\text{g/ml}$), low ($\leq 2 \mu\text{g/ml}$) or undetectable concentrations (hypo γ) of type III specific antibody. Directed migration values shown represent the mean \pm S.D. of mean values determined for a minimum of three experiments performed on each serum obtained from (n) healthy adult donors. Purified IgG at various dilutions was added to untreated and heated hypogammaglobulinemic serum.

with type III GBS strains M781 and M803 (data not shown). Mean directed migration \pm S.D. values for untreated moderate-to-high antibody containing sera ($70 \pm 9 \mu\text{m}$) were significantly greater than those of six low antibody sera ($37 \pm 8 \mu\text{m}$) or hypogammaglobulinemic serum ($27 \pm 8 \mu\text{m}$) ($P < 0.001$ for both). Determinations in high or low antibody sera (but not hypogammaglobulinemic serum) were significantly increased compared to respective heat-treated samples ($P < 0.01$ by paired "t" test). Among each serum group, mean values for heated sera were comparable to those of sera incubated in the absence of GBS. In addition, for representative test sera containing high, low, or undetectable antibody concentrations, anti-C5 preincubation diminished CTG values to approximately those noted in heat-inactivated sera.

Values (mean \pm 1 S.D.) for MgEGTA-chelated moderate-to-high antibody sera ($58 \pm 8 \mu\text{m}$) were diminished compared to respective untreated sera values, but differences observed were not statistically significant ($P < 0.05$). These values were significantly ($P < 0.01$) increased when compared to respective heated sera (24 ± 6). In contrast, chemotaxigenic activities in chelated low antibody and hypogammaglobulinemic sera were comparable to those of respective heated sera ($P < 0.05$). Similar relationships were observed with C4D or normal GPS reconstituted with IgG.

The importance of specific antibody in promoting CTG by type III GBS was further emphasized by reconstitution experiments employing purified IgG (35 $\mu\text{g Ab/ml}$). Enhancement of chemotaxigenic activity in hypogammaglobulinemic serum to values approximately equal to those of high antibody adult sera was achieved with a 1:10 or a 1:20 dilution of this IgG preparation. In contrast, when IgG was added to heated hypogammaglobulinemic serum, no increments in activity were observed.

Immunospecificity of CTG by type III GBS. The specificity of antibody-augmented CTG with respect to types III (M732) or Ia (515) GBS is shown in Table I. Representative sera containing high or low specific antibody concentrations to type III or Ia capsular polysaccharides were incubated with heterologous or homologous organisms. Significantly greater CTG was derived by incubating type III organisms in high anti-III, low anti-Ia sera as

compared to high anti-Ia, low anti-III sera ($P < 0.01$), or compared to hypogammaglobulinemic serum ($P < 0.01$). Similarly, significantly greater CTG was observed when type Ia GBS were incubated in high anti-Ia + low anti-III sera as compared to that generated in high anti-III + low anti-Ia or low anti-III + low anti-Ia sera ($P < 0.01$).

CTG by type III GBS in neonatal sera. The contributions of specific antibody and complement to CTG by type III GBS were evaluated in cord sera from 19 neonates. Activity was compared to that of paired maternal samples and adult sera containing various concentrations of specific antibody. As shown in Table 2, normal total CH50 and normal concentrations of C3, C4, C5, and Factor B were demonstrated in all maternal and adult test sera. Among neonatal sera samples tested, mean CH50 values and mean concentrations of C3, C4, and Factor B were significantly diminished compared to healthy adult sera as has been previously reported (1, 38). No differences in mean CH50 values or C' component concentration were observed among adult or neonatal sera containing high as compared to low concentrations of specific antibody to type III GBS.

Total and heat stable chemotaxigenic activities of neonatal sera containing moderate-high (8.9–38.3 $\mu\text{g/ml}$) or low (0.1–1.8 $\mu\text{g Ab/ml}$) concentrations of specific antibody are shown in Figure 4 and are compared to those of respective adult sera groups. Mean total values for high or low antibody-containing neonatal sera groups were significantly ($P < 0.001$) diminished when compared to those of high antibody-containing adult sera. Mean total values in high antibody neonatal sera were comparable to those of low antibody adult sera ($P < 0.05$), but those for low antibody neonatal sera were significantly ($P < 0.01$) diminished compared to those of both adult serum groups; moreover, when differences between total and heat-stable activity were computed for each test serum, mean increments generated in low antibody neonatal sera ($7 \pm 6 \mu\text{m}$) were significantly less than in high antibody ($46 \pm 9 \mu\text{m}$) ($P < 0.001$) or low antibody ($15 \pm 4 \mu\text{m}$) ($P < 0.05$) adult sera, and significantly ($P < 0.05$) less than those in high antibody neonatal sera ($17 \pm 8 \mu\text{m}$).

Considerable heterogeneity among low antibody neonatal test sera was observed. Total activity as assessed in this experimental

system ranged from 10–34 μm for the 16 low antibody neonatal sera tested. Individual values among this group were not directly related to serum concentrations of specific antibody; thus, variable activity observed among low antibody neonates may be related to differences in serum complement function. The lowest individual values (both total activity and increment) were observed in a neonatal sample containing the lowest CH50 titer among all sera tested.

As shown in Table 3, representative low antibody containing neonatal sera were reconstituted with purified IgG and/or complement (hypogammaglobulinemic adult sera). In those experiments, hypogammaglobulinemic serum (0.25 ml) was incorporated into opsonic mixtures containing test sera (0.25 ml) and 5×10^8 organisms (0.5 ml). When exogenous complement in addition to purified IgG (but not either individually) were added to reaction mixtures, the chemotaxigenic activities of neonatal sera were comparable to that of high antibody adult sera.

Additional influence of neonatal PMN motility on chemotaxis by type III GBS. To further study the capacity of type III GBS to effect leukocyte mobilization in neonates, the influence of antibody and/or complement in neonatal sera was assessed with respect to adult and neonatal PMNs (Fig. 5). Mean chemotaxigenic activity for healthy adult PMNs (expressed as mean % of high antibody adult sera values) in high antibody maternal sera (102%) was significantly greater than that generated in low antibody maternal sera (55%), high antibody neonatal sera (48%), or low antibody neonatal sera (28%) ($P < 0.001$). Further, mean low

Table 1. Immunospecificity of chemotaxis by type III or Ia group B Streptococcus

Test serum	Specific antibody concentration in test serum ($\mu\text{g/ml}$)		Chemotaxigenic activity ($\mu\text{m}/80 \text{ min}$)	
	Anti-III	Anti-Ia	Type III GBS (M732)	Type Ia GBS (515)
#1	29.1	<1	65 ¹	42
#2	<0.3	6.4	37	69
#3	<0.3	<1.8	34	37
#4 ²	35	<1	70	42

¹ Represents mean values of three experiments performed with each test serum.

² Test serum #3 reconstituted with specific IgG.

Table 2. Serum complement determinations in neonatal and adult test sera

Serum source	CH50 (unit/ml)	C3 (mg/dl)	C4 (mg/dl)	C5 (mg/dl)	Factor B (mg/dl)
Adult sera (13)	317 \pm 26 ¹	108 \pm 14	23 \pm 6	13.5 \pm 4	33 \pm 6
Maternal sera (5)	316 \pm 46	115 \pm 14	26 \pm 14	16 \pm 4	39.5 \pm 6
Hypogammaglobulinemic serum (1)	309	118	22	14	32
Neonatal sera (22)	263 \pm 61 ²	85 \pm 8 ²	12.4 \pm 4 ³	12 \pm 1.5	15 \pm 3.5 ³

¹ Represents the mean \pm 1 S.D. of *n* individual values.

² Significantly less than adult sera values ($P < 0.01$).

³ Significantly less than adult sera values ($P < 0.001$).

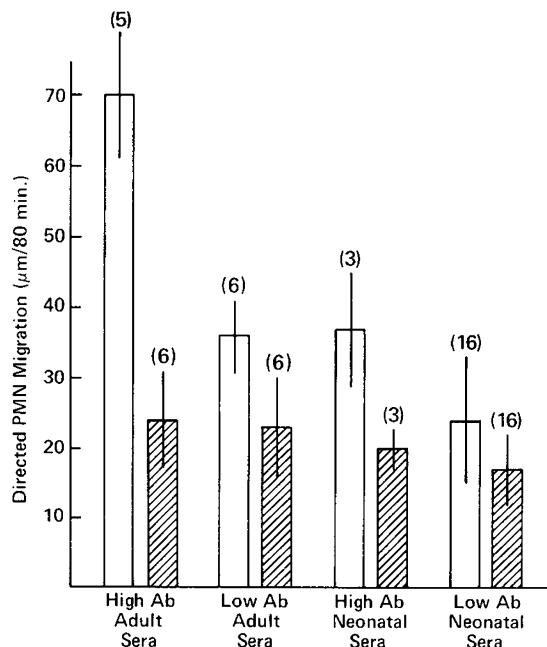


Fig. 4. Chemotaxis by type III GBS in untreated (□) or heat-inactivated (▨) adult or neonatal sera containing high or low concentrations of specific antibody. Directed migration values represent the mean \pm 1 S.D. of mean values from a minimum of three experiments performed on each serum from (*n*) donors.

Table 3. Comparison of chemotaxigenic and opsonic activity for type III group B streptococcus in neonatal sera

Serum source	n	RABA ($\mu\text{g Ab/ml}$)	Chemotaxis		Aggregometry		Opsonic-chemiluminescence	
			$\bar{x} \pm 1 \text{ S.D.}$ ($\mu\text{m}/80 \text{ min}$)	$\bar{x}\%$ High antibody adult sera	\bar{x} ZAP Units	$\bar{x}\%$ High antibody adult sera	\bar{x} AUC $\times 10^6$	$\bar{x}\%$ High antibody adult sera
High antibody neonate	2	8.9–38.3	34 \pm 10	49	ND		105	56
Low antibody neonate	6	0.3–1.8	24 \pm 6	36	6	33	86	45
Low antibody neonate + IgG ¹	4	35 ¹	54 \pm 8 ²	81	13	70	111	59
Low antibody neonate + C' ³	4	0.3–1.8	37 \pm 8 ⁴	55	13	70	116 ⁴	61
Low antibody neonate + C' ³ + IgG ¹	4	35 ¹	71 \pm 8 ²	102	23	127	163 ²	86

¹ Reconstituted with purified specific IgG (35 μg antibody/ml).

² $P < 0.001$ compared to low antibody neonatal sera.

³ C', untreated serum from hypogammaglobulinemic patient ($<0.3 \mu\text{g}$ antibody/ml).

⁴ $P < 0.01$ compared to low antibody neonatal sera.

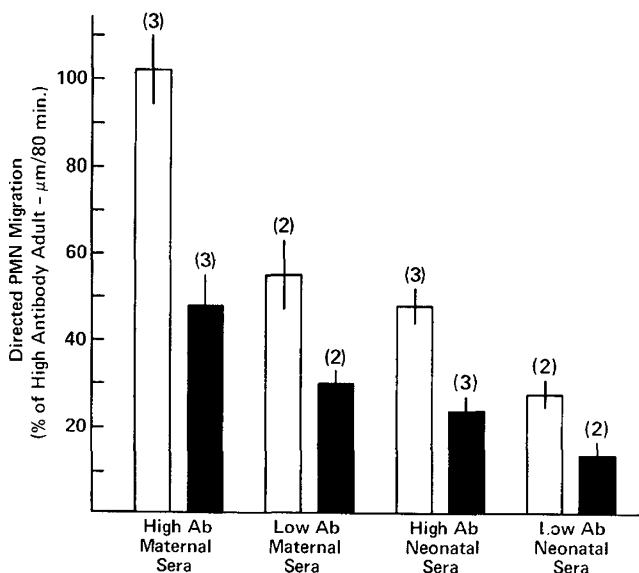


Fig. 5. Chemotaxis by type III GBS in paired maternal-neonatal sera containing moderate-to-high (4.8–41.8 $\mu\text{g/ml}$) or low ($\leq 2 \mu\text{g/ml}$) concentrations of specific antibody. GBS-serum supernatants were employed together with healthy adult PMNs (□), or neonatal PMNs (■). Values shown represent the mean \pm 1 S.D. values of experiments performed on (*n*) sera.

antibody neonatal sera values were significantly ($P < 0.001$) diminished compared to all other test sera groups. More striking differences were evident when the same opsonic mixtures were utilized to stimulate locomotion of neonatal PMNs. Directed migration values for neonatal PMNs ranged from 25–40% of adult PMN migration scores in response to the same opsonic supernatant. Values for high or low antibody neonatal sera reacted with neonatal PMNs were 24% and 14% of values for high antibody maternal sera reacted with adult PMNs, respectively ($P < 0.001$). Values for all low antibody neonatal serum + neonatal PMN mixtures were diminished compared to all reaction mixtures employing maternal sera and/or adult PMNs.

Characterization of type III GBS chemotaxigenes. To confirm that C5a generation accounts for the majority of chemotactic activity derived from GBS serum interactions, investigations of the PMN aggregating and/or adherence promoting activities of opsonic supernatants were performed (1, 40). As in chemotaxis experiments, high antibody adult sera ($n = 5$) values ($\bar{x} = 18$ ZAP units), hypogammaglobulinemic sera ($\bar{x} = 7$ ZAP units), or low antibody neonatal sera ($\bar{x} = 6$ ZAP units). Approximately

85% of aggregating activity in all test sera was neutralized by anti-C5 pre-incubation, and no aggregating activity was demonstrable in reaction mixtures employing heat-inactivated sera or GBS organisms alone. In control experiments, no neutralization of activity by anti-albumin was observed. Reconstitution of hypogammaglobulinemic adult serum with purified IgG increased its aggregating activity from 6–21 ZAP units, but no enhancement of activity was apparent when IgG was added to the same serum after heat inactivation. As shown in Table 3, mean activity observed in six representative low antibody neonatal sera reactants increased from 6–13 ZAP units with the incorporation of either specific IgG or exogenous complement, and increased to 23 ZAP units when both IgG and complement were added.

The relative capacity of representative type III GBS-serum reactants to enhance PMN adherence for protein-coated glass substrates was also directly related to their chemotaxigenic and aggregating activities. Increased adherence values were observed with incorporation of high antibody adult serum ($n = 5$) ($\bar{x} = 68\%$) as compared to values achieved with low antibody adult sera ($n = 4$) ($\bar{x} = 46\%$), low antibody neonatal sera ($n = 5$) ($\bar{x} = 33\%$) and GBS organisms alone ($\bar{x} = 27\%$). Anti-C5 serum (but not anti-albumin) neutralized the adherence promoting capacities of these test sera.

Relationship between chemotaxigenic and opsonic activity generated in serum by type III GBS. A previously described phagocytosis-CL assay (4) was utilized to quantitate opsonins for type III GBS (M732) in selected test sera employed in CTG assays. Opsonic CL activities generated in these test sera were compared to respective chemotaxigenic and aggregating activities (Table 3). The relative CL, chemotaxigenic, and aggregating activities among test sera were comparable. Values for each functional assay in both adult and neonatal sera were directly related to the serum concentration of specific antibody. Mean CL values noted in low antibody-containing neonatal sera was significantly ($P < 0.01$) diminished compared to adult sera containing high, low, or undetectable concentrations of specific antibody. As observed in CTG and aggregometry experiments, reconstitution of opsonic activity in hypogammaglobulinemic adult serum was also achieved with addition of purified IgG. As has been previously described (4), the majority of opsonic activity in test sera remained after MgEGTA chelation, indicating the importance of the alternative complement pathway in generation of opsonins for type III GBS (M732). These studies indicate that the opsonic and chemotaxigenic requirements for type III GBS are similar, and that abnormalities of both functions observed in neonatal sera are related to quantitative deficiencies of specific anticapsular antibody and functional abnormalities of serum complement.

Generation of chemotactic activity from purified C5 preparations. A partially purified C5 preparation was reacted with log phase,

type III GBS and with the supernatants of log or lag phase organisms. For these experiments, the final C5 concentration was adjusted to that of 25% (v/v) serum. Supernatants of these reaction mixtures were employed in Boyden chambers in the usual manner. Only minimal chemoattractant activity (-3 to $+2 \mu\text{m}/80 \text{ min}$) as compared to C5 incubated in the absence of GBS and essentially no aggregating activity was observed in these preparations. These findings suggest that direct (proteolytic) activation of C5 by an agent(s) elaborated by type III GBS does not significantly contribute to overall CTG in human serum.

DISCUSSION

The present study was designed to define chemotaxigenic requirements in serum for type Ia and type III strains of GBS and to evaluate the possibility that impaired CTG by GBS may be of pathogenic significance in neonates. Our studies demonstrate that serum complement-derived peptides (C5a and C5a des-arg) (12, 18, 42) account for the majority of chemotactic activity derived from GBS-serum interactions, and that chemotactic factors elaborated by GBS independent of serum are quantitatively of less overall importance. These findings are in accord with observations previously reported for other bacterial pathogens (24, 48, 49).

Our findings indicate that type specific antibody is required for maximal CTG by the GBS serotypes evaluated; low antibody concentrations in adult or neonatal serum samples significantly limited their chemotaxigenic capacity. Dose-dependent enhancement of activity by type III GBS in hypogammaglobulinemic serum was observed upon addition of an IgG preparation containing a high concentration of antibody to the native type III polysaccharide of GBS. Parallel findings were observed with respect to opsonic and aggregating activities when this IgG preparation was restored to hypogammaglobulinemic adult serum or low antibody-containing neonatal serum.

The requirement for serum complement in facilitating CTG by GBS was shown by the abrogation of C5a generation in heated sera and by the failure of specific IgG to restore activity when added back to heat-inactivated sera or complement deficient neonatal sera. The requirements of specific antibody and complement for maximal CTG by GBS are in accord with those reported for other pathogenic bacterial pathogens. CTG in fresh sera by washed *E. coli* organisms or *Salmonella enteritidis* endotoxin has been shown by depend upon the presence of type-specific antibody (24). Classical complement components, IgG, and an intact alternative complement pathway are also required to allow maximal CTG in sera by purified cell walls or peptidoglycan moieties of staphylococci (10, 39).

ACP-dependent CTG by type III GBS was demonstrated by employing MgEGTA chelation and C4 deficient GPS containing varying concentrations of specific antibody. Generation of C5a in chelated sera was delayed, but that achieved after 45-min incubation intervals was only slightly diminished compared to untreated sera. Activity generated in chelated or C4D GPS was directly related to the concentration of specific antibody in the reaction mixture. Antibody facilitation of ACP activation by type III GBS has been previously described in studies of serum opsonins for this serotype (16). Our present studies indicate that type III GBS generate quantitatively similar opsonic and chemotaxigenic activity in serum via the ACP and that both functions are facilitated by specific antibody when complement function is intact.

These and other studies indicate that ACP-mediated C5a generation may represent an important host defense mechanism. ACP-derived CTG in serum or plasma has been reported for endotoxins of a variety of gram negative organisms and for encapsulated or nonencapsulated *Cryptococcus neoformans* (16, 28, 41, 49). Of interest is the observation that polysaccharide capsules of *C. neoformans* impair opsonophagocytosis via the ACP while allowing generation of C5a by GBS was not explored in the present study but our data do not indicate a dissociation between serum opsonic and chemotaxigenic activation by type III GBS.

Direct proteolytic attack on C5 by bacterial proteases represents a separate mechanism whereby bacteria can generate chemotactic activity in serum (49). Proteases directly stimulating chemotactic activity have been identified for other streptococcal species and for *Serratia marcescens* (45, 47), and elaboration of proteases by GBS has been previously reported (44); however, our studies failed to demonstrate significant generation of C5a from a partially purified C5 preparation directly by GBS organisms or by filtrates of GBS suspensions.

The contribution of impaired CTG to the pathogenesis of GBS infection in human neonates or other high risk populations is unknown. Diminished CTG has been documented in inherited or acquired deficiency of C1r, C4, C2, C3, C5 and C3bINA (2, 3), systemic lupus erythematosus (3), diabetes mellitus (34), hypogammaglobulinemia (13), and in nephrotic syndrome associated with low serum levels of Factor B (6). The present study demonstrates that CTG by type III GBS in sera of fullterm healthy neonates is impaired. More striking abnormalities of CTG could exist for premature neonates because IgG concentrations and maturation of complement function in neonatal sera are directly related to gestational age (17, 26).

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52. This research was supported in part by the U. S. Department of Agriculture Children's Nutrition and Research Center, Texas Children's Hospital, Houston, Texas, the American Heart Association, Texas Affiliate, grants AI 13249 and AI 19031 from the National Institute of Allergy and Infectious Diseases, and Biomedical Research Support Grant 10256 to the Baylor College of Medicine.
53. Received for publication May 7, 1982.
54. Accepted for publication September 23, 1982.