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Role of Neutrophil Receptors in Opsonophagocytosis of Coagulase-Negative Staphylococci

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The role of neutrophil complement receptors in the opsonophagocytosis of 10 strains of coagulase-negative staphylococci was investigated. Polymorphonuclear leukocytes from adults as well as term and premature newborn infants were tested with normal human serum, adult hypogammaglobulinemic serum, and pooled premature infant serum in an opsonophagocytic assay. Neutrophils from premature infants demonstrated significantly lower killing capacity (62%) than neutrophils from adults (86%) or term infants (84%; $P < 0.02$). Maximum inhibition of opsonophagocytosis by adult or infant neutrophils occurred with an FcIII receptor blockade (80%), whereas a blockade of complement receptors produced minimal inhibition. Opsonophagocytic activity for the coagulase-negative staphylococci was not influenced by the serum source but was influenced by reducing the serum concentration below 5%. Abrogation of the complement activity of normal human serum by heating or the addition of ethylenediamine tetraacetate reduced opsonophagocytosis by 100 and 96%, respectively, whereas selective inhibition of the classical complement pathway reduced opsonophagocytosis by only 40%. Thus, opsonophagocytosis of coagulase-negative staphylococci by human sera appears to be mediated primarily by neutrophil Fc receptors, but complement is also required. The inefficiency of these interactions with neutrophils from premature infants may partially explain the enhanced susceptibility of very-low-birth-weight neonates to disseminated, coagulase-negative staphylococcal infections.

The coagulase-negative staphylococci (CONS) are a group of 21 species among which the most frequent human pathogen is *Staphylococcus epidermidis*. They have become a prominent cause of nosocomial infections associated with indwelling devices such as central venous catheters, central nervous system shunts, prosthetic cardiac valves, and peritoneal dialysis catheters (22). CONS infections also are encountered in bone marrow transplant recipients (16) and cancer patients with neutropenia (13). Among neonates born prematurely, especially those weighing less than 1,500 g at birth, CONS are the leading cause of late-onset bacteremia (18, 19, 23) and also are implicated as pathogens in necrotizing enterocolitis (17).

Previous studies of CONS have reported that both complement and immunoglobulin are required for opsonophagocytosis by human sera and adult polymorphonuclear leukocytes (PMNL) (5, 30). However, the role of neutrophil receptors in promoting these interactions has not been delineated. The purpose of our experiments was to compare the opsonophagocytosis of CONS strains isolated from bacteremic premature infants by human sera in the presence of adult or newborn (term and premature) PMNL. Our goal was to study PMNL receptors in the opsonophagocytosis of CONS so that a better understanding of the unique susceptibility of premature infants to disseminated CONS infections might be gained.

MATERIALS AND METHODS

Collection and preparation of sera. All sera were separated from whole blood obtained by venipuncture and were processed to preserve endogenous complement activity. Normal human serum samples (NHS) from five healthy adults were pooled. Adult hypogammaglobulinemic serum (AHG) was collected from a patient with common variable immunodeficiency (immunoglobulin G [IgG], 140 mg/dl). Serum samples were obtained from two healthy premature neonates (mean gestation, 28.5 weeks; birthweight, 720 g; age, 63 days) and pooled (pooled premature neonate serum [PPS]) (IgG, 77 mg/dl). Whole blood from each individual was allowed to clot at room temperature and centrifuged at 4°C. Separated sera were stored in aliquots at −70°C within 1 h of collection. The comparative antibody content was determined by whole-cell enzyme-linked immunosorbent assay (ELISA). Heat inactivation was accomplished by heating NHS to 56°C for 30 min. The extent of complement consumption was determined by a total hemolytic complement consumption assay (CH_{50}) with sheep erythrocytes before and after incubation of NHS, AHG, or PPS with CONS (10, 15). These experiments were performed employing an inoculum of 10^8 CONS in isotonic veronal buffer (pH 7.5) with 0.1% gelatin–0.15 mM $CaCl_2$–0.5 mM $MgCl_2$ incubated for 60 min with 50% serum.

Isolation of neutrophils. PMNL were obtained either from adult volunteers or from the placentas of healthy term (mean gestation, 39 weeks; birthweight, 3,330 g) or premature (mean gestation, 31 weeks; birthweight, 1,410 g) newborns within 20 min of birth. The fresh whole blood was anticoagulated with citrate-phosphate-glucose solution (Abbott Laboratories, North Chicago, Ill.) and dextran sedimented before purification over Ficoll-Hypaque gradients. Isolated PMNL were resuspended in Dulbecco's phosphate-buffered saline (dPBS; pH 7.4) (GIBCO, Grand Island, N.Y.) with 0.2% dextrose to a mean working concentration of 4×10^6 PMNL per ml (range, 1.3×10^6 to 7×10^6 PMNL per ml). For each experiment, unstained or Wright-stained leukocytes were manually counted; this procedure verified that PMNL preparations, including those from placentas, were ≥95% pure. For experiments involving inhibition of neutrophil complement receptor 1 (CR1), erythrocytes were elim-

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TABLE 1. MAb and their targets

MAb (source)	Antigen	Target
CD35 (Becton-Dickinson, Mountain View, Calif.)	CD35	CR1, C3b receptor
leu 15 (Becton-Dickinson)	CD11b	CR3, iC3b receptor
OKM1 (F. M. Schmalsteig)	CD11b	CR3, lectin-like receptor
leu 11b (Becton-Dickinson)	CD18	FcRIII

inated by hypotonic lysis prior to the addition of PMNL to the reaction mixture.

Bacteria. Ten strains of the CONS described by Patrick et al. (23) were evaluated. Each strain had been isolated from the bloodstream of a premature neonate with symptoms consistent with the diagnosis of staphylococcal bacteremia. Five infants had bacteremia without a documented focus persisting for a mean of 9 days, while the remainder had bacteremia that resolved promptly after the initiation of appropriate antimicrobial therapy. Biochemical analysis of CONS, performed by using a Vitek Automicrobial System (Vitek Systems, Inc., Hazelwood, Mo.), determined that eight strains were *S. epidermidis* and two were *S. cohnii*. No differences between the persistent and nonpersistent CONS strains were noted with respect to antimicrobial susceptibility. The five persistent strains included the two *S. cohnii* isolates. Each strain was susceptible to vancomycin. Neither plasmid profile analysis, performed by Patrick et al. (23) by the method of Parisi and Hecht (20), nor phage typing with 17 phages (21) had distinguished a unique biotype among the strains of CONS causing persistent bacteremia. With the use of sodium dodecyl sulfate-polyacrylamide gel electrophoresis, there were no proteins identified as unique to either persistent or nonpersistent CONS (23). Finally, slime layer analysis, performed by Patrick et al. (23) by the method of Christensen et al. (3), identified two of the persistent and three of the nonpersistent strains as slime producers. The original isolates were stored as aliquots in 20% glycerol–trypticase soy broth at −70°C. For each experiment, an aliquot was thawed, inoculated into trypticase soy broth, incubated overnight at 37°C, and adjusted to an optical density (OD) resulting in an inoculum of 1×10^7 to 2×10^7 CFU/ml.

Antibodies. Monoclonal antibodies (MAb) to CR1 (CD35), to complement receptor 3 (CR3) (leu 15), and to the FcIII receptor (leu 11b) were purchased commercially (Table 1). MAb OKM1, also directed to CR3, was purified from murine ascites fluid by gradient elution by using a high-performance liquid chromatography hydroxylapatite (Bio-Rad, Richmond, Calif.) ion-exchange column (a gift from Frank Schmalsteig, Galveston, Tex.). MAb to soy bean lectin, mouse IgG2a, was purchased from Coulter Immunology (Hialeah, Fla.) for use as a control. Commercially obtained MAb were dialyzed against 0.01 M PBS (pH 7.4) containing 0.2% bovine serum albumin (Armour Pharmaceutical Co., Kankakee, Ill.) to remove sodium azide. Immunofluorescence flow cytometry was employed by the methods of Anderson et al. (1) to determine the saturating concentrations of each MAb for freshly isolated PMNL. From these analyses, concentrations of MAb in our experiments were 10 μg/ml for OKM1 and leu 15, 5 μg/ml for CD35, and 2.5 μg/ml for leu 11b. When combinations of MAb were employed, the final concentration of each was the same as that when used alone. Human immunoglobulin modified for intravenous use (IVIG) (Gammagard lot no. 880120AG11; Hyland Laboratories, Glendale, Calif.) was diluted from lyophilized material and used at a concentration of 600 mg/dl. For experiments in which both the classical and the alternative complement pathways were blocked, 10 mM EDTA was employed, and for a preferential blockade of the classical complement pathway, 8 mM magnesium–ethyleneglycol–tetraacetate (MgEGTA) was used.

Opsonophagocytic assay. An assay described previously (7) was modified to achieve a bacterium-to-PMNL ratio range in the reaction mixture of 3:1 to 5:1. The opsonophagocytosis of CONS by PMNL from various sources in the presence or absence of MAb was determined. The reaction mixture contained 100 μl of serum, 50 μl each of PMNL and bacterial suspension, and 100 μl of dPBS or of the saturating concentration of MAb in dPBS. Controls for each experiment included tubes lacking PMNL or serum and MAb without PMNL, each of which resulted in no reduction in the initial inoculum of CONS. The reaction mixture was incubated at 37°C with end-over-end rotation for 60 min. The results were expressed as the bactericidal index (BI) (7), calculated as the percent reduction in the initial inoculum in the reaction mixture at 60 min. Quantitation of the inhibitory effect of the blocking antibodies was expressed as the percent inhibition of opsonophagocytosis. Unless otherwise specified, the results represented the means of four experiments performed by using four different bacterial strains.

ELISA. A whole-cell ELISA was carried out by incubating an overnight growth of a CONS non-slime-producing strain resuspended in ELISA coating buffer, pH 9.0, in wells of polystyrene microtiter plates (Dynatech Laboratories, Inc., Alexandria, Va.). After being washed, dilutions of test sera in PBS-Tween 20 were incubated at 4°C for 2 h. Successive washings and the addition of alkaline phosphatase-conjugated goat anti-human IgG (Sigma Chemical Company, St. Louis, Mo.), *p*-nitrophenyl phosphate substrate (Sigma), and 3 N NaOH were carried out. The optical density at 405 nm (OD_{405}) was read on an enzyme immunoassay reader, model EL307 (Bio-Tek Instruments, Inc., Burlington, Vt.).

Statistics. Data were analyzed by the Student unpaired *t* test (two tailed) with the Bonferroni correction when multiple comparisons were analyzed (9).

RESULTS

Preliminary experiments determined the saturating concentration of each MAb for its respective PMNL receptor. Immunofluorescence flow cytometry indicated saturation at plateaus ranging from 1.2 to 10 μg/ml. A representative dose-dependent binding curve for leu 15 with adult PMNL is shown in Fig. 1. To correlate functional activity with flow cytometry, adult PMNL were incubated in the presence of NHS and MAb in concentrations ranging from 0.04 to 20 μg/ml. These experiments revealed a plateau of inhibition for leu 11b at 0.6 to 1.3 μg/ml for CONS (Fig. 2). In subsequent experiments, leu 11b was employed at a concentration of 2.5 μg/ml. Since OKM1, CD35, and leu 15 demonstrated minimal inhibition for CONS, a type III group B *Streptococcus* isolate was used as a control organism to document the inhibition of receptor function. Blockade of the CR3 receptor with OKM1 and leu 15 inhibited opsonophagocytosis by 51 and 98%, respectively, whereas the CR1 blockade with CD35 was 38%. Experimentation with this organism has proven that there is dependence on the complement freceptor function for ingestion and that a blockade of these receptors results in the inhibition of this process (28).

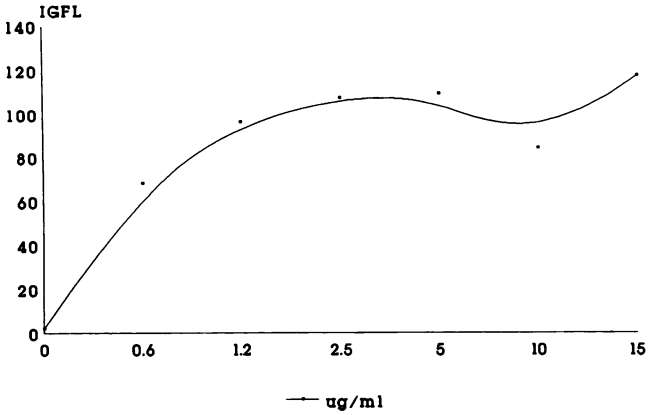

FIG. 1. Dose-response curve by immunofluorescence flow cytometry showing the saturation of PMNL receptors by leu 15 at a concentration of 2.5 μg/ml. IGFL, integral fluorescence reported as mean channel fluorescence.

Functionally, inhibition was maximal at concentrations from 5 to 10 μg/ml employed in the assay.

Effect of persistence or slime production on opsonophagocytic activity of CONS. Initial experiments were performed to compare the five CONS causing persistent bacteremia to five nonpersistent strains. No significant differences in the opsonophagocytosis of these two groups of isolates were observed when NHS was tested with PMNL from adults or term neonates (Table 2). Similar results were obtained (data not shown) when PMNL from premature infants were employed. The 10 strains were then regrouped according to slime production. When the five slime-producing strains were compared with the five non-slime-producing strains by using adult or term PMNL and NHS, no opsonophagocytic differences were detected. Leu 11b did effect significantly greater inhibition of opsonophagocytic activity for slime (92 ± 12) than non-slime-producing strains when term infant PMNL and NHS were tested. However, inhibition of non-slime-producing strains by leu 11b was still substantial (57 ± 19%). Thus, in subsequent experiments, the results for all 10

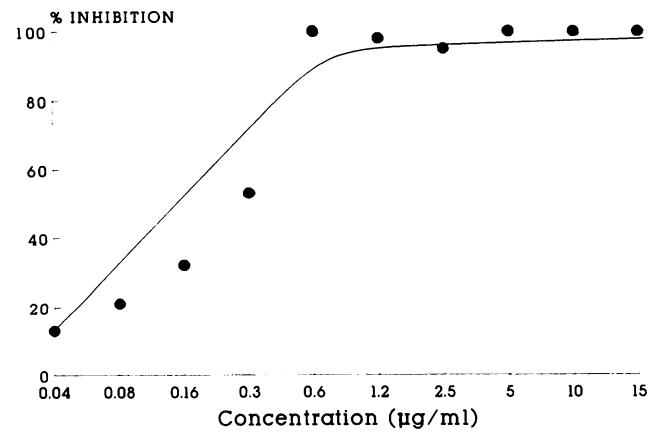

FIG. 2. Dose-response inhibition of opsonophagocytosis of CONS by leu 11b. Adult PMNL were incubated with various concentrations of leu 11b, and the percent inhibition of intracellular killing of CONS was determined. A plateau for inhibition was observed at 0.6 to 1.3 μg/ml.

TABLE 2. Comparison of opsonophagocytosis, expressed as the percent inhibition of BI, for CONS causing persistent or nonpersistent bacteremia by PMNL from adults or term neonates

MAb (concn, μg/ml)	Mean (SD) % inhibition of BI[a]			
	Adult PMNL		Term neonate PMNL	
	P	NP	P	NP
leu 11b (2.5)	77 (15)	83 (13)	69 (31)	81 (15)[b]
CD35 (5)	1 (2)	5 (9)	1 (1)	12 (14)
OKM1 (10)	8 (5)	3 (3)	4 (4)	11 (11)

[a] Values in parentheses reflect one standard deviation. For adult and term neonate PMNL, the initial BI was 87 ± 9% and 82 ± 9%, respectively, for persistent (P) strains and 84 ± 12% and 86 ± 9%, respectively, for nonpersistent (NP) strains.

[b] Difference in BI, not significant ($P = 0.5$; unpaired t test, mean of five experiments).

CONS strains were combined when PMNL from adults and term and premature neonates were compared.

Influence of source of serum and PMNL on opsonophagocytosis. The influence of the serum and PMNL sources on the opsonophagocytosis of CONS was then determined (Table 3). The mean BI with NHS as the serum source was 86 and 84%, respectively, when adult and term neonate PMNL were tested. It was significantly lower (62%) when PMNL from premature infants were used ($P < 0.02$). These results suggested a possible functional deficiency in PMNL from premature infants. When AHG was tested with adult PMNL, the mean BI was 75%, a value significantly lower than that observed with NHS ($P < 0.01$). At a uniform serum dilution (1:400), NHS demonstrated a higher OD (1.528) in a whole-cell ELISA than that of AHG or PPS (0.177 and 0.334, respectively). The higher OD of PPS compared with that of AHG suggested that PPS might contain a higher amount of CONS-specific antibody. However, when adult or term infant PMNL were tested with PPS, the BIs were similar (79 and 84%, respectively).

Role of complement and complement receptors in opsonophagocytosis of CONS. The initial total hemolytic complement activity in NHS (464 U/ml), AHG (484 U/ml), and PPS (232 U/ml) indicated the known decreased activity in sera from premature infants. NHS and PPS displayed greater than 98% consumption during opsonophagocytosis with CONS, whereas consumption by AHG was 30%. Consumption by sera maintained at 4 or 37°C without CONS was negligible. Since complement activation by the sera employed was adequate, the effect of inhibition of PMNL complement receptors by saturating concentrations of MAb was then tested. Inhibition of CR1 by CD35 effected a mean of only 3% inhibition of opsonophagocytic activity by adult

TABLE 3. Influence of serum and PMNL source on BI for CONS

Serum source	Mean (SD) BI for PMNL from[a]:		
	Adults	Term newborns	Premature infants
NHS	86 (10)	84 (9)	62 (12)[b]
AHG	75 (8)[c]	82 (6)	NT
PPS	79 (19)	84 (7)	NT

[a] Values in parentheses reflect one standard deviation. NT, not tested.

[b] $P < 0.03$ (Bonferonni t test), compared with adult or term newborn PMNL.

[c] $P < 0.01$ (Bonferonni t test), compared with NHS.

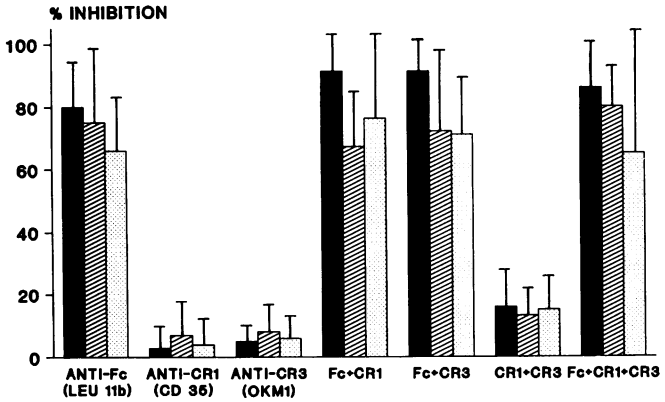

FIG. 3. Percent inhibition of the BI for CONS strains with NHS and PMNL from adults (■), term infants (▨), or premature infants (▥) when MAb to neutrophil receptors FcIII (leu 11b), CR1 (CD35), or CR3 (OKM1) were employed alone or in combination. Significant differences were observed when both FcRIII and CR1 were inhibited on adult versus term PMNL ($P < 0.004$) and when FcRIII and CR3 were inhibited on adult versus premature PMNL ($P < 0.02$).

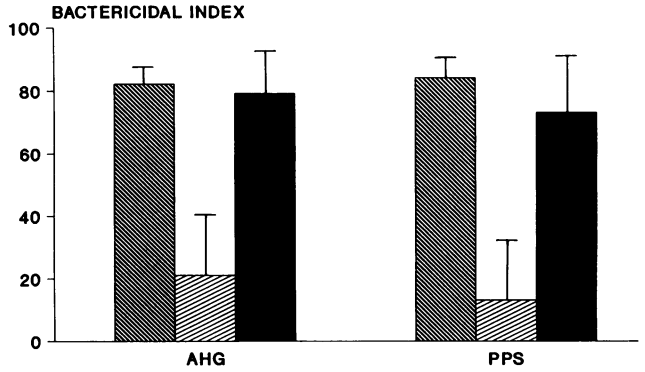

FIG. 4. Opsonophagocytosis of CONS, expressed as the percent BI, by PMNL from term infants in the presence of hypogammaglobulinemic serum (AHG) or pooled premature serum (PPS) with or without added IVIG. ▧, 33% serum; ▨, 1% serum; ■, 1% serum plus IVIG.

PMNL, 7% by term infant PMNL, and 4% by premature infant PMNL when NHS was used as the serum source (Fig. 3). When AHG was employed, inhibition remained at only 5% (the mean of two experiments) with adult PMNL (data not shown). Inhibition of the CR3 receptor by OKM1 gave results similar to those for CR1 (Fig. 3). With AHG and adult PMNL, the use of OKM1 resulted in 8% inhibition of opsonophagocytosis. Since OKM1 has been shown to selectively block the CR3 sugar or lectin-like binding site on PMNL, experiments also were performed by using leu 15, a MAb shown to inhibit specifically the iC3b binding site of CR3 (2, 31, 32). With leu 15, there was 8% inhibition by adult PMNL and 5% inhibition by term infant PMNL. Regardless of the source of PMNL, maximum inhibition did not exceed 16% when both the CR1 and CR3 receptors were blocked (Fig. 3).

Role of the FcIII receptor in opsonophagocytosis of CONS. With NHS, the mean inhibition of opsonophagocytic activity by leu 11b was 80% (Fig. 3). The degree of inhibition was not significantly different when PMNL from term or premature infants was compared with that from adults ($P = 0.14$). When the immunoglobulin concentration was limited by using AHG or PPS and adult PMNL, inhibition increased to 97 and 100%, respectively (data not shown).

Combination of FcIII and complement receptor inhibition. The results shown above suggested an important role for Fc receptors in the opsonophagocytosis of CONS. To assess the possibility that complement receptor interactions enhanced Fc receptor-mediated opsonophagocytosis, MAb were used in combination (Fig. 3). Experiments with adult PMNL and NHS indicated a mean of 91% inhibition when leu 11b (FcRIII) and CD35 (CR1) were combined. The combination of leu 11b and OKM1 (CR3) gave identical results. This extent of inhibition was not significantly different from that resulting from FcRIII blockade alone ($P = 0.1$). However, less inhibition was observed when combinations of MAb were tested with term infant or premature infant PMNL. Compared with adult PMNL, the combination of FcRIII and CR1 inhibited opsonophagocytosis by 67% by term infant PMNL ($P < 0.004$) and 76% by premature infant PMNL ($P = 0.35$). The combination of FcRIII and the CR3 blockade effected significantly greater inhibition of adult PMNL (91%) than premature infant PMNL (71%; $P < 0.02$). No further enhancement of inhibition was observed when FcRIII, CR1, and CR3 blockades were combined.

Role of immunoglobulin. Since AHG or PPS were both able to promote substantial opsonophagocytosis of CONS, we examined the effect of limiting Fc interactions by serum dilution. When serum concentrations of between 1 and 33% were tested, opsonic activity was limited at or below a concentration of 5%. At 1%, the mean opsonophagocytosis by AHG or PPS was 21 and 13%, respectively (Fig. 4). The addition of IVIG restored the BIs to 90 and 83% of the original values for AHG and PPS, respectively. A reduction in opsonophagocytosis with NHS (IgG, 960 mg/dl) was observed when the concentration reached 1% or less (28 mg/dl in the reaction mixture) (data not shown). Although immunoglobulin replacement restored the opsonophagocytic activity under these in vitro conditions, heating of NHS or AHG reduced activity by 99 and 100%, respectively, and the addition of immunoglobulin to reaction mixtures did not restore activity.

These results confirmed an absolute requirement for heat-labile opsonins in promoting phagocytosis and the intracellular killing of CONS. Therefore, EDTA or MgEGTA was used to further define the role of complement in mediating this activity. EDTA abrogated opsonophagocytosis (96 ± 1%) by NHS and adult PMNL; the addition of IVIG did not influence this. MgEGTA reduced the BI of NHS by 40 ± 10% (Fig. 5). Inhibition was significantly less (28 ± 5%) when IVIG was added in addition to MgEGTA ($P < 0.05$). By using AHG, opsonophagocytosis was inhibited significantly more (78 ± 7%) than in experiments with NHS ($P < 0.005$). The addition of IVIG to AHG reduced the inhibition to 55 ± 13%.

DISCUSSION

The preceding experiments were designed to assess differences in receptor utilization by PMNL from adults and term or premature neonates by using well-characterized strains of CONS. The 10 strains included 5 that had been isolated from neonates with persistent bacteremia and 5 from neonates with nonpersistent bacteremia (23). The strains were heterogeneous with respect to slime production. Since initial

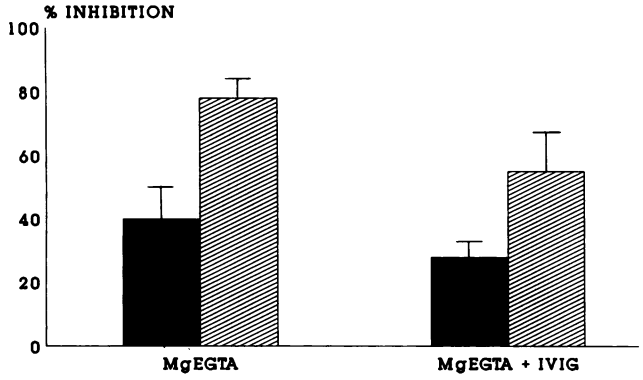

FIG. 5. Percent inhibition of the BI for CONS strains by NHS (■) or AHG (▨), by using MgEGTA with or without added IVIG. Inhibition was significantly less when IVIG was added to NHS ($P < 0.05$) compared with MgEGTA alone.

experiments revealed that the ability to cause persistent bacteremia or to produce slime did not influence the opsonophagocytosis of these CONS by NHS, results for all 10 strains were combined for subsequent experiments.

Our results indicated that FcRIII of human neutrophils plays a major role in the opsonophagocytosis of CONS by human sera. However, this opsonophagocytic activity was significantly decreased when neutrophils from premature infants were compared with those from adults or term infants. Although others have shown that PMNL from stressed neonates have reduced phagocytosis of type III group B streptococci when compared with those from healthy newborns (27), the mechanism for this impairment has not been elucidated. We found that Fc receptor-mediated inhibition of opsonophagocytosis of CONS was maximal when adult, rather than term infant or premature infant, PMNL were studied. This occurred when only the Fc receptor was blocked or when its inhibition was combined with a complement receptor blockade. This finding may reflect reduced Fc receptor expression by neutrophils from newborns. While Pross et al. (24) noted no difference in the percent of adult or term infant PMNL bearing Fc receptors, others have recently reported reduced Fc receptor expression, chemotaxis, and adherence in neonatal PMNL (14, 29). Our results support these latter observations, since PMNL from premature and term newborns had decreased Fc receptor function for a known bacterial pathogen.

Blockade of CR1 or CR3 with MAb has been shown to inhibit the opsonophagocytosis of group B streptococci and *Klebsiella pneumoniae* (28, 33). For example, Smith et al. (28) demonstrated that two inhibitors of CR3, OKM1 and leu 15, inhibited opsonophagocytosis of type III group B streptococci by 51 ± 7% and 27 ± 15%, respectively. Yang et al. (33), in experiments in which two MAb, leu 15 and CD35, were tested with *K. pneumoniae*, demonstrated inhibition of opsonophagocytosis by 85 and 86%, respectively. To investigate the possibility that neonatal PMNL were more dependent on complement than FcIII receptors for the opsonophagocytosis of CONS than were adult PMNL, complement receptor inhibition experiments were carried out. We found nominal inhibition (less than 10%) irrespective of the PMNL source when CR1 receptors were saturated by using the MAb CD35. The control for the ability of CD35 to block CR1 was group B streptococci. Since CD35 inhibited phagocytosis of group B streptococci by only 38%, the possibility exists that CD35 is sensitive to competition with C3b bound to CONS. Alternatively, it is possible that immunoglobulin bound to Fc receptors is highly efficient at depositing C3b onto CONS, where it can displace CD35 bound to CR1.

Similarly, when OKM1 was used to saturate PMNL CR3, low inhibition was noted. However, since OKM1 has been shown to interact with a lectin-like receptor rather than with the iC3b binding site of CR3 (25, 31, 32), experiments were repeated with leu 15, which is specific for the functional epitope of CR3 critical to iC3b docking (2, 25). Again, the inhibitory effect was minimal. Only the FcRIII blockade alone or in combination with complement receptor inhibition effectively reduced opsonophagocytosis.

Since FcRIII inhibition was the most potent suppressor of opsonophagocytosis of CONS, we investigated whether the concentration of immunoglobulin might have a role in limiting opsonization. The opsonophagocytic assay was modified so that the percent of serum ranged from 1 to 33%. With NHS, a reduction in opsonophagocytosis occurred only when the serum concentration was limited to 1%, whereas, with adult hypogammaglobulinemic or pooled sera from premature infants, this effect was observed at a concentration of 5% or less. These findings are in accord with the report by Clark and Easmon (5), who noted good opsonic activity at a 2.5% concentration of adult serum. Fleer et al. (8) demonstrated that the minimum concentration of premature infant serum for optimal opsonization was 15% and that lower serum concentrations were associated with reduced opsonic activity. In our experiments, the addition of IVIG restored opsonophagocytosis, confirming the importance of the interaction between immunoglobulin and FcIII receptors for efficient opsonophagocytosis of CONS. Similar results have been demonstrated in studies employing peritoneal dialysis effluents and hypogammaglobulinemic serum, each of which contains low levels of immunoglobulin and complement (4, 30).

Taken together, these results prove the importance of the interaction between immunoglobulin and the neutrophil FcRIII in the opsonization, phagocytosis, and intracellular killing of CONS. However, immunoglobulin alone would not promote opsonophagocytosis. Heat inactivation of human serum has been previously shown to inhibit its opsonic activity for CONS (4, 5, 8). In our experiments, heating of NHS ablated opsonophagocytosis even when immunoglobulin was added. Even though complement receptor blockers had little effect on opsonophagocytosis, complement was required for opsonophagocytosis to occur. This has been recognized previously with CONS (33) and also with K1 strains of *Escherichia coli*, *S. aureus*, *Streptococcus pyogenes*, *Salmonella enteriditis*, *K. pneumoniae*, and *Pseudomonas aeruginosa* (11, 12). In studies with premature infant serum, it was shown that IVIG activates both complement pathways to deposit C3 on the bacterial surface and that the optimal opsonic activity of IVIG is mediated by complement activity (26). Possibly, the mechanism by which FcR-bound immunoglobulin functioned in our experiments was to facilitate C3b deposition onto CONS. Experiments with MgEGTA suggested, as shown by others, that the alternative complement pathway had an important role in the opsonophagocytosis of CONS (5, 8, 30). When hypogammaglobulinemic serum was treated with MgEGTA, even greater inhibition occurred. Possibly this occurred because patients with common variable immunodeficiency have a greater dependence upon the classical pathway for opsonophagocytosis of CONS than do healthy patients. This use of the

classical pathway may be due to deficient activation of the alternative pathway in patients with hypogammaglobulinemia (6). Of patients with hypogammaglobulinemic states, premature infants are the most likely to develop systemic infection with CONS. Prolonged hospitalization, requirements for indwelling intravascular devices, low serum complement levels, and the immaturity of the PMNL function may each contribute to their enhanced susceptibility. Administration of intravenous immunoglobulin to premature infants may correct their IgG deficiency as well as assist in correcting the function of the alternative complement pathway in the opsonization of neonatal pathogens (26).

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