Soluble Human Complement Receptor Type 1 Inhibits Complement-Mediated Host Defense

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Received 22 April 1994/Returned for modification 17 May 1994/Accepted 13 June 1994

Soluble complement receptor type 1 (sCR1) is a powerful inhibitor of complement activation. Because of this ability, sCR1 may prove to be an important therapeutic agent that can be used to block the immunopathologic effects of uncontrolled complement activation in a variety of clinically significant disorders. Although several previous studies have examined the ability of sCR1 to inhibit complement-mediated immunopathologic damage, there is no information on its ability to interfere with the host’s defense against infection. In the current experiments sCR1 exerted a concentration-dependent inhibitory effect on the phagocytosis of Streptococcus pneumoniae by human polymorphonuclear leukocytes in vitro. Not only did sCR1 inhibit complement-dependent opsonization of the pneumococcus but at higher concentrations it also inhibited the ingestion of bacteria which had been previously opsonized. Furthermore, when rats were injected with sCR1, it inhibited both their serum hemolytic activity and serum opsonic activity in a dose-dependent fashion. Finally, for rats treated with sCR1, the 50% lethal dose was also shown to be significantly lower than that for control animals after intravenous challenge with S. pneumoniae and Pseudomonas aeruginosa. These data demonstrate that sCR1 significantly inhibits complement-mediated host defense against bacterial infection.

The complement system is composed of a series of serum proteins and cellular receptors which serve as important mediators of host defense and inflammation. When the activation of complement is controlled and directed against invading microorganisms, it plays an important role in resistance to infection. However, when its activation proceeds in an uncontrolled fashion or is directed against the host, the complement system can cause immunopathologic damage and can be detrimental to the host.

Complement receptor type 1 (CR1, CD35) is found on primate erythrocytes and leukocytes and has specificity for the activated complement components C3b and C4b (3). It has the ability to inhibit activation of both the classical and alternative pathways of the complement system (1, 2, 7, 12). Recently, a soluble form of the receptor has been produced by recombinant DNA technology (12). Soluble complement receptor type 1 (sCR1) lacks the transmembrane and cytoplasmic domains of its membrane-bound parent molecule but retains its ability to inhibit the complement system (12). In vivo studies have shown that sCR1 inhibits the immunopathologic damage mediated by complement in animal models of myocardial infarction (6, 12), intestinal ischemia (5), cardiopulmonary bypass (4), the reversed passive Arthus reaction (15), and the adult respiratory distress syndrome (9).

Because of its potent ability to inhibit the complement system, sCR1 may prove to be an important therapeutic agent that can be used to block the immunopathologic effects of uncontrolled complement activation in a variety of clinically significant disorders. Although several previous studies have examined the ability of sCR1 to inhibit complement-mediated immunopathologic damage (4, 5, 6, 9, 12, 15), to date no study has looked at the ability of sCR1 to interfere with host defense against infection.

MATERIALS AND METHODS

Buffers. A five-times-concentrated stock solution of Veronal-buffered saline (VBS; pH 7.4) was prepared as described previously (8). From this stock solution, VBS (ionic strength, 0.147) and VBS containing 0.15 mM CaCl2, 0.5 mM MgCl2, and 0.5% bovine serum albumin (BSA; Sigma, St. Louis, Mo.) (VBS2+–BSA) were prepared.

Recombinant human sCR1. Recombinant human sCR1 is a 240-kDa glycoprotein which consists of the extracellular domains of human sCR1 (12). sCR1 was expressed by stably transfected Chinese hamster ovary cells and was purified from conditioned medium to greater than 99% purity. sCR1 (BRL 55730, TP10-HD) was supplied lyophilized by the Department of Pharmaceutical Science and Technology, SmithKline Beecham Pharmaceuticals. It was reconstituted to a concentration of 5 mg/ml.

Bacteria. Streptococcus pneumoniae type 25 (Pn25) was obtained from a blood culture of a patient with sickle-cell disease (14). The methods of storage, mouse passage, and quantitation were as described previously (14). To prepare Pn25, a drop of the stock culture from defibrinated rabbit blood was placed in 5 ml of brain heart infusion (BHI; Difco Laboratories, Detroit, Mich.) containing 10% sheep serum (SS; Pelfreeze, Rodger, AK) (BHI-SS) and incubated for 9 h at 37°C. The culture was then diluted 1/10 in fresh BHI-SS, reincubated at 37°C for 9 h, and rediluted 1/10 in fresh BHI-SS. After an additional 9-h incubation at 37°C, the bacteria were in log-phase growth and were harvested for use. The bacterial cultures to be used in the phagocytic assays (see below) were washed in VBS three times, counted in a Petroff-Hauser chamber, and adjusted to 125 × 10⁹ bacteria per 0.025 ml. Bacterial cultures to be used in the 50% lethal dose (LD₅₀) studies (see below) were concentrated 40-fold by centrifugation.
tion and were then serially diluted 3-fold in trypsic soy broth (TSB; Difco, Detroit, Mich.).

*Pseudomonas aeruginosa* ATCC 19660, isolated from the blood of a burn patient, was purchased from the American Type Culture Collection, Rockville, Md. The bacteria also underwent passage in mice and were stored in trypsic soy broth (TSB) with 20% glycerol at −70 °C. In preparation for challenge, a drop of the bacterial stock was added to TSB and the mixture was incubated for 9 h at 37 °C. After two successive 9-h passages, the bacteria were prepared for LD<sub>50</sub> studies as described above.

**Polymorphonuclear leukocytes.** On the day of each phagocytic assay, venous blood was collected from healthy human volunteers into a heparinized syringe. Erythrocyte sedimentation with dextran was performed as described previously (14). The leukocyte-rich plasma was layered over lymphocyte separation medium (Litton Bionetics-Organon Teknika, Irving, Tex.), and the mixture was centrifuged at 300 × g for 30 min at room temperature (11). The pellet containing the polymorphonuclear leukocytes was resuspended in a small quantity of VBS<sup>2+</sup>-BSA, and contaminating erythrocytes were lysed by adding 7.5 ml of distilled water. After 20 s of agitation, 2.5 ml of 3.6% saline was added to reverse the hypotonicity. This suspension was recentrifuged at 300 × g for 10 min, and the pellet was resuspended in VBS<sup>2+</sup>-BSA and was subsequently maintained at 4 °C. The cells were counted with a hemocytometer and diluted to the desired concentration.

**Animals.** All animal experiments were carried out with male Sprague-Dawley rats (weight, 100 to 150 g) obtained from Harlan Sprague-Dawley, Indianapolis, Ind.

**Serum collection.** Blood was obtained from the tail vein of rats and was allowed to clot at room temperature, and the serum was separated and stored at −20 °C.

**Hemolytic assay of complement.** The rat sera were tested for total hemolytic complement activity (50% hemolytic complement [CH<sub>50</sub>]) as described previously (8).

**Serum opsonizing activity.** The opsonic assay was performed as described previously (14). Briefly, aliquots of the leukocyte suspension containing 12.5 × 10<sup>6</sup> cells were centrifuged in propylene tubes at 300 × g for 10 min. The supernatant was discarded, and the cells were resuspended in 20% test serum in 0.4 ml of VBS<sup>2+</sup>-BSA. A total of 125 × 10<sup>6</sup> Pn25 contained in 0.025 ml was added, and the mixture was rotated at 12 rpm end-over-end in a multipurpose rotator (Scientific Industries, Bohemia, N.Y.) for 60 min at 37 °C. Thin smears were made on glass slides, heat fixed, and stained with methylene blue. Serum opsonic activity was measured as percent phagocytosis, which was determined by counting 200 polymorphonuclear cells and scoring the percentage of cells with ingested bacteria. Controls included bacteria and phagocytic cells, and 20% serum containing 0.01% EDTA. In each case the percent phagocytosis was 1% or less.

In some experiments, aliquots of 0.075 ml of VBS<sup>2+</sup>-BSA containing 125 × 10<sup>6</sup> bacteria were preincubated at 37° C in 20% rat serum containing various concentrations of sCR1 or in 20% rat serum alone diluted with 0.35 ml of VBS<sup>2+</sup>-BSA. After 30 min of rotation, 1 ml of VBS was added and the mixture was centrifuged for 10 min. The supernatant was discarded and the bacteria were resuspended in 0.2 ml of VBS<sup>2+</sup>-BSA containing various concentrations of sCR1 or in 0.2 ml of VBS<sup>2+</sup>-BSA alone. An additional 0.2 ml of VBS<sup>2+</sup>-BSA containing 12.5 × 10<sup>6</sup> polymorphonuclear leukocytes was added, and the mixture was rotated at 12 rpm for 60 min at 37° C and was treated as described above.

**Bacterial challenge.** Groups of seven animals each were injected via the tail vein with the desired dose of either sCR1 or an equivalent volume of saline. Twenty minutes later, the rats were challenged with 0.3 ml of various concentrations of the desired bacteria. The animals were observed at 12-h intervals for 1 week, and the deaths were recorded. The LD<sub>50</sub> was calculated by the method of Reed and Muench (10).

**RESULTS**

**Effect of sCR1 on in vitro phagocytosis.** Since the complement system is known to play a critical role in the phagocytosis of certain bacteria, we examined the effect of sCR1 on the phagocytosis of Pn25. When sCR1 was added to the phagocytic mixtures containing 20% test serum, it exerted an inhibitory effect on the phagocytosis of Pn25 by human PMNs in a concentration-dependent fashion (Fig. 1). At all concentrations greater than 10 μg/ml, nearly complete inhibition of phagocytosis was seen.

**Effect of sCR1 on opsonization and ingestion.** Complement-mediated opsonization can be separated into two stages: opsonization of the bacteria by activated C3b and ingestion of the opsonized bacteria by the phagocytic cell. To investigate in which stage sCR1 inhibits phagocytosis, we added sCR1 to each of the two stages of our phagocytic assay. First, we examined the effect of sCR1 on the activation of complement and its opsonization of bacteria. Pn25 was preincubated in 20% serum containing various concentrations of sCR1 for 30 min or in 20% serum which did not contain sCR1, and the bacteria were washed free of the serum and sCR1 and were then incubated by rotation in the presence of buffer and phagocytic cells. We also examined the effect of sCR1 on the ingestion of preopsonized bacteria by the phagocytic cell. In this case, bacteria were preincubated in 20% test serum which did not contain sCR1 (as described above), washed free of serum, and then incubated in the presence of either buffer or various concentrations of sCR1 and the phagocytic cells.

sCR1 was shown to inhibit both opsonization and ingestion in a concentration-dependent fashion (Fig. 2). This inhibitory effect was more pronounced when sCR1 was added during the opsonization of bacteria. For example, whereas a concentration of 10 μg/ml added during opsonization caused a 93%
inhibition of phagocytosis, a 100-fold greater concentration of sCR1 added after opsonization resulted in only 76% inhibition.

In vivo effect of sCR1 on serum hemolytic and opsonic activities. Groups of five rats each were injected with various doses of sCR1 and were bled at given time intervals after injection, the serum was pooled, and the CH50s were determined (Fig. 3). One hour after administration of the sCR1, the sera of those rats that received the higher dose of sCR1 (25 mg/kg) had a CH50 which was only 6% of the pretreatment level. One day following treatment, the CH50 was still significantly depressed (29% of the pretreatment levels) and did not approach normal levels until day 4 (85% of pretreatment levels). The sera of rats receiving a lower dose of sCR1 (10 mg/kg of body weight) showed a smaller decrease in CH50 after 1 h (17% of pretreatment levels) and approached normal levels much more quickly.

The ability of pooled serum obtained from the groups of rats that had been treated with sCR1 to support opsonization of Pn25 was also tested. The sera of rats receiving sCR1 at the highest dose (25 mg/kg) had only 3% of their pretreatment opsonizing activity at 1 h after injection, and the opsonizing activity of serum did not approach normal levels until 1 day later (Fig. 4). The opsonizing activity of the sera of rats that received a lower dose (10 mg/kg) was inhibited most at 20 min (23% of pretreatment levels) and began to approach normal levels by 24 h.

Bacterial challenge of sCR1-treated animals with S. pneumoniae. Since sCR1 inhibited both opsonization and ingestion of the pneumococcus both in vitro and in vivo, we investigated whether sCR1-treated rats were more susceptible to infection. Groups of seven rats each were injected with the desired dose of sCR1 and were then challenged intravenously with Pn25 30 min later. Table 1 lists the LD50s found for each dose of sCR1. At 10 mg/kg there was a fourfold difference between the LD50s for those rats receiving sCR1 and those rats receiving saline ($P < 0.05$). This effect was even more pronounced at a higher dose of sCR1. For those rats receiving 25 mg of sCR1 per kg, the LD50 was eightfold lower than that for control animals ($P < 0.05$) (Table 1 and Fig. 5).

Bacterial challenge of sCR1-treated animals with P. aeruginosa. To determine whether rats treated with sCR1 were more

### Table 1. LD50s for rats treated with sCR1 and challenged with bacteria

<table>
<thead>
<tr>
<th>Bacterium</th>
<th>sCR1 dose (mg/kg)</th>
<th>LD50 (±2 SD)$^*$</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Control rats</td>
</tr>
<tr>
<td>Pneumococcus</td>
<td>10</td>
<td>$(8.5 ± 2.8) \times 10^8$</td>
</tr>
<tr>
<td></td>
<td>25</td>
<td>$(8.9 ± 1.0) \times 10^8$</td>
</tr>
<tr>
<td>Pseudomonas</td>
<td>25</td>
<td>$(11.0 ± 2.6) \times 10^7$</td>
</tr>
</tbody>
</table>

* $P < 0.05$ for comparisons between both groups.
sCR1 has been shown to be a powerful inhibitor of complement activation (1, 2, 7, 12). It inhibits the activation of C3 and C5 by interfering with the assembly and expression of the C3 and C5-cleaving enzymes of both the classical and alternative pathways. Because it is such an efficient inhibitor of complement activation, it may be of value as a pharmacologic agent that can be used to inhibit complement-mediated damage in a number of disease states. In fact, it has already been shown to be effective in suppressing complement-mediated immunopathologic damage in animal models of postischemic myocardial infarction (6, 12), intestinal ischemia (5), cardiopulmonary bypass (4), the reversed passive Arthus reaction (15), and acute respiratory distress syndrome (9). The present study provides evidence that sCR1 may also have detrimental effects on a patient’s complement-mediated host defense against bacterial infections.

Complement plays a critical role in the opsonization of a variety of bacteria, including the pneumococcus (13). It has previously been established that sCR1 inhibits the hemolytic activity of both the classical and alternative pathways (1, 2, 7, 12). Our study extends these observations by showing that sCR1 inhibits opsonization of the pneumococcus both in vitro and in vivo. When rats were treated with sCR1 in vivo, their serum opsonizing activity was inhibited in a dose-dependent fashion. Whereas a dose of 10 mg of sCR1 per kg resulted in a 67% inhibition of serum opsonizing activity 1 h after administration, the higher dose of 25 mg/kg inhibited the serum opsonizing activity nearly completely at the same time point. The fact that sCR1 inhibits the complement system in a dose-dependent manner will undoubtedly prove to be an important consideration in the dose of the drug that may be administered, particularly to patients already at an increased risk for infection, i.e., burn patients and postoperative patients.

The results of the present study also indicate that sCR1 interferes with the ingestion of bacteria that carry opsonically active C3b on their surfaces. When sCR1 was added to phagocytic mixtures containing bacteria that had already been preopsonized with C3b, the ingestion of the bacteria by granulocytes was inhibited in a dose-dependent fashion. It should be noted, however, that much higher doses of sCR1 were necessary to inhibit ingestion of opsonized bacteria than were needed to inhibit opsonization. It is possible that sCR1 binds to the C3b molecule on the surface of the bacteria and competitively inhibits the recognition of opsonized bacteria by the phagocytic cell.

Finally, rats treated with sCR1 had increased susceptibilities to challenge with two different bacterial species, S. pneumoniae and P. aeruginosa. In each instance, for animals treated with sCR1, the LD$_{50}$s were lower when the animals were challenged with bacteria intravenously and the effect was more prominent with the higher dose of sCR1 (25 mg/kg). Although the studies described here demonstrated that sCR1 interferes with the host’s defense against bacterial infection, it is possible that the effect would be less pronounced if the animals were challenged by different routes, e.g., by inhalation.

It is not clear if the effect of sCR1 on host defense will interfere with its potential clinical utility in preventing complement-mediated immunopathologic damage. It is difficult to predict to what extent sCR1 would interfere with host defense in humans who might receive this pharmacologic agent. In this regard, it is interesting that human sCR1 inhibits the lysis of antibody-sensitized sheep erythrocytes by human serum to a greater degree than it does rat serum (12). Thus, it is possible that its effects on host defense will be greater in humans than in rats. Nevertheless, the results of the current study do not necessarily preclude the use of sCR1 in humans. Its effect on the complement system is relatively short-lived, is dose dependent, and should not interfere with other mechanisms of host defense. Thus, its benefit in certain clinical situations characterized by complement-mediated damage may outweigh any potential risks.

ACKNOWLEDGMENT
This work was supported in part by NIH grant HL-47191.

REFERENCES