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High Dose Intravenous Immunoglobulin Does Not Affect Complement-Bacteria Interactions

Eric Wagner,* † Jeffrey L. Platt,* †‡ and Michael M. Frank†‡

Pooled IgG preparations for i.v. use (IVIg) have been shown to possess anticomplementary activity in autoimmune and systemic inflammatory diseases. Both in vitro and in vivo, IVIg is a preferential acceptor of activated C4 and C3, thus diverting complement activation from the target surface. We explored the effect of IVIg on complement-bacteria interactions in an attempt both to determine the safety of IVIg preparations in relation to natural immunity to bacteria and to extend our knowledge of the physiologic mechanism of action of IVIg. Using both complement-sensitive and complement-resistant bacterial strains, we investigated the effect of IVIg on C3 binding to bacterial surfaces. In all cases, whether complement could be directly activated by bacteria through the classical or the alternative pathway, IVIg had no effect on the amount of C3 bound to bacteria. In addition, IVIg did not inhibit complement-dependent bacterial lysis. Interestingly, increasing concentrations of IVIg induced an increase in C1q binding, suggesting the presence of low affinity complement-fixing antibacterial Abs in certain preparations. Using serum samples from patients treated with IVIg, complement binding to and lysis of complement-sensitive bacterial strains were not modified as compared with normal controls and pretreatment samples, although a decrease in C3 binding to sensitized human erythrocytes was observed. Our data suggest that IVIg does not affect direct complement-bacteria interactions, although it is a potent agent to use for diversion of complement activation on sensitized target surfaces. The Journal of Immunology, 1998, 160: 1936–1943.

IgG preparations for i.v. use (IVIg) preparations are used increasingly in the treatment of primary and secondary immunodeficiencies (1) and autoimmune and systemic inflammatory diseases (2). Although IVIg has been successfully used to treat patients with such illnesses as Kawasaki disease (3) and idiopathic thrombocytopenic purpura (4), the mechanism of action of IVIg is incompletely understood. IVIg was proposed to exert its action through Id-anti-Id interactions, blockade of Fc receptors, modulation of cytokine synthesis, or interference with T and B cell functions (5).

On the basis of findings that C3 preferentially binds to IgG on a bacterial surface and not to the bacterium itself (6), we proposed some years ago that IVIg may act as a preferential acceptor of activated C3 (7). In support of this hypothesis, IVIg was shown to interfere with complement-dependent in vivo tissue damage such as Forssman shock (8). The survival of guinea pigs in which shock was induced was significantly prolonged by administration of IVIg as a single bolus dose of 1.8 g/kg, although the serum levels of C3 were unchanged as compared with control animals. In other experiments, IVIg markedly reduced C3 uptake on sensitized erythrocytes and abrogated deposition of complement components into the target tissue (lungs). More recent experiments showed that IVIg blocks complement deposition on cardiac endothelial cells in a xenograft model in which porcine hearts are transplanted into baboons (9). In this model, hyperacute xenograft rejection is completely inhibited. In addition, IVIg blocked deposition of activated C3 onto target tissues in patients with dermatomyositis, thus proving complement-regulatory activity of IVIg in a clinical setting (10). We clearly demonstrated recently that fluid phase monomeric IgG blocks deposition of C4 onto sensitized sheep erythrocytes and blocks C4 deposition on immune complexes (11). This suggests that the anticomplementary effect of IVIg may act on complement proteins other than C3 in the cascade.

The earliest description of complement focused on its ability to interact with bacteria (12) and provide an element of natural immunity. Since IVIg can block complement from depositing onto target surfaces, it was possible that IVIg might interfere with direct complement binding to bacteria or other microorganisms. Given the fact that many patients who receive IVIg treatment are critically ill, it was important to determine whether opsonization of bacteria, critical to normal host defense processes, was inhibited by IVIg use. The aim of the present study was to investigate the effect of IVIg on complement-bacteria interactions through both the alternative and the classical pathways, answering this important question and providing information on its mechanism of action in complement regulation. Since C3 is a key component in host defense against bacteria through several mechanisms (clearance, opsonophagocytosis), we focused on the ability of IVIg to modify the pattern of C3 deposition on a bacterial surface.

Materials and Methods

Bacterial strains

Strains sensitive and resistant to complement-mediated lysis were examined in this study. Since complement-mediated opsonization of strains sensitive and resistant to lysis is of critical importance, complement binding to the various strains was examined as well. Salmonella minnesota Re595

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Abbreviations used in this paper: IVIg, pooled IgG for i.v. use; VBS, isotonic Vero-

nal buffer; VBS2−, 0.5 mM MgCl2 and 0.15 mM CaCl2; GVBS2−, VBS2− with 0.1% gelatin; EDTA-GVBS, GVBS2− with 10 mM EDTA; EGTA-GVBS, GVBS2− with 8 mM EGTA and 5 mM MgCl2; C′ EDTA, EDTA-inactivated human serum; AH50 units, titer at which 50% of erythrocyte lysis occurs.

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(ATCC #49284) was used as a complement-sensitive bacterial strain. The complement-resistant bacterial strains that were used were a clinical isolate of *Listeria monocytogenes* obtained from the Clinical Microbiology Laboratory at Duke University Medical Center and *Escherichia coli* 0111A (ATCC 29552). A methicillin-sensitive clinical isolate of *Staphylococcus aureus* was obtained from the Clinical Microbiology Laboratory at Duke University Medical Center. Each strain was grown in an appropriate medium, and the growth curve was established. Before use, each bacterial strain was grown to mid-log phase and tested immediately to ensure susceptibility to complement-mediated lysis in the complement-sensitive strain (13).

**Complement buffers and sera**

Isotonic Veronal buffer (VBS) containing 0.5 mM MgCl<sub>2</sub> and 0.15 mM CaCl<sub>2</sub> (VBS<sup>-</sup>), 0.1% gelatin (GVBS<sup>-</sup>), or 10 mM EDTA and 0.1% gelatin (EDTA-GVBS<sup>-</sup>) was used (14). Isotonic Veronal buffer<sup>+</sup> containing 8 mM EGTA and 5 mM MgCl<sub>2</sub> (EGTA-GVBS) for alternative pathway activation assays was prepared according to a standard procedure (15).

Fresh frozen serum samples from three healthy individuals were used as a source of human complement. In some assays, serum was preadsorbed with the bacteria to be tested to remove pre-existing reactive Abs (1 ml of serum adsorbed three times with 10<sup>10</sup> CFU of mid-log-phase-grown bacteria, on ice for 30 min) (16). To assess the effect of IVIg treatment in vivo, serum samples from patients treated with low dose IVIg (immunodeficiency) or high dose IVIg (idiopathic thrombocytopenic purpura) were obtained after suitable informed consent. IgG serum levels were measured using a radial immunodiffusion assay according to the manufacturer’s instructions (The Binding Site, Birmingham, U.K.), and the samples were used untreated to assess complement interactions with bacteria and lysis of sensitive strains.

**Development of polyspecific Abs to complement-resistant bacteria**

To determine the effect of IVIg on complement interaction with sensitized bacteria, the complement-resistant bacterial strain *L. monocytogenes* was used for the production of specific rabbit antiserum according to a previously published method (17). Briefly, bacteria were grown to mid-log phase, washed three times in 0.9% sterile NaCl, and resuspended at a concentration of 10<sup>7</sup> CFU/ml. The organisms were then boiled for 2.5 h and diluted 1:10 in sterile physiologic saline. One-milliliter samples of this concentration of 10<sup>9</sup> CFU/ml. The organisms were then boiled for 2.5 h. The organisms were then boiled for 2.5 h.

**Complement uptake onto bacteria and bacterial viability**

As stated earlier, bacteria were grown to mid-log phase for use in all assays. Cells were washed in the assay buffer three times and resuspended to achieve a final concentration of ~5 × 10<sup>7</sup> CFU/ml in a final reaction volume of 500 µl. Different concentrations of IVIg were added to bacteria before incorporation of a source of human complement (20% human serum). BSA was used as a control in each experiment. Additional controls included bacteria incubated with buffer alone or a source of complement that was inactivated by treatment with EDTA (C<sup>-</sup>-EDTA). Bacteria were then incubated with mixing at 37°C for 1 h. At the end of the incubation time, 100 µl were removed from each tube and serially diluted in peptone water. Fifty microfilters of these dilutions were then plated onto agar plates and incubated at 37°C overnight for bacterial viability determination. The remaining reaction volume was diluted in EDTA-GVBS and washed three times in the same buffer. Then, 125<sup>i</sup>-labeled goat anti-human C3 or Clq IgG diluted in EDTA-GVBS (2 µg/tube) was added before a 45-min incubation at 4°C. Cells were then washed three times in EDTA-GVBS, and the pellet was counted in a gamma spectrometer (Cobra II, Packard, Meriden, CT). All experimental results were expressed as a percentage of maximal labeled Ab binding to complement components bound to the bacterial surface (human serum alone). For comparison, nonspecific C3 uptake was examined with bacteria incubated with buffer alone or with C<sup>-</sup>-EDTA. This is expressed as a percentage of maximal anti-human C3 uptake by bacteria (human serum alone).

**The erythrocyte as a model for the evaluation of the anticomplementary activity of IVIg**

In parallel to complement binding assays using bacteria, we examined the effect of IVIg on complement binding to sensitized erythrocytes, which was previously shown to be an adequate model for the study of the anticomplementary activity of IVIg (10, 11). To allow for the same conditions under which assays with bacteria were performed, the sensitized human erythrocyte model was chosen. Briefly, washed human blood group O<sup>+</sup> erythrocytes were sensitized with a rabbit anti-human red blood cell IgG (ICN Pharmaceuticals Inc., Aurora, OH). Then, 1 × 10<sup>8</sup> sensitized erythrocytes were incubated with various concentrations of IVIg diluted in GVBS<sup>-</sup> along with autologous serum or IVIg-treated patient serum samples diluted 1:5. The deposition of C3 onto erythrocytes was measured by using a goat anti-human C3 IgG labeled with 125<sup>i</sup> as described above.

To determine the effect of IVIg on the alternative pathway of complement activation, a rabbit erythrocyte model was used. Rabbit erythrocytes were washed and resuspended in EDTA-GVBS at 7.5 × 10<sup>7</sup> cells/ml. Ten microliters of this suspension were added to wells of a U-bottom 96-well microtiter plate. Serial dilutions of human serum adsorbed with rabbit erythrocytes and different concentrations of IVIg were added in a 100-µl total reaction volume before a 30-min incubation at 37°C. The reaction was then stopped with 100 µl of EDTA-GVBS. The plate was centrifuged at 850 × g for 15 min to remove nonbound C3 deposition. Total cell viability was determined using an ELISA plate reader (Molecular Devices, Menlo Park, CA). The ability of IVIg to inhibit alternative pathway-dependent complement lysis of rabbit erythrocytes was measured and expressed as AH<sub>50</sub> units per ml of serum (titer at which 50% of erythrocyte lysis occurs).

**Results**

Several bacterial strains activated the alternative pathway in systems in which Ab had been removed by adsorption. C3 deposition via direct activation of the alternative pathway of complement was measured using *S. minnesota* Re595, *E. coli* 0111A, and *Staphylococcus aureus*. As shown in Figure 1, IVIg at concentrations of 1 to 40 mg/ml failed to inhibit C3 binding to *S. minnesota* Re595 (Fig. 1A). Furthermore, bacterial lysis of this bacterial strain, known to be complement sensitive, was not affected by IVIg or by a control protein, BSA, given that >99% of cell lysis was observed in each case (data not shown). Although an appropriate buffer was used to measure C3 deposition through the alternative pathway of complement activation, adsorption procedures were performed to rule out the possibility that complement might be activated by antibacterial Abs in the IVIg preparation. We observed <20% inhibition of C3 binding to the bacterial surface (Fig. 1B), and bacteria were efficiently lysed by a source of human complement, regardless of the dose of IVIg used (not shown).

We also tested strains of bacteria that activate the alternative pathway of complement without being sensitive to lysis. Shown in Figure 2 is a representative experiment involving *E. coli* 0111A. Treatment with increasing concentrations of IVIg did not decrease the amount of C3 bound to the bacterial surface as compared with a control protein (BSA) (Fig. 2A). In addition, cell viability even decreased slightly when high doses of IVIg were used (Fig. 2B), suggesting that despite extensive adsorption procedures, low affinity Abs persisted in the preparation, triggering activation of complement on the bacteria. A methicillin-sensitive clinical isolate of *Staphylococcus aureus* was also tested for the ability of IVIg to modify complement activation. As shown in Figure 3, C3 binding to the bacterial surface was inhibited by ~30% when a very high, nonphysiologic concentration of IVIg was used (40 mg/ml); however, a similar level of inhibition was observed with the control.
protein (BSA). This bacterial strain showed great variability in its C3 binding capacity from one experiment to the other. However, there was no difference in C3 binding when comparing IVIg with BSA. Consistent with the known resistance to human complement-mediated lysis, this clinical isolate of *Staphylococcus aureus* did not exhibit sensitivity to human complement activation in the presence of either IVIg or BSA.

IVIg had no effect on the human alternative pathway of complement activation in a model based on the lysis of rabbit erythrocytes by human serum (Fig. 4). IVIg at different concentrations was unable to modify the pattern of lysis of rabbit erythrocytes by human serum. Although some changes are noted in AH50 titers, no significant consumption of alternative pathway activity could be demonstrated, perhaps reflecting the fact that relatively small numbers of complement molecules are utilized in this lytic reaction as is true in lysis of sheep erythrocytes.

**Ab-independent classical pathway activation**

Certain bacterial strains activate directly the classical pathway of complement in the absence of Ab. In addition to activating the alternative pathway, *S. minnesota* Re595 activates the classical pathway directly through binding of C1q. To test this pathway, human serum was heated at 50°C to inactivate factor B, hence destroying alternative pathway activity. The serum sample was further adsorbed with the bacteria to favor Ab-independent complement activation. This treatment did not modify the classical pathway-activating potential of serum samples as determined by a standard total classical pathway hemolytic activity assay (CH50 assay) (not shown). Figure 5 displays the effect of IVIg on C3 binding to a bacterial surface through the classical pathway. IVIg did not significantly inhibit complement deposition on the target bacteria (Fig. 5A), nor did it prevent bacterial lysis induced by complement activation (Fig. 5B).

Some investigators proposed that the anticomplementary activity of IVIg resides in its ability to bind C1q, diverting the attachment of this component to target surfaces (18, 19). We investigated the effect of IVIg on C1q uptake onto bacterial strains that can activate the classical pathway directly. As demonstrated in Figure 6, IVIg did not reduce the amount of C1q bound to bacteria. In fact, the amount of C1q bound to the bacterial surface increased considerably with increasing concentrations of IVIg, despite multiple cycles of adsorption of IVIg with bacterial pellets. The presence of low affinity antibacterial Abs could account for the increased binding seen in our experiments.
Ab-dependent classical pathway activation

Since IVIg had no effect on direct C3 binding to bacterial surfaces, it was essential to determine whether complement would be diverted from sensitized erythrocyte surfaces using our reagents, as previously described by us in a sheep erythrocyte model (11) and in a human model (10). Using sensitized human erythrocytes as a target for complement activation, IVIg yielded a dose-dependent inhibition of C3 binding with increasing concentrations (Fig. 7). In keeping with earlier results, this suggests that IVIg exerts its anticomplementary activity on sensitized targets primarily through the classical pathway.

To determine whether IVIg can modify complement interactions with Ab-sensitized bacteria, a strain resistant to complement-mediated lysis was used. Abs specific to *L. monocytogenes* were raised in rabbits and used as sensitizing agents capable of activating human complement and depositing C3. Although not as striking as in the sensitized human erythrocyte model, IVIg reduced C3 binding to the bacterial surface in a dose-dependent manner (Fig. 8).

In vivo effect of IVIg on complement binding to bacteria and to sensitized human erythrocytes

To assess the impact of IVIg treatment in patients on complement-bacteria interactions, we evaluated serum samples collected at different time points before and after initiation of IVIg treatment. Two patients with idiopathic thrombocytopenic purpura treated with 2 g/kg of IVIg in a single bolus dose infused over 3 h were screened as were three patients with immunodeficiency who were given 400 mg/kg IVIg in a single dose. In patients with idiopathic thrombocytopenic purpura, serum samples were collected before infusion, at 30 and 60 min following the start of infusion, at the end of the infusion procedure (3 h following start of infusion), and at 30 and 60 min following the end of infusion. Serum samples were collected from patients with immunodeficiency before infusion, at the start of the infusion, and 1 h and 2 h post-i.v. infusion. Binding of C3 to a complement-sensitive strain (*S. minnesota* Re595), and the resultant lysis were measured. As depicted in Figure 9, serum IgG levels rose sharply to approximately three times the baseline level at the end of i.v. infusion of a high dose of IVIg in patients with idiopathic thrombocytopenic purpura (Fig. 9A). In
patients with immunodeficiency, however, i.v. injection of IVIg resulted in less than a twofold increase in serum IgG concentration (Fig. 9B). Importantly, serum samples from patients with both idiopathic thrombocytopenic purpura and immunodeficiency failed to show any decrease in C3 binding to *S. minnesota* Re595 despite the presence of IVIg in the circulation (Fig. 10). In some instances (patient 1, idiopathic thrombocytopenic purpura group, Fig. 10A), IVIg preparations increased the amount of C3 deposited on the bacterial surface, suggesting the presence of Abs with antibacterial specificity within the preparation used. In viability assays, all the serum samples were able to induce complete lysis of *S. minnesota* Re595, whether IVIg was present in the circulation or not, which were in accordance with normal human sera tested with the same organisms (not shown).

To verify that IVIg treatment had an effect on complement activation on sensitized targets, we tested patient serum samples for ability to deposit complement component C3 onto human sensitized erythrocytes. Following the infusion period, the extent of C3 binding to human sensitized erythrocytes decreased compared with pretreatment values in patients with idiopathic thrombocytopenic purpura treated with a high dose of IVIg (Fig. 11A). No more than 25% inhibition of C3 binding was observed but, although an obvious trend toward decrease in C3 binding was observed, statistical significance was not reached, given the high error values. In patients with immunodeficiency treated with a smaller dose of IVIg,
there was no significant effect of IVIg on C3 binding to human erythrocytes during the post-treatment period in two patients (Fig. 11B). However, in one patient, a 20% inhibition of C3 binding was observed at 2 h postinfusion.

Discussion
Our original observation that anticapsular IgG on a pneumococcal surface may bind 30% of C3 deposited on the bacterium (6) led to the hypothesis that IgG is a preferential acceptor of activated C3 fragments (7). The demonstration of specific C3-binding sites on the IgG heavy chain further strengthened this hypothesis (20). In vitro and in vivo experiments have shown that monomeric IgG has anticomplementary activity and diverts complement binding from Ab-sensitized target surfaces. In vitro, IVIg inhibited the uptake of C4 and C3 onto sensitized erythrocytes (11, 21). More strikingly, IVIg proved to be very potent in blocking the lethal complement-dependent Fossman reaction in guinea pigs (8) and in prolonging pig heart xenotransplant survival in baboons, thus abrogating complement-dependent hyperacute rejection of discordant xenografts (9). In humans, IVIg prevented C3b and C5b-9 deposition onto endomyosial capillaries in patients with dermatomyositis whereas serum complement levels remained unchanged (10). IVIg was also efficient in reducing C3 binding to erythrocytes in patients with autoimmune hemolytic anemia (22). In an in vitro model of complement activation induced by heat-aggregated IgG, Lutz et al. (23) reported that in addition to preventing the deposition of activated C3 fragments from the activating immune aggregate via competition for C3b binding, high dose IgG may increase by two- to threefold the rate of C3b inactivation by factors H and I. Therefore, at high doses, IgG would seem to favor C3b cleavage by factors H and I, thus losing its protective ability toward C3b, as we reported (24).

Since IgG is a potent regulator of complement activation, it might interfere with the main role of complement: interaction with microorganisms to provide a first line of defense against potential pathogens via opsonization or direct lysis. We investigated the ability of IVIg to potentially modify direct complement interactions with bacterial strains through both the alternative and the classical pathway. In our experiments, IVIg did not down-regulate C3 binding on bacterial surfaces on which complement was directly activated through the alternative or the classical pathways in the absence of specific Ab. IVIg did not allow survival of complement-sensitive bacterial strains. These observations raise the possibility that IVIg down-regulates complement action only if complement is activated by Abs bound to target cells. They also raise the possibility that IVIg does not simply act in the fluid phase. For example, IVIg may exert its action via Fc-Fc interactions between sensitizing Abs bound to the target and IgG molecules in the
IVIg preparation. IVIg may also act via low affinity binding to the target surface that does not lead to effective complement activation but allows the IgG to compete successfully with the target surface for binding of activated complement proteins. In a xenotransplant model, our group demonstrated inhibition of complement binding to target tissues even though natural IgM that are responsible for xenograft rejection were still present on the tissue (9). Interestingly, one of us (J.L.P.) recently showed complement-modulatory activity of xenoreactive IgG in an in vitro xenotransplant model using porcine aortic endothelial cells (25). Ig of IgG2 isotype reacting specifically with the galactose(α1,3)galactose epitope competed with natural IgM binding and prevented binding of C1q on the target cell surface. Although the low concentration of xenoreactive IgG in IVIg preparations cannot solely account for the anticomplementary activity seen in xenotransplant models, this observation raises the possibility that competition might occur between IgG2 present in some IVIg preparations and harmful complement-binding Abs on the surface of target cells in some situations.

Extensively adsorbed IVIg, at increasing concentrations, induced an increase in C1q binding to \textit{S. minnesota} Re595 via classical pathway activation (Fig. 5 to 6). This suggests that low affinity Abs reacting with the bacterial strain of interest were still present in the preparation. In fact, the first use of IVIg in humans was to increase the Ig level in patients with immunodeficiencies to prevent harmful infections (1). In addition, IVIg is used in sepsis therapy related to nosocomial infections (26). In numerous reports, IVIg preparations are reported to contain bacteria-specific Abs with opsonic ability. Whereas IVIg is helpful for proper opsonization of certain bacterial strains (27–29), it is insufficient for other strains (30). Thus, in the pool of IgG molecules present in IVIg, there are specific Abs to some common bacteria but not to others. In the case of IgG supplementation in patients with immunodeficiency or sepsis, screening for opsonic Abs specific for harmful bacteria is mandatory and is routinely done by the manufacturer. In fact, IVIg provided protection against pneumococcal infection in patients with Ig deficiency, although serum antcapsular Abs could not be detected following treatment (31). If IVIg is used to prevent complement-mediated damage in patients with autoimmunity, opsonic Abs in the preparation used would be an advantage since, as demonstrated here, IVIg does not interfere with the ability of complement to bind to the bacterial surface. This is well demonstrated by the fact that serum samples from patients treated with IVIg are able to induce complement activation on a sensitive bacterial strain and its subsequent lysis (Fig. 10). Our observations are in accord with a recently published report examining the effect of IVIg treatment in neonates on IgG and C3 binding to type III group B \textit{Streptococcus} and \textit{E. coli} K1 (32). The authors did not observe any difference in the amount of C3 bound to a bacterial surface between pre- and postinfusion serum samples. Although bacteria-specific Abs were present in the IVIg preparations, they did not induce any decrease in complement activation on the target bacterium. It can be noted that there is a discrepancy between C1q binding (Fig. 6), C3 binding (Fig. 5A) to \textit{S. minnesota} Re595, and lysis in our assays. C3 binding did not increase with increasing concentrations of IVIg, as did C1q binding. However, we have shown that there can be a complete dissociation between the amount of complement components deposited on the surface of bacteria and bacterial lysis (33). In addition, on a bacterial surface in a complex mixture of complement protein and regulatory molecules, there need not be a 1:1 correspondence between the amount of one complement component bound and that of another.

The effect of IVIg resides in its ability to divert deposition of C4, C3, and possibly C1q away from sensitized targets. In Figure 7, we clearly show that IVIg can block C3 deposition onto human sensitized erythrocytes. If one considers the total amount of IgG in our assay system, which uses 20% serum (~6 mg/ml following IVIg treatment in patients with idiopathic thrombocytopenic purpura) (Fig. 9), idiopathic thrombocytopenic purpura patient serum samples show about the same percentage of inhibition of C3 binding as a control serum to which an external source of IVIg is added (Fig. 7). In these studies, inhibition of C3 binding was ~25%. This finding differs from previously published data in which inhibition was more extensive (10). Many differences including disease state, time of blood collection, and test system account for the differences observed. To investigate whether it is possible to inhibit C3 binding to sensitized bacteria, we produced rabbit polyclonal Abs against \textit{L. monocytogenes}, a strain resistant to the lytic activity of human complement. After sensitization, \textit{L. monocytogenes} showed reduced C3 binding after exposure to 20% human serum at increasing concentrations of IVIg as compared with BSA (Fig. 8). The inhibition of C3 uptake was not as striking as that observed in the sensitized human erythrocyte model. This may be because \textit{L. monocytogenes} can directly bind C3, although the bacterium is not affected by complement activation on its surface and survives and even grows in the presence of a complement source (34). This observation raises the possibility that IVIg could have a negative effect on complement binding to Ab-sensitized bacteria. Whether IVIg can interfere with complement binding to sensitized bacteria and prevent proper opsonization remains to be studied.

The lack of regulation of complement activation on bacterial surfaces suggests an extra advantage in using IVIg in complement-mediated diseases. Several complement inhibitors are now being developed to inhibit the deleterious effects of uncontrolled complement activation. One interesting agent presently in its first phase of clinical trial in patients with severe burns is scCR1 (35). This soluble form of membrane complement receptor type 1 is a potent inhibitor of the complement cascade that acts at the level of C4 and C3. Still, this promising agent inhibited opsonization and phagocytosis of \textit{Streptococcus pneumoniae} and reduced the lethal dose of bacteria in a rat model (36). Although phagocytosis experiments were not performed in our study, deposition of complement components on bacteria was unaffected by IVIg, suggesting that interaction between complement fragments onto bacteria and complement receptors on phagocytes would not be perturbed. IVIg would thus represent a potentially important therapeutic tool in complement-mediated diseases that would be of little infectious risk for the patient.

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