Antibody-Independent and -Dependent Opsonization of Group B Streptococcus Requires the First Component of Complement C1

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The role of the classical complement pathway and specifically the first component, C1 in antibody-independent opsonization of type Ia group B Streptococcus (GBS) was investigated. For these studies a radiolabeled bacterial uptake assay was developed that was dependent on time and bacterial concentration and that required an intact classical complement pathway. To directly investigate the role of C1 in opsonization of type Ia GBS, C1 was isolated by chromatography on an immunoglobulin G (IgG) affinity column and further purified by molecular sieve chromatography on an Ultrogel AcA 22 column. When normal human serum was adsorbed with 10⁶ CFU of type Ia or III GBS, the serum opsonic capacity diminished (33 to 34%) for type Ia GBS compared with unadsorbed serum. Preincubation of the bacteria with purified C1 (10⁴ U of C1 per ml) restored the opsonizing capacity of the adsorbed serum. A C1-depleted serum was prepared from the nonadherent fractions of the CH-Sepharose 4B IgG column which only contained 5 U of C1 per ml. Substitution of C1-depleted reagent for normal serum in the uptake assay resulted in dramatic decreases in the opsonization of type Ia GBS, but opsonization could be restored by preincubation of the bacteria with purified C1. Heat-inactivated C1-depleted serum did not support opsonization of type Ia GBS, even with the addition of C1. Preincubation of type Ia GBS with heat-inactivated hyperimmune sera did not result in opsonization of type Ia GBS in the presence of C1-depleted serum. However, opsonization could be restored by the addition of C1, and the effects of C1 and antibody were additive. These results indicate the critical role of C1 in direct activation of the classical complement pathway by type Ia GBS and in antibody-mediated opsonization of the bacteria.

The classical complement pathway is a critical element in immunity to extracellular pathogens such as group B Streptococcus (GBS). One serotype of GBS, type Ia, has been documented to activate directly the classical complement pathway in human serum (4, 9). In the initial observation, 18 fresh clinical isolates of type Ia GBS were opsonized and killed by incubation with leukocytes and sera devoid of type-specific antibody (4). The mechanism of direct activation by the bacteria was not examined, but there was indirect evidence for the role of the classical complement pathway binding and triggering component, C1. In opsonophagocytosis of type Ia GBS there was an absolute requirement for C2, a substrate of C1 (9). Furthermore, the opsonization of type Ia GBS could be decreased by incubation of the serum complement source with the chelating agent, MgEGTA, which depletes serum of C1 activity (4, 9). Other evidence for the role of C1 in the opsonization of type Ia GBS have come from our studies on complement activation by purified GBS capsular polysaccharides (15). When human serum was incubated with purified GBS capsular polysaccharides, complement-mediated opsonophagocytic killing of type Ia GBS was dramatically decreased. Measurement of the complement component levels in the serum after incubation with the polysaccharides revealed alterations in functional levels of both C1 and C4. Results of these previous studies have implicated the requirement for C1 in the opsonization of type Ia GBS through direct activation of the classical complement pathway and have led us to our present investigations.

We developed a radiolabeled bacterial uptake assay to define the mechanism by which type Ia GBS directly activates the classical complement pathway. This opsonization assay requires an intact classical pathway and similar ratios of reactants, as were required in the opsonophagocytic killing assay described earlier. To investigate directly the role of C1 in initiating the opsonization of type Ia GBS, we purified C1 from serum and prepared a C1-depleted reagent. Results of studies with the C1-depleted reagent revealed decreased opsonization of type Ia GBS, which could be restored to normal levels with the addition of purified C1. In further studies C1 was also found to be a necessary component for the optimal opsonization of type Ia GBS in the presence of type-specific antibody.

MATERIALS AND METHODS

GBS strains and growth conditions. Type Ia strain 515 and type III strain M732, both originally clinical isolates, were obtained from the culture collection at the Channing Laboratory. The organisms had been stored at −70°C in 0.5 ml of Todd-Hewitt broth (THB; Difco Laboratories, Detroit, Mich.). For the opsonophagocytic assay, GBS were grown into early log-phase, pelleted at 8,000 × g, and suspended in minimal essential medium (MEM) to achieve a concentration of 2 × 10⁶ CFU/ml of MEM (9). For adsorption experiments bacteria were grown as described above, and a small fraction was diluted serially for determination of the CFU. The remainder of the suspension was pelleted at 8,000 × g, washed once in saline, lyophilized, and stored in dessicated form at room temperature (23°C) (9). Bacteria were quantitated by assuming that the total yield in weight corresponded to the total number of CFU before lyophilization.

Human sera. Normal human serum was obtained from the blood of healthy volunteers that worked in the laboratory. The blood was collected for 30 min at room temperature. After centrifugation for 10 min at 4°C, the serum was removed, divided into aliquots, and frozen at −70°C until use. Com-
plemement activity in selected sera was inactivated by heating at 56°C for 30 min.

Preparation of rabbit antiserum. Type 1a antiserum was prepared in New Zealand White rabbits by immunization with Formalin-killed strain 515 group in pH-titrated THB (12).

Antibody concentrations. Antibody levels in serum to the type-specific and group B antigen of GBS were measured in the radioactive antigen binding assay (5).

Immunoglobulin purification. Serum was fractionated from human plasma (Northeast Region American Red Cross) by the addition of CaCl₂ (final concentration, 0.02 M) and by precipitation with ammonium sulfate to a final concentration of 33%. After centrifugation at 8,000 × g, the pellet was dissolved in and dialyzed against 0.05 M Tris buffer (pH 7.9). The immunoglobulins were applied to a DEAE-Sephacel column (4.8 by 21 cm) equilibrated with the same buffer. The protein peak from the fractions not retained by the column was pooled, concentrated, dialyzed against the starting buffer, and frozen at −70°C. This protein peak contained two major bands, the heavy and light chains of immunoglobulin G (IgG) (molecular weights of 55,000 and 25,000 respectively) under reducing conditions assessed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis.

Complement reagents. The following buffers were used in the hemolytic assays: (i) GVB, isotonic Veronal-buffered NaCl (pH 7.5), containing 0.1% gelatin (16); (ii) GVB++, GVB with 0.15 mM CaCl₂ and 0.5 mM MgCl₂ (iii) DGVB++, 1 part GVB + 1 part 5% glucose with 0.15 mM CaCl₂ and 0.5 mM MgCl₂ and (iv) GVB-0.04 M EDTA, GVB containing 0.04 M EDTA. Functionally purified guinea pig C2 was prepared from guinea pig sera (18). MEGGTA buffer (GVB with 16 mM MgCl₂ and 48 mM EGTA) was dialyzed 1 part to 7 parts with serum (9).

C₁ purification and C₁-depleted reagent. The purification utilized the procedure of Medicus and Chapuis (17) and was based on the ability of C₁ to bind immobilized IgG. Human immunoglobulin was coupled to a CH-Sepharose 4B column using carbodiimide (Pharmacia Fine Chemicals, Piscatway, N.J.). The running buffer for the column (2.4 by 9 cm) was composed of one part GVB++ and one part GVB++ containing 1.4 M sucrose (16) with 3 × 10⁻⁵ M p-nitrophenyl guanidinobenzoate (NPGD) (Sigma Chemical Co., St. Louis Mo.). Serum (100 ml) diluted 1:2 with running buffer was applied to the column at 4°C, and the fractions that were not retained were collected and utilized as the C₁-depleted reagent. After the column was washed extensively with GVB++, C₁ was eluted with 0.4 M NaCl in GVB++. The eluate was concentrated and applied to an Ultrogel AcA 22 column (2.6 by 75 cm) (LKB Instruments, Inc., Rockville, Md.) in the same buffer. The protein fractions containing C₁ hemolytic activity, as determined by hemolytic titration, were pooled and concentrated in an Amicon ultrafiltration unit and stored at −70°C. These fractions were free of IgG, IgM, C₄, and C₁ inhibitor (C₁ NIH) by double immunodiffusion with specific commercial antisera (Atlantic Antibodies). The C₁-depleted reagent was concentrated to the original volume of the serum and frozen at −70°C.

Complement assays. The activity of human C₁ was measured with sheep erythrocytes sensitized with C₄ (EAC₄ cells) (Cordsis Laboratory, Miami, Fla.), purified guinea pig C₂, and guinea pig serum diluted 1:20 in GVB-0.04 M EDTA (18). The degree of hemolysis was quantitated by measurement at an optical density of 414 nm, and the number of hemolytic sites per cell (Z) was calculated (16). In complement assays, one functional hemolytic unit (Z = 1.0) of a specific component is defined as the concentration of this component which lyses 63% of the hemolytic intermediate (16).

Radiolabeled bacterial uptake assay. Type 1a strain 515 was grown in 100 ml of THB into early log phase, at which time 50 mCi of [³H]sodium acetate (New England Nuclear Corp., Boston, Mass.) was added. Growth was continued until the culture no longer required NaOH to maintain a neutral pH. The organisms were pelleted at 8,000 × g and washed once. The pellet was suspended in 10 ml of THB, divided into aliquots and frozen at −70°C. The specific activity of the organisms was 0.04 cpm/CFU.

The reaction mixture for the uptake assay contained ³H-labeled type 1a GBS (5 × 10⁶ CFU, except in the dose-response experiment), 2 × 10⁶ polymorphonuclear leukocytes (PMNs), and 0.1 ml of human serum in a total of 0.4 ml of MEM (GIBCO Laboratories, Grand Island, N.Y.). The controls included heat-inactivated serum (56°C for 30 min) and samples without serum or cells (background cpm). The samples were incubated for 60 min (except in the time-response experiment) at 37°C in a shaking water bath and transferred to 25-mm polypropylene filters (10 μm porosity; Gelman Sciences, Inc., Ann Arbor, Mich.) on a Büchner funnel. After the filters were washed extensively with saline, they were transferred to scintillation vials, and 1 ml of protosol (New England Nuclear) was added. After overnight digestion, 10 ml of liquifluor toluene (New England Nuclear) was added, and the samples were counted in a Packard Tri-Carb 4530 (Packard Instrument Co., Downers Grove, Ill.). The results were expressed as the percentage change in counts associated with PMNs compared with a normal serum control according to the following formula: (experimental cpm - background cpm)/(control cpm - background cpm) × 100.

Adsorption of serum with bacteria or MEGGTA. In some experiments sera were first adsorbed with bacteria. Lyophilized strain 515 or M732 were diluted in 0.01 ml of saline and mixed with human serum to yield 10⁹ CFU/ml of serum. The control was to add an equivalent amount of saline to the serum instead of bacteria. After adsorption for 30 min on ice, bacteria were removed by centrifugation, and the adsorbed sera were used in the uptake and killing assays described above.

In studies in which the classical pathway of complement was inhibited by chelation with MEGGTA, the experimental procedure was modified to include a preopsonization step to avoid toxicity to the leukocytes (9). Serum diluted in either MEGGTA or GVB++ (control) were incubated for 5 min at 37°C, and the bacteria were added (0.1 ml of a suspension of 2 × 10⁶ CFU/ml). The mixture was opsonized for 30 min at 37°C with end-over-end rotation and chilled, and the organisms were removed by centrifugation, washed once, and suspended in MEM for the uptake assay.

Preincubation of type 1a GBS with antiserum or C₁. Hyperimmune rabbit antiserum at dilutions of 1:10 to 1:1,000 in MEM was mixed with type 1a GBS (0.1 ml of serum per 5 × 10⁶ CFU). After undergoing incubation for 10 min at 4°C, the bacteria-antibody mixture was used in the uptake assay.

In experiments requiring purified C₁ (10⁴ U of C₁ per 5 × 10⁶ CFU), bacteria were incubated at 4°C for 10 min. The bacteria-C₁ mixture was used in the uptake assay.

RESULTS

Characterization of the complement-mediated bacteria-PMN association assay. In the development of an experimental system to examine the role of specific complement
components in the opsonization of type Ia GBS, a critical variable has been the ratio of bacteria to leukocytes. Results of previous studies from our laboratory have employed a bacteria to leukocyte ratio of 3:1. To establish the optimum proportions of these reactants in a complement-dependent radiolabeled bacterial uptake assay, increasing numbers of $^3$H-labeled type Ia GBS were incubated with PMNs and normal human serum for 30 min. Bacterial uptake by leukocytes increased dramatically in direct relation to the number of organisms in the reaction mixture (Fig. 1). As assessed by specific activity when $5 \times 10^8$ CFU were in the reaction mixture, $4 \times 10^5$ CFU were associated with leukocytes in the presence of normal human serum. To assess the role of complement in this uptake assay, the serum was first heat-inactivated at 56°C for 30 min and used in the uptake assay with the same bacteria to leukocyte ratio. This complement depletion resulted in a large decrease in the association of bacteria and leukocytes (Fig. 1).

The classical complement pathway is required for opsonophagocytic killing of type Ia GBS in the absence of type-specific antibody (4, 9). To determine whether the complement-mediated uptake of $^3$H-labeled type Ia GBS by leukocytes is also due to the classical pathway, serum was diluted with MgEGTA buffer and used as the complement source in the uptake assay. This resulted in a $84 \pm 2\%$ decrease in association of $^3$H-labeled type Ia GBS with leukocytes. C1 is the complement component most affected by the availability of Ca$^{2+}$. Therefore, the radiolabeled uptake assay requires the classical complement pathway, most likely C1.

Opsonophagocytosis of type Ia GBS is a time-dependent phenomena. To determine the optimal incubation time for the complement-mediated opsonization and uptake of $^3$H-labeled type Ia GBS, bacteria and leukocytes were incubated with normal human serum for various periods of time. When the amount of radioactivity associated with the cells was measured, there was a direct relation between the time of incubation and the amount of type Ia GBS associated with the cells. In contrast to this time relation with active complement was the association of bacteria with PMNs in the presence of heat-inactivated serum (Fig. 2).

**Complement and antibody requirements for type Ia opsonization.** To assess the relative contribution of both complement and antibody in opsonization of type Ia GBS, we employed heat-inactivated hyperimmune type Ia rabbit antiserum as an antibody source. $^3$H-labeled type Ia GBS were preincubated with dilutions of type Ia antiserum and incubated in the uptake assay with normal human serum and PMNs. There was an increase in association of $^3$H-labeled type Ia GBS with leukocytes in direct relation to the concentration of type-specific antiserum in the reaction mixture (Table 1). The use of a heat-inactivated complement source along with antibody resulted in greatly reduced association of bacteria with leukocytes compared with the same antibody with an intact complement source.

**Purification of human C1.** The chelation studies with MgEGTA (see above) demonstrated that antibody-independent opsonization of type Ia GBS in the uptake assay

![FIG. 1. Uptake of type Ia GBS by leukocytes in the presence of (●) or heat-inactivated serum (○). Association of the highest concentration of CFU ($10^7$) with leukocytes was employed as 100%. Mean ± SD values of three experiments are plotted at each point.](http://iai.asm.org)

![FIG. 2. Increase in the uptake of type Ia GBS by leukocytes with increasing time of incubation. The complement source was normal (●) or heat-inactivated serum (○). Association of bacteria with leukocytes at 90 min was taken as 100%. Mean ± SD values of three experiments are plotted at each point.](http://iai.asm.org)

**TABLE 1. $^3$H-labeled type Ia GBS associated with leukocytes in the presence of complement or type Ia antiserum or both**

<table>
<thead>
<tr>
<th>Complement source (human serum)</th>
<th>Heat-inactivated type Ia antiserum at the following dilutions:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>No antiserum</td>
</tr>
<tr>
<td>Normal</td>
<td>48 ± 4</td>
</tr>
<tr>
<td>Heat-inactivated</td>
<td>0</td>
</tr>
</tbody>
</table>

*Percentage of $^3$H-labeled type Ia associated with leukocytes. Association of bacteria with leukocytes with a 1:10 dilution of antiserum was taken as 100%.
TABLE 2. ³H-labeled type Ia GBS associated with leukocytes after adsorption of complement source with bacteria and effect of addition of C1

| Adsorbing strain | Percent type Ia GBS under the following adsorption conditions: |
|------------------|--------------------------------------------------|--------------------------------------------------|--------------------------------------------------|
|                  | No adsorption | Adsorption with GBS | Adsorption with GBS with subsequent addition of C1 |
| None             | 100           |                     |                                                  |
| Type Ia          | 67 ± 8        | 114 ± 16            |                                                 |
| Type III         | 66 ± 8        | 97 ± 7              |                                                 |

* Percentage of ³H-labeled type Ia associated with leukocytes.

requires an intact classical complement pathway, specifically the first component, C1. The functional role of C1 in the opsonization of type Ia GBS was studied by purifying C1 from normal human serum by immunoabsorption on a CH-Sepharose 4B IgG affinity column. C1 was eluted from the column with GVB** containing 0.4 M NaCl and further purified on Ultrogel AcA 22. Figure 3 represents a typical protein profile obtained from the fractions collected from the gel filtration column. Fractions were tested for functional C1 activity by hemolytic titration with EAC4 cells. The peak of C1 activity coincided with a small protein peak at fraction 64 and contained 2,400 U of C1 per ml.

C1 requirements for opsonization of type Ia. In previous studies of complement activation by type Ia GBS, we were unable to adsorb serum with GBS to demonstrate the complete antibody independence of the system. Although attempts at adsorption with whole bacteria resulted in decreased opsonization, we were unable to determine whether this was due to a decrease in C1 or to a decrease in type-specific antibody. This altered opsonic capacity occurs after adsorption of serum with either type Ia or III GBS. The observed decreased opsonic activity with both GBS serotypes suggested that the effect was not the result of adsorption of a specific antibody but perhaps the loss of another opsonin such as a complement component. Serum was adsorbed for 10 min on ice with 10⁶ CFU of type Ia or III GBS per ml of serum. After removal of the bacteria by centrifugation, the preadsorbed sera were used as the complement source in the uptake assay and were found to have a diminished opsonic capacity for ³H-labeled type Ia GBS of 33 to 34% (Table 2).

To determine whether C1 had been affected by the adsorption, purified C1 was employed. ³H-labeled type Ia GBS were incubated with purified C1 and then used in the uptake assay. Use of these C1-incubated bacteria with the adsorbed serum as the complement source in the uptake assay resulted in no decrease in association of the ³H-labeled type Ia GBS with the leukocytes compared with the normal unadsorbed serum control (Table 2). Thus, GBS appears to adsorb a critical complement component out of normal human serum, diminishing the opsonic capacity of the serum, but this activity could be replenished by the addition of purified C1.

The extensive adsorption of our complement source with whole GBS (10⁹) coupled with the complete restoration of the opsonization capacity of this adsorbed serum by pure C1 is strong evidence for the direct activation of C1 by GBS in the absence of antibody.

C1-depleted serum was also employed as a complement source, instead of normal human serum, in the uptake assay to prove that C1 was a critical component in the classical pathway-mediated opsonization of type Ia. This C1-depleted reagent was prepared from the nonadherent fractions of the CH-Sepharose 4B IgG column and contained only 5 U of C1 per ml by hemolytic titration with EAC4 cells compared with 80,000 U per ml for the normal serum control. Substitution of the C1-depleted reagent in the uptake assay compared with normal human serum resulted in dramatic decreases in the association of ³H-labeled type Ia GBS with PMNs (Fig. 4, lane A versus lane B). Incubation of ³H-labeled type Ia GBS with purified C1 and the subsequent addition of C1-depleted serum and PMNs resulted in the reassociation of bacteria with the leukocytes (Fig. 4, lane C).

To determine whether complement components after C1 are required for association of bacteria with leukocytes, C1-opsonized bacteria, heat-inactivated C1-depleted serum, and leukocytes were employed in an uptake assay. This combination resulted in only 8% association of ³H-
labeled type Ia GBS with leukocytes, suggesting that other heat-sensitive complement components besides C1 are required for opsonization of type Ia GBS, in the absence of type-specific antibody.

C1 and antibody-mediated opsonization. The role of C1 in antibody-mediated opsonization of type Ia GBS was also investigated. 3H-labeled type Ia GBS were opsonized with undiluted, heat-inactivated, hyperimmune rabbit antisera and used in the uptake assay with C1-depleted serum as the complement source (Fig. 4, lane D). This did not increase the uptake of type Ia bacteria when compared with the C1-depleted normal human serum control (Fig. 4, lane B). In contrast, if the bacteria were first opsonized with antibody and then with purified C1, opsonization was restored (Fig. 4, lane E).

To determine whether the observed increase in association of bacteria with PMNs was due to both C1 and antibody, C1 was diluted to allow the additive effects of C1 and antibody to be examined. The association of type Ia GBS and leukocyte was 28 ± 5% in C1-depleted serum with undiluted type Ia antibody. When 3H-labeled type Ia GBS were incubated with a 1:4 dilution of our C1 (2.5 × 10^7 U/5 × 10^7 CFU) but without antibody, there was 39 ± 0.5% association of bacteria with the leukocytes in the presence of C1-depleted serum. However, if 3H-labeled type Ia GBS were opsonized with antibody and C1, the association of the bacteria increased to 55 ± 3%. Therefore, C1 is required for both antibody-independent and -dependent opsonization of type Ia GBS.

**DISCUSSION**

Results of our previous studies have documented the role of the classical complement pathway in opsonophagocytic killing of type Ia GBS (4, 9, 15). The initial observation made by Baker et al. (4) was that when 18 fresh clinical isolates of type Ia GBS were incubated with leukocytes and serum devoid of type-specific antibody, the bacteria were opsonized and killed. The role of the classical complement pathway in opsonization of type Ia GBS was documented by the dramatic decreases in opsonophagocytosis of a representative clinical isolate of type Ia GBS, strain 515, in serum deficient in C2 or chelated with MgEGTA (4, 9). Results of these studies indicate that C1 has a role in initiating opsonization because C1 is the component most affected by MgEGTA chelation (14). In examining whether type Ia GBS and C1 could directly interact, a C1 transfer assay was used (8, 9). This assay measures the capacity of an organism to bind purified C1 and transfer it to recipient indicator cells, sheep erythrocytes sensitized with C4. It was found that binding and transfer of C1 was directly related to the number of bacteria that interacted with C1 (9). Additional evidence for the role of C1 in initiating opsonization of type Ia GBS came from our studies on complement activation by purified GBS capsular polysaccharides (15). When human serum was incubated with purified GBS capsular polysaccharides, complement-mediated opsonophagocytic killing of type Ia GBS was dramatically decreased. Measurement of the complement component levels in the serum after incubation with polysaccharides revealed alterations in functional levels of both C1 and C4. Results of these studies implicate the requirement for C1 in initiating opsonization of type Ia GBS and led us to our present investigations of the direct requirement for C1 in opsonization of these bacteria.

To study the role of C1 in antibody-independent opsonization of type Ia GBS, we developed a radiolabeled bacterial uptake assay. This opsonization assay requires an intact classical complement pathway and similar ratios of reactants, as are required in the opsonophagocytic killing assay described previously (4, 9, 15). The advantage of the uptake assay over the opsonophagocytic killing assay is that it separates the first phase of opsonophagocytosis (opsonization) from the second phase (intracellular killing). The separation of these phases is important in view of the findings of other workers that opsonins are important for both steps of opsonophagocytosis. For example, the presence of C3b on the surface of *Staphylococcus aureus* and *Escherichia coli* was found to be necessary for the intracellular killing of these bacteria (13). Although this is the first antibody-independent, complement-dependent, radiolabeled bacterial uptake assay described for GBS, other workers have reported on antibody-dependent radiolabeled bacterial uptake assays. These include the study of opsonization of 3H-labeled type Ia GBS by alveolar macrophages (2) and the uptake of 3H-labeled type III GBS by leukocytes (1). The uptake of type Ia GBS by macrophage in the presence of very low concentrations of type-specific antibody did not require complement (2); however, the optimal uptake of type III GBS by leukocytes required a competent classical complement pathway in addition to type-specific antibody (1).

The role of antibody in opsonization was also examined in our assay by using hyperimmune rabbit antisera as an opsonic source. Our results indicate that high levels of hyperimmune rabbit antisera would opsonize type Ia GBS, but never to the extent that occurs if the complement cascade is intact. Furthermore, the combination of both antibody and an intact complement cascade resulted in the greatest opsonization of type Ia GBS. These findings were similar to those of other workers investigating opsonophagocytic killing of type Ia GBS. Results of studies by both Hemming et al. (11) and Baltimore et al., (6) have shown that optimal killing of type Ia GBS occurs in the presence of hyperimmune rabbit antisera and requires an intact complement cascade.

To directly investigate the role of the classical complement pathway and, specifically, the role of C1 in initiating opsonization of type Ia GBS, purified C1 was necessary. The use of an IgG affinity column to purify C1 takes advantage of the immunochromatic recognition of IgG by C1 (7, 17). Throughout the isolation procedure the enzyme inhibitor *p*-nitrophenyl guanidinobenzoate was included in the buffers to maintain C1 in the inactive state. The subcomponents C1r and C1s in the native state can be differentiated from the active subcomponents by sodium dodecyl sulphate-polyacrylamide gel electrophoresis under reducing conditions (3, 17). Electrophoretic analysis of the reduced C1 sample revealed some cleavage of the C1r and C1s zymogens. This study does not elucidate how C1 becomes functional to initiate activation of the classical pathway resulting in the opsonization of type Ia GBS.

The complement source employed to investigate classical complement pathway activation by type Ia GBS in this and previous studies has been normal or antibody-deficient serum (4, 9). Use of this complex reagent raised the question of whether an undetected antibody to type Ia GBS was present in very low concentrations in the serum and whether this antibody was mediating activation of the classical complement pathway. In the present study, we exhaustively adsorbed the serum complement with two serotypes of GBS by a technique which should adsorb out any undetectable antibody. Even though this treatment resulted in decreased opsonic capacity of the serum, the opsonic capacity of the serum could be restored by the addition of purified C1. We
believe this is good evidence for the direct activation of C1 by type Ia GBS in the absence of type-specific antibody.

The finding that type Ia GBS adsorbs a component critical for opsonization out of serum correlates well with our previous findings by use of a C1 transfer assay in which type Ia GBS bound and transferred C1 (9). It is interesting to speculate that the component that adsorbs C1 is also the C1 binding and triggering site on the surface of type Ia GBS. Although we have not identified the activating site on the surface of type Ia GBS, our previous data on complement activation by purified types Ia and III GBS capsular polysaccharides may be relevant (15). When these polysaccharides are incubated in human serum, C1 is activated and C4 is depleted, decreasing the opsonic capacity of the serum for type Ia GBS. Whether the capsule on the surface of type Ia GBS is the site for complement activation or for the adsorption of opsonic capacity from the serum must be examined.

In our present investigations we present the first direct evidence for the participation of C1 in antibody-independent opsonization of type Ia GBS. We showed that a C1-depleted reagent did not support opsonization of the organism but that opsonization could be restored by the addition of physiologically relevant amounts of purified C1. These findings also revealed that it is not just the C1 components but a functionally active C1 which is necessary for the initiation of opsonization. With heat-inactivated, C1-depleted serum as the complement source, C1 could not restore opsonic activity. Therefore, it is not only C1 that is necessary to lead to opsonization of the organisms but, more importantly, a functional C1 which can participate with later complement components for opsonic activity, presumably through C3b deposition. This finding is not surprising in view of our previous data showing the requirements for C2 in opsonization of type Ia GBS (9). Because C1 is the binding and triggering component of the classical pathway, it appears that type Ia GBS may directly activate the classical pathway by a mechanism similar to an immune complex, that is, through binding and activation of C1. The failure of C1-depleted serum to initiate opsonization and the restoration of this activity with purified C1 suggests that direct classical pathway activation by GBS is not occurring by binding of the C1 INH nor cleavage and activation of C4 and C2. Results of our previous studies left these questions unanswered (4, 9, 15).

We have now documented the initial role for C1 in direct activation of the classical complement pathway by type Ia GBS. Furthermore, we presented data on its critical role in antibody-dependent opsonization of type Ia GBS. In these experiments the addition of large amounts of hyperimmune rabbit antiserum to C1-depleted serum did not restore opsonization of type Ia GBS. However, opsonization could be restored by inclusion of C1 with the antibody. Furthermore, C1 and antibody in combination resulted in greater restoration of opsonic activity to C1-depleted serum that either component alone. This supports the role of the classical complement pathway in the enhancement of antibody-mediated opsonization of type Ia GBS.

The significance of our findings on the requirement of C1 for initiating opsonization of type Ia GBS may be related to investigations on complement deficiencies in neonatal sera. Complement levels have been measured in neonatal sera, and these findings were correlated with opsonophagocytic capacity of the sera for type Ia GBS (10). Deficient opsonophagocytic activity for type Ia GBS was correlated with low 50% hemolytic component levels and depressed classical complement pathway components C1q and C4. This is additional evidence for the role of C1 in initiating opsonization of type Ia GBS and points out the possible relation between decreased C1 levels in neonates and susceptibility to GBS disease.

ACKNOWLEDGMENTS

This work was supported by National Research Service Award NIH 1F32AI-16874 from the National Institutes of Health and the Charles H. Hood Foundation.

We thank Roger Spingarn for technical assistance and Loreen Carr for assistance in the preparation of this manuscript.

LITERATURE CITED