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*J Immunol* 1999; 163:2761-2768;  
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Role of Complement Component C1q in the IgG-Independent Opsonophagocytosis of Group B Streptococcus

Peter Butko, Anne Nicholson-Weller, and Michael R. Wessels

We investigated the role of complement component C1q in the IgG-independent opsonophagocytosis of type III group B Streptococcus (GBS) by peripheral blood leukocytes. We report that C1q binds to type III GBS both in normal human serum deficient in IgG specific for type III capsular polysaccharide and in a low-ionic strength buffer. The dissociation constant \( K_d \) ranged from 2.0 to 5.5 nM, and the number of binding sites \( B_{\text{max}} \) ranged from 630 to 1360 molecules of C1q per bacterium (CFU). An acapsular mutant strain of GBS bound C1q even better than the wild type, indicating that the polysaccharide capsule is not the receptor for C1q. In serum, binding of C1q to GBS was associated with activation of the classical complement pathway. However, normal human serum retained significant opsonic activity after complete depletion of C1q, suggesting that the serum contains a molecule that is able to replace C1q in opsonization and/or complement activation. Mannan-binding lectin, known to share some functions with C1q, appeared not to be involved, since its depletion from serum had little effect on opsonic activity. Excess soluble C1q or its collagen-like fragment inhibited phagocytosis mediated by normal human serum, suggesting that C1q may compete with other opsonins for binding to receptor(s) on phagocytes. We conclude that, although C1q binds directly to GBS, C1q binding is neither necessary nor sufficient for IgG-independent opsonophagocytosis. The results raise the possibility that additional unknown serum factor(s) may contribute to opsonization of GBS directly or via a novel mechanism of complement activation. The Journal of Immunology, 1999, 163: 2761–2768.

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roup B streptococcus (GBS) are the most common cause of neonatal sepsis and meningitis in the United States. Isolates of GBS associated with human infection produce an extracellular capsular polysaccharide; variations in the monosaccharide composition and pattern of glycosidic bonds in the polysaccharide repeating unit define several known serotypes (1–6). Serotype III is the one most often associated with invasive neonatal disease (7). Deficiency in maternal IgG specific for the capsular polysaccharide correlates with susceptibility of neonates to GBS infection (8). In the absence of specific Ab, sialic acid residues in the type III polysaccharide inhibit activation of the alternative pathway of complement, rendering the organisms resistant to alternative pathway-mediated opsonophagocytosis (9). Abs to the capsular polysaccharide overcome this inhibitory effect, and the amount of type-specific anti-polysaccharide IgG correlates with the efficiency of opsonophagocytic killing of GBS in vitro (10). The observations summarized above support a critical role for Abs to the capsular polysaccharide in alternative pathway-mediated opsonophagocytic killing of type III GBS (9, 11). In addition, however, evidence from several studies indicates that normal human serum (NHS) deficient in capsular polysaccharide-specific Abs can opsonize GBS for phagocytosis via the classical complement pathway (C5b-9). NHS containing low levels (0.4 µg/ml) of IgG specific for type III polysaccharide mediated significant opsonic killing of type III GBS (10). Opsonic activity of the serum could be abolished or reduced by various conditions or treatments: deficiency in complement C2, heat inactivation, preabsorption with living bacteria, or chelation with MgEGTA. Similar results were obtained with both type III and type Ia GBS (11, 12). Furthermore, incubation of type Ia GBS with NHS led to consumption of C3 and C4 complement proteins, and the bacteria directly bound purified C1q, the first component of CCP, in the absence of Ab (13). Removal of polysaccharide sialic acid residues by neuraminidase treatment resulted in decreased binding of C1 to type Ia GBS, and the GBS-C1 interaction could be inhibited by F(ab')2 with specificity for polysaccharide (14). These results indirectly supported the role of capsular polysaccharide as the target for C1 binding and, moreover, indicated that GBS can directly activate CCP without the need for formation of Ab-Ab complexes. Direct activation of CCP by bacteria is unusual, but not unprecedented: Ab-independent binding of C1q was reported for Legionella pneumophila (15), Klebsiella pneumoniae (16), Escherichia coli (17), and Salmonella minnesota (18). The goal of the present investigation was to better define the role of C1q and CCP in the cellular and molecular interactions responsible for IgG-independent opsonophagocytic killing of type III GBS.

Materials and Methods

Buffers

The following buffers were used in this study: GVB, isotonic veronal-buffered saline (pH 7.5), containing 0.1% (w/v) gelatin; GVB\(^{1+} \), GVB with 0.15 mM CaCl\(2 \), and 0.5 mM MgCl\(2 \), GVB\(^{2+} \), GVB with 10 mM EDTA; DGVB, GVB diluted with an equal volume of 5% d-glucose in water; DGVB\(^{1+} \), GVB\(^{1+} \) diluted with an equal volume of 5% d-glucose in water containing the same concentration of Ca\(^{2+} \) and Mg\(^{2+} \); DGVB\(^{2+} \), GVB\(^{2+} \) diluted with an equal volume of 5% d-glucose in 10 mM EDTA;
of GBS (10^8 CFU/ml) in a total volume of 0.25 ml. The buffer (either
ity. The bacteria were then pelleted by microcentrifugation, and a 50-

mixture by gel filtration on a Sephacryl S-200 column. SDS-PAGE was
ing to

C1q, which resulted in the sp. act. of 0.06 mCi/mg of protein, correspond-
purification of C1q with 125 I was performed with the Iodobead sys-

Binding of iodinated C1q to GBS

Concentrations from 0.1 to 20 μM of 125I-C1q were added to a suspension of GBS (10^6 CFU/ml) in a total volume of 0.25 ml. The buffer (either DGBV or PBS/gelatin) contained 0.1% gelatin to diminish nonspecific binding of C1q. After 15 min of incubation at 37°C, the samples were vortexed, and 50-μl aliquots were taken to determine the total radioactivity. The bacteria were then pelleted by microcentrifugation, and a 50-μl aliquot of the supernatant was used to determine the free (unbound) C1q. The bound C1q was calculated as: total C1q − free C1q. Alternatively, the bound C1q was quantified directly by counting the radioactivity in the bacterial pellet after two washes with DGBV. Results of both methods were similar; they were pooled together for statistical evaluation of data. Radioactivity was measured on a Beckman (Fullerton, CA) 5500 counter.

Opsonophagocytosis assay

Opsonophagocytosis was assayed essentially according to Baltimore et al. (27). GBS (10^6 CFU) were mixed with 3 × 10^6 PMN in MEM, total concentration of the specific anti-capsular polysaccharide IgG (0.15 ± 0.03 μg/ml) was determined by ELISA (28) in three independent measurements during the course of this work.

One part of the serum (NHS or unabsorbed serum) was immediately stored in aliquots at −70°C. Another part of the serum was “absorbed” with GBS as follows. Approximately 10^6 CFU of GBS were harvested by centrifugation of a 50-ml overnight broth culture, washed with PBS, and incubated with 3–5 ml of serum at 4°C. The bacteria were then pelleted, and the serum was filtered through a 0.45-μm Acrodisc filter (Gelman Sciences, Ann Arbor, MI) and stored. In some experiments, absorption was conducted in the presence of 5 mM MgEGTA. There was no difference in opsonic potency of the serum, whether it was or was not filtered and whether it was absorbed with live or dead (heated to 60°C) bacteria. The third part of serum was heat-inactivated (56°C for 30 min).

In some experiments, serum was selectively depleted of C1q and/or mannann-binding lectin (MBL) by affinity chromatography. A weakly acidic cation-exchange resin Bio-Rex 70 was used to deplete C1q, a highly basic protein (24). Serum was chelated with 5 mM EDTA, applied to the column, and eluted with 50 mM phosphate buffer containing 82 mM NaCl and 2 mM EDTA (pH 7.4). The degree of C1q depletion was determined by a C1q activity assay, as described below.

Depletion of MBL was achieved by passing the serum through an immobilized mannan column (Sigma) with the elution buffer containing 50 mM Tris-HCl, 1 M NaCl, 20 mM CaCl_2 (pH 7.8). Before use, the MBL-depleted serum was dialyzed (tubing Spectra/Por 3, m.w. cut-off 3500; Spectrum, Houston, TX) against PBS to remove high salt. The MBL concentrations in coded serum samples were determined by ELISA in the laboratory of Dr. Alan Ezekowitz, then at Children’s Hospital (Boston, MA).

Assembly of the bacteria-antibody complex

Purification, enzymatic digestion, and radiolabeling of human C1q

C1q was purified essentially according to Tenner et al. (24). Briefly, out-
dated human plasma, obtained from the Brigham and Women’s Hospital (Boston, MA) blood bank, was ultracentrifuged to remove lipids and clot-
ted. The serum was applied to a 25-ml Bio-Beads SM-2 column (Bio-Beads, Richmond, CA) cation-exchange column; the C1q-containing fractions were concentrated by ammonium sulfate precipitation and gel-filtered on a cation-exchange column; the C1q-containing fractions were concentrated by ammonium sulfate precipitation and gel-filtered on a Sephacryl S-200 column (Amersham Pharmacia Biotech, Piscataway, NJ). The resulting C1q was concentrated and frozen in aliquots. Purity of the final preparation was assessed to be 90–95% by SDS-PAGE.

The N-terminal collagen-like region of C1q was prepared by pepsin digestion of C1q at pH 4.5 (25) for 20 h. After elevating pH to 7.5 to stop the digestion, the collagen-like fragment was purified from the reaction mixture by gel filtration on a Sephacryl S-200 column. SDS-PAGE was used to assess the fragment’s identity and purity.

Radiolabeling of C1q with 125 I was performed with the Iodobead sys-
tem (Pierce, Rockford, IL): one bead was incubated with 0.2 mg C1q and 0.02 mCi of 125I (Amersham, Arlington Heights, IL) in 0.25 ml of a 40 mM phosphate buffer (pH 7.4) for 5 min at room temperature. The reaction was terminated by removal of the bead from the solution. Iodinated C1q was separated from unbound 125I on a PD-10 gel filtration column (Pharmacia, Piscataway, NJ). Under these conditions, 71% of 125I was incorporated into C1q, which resulted in the sp. act. of 0.06 mCi/mg of protein, correspond-

Serum

Serum from a normal healthy volunteer with a very low level of IgG spe-
cific for type III capsular polysaccharide was used for all the studies. The concentration of the specific anti-capsular polysaccharide IgG (0.15 ± 0.03 μg/ml) was determined by ELISA (28) in three independent measurements during the course of this work.

Preparation of human polymorphonuclear leukocytes (PMN)

A plasma fraction enriched in PMN was prepared from heparinized blood by dextran sedimentation, as described (27).

ASSAY FOR THE ASSOCIATION OF GBS WITH PMN

GBS (3 × 10^6 CFU) were preincubated with C1q or anti-capsular polysaccharide IgG in DGBV for 30 min, washed twice with DGBV, and then mixed with 3 × 10^6 human PMN in MEM, total volume 0.5 ml. When desired, C1q or the anti-capsular polysaccharide IgG were added to the second suspension. After 20 min of incubation at 37°C, a 50-μl aliquot was dropped onto a microscope slide (Fisher Scientific) and spread. The specimen was heat-fixed and Gram-stained. Associated (i.e., adherent or ingested) bacteria were enumerated for 100 PMN, and the median number of GBS per PMN was determined.

ASSAY FOR THE ASSOCIATION OF GBS WITH PMN
Complement assays

Modified hemolysis assays were used to measure the activities of individual CCP components C1, C1q, C3, and C4 (21, 22, 24, 29).

Coupling of the type III capsular polysaccharide to the carbolink resin

Type III capsular polysaccharide was isolated, purified, and partially oxidized by treatment with sodium periodate (19). The oxidized polysaccharide was coupled to the Carbolink Gel (Pierce) containing hydrazide-active groups, according to the manufacturer’s instructions (see also O’Sullivan and Wilchek (30); 25 mg of polysaccharide were gently rotated with 8 ml of Carbolink in a 0.1 M sodium phosphate buffer (pH 7.0) in the presence of 60 mg of sodium cyanoborohydride for 18 h at room temperature. Efficiency of coupling was determined qualitatively by immunofluorescence microscopy and quantitatively by ELISA. In the first method, the Carbolink resin beads were incubated with a rabbit antiserum against type III capsular polysaccharide, followed by incubation with an FITC-conjugated goat IgG against rabbit IgG. The polysaccharide resin fluoresced brightly, whereas the control resin, with glyceraldehyde coupled instead of the polysaccharide, did not (data not shown). In the second method, the amount of polysaccharide remaining in the solution and in the washes after coupling was determined by inhibition ELISA using microtiter plates coated with polyclonal (Trp) and polysaccharide (31) or polysaccharide/human serum albumin conjugate (28). Approximately 15 mg of polysaccharide was found to be linked to 8 ml of the packed resin.

Determination of protein concentrations

Routine Pierce’s bicinchoninic acid assay was used with BSA as a standard. When a sample volume permitted it, absorption at 280 nm was measured and the concentration of C1q estimated using the molar extinction coefficient e of 2.742 × 104 cm−1 M−1, which was calculated from the published amino acid sequence (32) according to the formula e = 5700nTrp + 1300nTy r, where nTrp and nTyr are the numbers of tryptophans and tyrosines, respectively, in the protein (33). This value is within 2% of that determined by Reid et al. (34) using a value of 410,000 for the m.w. of C1; if the currently accepted value of 460,000 is used for the m.w. of C1q, the calculated extinction coefficient is −14% less than that determined by Reid et al (34).

Data analysis

Nonlinear curve fitting and statistical analyses were performed with the Prism and Instat software (GraphPad Software, San Diego, CA).

Results

Involvement of the CCP

Preliminary testing of serum samples from several healthy volunteers confirmed previous observations that NHS containing very low concentrations of specific Abs to the capsular polysaccharide mediated 60–99% killing of type III and type Ia GBS by peripheral blood leukocytes in an in vitro assay of opsonophagocytosis (11–13). Serum depleted of 98% of its IgG by passage over a protein A affinity column mediated 89% killing of type III GBS, compared with 97% before IgG depletion (M. B. Marques and M. R. Wessels, unpublished observation). In the present investigation, one serum selected for study was found to contain 0.15 ± 0.03 μg/ml of IgG specific to type III GBS capsular polysaccharide by quantitative ELISA (28). Opsonic activity of the serum could be removed by absorption with type III GBS, which resulted in classical pathway activation and depletion from the serum of C3 and C4 (Table I). However, sufficient complement activity remained in the absorbed serum to support >90% opsonophagocytic killing of type III GBS in the presence of capsular polysaccharide-specific Abs (1% rabbit antiserum to type III polysaccharide-tetanus toxoid conjugate vaccine). To rule out the possibility that the opsonic activity of the NHS was due to the small amount of specific Abs, we tested whether opsonic activity of the GBS-absorbed serum could be restored by adding a sample of the same serum not absorbed with GBS but previously heated to 56°C to inactivate complement but preserve Abs. In this experiment, unabsorbed serum mediated 61 ± 4% kill (mean ± range, n = 2), 0 ± 0% kill after absorption, and 5 ± 2% kill after adding heat-inactivated, unabsorbed serum to the absorbed serum. These results indicate that the amount of specific Abs in the serum used for the present studies was not sufficient to support opsonophagocytic killing under the conditions used in the assay.

Binding of C1q to GBS

The cascade of C3 activation usually starts with binding of its first component C1q to the activating entity, which can be an Ag-Ab complex or a polymeric molecule. Therefore, we examined the ability of C1q to bind to the GBS surface.

The binding of native, unlabeled C1 to GBS was determined in modified C1 transfer experiments (21). GBS were first exposed to serum, washed, and then mixed with sensitized sheep erythrocytes carrying the C4b component of complement (EAC4). Under favorable conditions, namely higher ionic strength, any C1 previously adsorbed on GBS would transfer onto the EAC4 cells and, upon addition of the other CCP components, would cause their hemolysis. The data in Table II show that incubation of GBS with NHS resulted in deposition of C1 on the bacterial surface, reflected in subsequent transfer to EAC4.

The previous experiment, similar to that of Eads et al. (13), provided a qualitative demonstration that C1q (in C1) bound to GBS. A rigorous biochemical binding assay using radiolabeled C1q was employed to obtain quantitative characteristics of the binding. Fig. 1 documents binding of radiolabeled C1q to the GBS strain M781 in buffers of normal (PBS) and decreased (DGVB) ionic strength. Binding parameters were obtained by nonlinear curve fitting to the data points. The dissociation constants Kd were 2.0 ± 0.8 nM and 5.6 ± 2.0 nM, while Bmax values were 2400 ± 200 C1q/CFU and 1600 ± 200 C1q/CFU in DGVB and PBS, respectively. Thus, increasing ionic strength decreased binding of C1q to GBS.

Competition binding experiments were performed to demonstrate that labeled C1q bound to the same sites as native, unlabeled ligand and to define the region of the C1q molecule involved in binding. Binding of labeled C1q was measured in the presence of various concentrations of unlabeled intact C1q or a 170-kDa fragment of C1q representing the collagen-like domain of the protein, C1qα (Fig. 2). The data were fitted with the one-site competition equation y = 100/[1 + 10(log x − log C)], where y is the degree of

Table I. Relative activities of C3 and C4 and bactericidal index of NHS after absorption with GBS in the presence or absence of 5 mM EGTA

<table>
<thead>
<tr>
<th>Serum</th>
<th>C3 (%)</th>
<th>C4 (%)</th>
<th>BI (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Unabsorbed</td>
<td>100</td>
<td>100</td>
<td>88</td>
</tr>
<tr>
<td>Absorbed</td>
<td>29</td>
<td>13</td>
<td>23</td>
</tr>
<tr>
<td>EGTA-Absorbed</td>
<td>60</td>
<td>72</td>
<td>83</td>
</tr>
</tbody>
</table>

* Titer values in absorbed serum samples are expressed as a percentage of the value for unabsorbed serum.

Table II. C1 activity detected on the surface of GBS previously exposed to buffer or serum in the presence or absence of 5 mM EGTA

<table>
<thead>
<tr>
<th>GBS Incubated in:</th>
<th>C1 Titer (functional units)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Buffer</td>
<td>0</td>
</tr>
<tr>
<td>Serum</td>
<td>1560</td>
</tr>
<tr>
<td>Serum + 5 mM EGTA</td>
<td>10</td>
</tr>
</tbody>
</table>
binding in %. x is the concentration of the competing molecule, and C is a constant (called IC₅₀) denoting the concentration at which binding is 50%. The value of log C was found to be 7.17 ± 0.09. Taking into account the concentration of radiolabeled C1q (1.9 nM), the 50% inhibition of binding was reached at ~35-fold excess of unlabeled C1q. From Fig. 2, it is clear that C1q_coll is much less efficient in inhibiting the ¹²⁵I-C1q binding than the whole C1q molecule.

To examine how the presence of the polysaccharide capsule affects C1q binding, three GBS strains with various degrees of encapsulation were tested (Table III). One-way ANOVA showed significant differences in the B_max values: the acapsular mutant COH1–13 bound significantly more C1q molecules than its parent strain COH1 or the highly encapsulated strain M781. In addition, there was a statistically nonsignificant trend of stronger binding (decreasing K_d) with decreasing capsule expression.

**Opsonic activity of serum passed through polysaccharide-Carbolink column**

To test whether the pure capsular polysaccharide can deplete NHS of CCP activity and of opsonic power to the same extent as the whole GBS organism, we linked the polysaccharide to a Carbolink of CCP activity and of opsonic power to the same extent as the whole GBS. Before applying serum, both the polysaccharide and mock columns were washed with 0.1% gelatin in PBS to diminish nonspecific protein adsorption. After passage of the serum over the columns, C1q activity was found to be essentially the same in both: the titers were 143 and 125 for the polysaccharide and mock column, respectively. The opsonic power of the serum, however, was reduced to a modest extent: while untreated serum mediated 88% killing of type III GBS, serum passed over the polysaccharide column mediated 60% killing, and serum passed over the mock column (matrix without polysaccharide) mediated 81% killing (Fig. 3). By comparison, absorption of the serum with whole type III GBS organisms reduced killing to 6% in this experiment.

**Opsonophagocytosis in the absence of C1q and/or MBL**

In an attempt to further define the role of C1q in opsonophagocytosis of GBS by NHS, we measured the extent of bacterial killing with serum devoid of C1q. C1q was selectively and quantitatively removed from NHS by cation exchange chromatography on a column of Bio-Rex 70. After passage over the column, C1q activity remaining in the serum was <1% of that in the control, untreated serum. However, the C1q-depleted serum still supported opsonophagocytic killing at a level of 81% of that mediated by the control serum (Fig. 4). This result suggested that, apart from C1q, there is another molecule in NHS that is deposited on the surface of GBS and is able to either activate CCP or interact with a receptor on PMN, or both. Another serum protein implicated in classical pathway activation is MBL (previously called mannose- or mannan-binding protein, MBP). MBL has been shown to interact with bacterial polysaccharides (35) and activate CCP (36). To test the involvement of MBL in CCP activation and opsonophagocytic killing of GBS, NHS was depleted of MBL by affinity chromatography. A mannan affinity column removed >96% of MBL, but

<table>
<thead>
<tr>
<th>Strain</th>
<th>Encapsulation</th>
<th>K_d(nM)</th>
<th>B_max(C1q/CPU)</th>
</tr>
</thead>
<tbody>
<tr>
<td>M781</td>
<td>++</td>
<td>5.5 ± 0.9</td>
<td>960 ± 50</td>
</tr>
<tr>
<td>COH1</td>
<td>+</td>
<td>3.3 ± 1.0</td>
<td>630 ± 60</td>
</tr>
<tr>
<td>COH1–13</td>
<td>–</td>
<td>2.9 ± 0.6</td>
<td>1360 ± 90</td>
</tr>
</tbody>
</table>

*a The experiments were performed in DGVB as described in the text. The data are means ± SEM (n = 4).
Relative concentration of MBL (filled bars) and opsonic power (percent of GBS killed, or bactericidal index; hatched bars) of serum depleted of MBL (depl), absorbed with GBS (abs), and the control, untreated serum (unabs). The MBL concentration of unabsorbed serum, 1.68 μg/ml, was set to 100%. Values for bactericidal index represent means ± SEM of four experiments in duplicate.

The previous experiments demonstrated that C1q binds to the GBS surface. Therefore, we tested whether the killing activity of the GBS-absorbed serum could be restored by addition of C1q. The results (data not shown) were negative: C1q up to a concentration of 50 μg/ml failed to restore the killing activity of the GBS-absorbed serum. Moreover, we noticed in those experiments that excess C1q added to the control, untreated serum inhibited killing activity of that serum. When GBS were incubated with 10% NHS and PMN in the presence of increasing amounts of C1q, a concentration-dependent inhibition of opsonophagocytic killing was observed (data not shown). While no discernible effect on bactericidal index was seen up to ~40 μg/ml, opsonophagocytosis was inhibited by ~50% at 100 μg/ml C1q, which corresponds to 1 mg/ml of serum or ~10-fold excess over the usual C1q concentration in serum (Fig. 6).

Inability of C1q to act as a primary opsonin

To investigate whether C1q itself can act as an opsonin in the absence of other complement components, we preincubated GBS either with purified C1q (40 μg/ml) or 10% NHS (as a control) in DGVB \(^{+/-}\) and, after two washes with DGVB \(^{+/-}\), added PMN and continued incubation in the absence of serum for 1 h. In this assay (\(n = 14\)), the bactericidal index for GBS preopsonized with NHS was 60 ± 5, whereas those for GBS preopsonized with the pre-absorbed serum or with C1q alone were low and indistinguishable (12 ± 5 or 8 ± 5, respectively). Thus, there was very little, if any, phagocytic killing mediated by C1q in the absence of other serum components. Thus, C1q does not appear to act as a primary opsonin for GBS.

**FIGURE 4.** Relative levels of C1q activity (filled bars) and opsonic power (percent of GBS killed, or bactericidal index; hatched bars) of serum depleted of C1q (depl), absorbed with GBS (abs), and the control, untreated serum (unabs). The relative C1q activity was measured as the fraction of sheep erythrocytes lysed in a hemolysis assay (see Materials and Methods). The value for unabsorbed serum, 0.732, was set to 100%. The measurement was performed in duplicates; error bars represent the range between the two values.

**FIGURE 5.** Relative concentration of MBL (filled bars) and opsonic power (percent of GBS killed or bactericidal index (% kill)) of serum depleted of MBL (depl), absorbed with GBS (abs), and the control, untreated serum (unabs). The MBL concentration of unabsorbed serum, 1.68 μg/ml, was set to 100%. The value for unabsorbed serum, 0.732, was set to 100%. The measurement was performed in duplicates; error bars represent the range between the two values.

**FIGURE 6.** Opsonophagocytosis of type III GBS in 10% human serum without any treatment (un), in serum that had been preabsorbed with the same GBS strain (abs), and in the serum in the presence of 0.1 mg/ml C1q purchased from Quidel (C1qQuid), 0.1 mg/ml C1q purified in our laboratory (C1q), and 35 μg/ml collagen-like fragment of C1q (C1qcoll). Values represent means ± SEM of three independent measurements in duplicate.
C1q by itself may not be able to support opsonophagocytic killing in a serum-free system, but may participate in adherence of C1q-coated bacteria to PMN, thereby enhancing the effects of other opsonins. The role of C1q, alone or in conjunction with specific Ab, in the association of GBS with PMN was examined in the next experiment. The bacteria were first preincubated with either C1q or anti-capsular polysaccharide IgG and, after two washes, mixed with PMN in the presence of either the anti-capsular polysaccharide IgG or C1q, respectively. The GBS/PMN association was examined microscopically. The association data are compared with the bactericidal indices (BI) of the respective samples in Fig. 7. The negative control, labeled “no IgG/no C1q”, only contained GBS and PMN without added opsonins. This sample exhibited a BI of 12 ± 5, and the median number of GBS/PMN was 1.5. The sample labeled “C1q/no IgG” confirmed that, in the absence of other complement proteins and Ab, C1q is unable to support opsonophagocytosis of GBS: the BI was 15 ± 6 and median GBS/PMN 2, values not different from those of the negative control. However, when GBS were exposed to both C1q and the Ab, irrespective of the order of incubations, BI increased to ~35, and the median number of GBS/PMN increased above 3. It is interesting to compare the samples of the IgG-opsonized GBS mixed with PMN in the absence (“IgG/no C1q”) and presence (“IgG/C1q”) of C1q. The sample with C1q shows a slightly lower BI (30 ± 9 vs 47 ± 9, statistically NS), but higher association of GBS with PMN (3.5 vs 1). These results indicate that, although C1q does not promote phagocytic killing directly, it can act synergistically with IgG to enhance association of GBS with PMN.

Discussion

CCP plays an important part in innate immunity to GBS infection. Enhanced susceptibility to GBS infection was observed in knock-out mice deficient in C3 or in C4, findings that support a central role for CCP in host defense to this pathogen (39). NHS containing low concentrations of anti-capsular polysaccharide Abs mediated opsonophagocytic killing of type III GBS and type Ia GBS (11, 12). Opsonic activity was lost upon: 1) chelation of calcium, 2) absorption with GBS, or 3) heat inactivation. These observations suggested that NHS contains an opsonin, other than Ab, that binds to the surface of GBS. The calcium dependency indicated that the opsonin binding was itself calcium-dependent, or that the opsonin activated CCP (whose activation is calcium-dependent), or both.

In the present study, we investigated the role of C1q in IgG-independent opsonization of type III GBS. First, C1 activity was demonstrated on the bacteria exposed to NHS. Second, CCP components were depleted from NHS absorbed with the bacteria, presumably due to activation of CCP by deposited C1q. These data suggested a role for C1q in the IgG-independent opsonization of type III GBS. It was then quite surprising that serum completely devoid of C1q still exhibited significant opsonic power, a result that indicated that there must be another serum protein able to replace C1q in GBS opsonization and/or activation of CCP. One possibility is that, in addition to direct binding of C1q, C2 or C4 binds directly to GBS and activates the classical pathway; such a mechanism has been reported for pathogenic mycobacteria (40).

Another candidate protein is MBL, which, like C1q, has the capacity to activate CCP (35, 36). In our studies, however, levels of MBL did not correlate with the serum opsonic ability. Therefore, MBL appears not to be a major opsonic ligand for type III GBS. This conclusion is in accord with the previously reported low affinity of radioactively labeled MBL for GBS (41). The modest effect of depleting C1q and/or MBL leaves open the possibility that other serum factors may contribute to opsonophagocytosis of GBS as direct opsonins or in the activation of CCP.

Our data show that C1q binds to the surface of GBS. Binding was not inhibited by the collagen-like fragment of C1q, which indicates that C1q binds to GBS through the globular head region, a mode of binding that was observed for C1q deposition on other microorganisms (42). C1q binding alone did not mediate opsonophagocytic killing of GBS by PMN, as we incorrectly concluded from preliminary data (43). However, binding of C1q together with IgG to GBS promoted their association with PMN, which may enhance phagocytosis mediated by interactions between PMN and other opsonins, such as C3b and/or C4b.

The polysaccharide capsule is a conspicuous surface structure of GBS that has been identified as an important virulence factor (8, 10, 11, 44). High concentrations of soluble type III capsular polysaccharide in serum inhibit opsonophagocytosis of GBS (44). However, in our hands, even very high concentrations did not accomplish complete inhibition: 1.9 mg of polysaccharide per 1 ml of serum still allowed for 68% killing of the bacteria (data not shown). One could argue that the soluble polysaccharide conformation and/or affinity constant for complement proteins may differ from those of the polysaccharide on the GBS surface. Indeed, polysaccharide immobilized on Carbocilk beads, perhaps a better model of GBS capsule than soluble macromolecules, decreased the serum’s bactericidal index from 88 to 60. However, absorption of serum with whole type III GBS organisms reduced killing to 6% in the same experiment. Thus, interaction between a serum molecule and the GBS capsule accounts for at most 32% (= 1 – 60/88) of the opsonophagocytic killing of GBS. It is reasonable to expect nonspecific electrostatic binding between the negatively charged polysaccharide and the positively charged subcomponent C1q of the first component of CCP. However, a polysaccharide affinity column did not deplete C1q when compared with serum passed over a mock column. This finding is consistent with that of Baker et al. (12), who reported evidence of C1q binding by immunofluorescence to a moderately encapsulated type Ia strain, but no detectable C1q deposition on a highly encapsulated strain. Our own preliminary experiments attempting to measure the interaction between C1q and the polysaccharide using surface plasmon resonance, electron microscopy, and ELISA all failed to demonstrate...
appreciable binding (our unpublished observations). Our data show that the acapsular GBS mutant COH1–13 binds C1q even better than the encapsulated strains COH1 and M781. The Kₐ values of the C1q/GBS interactions in our experiments (3–6 nM) are very similar to those describing interaction between C1q and a Klebsiella porin (1.5 nM) (42). The data suggest that the target of C1q on the surface of GBS is probably not the capsular polysaccharide, but rather a protein, as is the case for other microorganisms (15–18, 45). The capsule, on the contrary, seems to protect the bacteria against direct deposition of C1q on their surface.

While C1q did not support phagocytosis in the absence of other opsonins, inhibition of opsonophagocytic killing of GBS by an excess of free C1q suggests a direct interaction of C1q with a receptor on PMN. C1q binding function has been described for PMN (46, 47), although the molecular identity of the receptor(s) is an area of ongoing investigation (48). In our studies, Abs to C1qRp had no effect on serum-mediated opsonophagocytosis of GBS; however, these Abs also failed to inhibit C1q-stimulated oxidative burst in neutrophils, so it is not clear that they can block C1q binding to C1qRp on this cell type (20). Another candidate receptor for C1q is complement receptor type 1 (CR1, CD35), a membrane protein recently shown to bind C1q in addition to its previously described ligands C3b and C4b (37). Abs to CR1 and to CR3 inhibit opsonophagocytosis of GBS in NHS (49, 50). An appealing hypothesis to explain the inhibition of phagocytic killing by excess C1q observed in the present study is that free C1q (in contrast with C1q deposited on the bacterial surface) may compete with other opsonins for binding to CR1 on PMN. Inhibition of the interaction between GBS-bound C3b and CR1 would be expected to interfere with phagocytic killing, since CR1 (together with complement receptor type 3, CR3) has been shown to mediate opsonophagocytosis of GBS both in the presence and absence of antcapsular Abs (49, 50). Another potential mechanism through which excess C1q could inhibit opsonophagocytosis is by competing with C1 for binding to GBS, thereby preventing classical pathway activation.

Our results indicate that C1q plays a role in opsonization and phagocytosis of GBS by at least two mechanisms operating in parallel. C1q binds directly to the GBS surface, a consequence of which is CCP activation with deposition of C3b. In addition, bound C1q may act synergistically with IgG to increase the association between GBS and PMN, thereby facilitating opsonophagocytosis mediated by IgG and C3b. In the absence of specific Abs, C1q deposited on the GBS surface (in contrast to soluble C1q) could enhance the association of GBS with PMN, perhaps through interaction with the CR1 complement receptor. Excess free C1q may inhibit opsonophagocytic killing of GBS opsonized with C3b or other opsonins by blocking CR1 on PMN or by interfering with classical pathway activation. Neither C1q nor MBL is required for opsonophagocytosis of GBS. This observation suggests that additional serum factors, which previously were not thought to have this function, may opsonize GBS directly or through a novel mechanism of CCP activation. It is possible that these factors could be found among members of the collectin or ficolin families of serum proteins (51–53).

Acknowledgments

We thank Marisa Marques for performing preliminary experiments that contributed to the development of this work, Hilde-Kari Guttmersen and Anne-Karin Brøtgen for determining the levels of specific anti-capsular polysaccharide IgG, Judith Epstein and Debrah Weiner for determining the concentration of MBL in the serum we used, Andrea Tenner for the gift of mAbs R3 and R139, and Dennis L. Kasper for enlightening discussions.

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