Effect of Group B Streptococcal Type-Specific Antigen on Polymorphonuclear Leukocyte Function and Polymorphonuclear Leukocyte-Endothelial Cell Interaction

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ABSTRACT. Neonatal group B streptococcal pneumonia is a severe disease, often resulting in death. Autopsy findings resemble those of hyaline membrane disease. Numerous organisms may be seen in the alveoli, but few polymorphonuclear leukocytes (PMNs) are found in the areas of bacterial invasion. Aggregated PMNs are often found, however, in the pulmonary capillaries. This study was designed to explore the effect of the group B streptococcal (GBS) type III antigen on PMN chemotaxis and PMN-endothelial cell interactions. Human PMNs were isolated and pretreated with 0.25 to 4 $\mu\text{g}/\text{ml}$ of GBS type III antigen prior to determining their chemotactic response to the chemoattractants formyl-methionyl-leucyl-phenylalanine, zymosan-activated serum, platelet-activating factor, and leukotriene B_4 . The GBS antigen caused a concentration-dependent inhibition of formyl-methionyl-leucyl-phenylalanine, zymosan-activated serum, and platelet-activating factor-mediated chemotaxis (% inhibition of 38.1 ± 4.0 , 55.5 ± 3.3 , $46.7 \pm 9.7\%$, respectively; $p < 0.01$). Leukotriene B_4 -mediated chemotaxis was not significantly depressed ($21.2\% \pm 7.7$ inhibition; NSD). Group B streptococcal antigen also inhibited formyl-methionyl-leucyl-phenylalanine-induced PMN adherence to endothelial cells in a concentration-dependent fashion when incubations were performed in the absence of serum. In contrast, incubation of GBS type III antigen with serum deficient in antibody to GBS resulted in a marked enhancement of PMN attachment to human endothelial cells. No significant enhancement of adherence was seen with the antigen in the presence of serum containing GBS type III antibody. These data suggest that the GBS type III antigen by itself may inhibit the influx of PMNs into the local site of infection in the alveoli. In contrast, when exposed to blood components within the vascular space, the antigen may promote PMN adhesion to the endothelium, potentially contributing to pulmonary hypertension, edema, and respiratory failure in this most fulminant of bacterial pulmonary infections of the neonate. (*Pediatr Res* 21: 517-523, 1987)

Abbreviations

GBS, group B streptococci

PMN, polymorphonuclear leukocytes

fMLP, formyl-methionyl-leucyl-phenylalanine

PAF, platelet-activating factor

ZAS, zymosan-activated serum

HBSS, Hanks' balanced salt solution

TCA, trichloroacetic acid

EC, endothelial cell

AB(+) serum, serum containing antibody to GBS

Ab(-) serum, serum deficient in GBS antibody

HBSS/A, HBSS with 0.5% human serum albumin

$\text{C5a}_{\text{desArg}}$, desarginated form of C5a

PGI_2 , prostacyclin; prostaglandin I_2

LTB_4 , leukotriene B_4

PBS, phosphate-buffered saline

Human disease caused by GBS was first described in 1938 (1). Since that initial report, GBS have been implicated as the cause of several forms of neonatal infection including septicemia, meningitis, and pneumonia (2-6). Pneumonia due to GBS is an extremely severe infection in the human neonate. The chest radiograph is often indistinguishable from that of hyaline membrane disease (6, 7). At autopsy, numerous organisms may be seen within the alveoli, but few phagocytic cells are present in the areas of bacterial invasion (6). In contrast, variable numbers of PMNs are found diffusely throughout the pulmonary capillaries in infants who die from group B streptococcal pneumonia or in experimental animals infused with streptococcal products (6-8).

One of the major virulence factors of GBS is the type III specific antigen (9-11). This antigen, which is composed of repeating polymers of glucose, galactose, glucosamine, and sialic acid (11), is produced during growth of the type III organisms and released in large quantities into the area surrounding bacterial growth (9, 10). Hemming *et al.* (12) have shown that infusion of a TCA extract containing the type III antigen of GBS-caused leukopenia, increased lung lymph flow, and pulmonary hypertension in sheep. Although the mechanism by which GBS exerts these effects is not clear, it is possible that the antigen alters neutrophil functions resulting in PMN-mediated vascular injury and an altered inflammatory response. In order to investigate this possibility, we examined the effect of the GBS type III antigen on neutrophil chemotaxis in response to the chemoattractants fMLP, C5a fragments in ZAS, PAF, and LTB_4 . In addition, we examined the effect of the GBS type III antigen on neutrophil-endothelial cell interactions.

MATERIALS AND METHODS

Bacterial cultures and antigen preparation. The type III GBS strain was isolated from the CSF of an infected human infant.

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The antigen was prepared using methods described by Hemming *et al.* (12) and Lancefield and Freiner (13). GBS type III was grown for 16–18 h at 37° C in 6–18 liters of enriched Todd-Hewitt broth. The cultures were centrifuged at 5000 rpm at 4° C for 30 min. The resulting pellet was washed and suspended in PBS and recentrifuged at 5000 rpm for 1 h at 4° C. The pellet was then suspended in cold 2.5% TCA and the pH adjusted to 2.0. This suspension was stirred overnight at 4° C, followed by centrifugation at 5000 rpm for 45 min at 4° C. The supernatant containing the antigen was then treated with cold 95% ethanol so that ethanol comprised 60% of the total volume and incubated at 4° C overnight. This ethanol fraction was centrifuged at 5000 rpm at 4° C for 45 min. The precipitate was separated from the supernatant and centrifuged at 3300 rpm for 30 min. The precipitate was resuspended in 0.5–1 ml of PBS and the pH adjusted to approximately 7.0. Purity of the type III antigen was determined using capillary precipitation methods and by counterimmunoelectrophoresis. The extract reacted only with type III rabbit antiserum. No group antigen was present. The antigen preparation was free of endotoxin when assayed by the limulus lysate assay and by examining its ability to directly stimulate PMN adhesion to nylon fiber columns. The antigen was stored at 4° C. Serial dilutions of the antigen were made in the control buffer appropriate for the experiment.

We also employed a highly purified type III antigen (a gift of Dr. Bascom Anthony, Harbor-UCLA Medical School) which had been isolated by column separation of concentrated culture supernatants (14). The concentration of the GBS antigen prepared by TCA and ethanol fractionation was determined by comparison with the known standard supplied to us by Dr. Anthony. In limited experiments, the highly purified type III antigen standard was also utilized in the functional assays. The results with both antigen preparations were essentially identical.

Complement assays. Complement consumption analysis was performed by incubating fresh, frozen GBS antibody-positive and antibody-negative serum with 20 µg/ml of the type III antigen for 60 min at 37° C. Total hemolytic complement levels in sera were determined before and after exposure to the antigen by a radial hemolytic technique (CH₁₀₀; Behring Diagnostics, La Jolla, CA) and by hemolytic titration with antibody-coated sheep red blood cells (CH₅₀) (15). The concentration of C5_{adesArg} in the sera following antigen incubation was determined with a commercially available radioimmunoassay kit (Upjohn, Kalamazoo, MI).

Human neutrophil isolation. Neutrophil suspensions were prepared by collecting peripheral venous blood from healthy human adult volunteers in heparin, 7.5 U/ml. The blood was allowed to settle in 1% dextran (Pharmacia, Piscataway, NJ) and the neutrophils were separated on Ficoll-Hypaque (Pharmacia) gradients. Contaminating red cells were removed by hypotonic lysis with 0.2% saline. The remaining neutrophils (98%) were resuspended to a concentration of 2×10^7 PMNs/ml in HEPES buffer with 1% bovine serum albumin for the chemotaxis assays. The cells were resuspended in HBSSIA (M.A. Bioproducts, Walkersville, MD) for endothelial cell-granulocyte adherence assays. Some of the experiments required pretreatment of the neutrophils with the GBS type III antigen. Appropriate volumes of the antigen were added to known volumes of the neutrophil suspensions and incubated at 37° C for 30 min prior to use in functional assays. In endothelial cell adherence assays, the neutrophils were radiolabeled with Indium 111 as described (16).

Neutrophil chemotaxis. Neutrophil chemotaxis was assayed using a multiwell micropore chemotaxis chamber (Neuroprobe, Cabin John, MD) with a 5 µ pore size filter (Millipore Corp., Bedford, MA) according to previously described methods (17–19). The bottom chambers were filled with 30 µl of chemoattractant or control buffer. The filters were inserted and then the upper chambers were filled with 50 µl of the appropriate neutrophil or neutrophil-GBS type III antigen suspensions.

Chambers were incubated at 37° C in a humidified chamber

with 5% CO₂ for 2 h. Filters were removed and stained with hematoxylin. Neutrophils that migrated completely through the filter were counted using a 10× ocular, a 45× objective, and a 5 × 5 mm photographic reticule. Chemotaxis was quantitated by determining the mean number of cells that migrated completely through the filter in 10 random fields. Assays were performed in triplicate on three or more occasions in most instances. Neutrophil viability was determined for neutrophils treated with control buffer and GBS type III antigen at the end of the incubation time using a trypan blue stain.

Chemoattractant. ZAS was prepared by incubating 10 ml of fresh human serum with 100 mg of washed zymosan (Schwarz/Mann, Biotech Division of ICN Biochemicals, Cleveland, OH) for 60 min at 37° C in a rotating rack. Following centrifugation, the clarified activated serum was stored in aliquots at –70° C. For use, the ZAS was diluted in HEPES buffer or HBSS to a concentration of 10%. fMLP (Sigma Chemical Co., St. Louis, MO) was prepared as a 10^{–5} M stock solution in HEPES buffer, stored at –70° C, and diluted to the appropriate concentration in HEPES buffer immediately before use. PAF was purchased from Avanti Polar Lipids, Inc. (Birmingham, AL). LTB₄ was a gift from Dr. Joshua Rokach (Merck-Frosst Canada, Inc., Quebec, Canada).

Endothelial cell isolation and culture. EC were isolated from human umbilical veins as previously described (16, 20). Briefly, the cells were isolated by collagenase perfusion of umbilical veins and suspended in medium 199 supplemented with 20% pooled human serum, L-glutamine, and antibiotics (M.A. Bioproducts, Walkersville, MD) (20). The cells were cultured in 35-mm polystyrene Petri dishes (Corning Glass Works, Corning, NY) in a 5% CO₂, 95% air atmosphere until confluence was reached (5–7 days). The media were changed every 72 h. Cells grown in this fashion were documented to be endothelial cells by several criteria including contact-inhibited monolayer morphology, the presence of von Willebrand factor, angiotensin-converting enzyme activity, and the production of PGI₂ in response to appropriate agonist stimulation (16, 20). These criteria taken together are unique for endothelial cells and are shared by no other cell type. Only tightly confluent, primary cultures were used for these experiments.

Adherence of labeled neutrophils to endothelial cell monolayers. The adherence of ¹¹¹In-labeled PMNs to EC monolayers was measured as described (16). The culture medium was aspirated, and the monolayers were washed with HBSS (37° C). Aliquots (0.9 ml containing 5×10^6 cells) of labeled PMN suspensions were layered over the endothelial monolayers. Control buffer, buffer containing an agonist (fMLP, etc., used as a positive control), or type III GBS antigen was then added (0.1 ml) and the suspensions were incubated in an atmosphere of 5% CO₂, 95% air at 37° C for varying times as indicated. After incubation, the neutrophils remaining in suspension were aspirated, the monolayers were washed, and the percentage of adherent PMNs was quantitated as described (16). The EC monolayers were also examined by phase contrast microscopy immediately before and immediately after incubation with PMNs to insure that the monolayers were intact and that the PMNs were adherent to the top surface of the confluent EC monolayers. In previous studies (16) we have also documented the adhesion by electron microscopy.

In some experiments, labeled neutrophils, or EC monolayers, were pretreated with various concentrations of the type III GBS antigen for 5 or 30 min at 37° C prior to the adherence assay. In additional experiments, whole human serum with or without type III-specific ELISA or opsonophagocytic antibody (determined as previously described) (21) was added (10% v/v) to labeled PMNs in the presence or absence of the type III antigen. Neutrophil adherence to the monolayers was then determined after varying time intervals.

Adherence of indium-labeled neutrophils to nylon fiber columns. Columns containing nylon fibers were constructed as

previously described (16) and warmed to 37° C. Aliquots of ^{111}In -labeled neutrophils (0.9 ml containing 5×10^6 PMNs) were mixed with control buffer or agonist solutions (0.1 ml) for 30 sec to 5 min at 37° C on a mixing platform. Then 1.0-ml volumes were added to the columns and allowed to drain by gravity at 37° C for 15 min. The radioactivity from the columns and the effluent were counted separately. The percentage of neutrophils adherent to the column was determined by dividing the radioactivity associated with the column by the sum of the radioactivities from the column and the effluent (15).

Neutrophil aggregation. The aggregation of neutrophil preparations suspended in HBSS/A in response to GBS type III antigen or agonists (fMLP, etc.) was measured as described using an aggregometer (22).

Data comparisons. All results were recorded as the mean $\pm$ SD. Statistical differences were determined using a paired Student's *t* test.

RESULTS

Effect of GBS type III antigen on neutrophil chemotaxis. We examined the GBS antigen that was employed with the more highly purified antigen (supplied by Dr. Anthony) by double immunodiffusion and electroimmunodiffusion against hyperimmune antiserum to the type III polysaccharide. As can be seen in Figure 1, lines of identity develop between the two antigens exposed to this antiserum. Moreover, immunoelectrophoresis of similar concentrations of the antigens against the hyperimmune rabbit antiserum yielded similar precipitin bands approximately the same distance from the antigen wells (Fig. 2). This indicates probable similarity of the charge and approximate molecular weight of the antigens.

As shown in Figures 3 and 4, the chemotactic activity of neutrophils was inhibited by treatment of the cells with the type III GBS antigen. The magnitude of inhibition varied with the chemotactic agent used as the stimulus. In response to 10^{-8} M fMLP the mean number of neutrophils that completely migrated

Fig. 2. Electroimmunodiffusion analysis of the purified type III antigen and the TCA extracted antigen. Equal quantities (10 $\mu\text{g}/\text{ml}$) of the TCA antigen were placed in the outside wells along with the purified type III antigen in the center well. The antigens were placed nearest to the cathode (top). A hyperimmune, rabbit type III specific serum was placed in the wells nearest the anode (bottom) and electrophoresis was carried out in barbital buffer (pH 8.8) at 6 milliamps per slide for 60 min.

Fig. 3. Effect of the type III GBS antigen on PMN chemotactic responses to 10% ZAS and fMLP (10^{-8} M).

Fig. 1. Analysis of immunologic identity of the TCA antigen and the column purified GBS type III antigen. Rabbit hyperimmune type III antiserum was placed in the center well. The purified type III antigen was placed in the top and bottom wells, while the TCA extracted type III antigen was placed in the left and right wells. A distinct line of identity is apparent between the two antigen preparations.

through the filter was 78.4 ± 12.1 (SD) when the cells were pretreated with control buffer. No significant effect on chemotaxis was observed when the cells were pretreated with 0.25, 0.5, or 1 $\mu\text{g}/\text{ml}$ of GBS type III antigen (Fig. 3). Significant inhibition was observed, however, with 2 and 4 $\mu\text{g}/\text{ml}$ of the GBS type III antigen ($p < 0.01$).

When 10% ZAS was used as the chemoattractant, the mean number of cells migrating completely through the filter was 54.8 ± 5.0 (SD) in the buffer control. Chemotaxis was inhibited

Fig. 4. Effect of the type III GBS antigen on PMN chemotactic responses to PAF (10^{-7} M) and LTB₄ (10^{-7} M).

following pretreatment of the neutrophils with 0.5 $\mu\text{g/ml}$ ($p < 0.01$), 1 $\mu\text{g/ml}$ ($p < 0.01$), and 2 $\mu\text{g/ml}$ ($p < 0.01$) of the type III antigen (Fig. 3).

Neutrophil chemotaxis in response to 10^{-7} M PAF was also significantly inhibited by pretreatment with GBS type III antigen at 2 and 4 $\mu\text{g/ml}$ (Fig. 4). The mean number of cells migrating completely through the filter after pretreatment with control buffer was 94.3 ± 5.1 (SD). This was compared with means of 55 ± 7.04 at 2 $\mu\text{g/ml}$ antigen ($p < 0.01$) and 50.2 ± 4.5 at 4 $\mu\text{g/ml}$ antigen ($p < 0.01$). These data demonstrate that the GBS type III antigen inhibits the chemotactic response to a potent lipid mediator of inflammation, PAF, in addition to the oligopeptide chemoattractants fMLP and C5a_{desArg}.

Chemotaxis in response to LTB₄, another potent lipid inflammatory mediator, was also reduced after pretreatment of the neutrophils with GBS antigens but the degree of reduction was not significantly different from control (Fig. 4). The mean number of cells migrating through the filter after incubation with buffer alone was 115 ± 7.6 (SD). At the maximum concentration of 4 $\mu\text{g/ml}$ of GBS type III antigen, the mean number of cells migrating through the filter was not statistically different (90.5 ± 8.8 ; $p > 0.05$). This corresponded to only $21.1 \pm 7.7\%$ inhibition. Trypan blue staining of GBS type III antigen-treated and buffer-treated neutrophils at the end of the incubation time indicated no increase in neutrophil death secondary to treatment with the GBS antigen. Neutrophil viability ranged from 92–99% for all samples. Thus, the inhibition of PMN chemotaxis by GBS type III antigen was not due to cytotoxic injury of the neutrophils.

Effect of GBS antigen on neutrophil-endothelial cell adherence. Because Hemming *et al.* (12) observed leukopenia in association with pulmonary vascular injury in sheep infused with GBS products, we sought evidence that the GBS type III antigen promoted neutrophil adhesion to other PMNs (aggregation), to inert surfaces (nylon fiber columns), or to endothelium. In preliminary experiments, whole live GBS, an ultrafiltrate of GBS, and GBS type III antigen alone did not increase neutrophil

adherence to nylon fiber columns and did not stimulate neutrophil aggregation when added in various concentrations to isolated neutrophils suspended in HBSS/A. These data indicated that GBS type III antigen alone did not directly stimulate cell-cell adhesion (aggregation) or the adhesion of isolated neutrophils to inert surfaces (16, 22).

We then proceeded to examine the effect of GBS type III antigen on what is perhaps a more relevant system, neutrophil adherence to human endothelial cell monolayers. In experiment I of Table 1, we determined the direct effect of the type III antigen on untreated PMN interaction with untreated endothelial cells. As shown, the type III antigen decreased adherence at 5 min while the known positive adherence agonist fMLP markedly enhanced attachment (Table 1, experiment I).

We next considered the possibility that pretreatment of the endothelial cells with the GBS type III antigen might promote neutrophil adherence, as do thrombin (16), interleukin-1 (23), and other agents including endotoxin (24–26). Pretreatment of endothelial cells with GBS type III antigen, followed by washing the monolayers prior to the addition of neutrophils, did not stimulate neutrophil adherence (Table 1, experiment II), as did the known positive agonist thrombin or fMLP. This result indicates that the GBS product does not directly enhance the adhesiveness of EC. Furthermore, it suggests that the lack of a proadhesive effect of the GBS type III antigen in experiment I (under conditions in which GBS type III antigen, PMNs, and endothelial cells were incubated together) was not due to the concomitant release of an inhibitor of PMN adhesion, such as PGI₂, by the endothelial cells (20).

In a third experiment, preincubation of PMNs with the GBS type III Ag for 30 min at 37° C also resulted in depression of adherence while the positive agonist fMLP increased adherence (Table 1, experiment III).

In another experiment, pretreatment of neutrophils with GBS type III antigen for 30 min at 37° C resulted in a concentration-dependent inhibition of fMLP-stimulated adhesion (Table 1, experiment IV). This result was similar to the effect of pretreatment of the neutrophils with GBS type III antigen on fMLP-stimulated chemotaxis, which also resulted in inhibition (Fig. 1). However, the concentration of antigen required for significant inhibition of adherence (20 $\mu\text{g/ml}$) was higher than that required to inhibit chemotaxis (2 $\mu\text{g/ml}$). The results of experiments I–IV in Table 1 indicated that GBS type III antigen did not directly stimulate PMN adherence to EC, and that it diminished chemotactic factor-stimulated adherence when the incubations were performed in aqueous buffer.

We next considered it possible that the effect of GBS type III antigen on PMN adherence to EC might be different in the presence of serum, since serum contains a variety of plasma-derived inflammatory mediators (16, 27–29). We examined, therefore, the effect of serum on neutrophil adherence in the presence or absence of GBS type III antigen. As shown in experiment V in Table 1 addition of whole human serum that contained antibody to the type III GBS antigen by ELISA employing a TCA extracted antigen and opsonophagocytic assay [Ab(+) serum; ELISA titer 1:1024] or serum that was deficient in antibody to type III GBS [Ab(–) serum; ELISA titer 1:16] caused greater adherence than did control buffer or GBS type III antigen alone (172 and 135–146% of control). Enhancement of PMN adherence to human endothelium by normal serum alone has been previously reported by Harlan *et al.* (30) and is probably due to the presence in normal serum of thrombin and other factors (23–26). Of note is the fact that these specimens contained essentially equivalent levels of fibronectin [AB(+) serum; 240 $\mu\text{g/ml}$ versus AB(–) serum 230; $\mu\text{g/ml}$] as determined spectrophotometrically. Addition of GBS type III antigen to Ab(–) serum further enhanced neutrophil adherence (315%) to more than 2-fold that of the level seen with AB(–) serum alone (146%). Moreover, the values remained elevated after 30 min (300%) of incubation. Similar results were seen when whole live GBS were

Table 1. *Effect of GBS type III antigen (Ag) on PMN adherence to human endothelial cells**

Experiment	Pretreatment of		Addition	Incubation time (min)	Adherence %	% of buffer control
	PMNs	EC				
I.	Untreated PMNs and EC		Buffer	5	5	100
			Ag 4 $\mu\text{g/ml}$	5	4	80
			Ag 20 $\mu\text{g/ml}$	5	3	60
			fMLP (10^{-6} M)	5	40	800
II.	Pretreated EC		Buffer	5	9	100
			Ag 4 $\mu\text{g/ml}$	5	7	78
			Ag 20 $\mu\text{g/ml}$	5	6	67
			Thrombin (2 U/ml)	5	30	333
			fMLP (10^{-6} M)	5	27	300
III.	Pretreated PMNs		Buffer	5	10	100
			Ag 4 $\mu\text{g/ml}$	5	8	80
			Ag 20 $\mu\text{g/ml}$	5	5	50
			fMLP (10^{-6} M)	5	55	550
IV.	Pretreatment of PMNs		Buffer	5	5	100
			fMLP (10^{-6} M)	5	35	700
			Ag 4 $\mu\text{g/ml}$	5	32	640
			Ag 20 $\mu\text{g/ml}$	5	18	360
V.	Effect of serum and GBS Ag		Buffer	5	13	100
			Buffer	30	11	100
			Ag 20 $\mu\text{g/ml}$	5	5	39
			Ag 20 $\mu\text{g/ml}$	30	7	64
			Ab(-) Serum	5	19	146
			Ab(-) Serum	30	15	135
			Ab(-) Serum Ag	5	41	315
			Ab(-) Serum Ag	30	33	300
			Ab(+) Serum	30	19	172
			Ab(+) Serum Ag	30	21	191
			fMLP (10^{-6} M)	5	59	454
			fMLP (10^{-6} M)	30	29	264

* Nine-tenths ml of a suspension containing 5.5×10^6 PMNs per ml was added to each monolayer and the percentage bound determined.

Table 2. *Effect of type III GBS antigen on complement consumption and generation of C5a_{desArg} as determined by radioimmunoassay (RIA)*

Incubation mixture	Total hemolytic CH ₁₀₀ Units	Complement CH ₅₀ Units	C5a by RIA (ng/ml)
Fresh antibody (+) serum	150	48	<10
Ab(+)serum + GBS Ag 20 $\mu\text{g/ml}$	56	4	56
Ab(-)serum	150	96	<10
Ab(-)serum + GBS Ag 20 $\mu\text{g/ml}$	150	96	<10

added to Ab(-) serum (data not shown). The enhanced PMN adherence in the presence of GBS type III antigen and Ab(-) serum approached the magnitude of adherence stimulated by fMLP at 5 min (454%) and exceeded it at 30 min (264%). In contrast, GBS type III antigen did not significantly stimulate

PMN adherence in the presence of Ab(+) serum (191%) above that with AB(+) serum (172%) alone. The stimulating effect of GBS type III antigen in the presence of Ab(-) serum was not due to the generation of C5a fragments, since the same preparation of GBS antigen did not cause complement activation in aliquots of the Ab(-) serum and did not generate C5a_{desArg} as determined by radioimmunoassay (Table 2). This is consistent with the recognized requirement for antibody in order for the sialic acid containing type III antigen to activate complement. The antibody-positive serum did activate complement in the presence of the type III antigen resulting in C5a generation. This did not, however, significantly enhance PMN-EC adherence suggesting that complement-derived components are not responsible for the enhancement we observed.

In an experiment designed to determine if the enhanced adhesion of PMN to EC in the presence of GBS type III antigen and Ab(-) serum was due to increased adhesiveness of the neutrophils or the EC, we incubated GBS antigen and 10% (Ab(-) serum with neutrophils and assessed their adherence to nylon fiber columns. Adherence in the presence of GBS antigen and Ab(-) serum was 160% of that in response to GBS type III antigen alone, suggesting that at least part of the effect resulted

from an increase in neutrophil adhesiveness, per se. The type III antigen had no effect on adhesiveness in the presence of antibody-positive serum.

DISCUSSION

The experiments described herein suggest that GBS type III antigen alters human neutrophil functional responses, and that the alteration may be influenced by other humoral mediators such as those found in serum. Our observations may be relevant to events that occur *in vivo* during episodes of group B streptococcal pneumonia and bacteremia which result in impaired PMN migration into the alveoli in the face of intravascular sequestration of these cells (6, 8).

We found that neutrophil chemotaxis in response to fMLP and C5a fragments in ZAS was inhibited in a concentration-dependent fashion by the GBS type III antigen. The mechanism of this inhibition is not clear. Neutrophil chemotaxis occurs in response to fMLP and C5a only after these peptides bind to specific cell surface receptors. Of interest is a recent report by Perez *et al.* (31), indicating that the plant lectin, wheat germ agglutinin, irreversibly inhibits human PMN chemotaxis to fMLP. This probably results from binding of the agglutinin to N-acetylglucosamine residues present in surface glycoproteins important in stimulus-response coupling in the PMN (31). This inhibition occurs even though there is no direct effect of the lectin to decrease labeled fMLP binding. Gray *et al.* (32) have shown that the type III antigen of GBS also binds directly to the wheat germ agglutinin through sialic acid. The structural relationships here suggest that there may be a competitive type of inhibition between the type III antigen and receptors important in stimulus-response coupling that results in the impaired response to fMLP. [In results similar to those reported (31) with the wheat germ agglutinin, however, we could show no direct effect of the GBS type III antigen on labeled fMLP binding to PMNs; data not shown.] Furthermore, the type III antigen inhibited chemotaxis in response to C5a and PAF, agents that have receptors separate from those for fMLP. These results suggest a pleiotropic inhibition of the PMN chemotactic response by the GBS type III antigen. We found, however, that the mean chemotactic response to LTB₄, a potent endogenous lipid mediator, was not significantly depressed by the type III antigen. The lack of a significant effect of the type III antigen on this mediator may have been due to the fact that the LTB₄ was the most potent chemoattractant that we used, or to the possibility that it activates cells in a unique manner (33).

One potential mechanism for the pleiotropic inhibition of PMN chemotaxis by the type III GBS antigen is the stimulation of markedly enhanced, irreversible adherence of the PMNs to surfaces resulting in their inability to migrate. We explored this possibility by examining the effect of the GBS antigen on PMN adhesiveness under a variety of conditions. We found that the type III GBS antigen did not directly stimulate PMN aggregation, adherence to nonbiologic surfaces (nylon fiber), or to human endothelium when incubations were carried out in buffer. Thus, the type III antigen does not directly enhance the adhesiveness of PMNs, as do fMLP, C5a_{desArg}, and certain other agonists. These results appear to exclude PMN hyperadhesiveness as the mechanism for impaired *in vitro* chemotaxis caused by the GBS antigen. Our experiments with endothelial monolayers (Table 1) also exclude a direct effect of the GBS type III antigen on endothelial cell adhesiveness such as that produced by alpha thrombin and leukotriene C₄ (16, 24).

Although GBS type III antigen did not stimulate neutrophil adhesion to endothelial cell monolayers alone, there was a rapid, potent, and prolonged stimulation of adherence in the presence of whole human serum lacking type-specific GBS antibody (Table 1). A similar effect was observed when live type III group B streptococcal organisms were utilized instead of the type-specific anti-

gen, in the presence of one or more serum components, alter neutrophil-endothelial cell interaction resulting in enhanced adherence. Whether this effect is due to a potentiation of a direct effect of an agent such as kallikrein on the neutrophil (27), an enhancement of the effect of a mediator such as thrombin on the endothelial cells (16), or another mechanism remains to be determined. The experiment showing that the GBS type III antigen in the presence of serum enhanced PMN adhesiveness to nylon wool columns suggests that the first thesis may be functional. The enhanced interaction between PMNs and endothelial cells as well as the increased adherence of PMNs to nylon wool in the presence of the type III antigen and Ab(-) serum did not appear to be due to activation of the complement cascade and the generation of C5a and C5a_{desArg}, since the type III antigen did not promote complement consumption or the release of C5a_{desArg} in serum which lacked antibody. Antibody appears to be an absolute requirement for complement activation with type III GBS or with the mildly extracted (2.5% TCA in the cold) sialic acid containing type III antigen (34).

Of interest was the fact that the type III antigen did not promote neutrophil adherence in serum that contained type-specific antibody when compared to the antibody-positive serum alone. These results are very similar to those of Wood and Gray (35) who found that type-specific antibody prevented platelet aggregation by plasma exposed to the type III antigen. This suggests that antibody may prevent the interaction of the antigen with the serum components responsible for promoting adherence, and is consistent with our earlier observations showing that antibody protects newborn rats from respiratory infections due to group B streptococci (21) and prevents the diffuse accumulation of PMNs throughout both lung fields observed in untreated, infected animals (36). This occurs even though there is complement activation and the generation of C5a by the interaction of antibody with the antigen. These data suggest that the mediator of the enhanced adherence is derived from factors other than those of the complement system.

Our experiments suggest that in the presence of serum components, the GBS type III antigen promotes neutrophil adherence to the endothelial surface. This event may result in pulmonary vascular injury (37) and may partially explain the leukopenia, pulmonary hypertension, and edema observed in human or experimental GBS pulmonary disease (6, 8, 12). Alternatively, release of this antigen in large amounts within the infected alveoli where serum components are not present in large quantities may actually decrease the influx of inflammatory cells through an effect to directly depress PMN chemotaxis. Together, these effects on neutrophil function may contribute to the altered inflammatory response and pathophysiology of group B streptococcal infection within the lungs of neonates.

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