INFECTION AND IMMUNITY, Dec. 1995, p. 4764–4769
0019-9567/95/$04.00+0

Vol. 63, No. 12

Role of C5a-ase in Group B Streptococcal Resistance to Opsonophagocytic Killing

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Received 2 June 1995/Returned for modification 9 August 1995/Accepted 6 September 1995

Type III group B streptococci (GBS) can be subdivided into three subtypes, RDP III-1, III-2, and III-3, on the basis of numerical analysis of *Hin*dIII restriction endonuclease digestion patterns (*Hin*dIII RDP) with their chromosomal DNAs. In the present study, the effect of C5a on opsonophagocytic killing of a representative strain from each RDP type was investigated by using a novel optical method for determining opsonophagocytic killing, and the effect of C5a-ase treatment of C5a on opsonophagocytic killing was also investigated. Prestimulation of polymorphonuclear leukocytes (PMNs) with C5a significantly increased opsonophagocytic killing of all three strains. The increase in killing was abolished by pretreating the C5a with GBS that express C5a-ase, a treatment that also destroyed the chemoattractant activity of the C5a. The kinetics of killing of the RDP III-2 strain differed from those of the other two strains. The survival of the RDP III-2 bacteria continued to decline over the entire 60-min incubation of the opsonophagocytic assay when PMNs were prestimulated with C5a or with C5a that had been inactivated with GBS C5a-ase (dC5a). In contrast, killing of the RDP III-1 and III-3 strains almost ceased after 20 or 60 min when PMNs were prestimulated with dC5a or C5a, respectively. A difference in bacterial killing between the III-2 strain and the III-1 and III-3 strains therefore became increasingly apparent with prolonged incubation time. The percentage of bacteria surviving in the extracellular fluid was approximately the same as the percentages of bacteria surviving in both intracellular and extracellular locations when PMNs were prestimulated with either C5a or dC5a. These data imply that the majority of bacterial killing occurred following phagocytosis and suggest that the enhanced killing of GBS following prestimulation of PMNs with C5a resulted from increased ingestion of the bacteria.

Type III group B streptococci (GBS) are one of the most common causes of neonatal bacterial infections. The major factor contributing to the virulence of type III GBS is the ability of the bacteria to avoid efficient ingestion by professional phagocytes. Resistance of type III GBS to phagocytosis results from inhibition of the alternative complement pathway by sialic acid of the type-specific polysaccharide (1, 5, 6, 12), thus resulting in decreased deposition of the opsonic fragments C3b and C3bi on the bacterial surface (12). The inhibition of C3 deposition by type III capsule can be reversed by antibody directed against the type III polysaccharide (12). Thus, the lack of type III-specific antibody is the best-defined factor contributing to neonatal GBS infections.

Efficient phagocytic killing of encapsulated type III GBS in the setting of a deficiency of specific antibody requires binding of the C3bi-coated GBS to the complement receptor CR3 (7). Both the number and function of CR3 are upregulated on the surfaces of polymorphonuclear leukocytes (PMNs) following stimulation of the PMNs by the complement-derived anaphylatoxin C5a (10, 11), suggesting that generation of C5a could increase phagocytosis of GBS. Many strains of GBS, however, express an enzyme, called C5a-ase, that rapidly inactivates C5a (9). While it has been proposed that the GBS C5a-ase contributes to the poor inflammatory response seen in neonatal infections (8, 9), the contribution of C5a-ase to GBS resistance to opsonophagocytosis has not been examined.

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We previously reported that type III GBS can be divided into three major subtypes, RDP III-1, III-2, and III-3, on the basis of *Hin*dIII restriction endonuclease digestion patterns (*Hin*dIII RDP) with chromosomal DNA (14). We also showed that bacteria from each of the RDP III types apparently differ in their susceptibilities to opsonophagocytosis in vitro, as measured by a chemiluminescence assay (16). In the present study, we investigated the effect of C5a on opsonophagocytic killing of a representative strain from each RDP type by using a novel optical method for determining opsonophagocytic killing, and we also determined the effect of C5a-ase treatment of C5a on opsonophagocytic killing.

MATERIALS AND METHODS

Bacterial strains. GBS isolates selected in a previous study (16) for resistance to opsonophagocytosis are listed in Table 1. Bacteria were cultured overnight in Todd-Hewitt broth (THB) (BBL Microbiology Systems, Cockeysville, Md.) and

TABLE 1. GBS strains tested and their characteristics

Strain	Alternative strain no.[a]	RDP type	LD_{50} for suckling rats (ng/g of body wt)	Ability of C5a-ase to inactivate C5a (% digestion)[b]
FM880557	14	III-1	52.0	98.5 ± 0.35
FM865043	19	III-2	591.6	98.5 ± 0.41
FM874391	24	III-3	90.6	98.7 ± 0.35

[a] Strain number used previously (16).
[b] For each strain, three estimations were made with the same PMN suspension, and the results are expressed as means ± standard deviations.

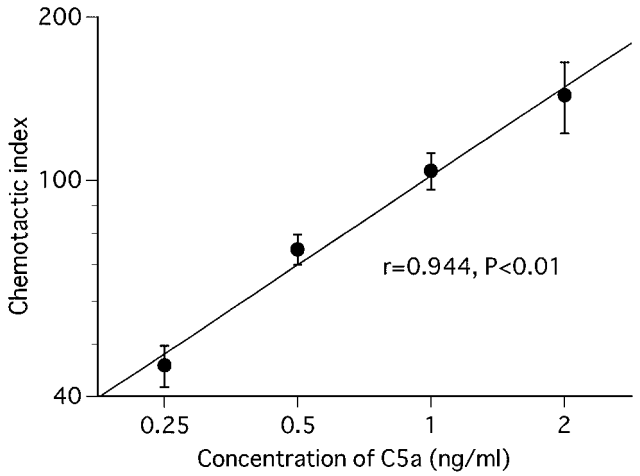

FIG. 1. Correlation between chemotactic index and C5a concentration. The chemotactic index for each C5a concentration was determined three times with the same PMN suspension, and the means ± standard deviations of the results are shown. The *r* and *P* values were calculated by linear regression analysis.

then inoculated at a 1:20 dilution into fresh THB and incubated at 37°C for 90 min. The GBS were washed twice and resuspended in Hanks' balanced salt solution (HBSS) to an optical density at 600 nm (OD_{600}) of 0.60 (0.60-OD_{600} suspensions). GBS used for determining bacterial dry weight were washed and resuspended in sterile water.

Determination of dry weight of bacteria. GBS were harvested into a tube from 40 ml of the 0.60-OD_{600} suspensions by repeated centrifuging. The GBS in the tube were weighed after lyophilization.

Determination of CFU. Serial 10-fold dilutions of the 0.60-OD_{600} GBS suspensions were prepared in sterile normal saline, and 1 ml of each dilution was poured into a plate and mixed with brain heart infusion agar. The numbers of colonies formed in the agar were counted after overnight incubation of the agar plates at 37°C.

TABLE 2. Bacterial dry weight and CFU of GBS suspensions

Strain	RDP type	Cell dry wt (μg/ml of 0.6-OD_{600} suspension)[a]	10^8 CFU/ml of 0.60-OD_{600} suspension)[a,b]
FM880557	III-1	167 ± 3.0	0.47 ± 0.08
FM865043	III-2	166 ± 3.0	0.89 ± 0.14
FM874391	III-3	172 ± 2.6	1.66 ± 0.12

[a] Expressed as means ± standard deviations of results from three determinations.

[b] There was a statistically significant difference ($P < 0.01$) between the three strains as determined by variance analysis.

Preparation of IgG containing type III-specific antibody. Immunoglobulin G (IgG) was isolated by affinity chromatography from pooled human sera by using protein G affinity medium (MAbTrap G; Pharmacia LKB Biotechnology AB, Uppsala, Sweden) according to the manufacturer's instructions and was then dialyzed with phosphate-buffered saline (pH 7.4). The IgG preparation (5 ml) was adsorbed twice at 37°C for 30 min with GBS harvested from 50 ml of a 0.60-OD_{600} suspension of asialo strain FM864237, possessing group B and protein R antigens and only a negligible amount of sialic acid (less than 0.2 μg/mg of bacteria [dry weight]) in order to remove anti-group B and anti-protein R antibodies. After centrifugation, the adsorbed IgG preparation was filter sterilized, lyophilized in aliquots, and stored at −80°C. The concentration of IgG antibody to type III capsular polysaccharide antigen was determined by K. Hoshina, Tokyo Teishin Hospital, with an enzyme-linked immunosorbent assay (ELISA) kit (Asahi Chemical Industry Co., Tokyo, Japan).

Preparation of serum as complement source. Sera used as complement sources were collected from healthy donors whose type III-specific IgG concentrations were less than 0.5 μg/ml. Pooled sera (5 ml) were adsorbed for 30 min in an ice-water bath with bacterial pellets (harvested from each of 50 ml of 0.60-OD_{600} suspensions) of the three GBS strains tested in order to remove antibodies directed against all antigens of the strains. After centrifugation and filter sterilization, the serum was adjusted to a concentration of 80% with HBSS

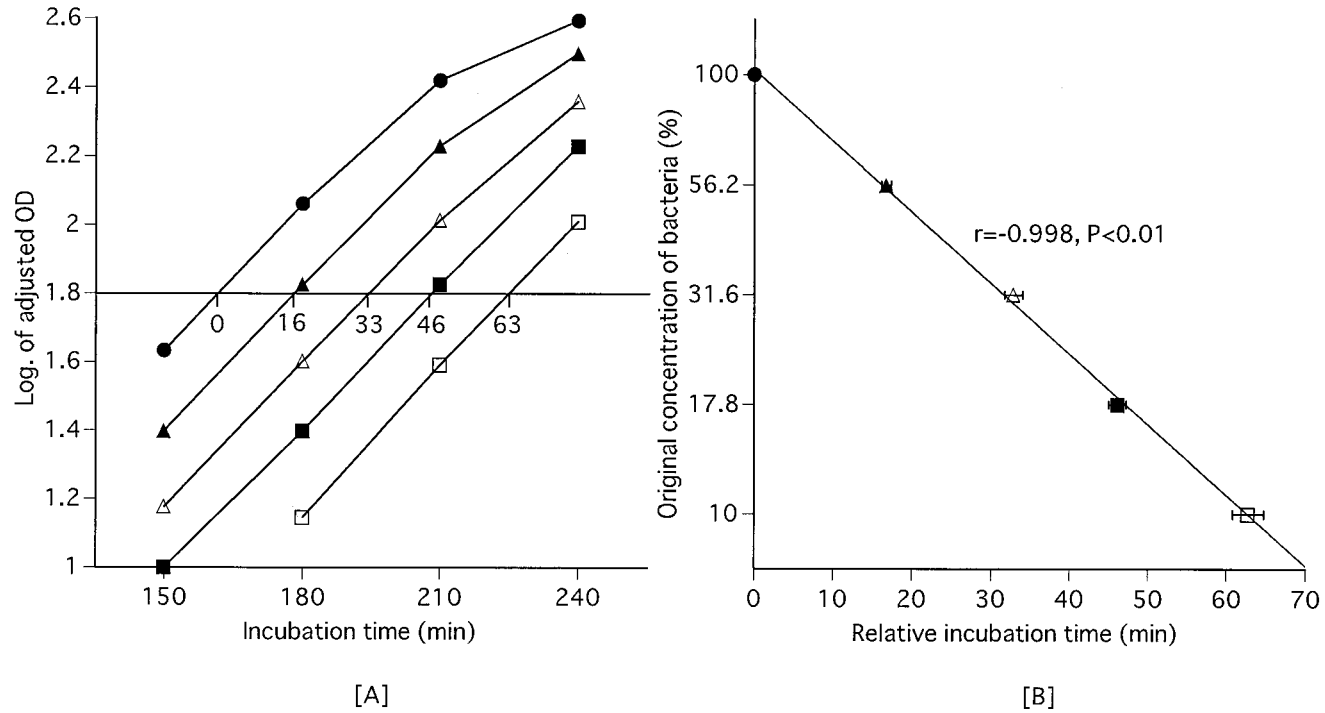

FIG. 2. (A) Growth curves of strain FM874391 suspensions with five original concentrations (100% [●], 56.2% [▲], 31.6% [△], 17.8% [■], and 10.0% [□]). An arbitrary incubation time of 0 was assigned when the log of the AOD of the 100% suspension reached 1.8. Relative incubation times for the other suspensions were taken from their growth curves. Numbers in the center indicate relative incubation times. (B) Correlation of original concentrations of bacteria with relative incubation times. The *r* and *P* values were calculated by linear regression analysis. Each symbol indicates the mean ± standard deviation of 12 determinations.

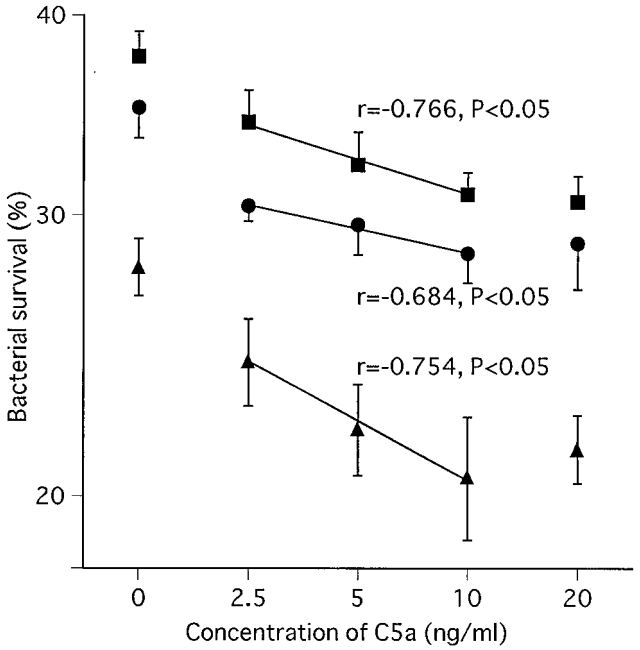

FIG. 3. Effect of prestimulating PMNs with various concentration of C5a on opsonophagocytic killing of strains FM880557 (RDP III-1) (■), FM865043 (III-2) (▲), and FM874391 (III-3) (●). GBS were preopsonized with type III-specific IgG and complement. A mixture of GBS and PMNs was incubated for 40 min. For each strain, the percent bacterial survival (intracellular and extracellular bacteria) was determined three times with the same PMN suspension, and the means ± standard deviations of the results are shown. The *r* and *P* values were calculated over the range of 2.5 to 10 ng/ml C5a for each strain by linear regression analysis.

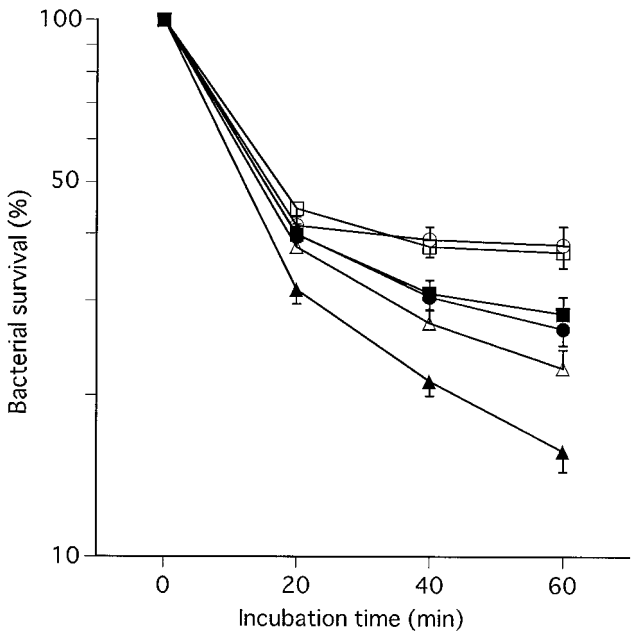

FIG. 4. Effect of C5a-ase on C5a-stimulated opsonophagocytic killing of strains FM880557 (RDP III-1) (squares), FM865043 (III-2) (triangles), and FM874391 (III-3) (circles). PMNs were prestimulated with either dC5a (open symbols) or 10 ng of C5a (closed symbols) per ml. GBS were preopsonized with type III-specific IgG and complement. The percent bacterial survival (intracellular and extracellular bacteria) was determined three times with the same PMN suspension, and the means ± standard deviations of the results are shown. There was a statistically significant difference ($P < 0.05$ by Student's *t* test) between bacterial survival with PMNs stimulated with dC5a and that with PMNs stimulated with C5a at 40 and 60 min for all strains.

and stored in aliquots at −80°C. Adsorption of antibody to the type III polysaccharide was confirmed by K. Hoshina by ELISA. The classical-pathway complement activity (50% hemolytic complement) was confirmed to be the same as that of untreated serum by the sensitized sheep erythrocyte lysis assay of Mayer (13).

Preparation of PMNs. Blood taken from healthy donors was heparinized (10 U of heparin per ml of blood), and PMNs were isolated by the gradient centrifugation method with Polymorphprep (Nycomed Pharm As, Torshov, Norway) according to the manufacturer's instructions and washed with 50% HBSS. The residual erythrocytes were removed by hypotonic lysis, and the PMNs were washed with HBSS and then resuspended in HBSS containing 0.4% human serum albumin (HSA) and 10 mM *N*-2-hydroxyethylpiperazine-*N*′-2-ethanesulfonic acid (HEPES) at a density of 2.0×10^6/ml or in medium 199 (Hanks' salt; Gibco BRL, Gaithersburg, Md.) containing 0.4% HSA and 10 mM HEPES at a density of 3.4×10^6/ml. Dye exclusion indicated that the viability of the PMNs was greater than 95%.

Preparation of dC5a with GBS C5a-ase. The method of Bohnsack et al. (4) was used for digestion of C5a with GBS C5a-ase and determination of PMN chemotaxis. Recombinant human C5a (1 ml of a 1.28-μg/ml solution; Sigma) in HBSS containing 0.4% HSA was mixed with the same volume of a suspension of GBS (OD_{600} = 1.2). The mixture was incubated at 37°C for 30 min with shaking, and the GBS were removed by centrifugation and filtration. The preparation of digested C5a (dC5a) was stored in aliquots at −80°C and was diluted eight times with HBSS containing 0.4% HSA before use.

The C5a-ase activity of GBS was quantitatively estimated by employing PMN chemotaxis to the dC5a preparation as an indication of C5a activity. PMN chemotaxis to either the dC5a preparation or the C5a standards containing 0.25, 0.5, 1.0, and 2.0 ng of C5a per ml of HBSS–0.4% HSA was determined in a blind well chamber (type 440900; Costar Co. Cambridge, Mass.) with a polyvinylpyrrolidone-free filter (pore size, 5 μm) (Nuclepore; Costar Co.) between the upper and lower wells. PMNs (50 μl of a solution of 2×10^6/ml in HBSS–10 mM HEPES–0.4% HSA) were added to the upper well of the chamber, and 200 μl of either the dC5a preparation or a C5a standard was added to the lower well. The chamber was incubated at 37°C for 90 min, and the filter was then stained with Giemsa stain. The number of PMNs that had migrated completely through 50 random 1,000× fields per filter (the chemotactic index) was determined by microscopy. The equation for the regression line between the concentrations of C5a standards and the chemotactic indexes was obtained, and the concentration of residual C5a in the dC5a preparation was estimated from the regression line. The ability of GBS C5a-ase to inactivate C5a was expressed as follows: percent digestion = (80 − residual C5a concentration [nanograms per milliliter])/80 × 100, where 80 is the original C5a concentration (in nanograms per milliliter).

Opsonization of GBS. The GBS suspensions (1 ml of 0.60-OD_{600} suspensions) were opsonized by being mixed with 1 ml of 50% serum in HBSS to act as complement source, 1 ml of type III-specific IgG (2.5 μg/ml), and 2 ml of HBSS and then incubation at 37°C for 30 min with shaking. The type III-specific IgG final concentration (0.5 μg/ml) corresponded to the level found in mothers of infected infants (2). Opsonized bacteria were washed twice, resuspended in HBSS, and then centrifuged at 150 × *g* at 4°C for 2 min to pellet the bacterial cell aggregates formed during the washing process. By adding HBSS, the concentration of the supernatant was adjusted to an OD_{600} of 0.074, which corresponded to the concentration of a 10-fold dilution of a 0.60-OD_{600} suspension on a spectrophotometer (Ultraspec Plus 4054; Pharmacia LKB Biochrom, Cambridge, United Kingdom). The opsonized GBS suspensions (100%) thus obtained were diluted with HBSS to prepare 56.2, 31.6, 17.8, and 10.0% suspensions, and all five of these suspensions were used as standards.

Opsonophagocytosis assay. For prestimulation, 50 μl of dC5a, 50 μl of various concentrations of C5a, recombinant human interleukin-1β (Intergen Co., Purchase, N.Y.), or recombinant human tumor necrosis factor alpha (Sigma) was added to 300 μl of a suspension of 3.4×10^6 PMNs per ml in medium 199 containing 0.4% HSA and 10 mM HEPES, and each mixture was incubated at 37°C for 5 min. The dC5a used for the prestimulation was prepared by exposure to each individual strain that was tested in the opsonophagocytosis assay. In some experiments, as indicated in Results, heterologous GBS were used for dC5a preparation. After prestimulation, 50 μl of a 100% opsonized GBS suspension was added to the mixture, which was incubated at 37°C for various times with shaking and then immediately cooled in an ice-water bath. To determine the percent bacterial survival (intracellular and extracellular bacteria) after opsonophagocytic killing, 3.6 ml of sterile water was added to the mixture, which was then thoroughly vortexed to destroy the PMNs. The destroyed PMNs were pelleted by centrifugation at 200 × *g* at 4°C for 5 min, and 1 ml of supernatant was transferred to a cuvette, mixed with the same volume of doubly concentrated THB, and incubated at 37°C for 4 h. During the incubation process, growth was monitored by recording OD_{640} values in the cuvettes with a spectrophotometer every 30 min. The five standard opsonized GBS suspensions (100, 56.2, 31.6, 17.8, and 10.0%) were treated in the same manner except that medium 199 containing 0.4% HSA and 10 mM HEPES was substituted for PMN suspensions. Growth curves were prepared by plotting the logarithms of the OD values multiplied by 1,000 (adjusted OD value [AOD]), where an arbitrary incubation time of 0 was

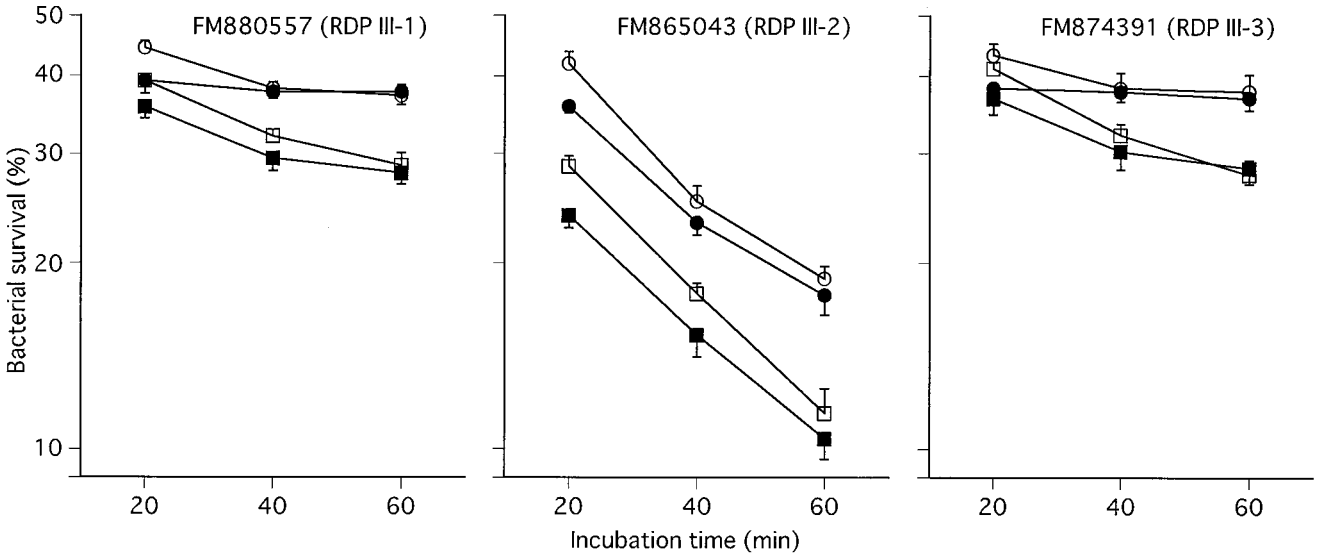

FIG. 5. Inhibitory effect of C5a-ase on C5a-stimulated opsonophagocytosis. PMNs were prestimulated with either dC5a (circles) or 10 ng of C5a (squares) per ml. GBS were preopsonized with type III-specific IgG and complement. The percentage of bacteria surviving in the extracellular fluid (closed symbols) was determined by using the supernatant after PMNs were removed, and the percentage of bacteria surviving in both intracellular and extracellular locations (open symbols) was determined by using the suspension after PMNs were destroyed and then removed. In each strain, three determinations were performed with the same PMN suspension, and the means ± standard deviations of the results are shown.

given for length of time required for the log AOD of the 100% standard suspension to reach 1.8. The relative incubation time, that is, the difference between the time it took for the log AOD of the 100% suspension to reach 1.8 and the time it took that of another standard suspension to reach 1.8, was determined for each of the remaining four standard suspensions. The equation for the regression line between the relative incubation times and the logs of the percent concentrations of the five standard suspensions was then obtained by the least-squares method. The log of the percent bacterial survival after opsonophagocytic killing was estimated from the regression line.

To determine the percent bacterial survival in the extracellular fluid, the bacterium-PMN mixture was treated in the same manner except that 3.6 ml of THB was substituted for water and the supernatant after centrifugation was mixed with an equal volume of THB. The integrity of the PMNs treated in this manner was confirmed by light microscopy.

Experimental infection. The method of Zeligs et al. (17) was used to infect suckling rats with GBS. Two-day-old Wistar suckling rats of both sexes with their mothers were obtained from Clea Japan Co., Tokyo, Japan. Groups of 10 to 12 9-day-old suckling rats were injected subcutaneously immediately cephalad to the tail with 10 μl of various dosages of either a GBS suspension or HBSS as a control per g of body weight. The 50% lethal dose (LD_{50}) was calculated by the method of Behrens and Kärbor (9a). The lethality kinetics (survival curve) of suckling rats infected with 1.0 LD_{50} of GBS was obtained from three observations per day for 5 days after injection.

RESULTS

Ability of GBS C5a-ase to inactivate C5a. Figure 1 demonstrates the correlation between the chemotactic index and the C5a concentration obtained with a single PMN suspension. The residual concentration of C5a in the dC5a preparation was estimated from the regression line, and the ability of GBS C5a-ase to inactivate C5a was calculated as percent digestion (Table 1). No differences between the C5a-ase activities of the three strains were found.

Determination of bacterial concentration in opsonophagocytic assay from an optical growth curve. Preliminary experiments were performed to determine the relationship between OD values and bacterial concentrations. For GBS strains FM874391, 865043, and 880557 in the exponential growth phase, bacterial dry weights and CFU are shown in Table 2. The bacterial dry weights were almost the same, whereas the CFU differed significantly between these three strains.

Figure 2 shows the growth curves of the five standard suspensions from strain FM874391. The correlation between the log of the original concentration of bacteria and the relative incubation time required to reach a log AOD of 1.8 was obtained. Similar results were obtained for strains FM865043 and FM880557. In the main study, therefore, the percent bacterial survival after opsonophagocytosis could be estimated from the regression line between the logs of percent concentrations of five standard suspensions and the relative incubation times.

Activation of opsonophagocytic killing by C5a. The effect of prestimulating PMNs with various concentrations of C5a (2.5 to 160 ng/ml) on opsonophagocytic killing of the three GBS strains was determined. For all three strains, bacterial killing was increased significantly at C5a concentrations of between 2.5 and 10 ng/ml (Fig. 3). In contrast, stimulation of PMNs with various concentrations of interleukin-1β and tumor necrosis

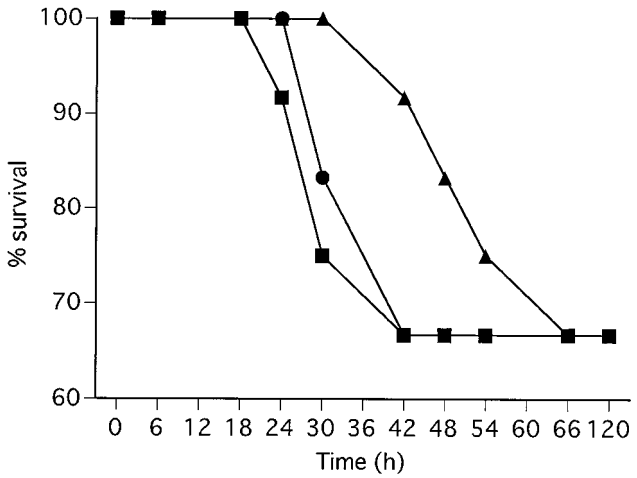

FIG. 6. Survival curves for 9-day-old suckling rats infected with 1.0 LD_{50} (Table 1) of strains FM880557 (RDP III-1) (■), FM865043 (III-2) (▲), and FM874391 (III-3) (●). Twelve rats were used for each strain.

factor alpha (125 to 8,000 pg/ml) had no effect on bacterial killing. Greater than 90% of the bacteria survived when opsonized with type III-specific IgG alone, in contrast to the results with antibody plus complement, and prestimulation of PMNs did not increase bacterial killing (data not shown).

Effect of C5a-ase on C5a-stimulated opsonophagocytic killing. The kinetics of opsonophagocytic killing after prestimulation with either C5a or dC5a is shown in Fig. 4. For each of the three strains, bacterial survival was greater when PMNs were prestimulated with dC5a than when they were prestimulated with C5a. This difference in bacterial survival was the same when dC5a prepared by exposing C5a to heterologous GBS was used to stimulate the PMNs. For strain FM865043 (III-2), a decline in bacterial survival persisted during the assay incubation period when the PMNs were prestimulated with either C5a or dC5a. In contrast, the decline in the bacterial survival of strains FM880557 (III-1) and FM874391 (III-3) almost ceased after 20 or 60 min when PMNs were stimulated with dC5a or C5a, respectively. A difference in bacterial killing between the III-2 strain and III-1 and III-3 strains therefore became increasingly apparent with prolonged incubation times.

C5a increases bacterial killing by increasing phagocytosis. The percentage of bacteria surviving in the extracellular fluid was compared with the percentage of bacteria surviving in intracellular and extracellular locations in order to clarify whether C5a activates ingestion of bacteria or intracellular killing (Fig. 5). The percentage of bacteria surviving in the extracellular fluid was approximately the same as the percentage of bacteria surviving in both intracellular and extracellular locations when PMNs were prestimulated with either C5a or dC5a. These data imply that the majority of bacterial killing occurred following phagocytosis and suggest that C5a increases bacterial killing by increasing the phagocytosis of the bacteria.

Experimental infection. The LD_{50}s of GBS strains in suckling rats and the survival curves for suckling rats infected with 1.0 LD_{50} of GBS are shown in Table 1 and Fig. 6, respectively. Strains FM880557 (III-1) and FM874391 (III-3) had LD_{50}s 1/6 to 1/11 of those of strain FM865043 (III-2), as well as more rapid lethal action.

DISCUSSION

In the opsonophagocytic assay developed by Baltimore et al. (3), the bactericidal activity of antibody directed against the type III capsule is determined by colony counts. Our preliminary experiments demonstrated that the bacterial CFU from suspensions with the same ODs differed significantly for the three strains tested, despite the fact that the bacterial dry weights were the same. It is conceivable that the differences in the CFU are due to differences in chain length or clump formation in the strains (15). Thus, the colony-counting technique is effective for comparing the number of viable bacteria within a single strain but may be less useful for comparing bacterial concentrations between strains that have different numbers of CFU in suspensions with the same OD. These observations led us to develop a technique whereby the bacterial concentration is determined from curves of ODs obtained during bacterial growth. Our results demonstrate that it is suitable to calculate the percent viable bacteria from the regression line between the original concentrations of standard suspensions and the relative incubation times taken to reach a given OD.

We employed this OD method to investigate the effect that C5a has on the in vitro PMN-mediated opsonophagocytic killing of three strains of type III GBS that differ in their *Hin*dIII RDP with chromosomal DNA. Prestimulation of PMNs with C5a increased opsonophagocytic killing of all three strains when the bacteria were opsonized with low concentrations of anti-type III antibody and complement. The increase in killing was abolished by pretreating the C5a with GBS that express C5a-ase, a treatment that also destroyed the chemoattractant activity of the C5a. The kinetics of killing of the RDP III-2 strain differed from those of the other two strains. The survival of the RDP III-2 bacteria continued to decline over the entire 60-min incubation in the opsonophagocytic assay when PMNs were prestimulated with either C5a or dC5a. In contrast, killing of the RDP III-1 and III-3 strains almost ceased after 20 or 60 min when PMNs were stimulated with dC5a or C5a, respectively. A difference in bacterial killing between the RDP III-2 strain and the RDP III-1 and III-3 strains therefore became increasingly apparent with prolonged incubation time. We hypothesized that C5a would increase the ingestion and killing of GBS, since C5a has been shown to upregulate the number and function of PMN CR3 (10, 11), an adhesive receptor on PMNs that is known to be critical for ingestion of GBS (7). The percentage of bacteria surviving in the extracellular fluid was approximately the same as the percentage of bacteria surviving in both intracellular and extracellular locations when PMNs were prestimulated with either C5a or dC5a. These data imply that the majority of bacterial killing occurred following phagocytosis and suggest that the enhanced killing of GBS following prestimulation of PMNs with C5a resulted from increased ingestion of the bacteria.

Bohnsack et al. (4) showed that GBS C5a-ase inactivates C5a preparations from humans, monkeys, and cows but does not inactivate those from rats or other animal species generally used as experimental models. Therefore, the differences in LD_{50} and survival seen in vivo with the three strains of GBS do not reflect the effect of C5a-ase destroying C5a chemotactic activity and decreasing the C5a-mediated opsonophagocytic killing. Nonetheless, our results show that the resistance to killing by the three strains in vitro correlates with the virulence of the three strains in a suckling rat model of GBS infection, with the RDP III-2 strain being less virulent than the RDP III-1 and III-3 strains. These results support the importance of efficient phagocytosis in host resistance to GBS infections.

Many strains of GBS, including the three strains used in these studies, express C5a-ase, and it has been proposed that GBS C5a-ase contributes to the virulence of GBS infections by reducing PMN recruitment to sites of GBS infection (9). The results of these studies suggest that C5a-ase could also contribute to the virulence of GBS by preventing C5a from stimulating enhanced killing of the bacteria by PMNs.

ACKNOWLEDGMENTS

We are extremely grateful to J. F. Bohnsack of the University of Utah for his kind comments and suggestions. We are also grateful to Vanessa A. M. Kipps and C. W. P. Reynolds for their revision of the English in this paper. We also thank Y. Ida and T. Maruko of Funabashi Medical Center for their cooperation.

REFERENCES

1. **Anderson, D. C., M. S. Edwards, and C. J. Baker.** 1980. Luminol-enhanced chemiluminescence for evaluation of type III group B streptococcal opsonins in human sera. J. Infect. Dis. **141:**370–381.
2. **Baker, C. J., D. L. Kasper, I. B. Tager, A. Paredes, S. Alpert, W. M. McCormack, and D. Goroff.** 1977. Quantitative determination of antibody to capsular polysaccharide in infection with type III strains of group B streptococcus. J. Clin. Invest. **59:**810–818.
3. **Baltimore, R. S., D. L. Kasper, C. J. Baker, and D. K. Goroff.** 1977. Antigenic specificity of opsonophagocytic antibodies in rabbit anti-sera to group B streptococci. J. Immunol. **118:**673–678.
4. **Bohnsack, J. F., J.-K. Chang, and H. R. Hill.** 1993. Restricted ability of group B streptococcal C5a-ase to inactivate C5a prepared from different animal species. Infect. Immun. **61:**1421–1426.

5. **Edwards, M. S., D. L. Kasper, H. J. Jennings, C. J. Baker, and A. Nicholson-Weller.** 1982. Capsular sialic acid prevents activation of the alternative complement pathway by type III, group B streptococci. J. Immunol. **128:**1278–1283.
6. **Edwards, M. S., A. Nicholson-Weller, C. J. Baker, and D. L. Kasper.** 1980. The role of specific antibody in alternative complement pathway-mediated opsonophagocytosis of type III, group B streptococcus. J. Exp. Med. **151:** 1275–1287.
7. **Edwards, M. S., M. R. Wessels, and C. J. Baker.** 1993. Capsular polysaccharide regulates neutrophil complement receptor interactions with type III group B streptococci. Infect. Immun. **61:**2866–2871.
8. **Hemming, V. G., D. W. McCloskey, and H. R. Hill.** 1976. Pneumonia in the neonate associated with group B streptococcal septicemia. Am. J. Dis. Child. **130:**1231–1233.
9. **Hill, H. R., J. F. Bohnsack, E. Z. Morris, N. H. Augustine, C. J. Parker, P. P. Cleary, and J. T. Wu.** 1988. Group B streptococci inhibit the chemotactic activity of the fifth component of complement. J. Immunol. **141:**3551–3556.
9a. **Kärbor, G.** 1931. Beitrag zur Kollektiven Behandlung pharmakologischer Reihenversuche. Arch. Exp. Path. Pharmakol. **162:**480–483.
10. **Kishimoto, T. K., M. A. Jutila, E. L. Berg, and E. C. Butcher.** 1989. Neutrophil Mac-1 and MEL-14 adhesion proteins inversely regulated by chemotactic factors. Science **245:**1238–1241.
11. **Kishimoto, T. K., R. S. Larson, A. L. Corbi, M. L. Dustin, D. E. Staunton, and T. A. Springer.** 1989. The leukocyte integrins. Adv. Immunol. **46:**149–182.
12. **Marques, M. B., D. L. Kasper, M. K. Pangburn, and M. R. Wessels.** 1992. Prevention of C3 deposition by capsular polysaccharide is a virulence mechanism of type III group B streptococci. Infect. Immun. **60:**3986–3993.
13. **Mayer, M. M.** 1961. Complement and complement fixation, p. 133–240. *In* E. A. Kabat and M. M. Mayer (ed.), Experimental immunochemistry. Charles C Thomas, Springfield, Ill.
14. **Nagano, Y., N. Nagano, S. Takahashi, K. Murono, K. Fujita, F. Taguchi, and Y. Okuwaki.** 1991. Restriction endonuclease digest patterns of chromosomal DNA from group B β-haemolytic streptococci. J. Med. Microbiol. **35:**297–303.
15. **Southwick, F. S., and T. P. Stossel.** 1986. Phagocytosis, p. 326–331. *In* N. R. Rose, H. Friedman, and J. L. Fahey (ed.), Manual of clinical laboratory immunology, 3rd ed. American Society for Microbiology, Washington, D.C.
16. **Takahashi, S., Y. Nagano, N. Nagano, K. Fujita, F. Taguchi, and Y. Okuwaki.** 1993. Opsonisation of group B streptococci and restriction endonuclease digestion patterns of their chromosomal DNA. J. Med. Microbiol. **38:** 191–196.
17. **Zeligs, B. J., C. D. Armstrong, J. B. Walser, and J. A. Bellanti.** 1982. Age-dependent susceptibility of neonatal rats to group B streptococcal type III infection: correlation of severity of infection and response of myeloid pools. Infect. Immun. **37:**255–263.

Editor: V. A. Fischetti