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J Immunol 1998; 160:475-484; 
http://www.jimmunol.org/content/160/1/475
Effect of C1 Inhibitor on Inflammatory and Physiologic Response Patterns in Primates Suffering from Lethal Septic Shock

Patty M. Jansen,* Bernd Eisele,+ Irma W. de Jong,* Alvin Chang,‡ Ulrich Delvos,+ Fletcher B. Taylor, Jr.,‡ and C. Erik Hack*§

We evaluated the effect of C1 inhibitor (C1-inh), an inhibitor of the classical pathway of complement and the contact system, on the physiologic and inflammatory response in baboons suffering from lethal Escherichia coli sepsis. Five animals pretreated with 500 U/kg C1-inh (treatment group; n = 5), followed by a 9-h continuous infusion of 200 U/kg C1-inh subsequent to bacterial challenge, were compared with five controls receiving E. coli alone. Of the treatment group, one animal survived and another lived beyond 48 h, whereas all control animals died within 27 h. In four of five treated animals, less severe pathology was observed in various target organs. C1-inh administration did not prevent the hemodynamic or hematologic changes observed upon E. coli infusion. The activation of fibrinolysis and the development of disseminated intravascular coagulation were essentially unaffected by C1-inh. However, C1-inh supplementation significantly reduced decreases in plasma levels of factor XII and prekallikrein and abrogated the systemic appearance of C4b/c, indicating substantial inhibition of activation of the contact system and the classical complement pathway, respectively. Furthermore, treated animals displayed a reduced elaboration of various cytokines including TNF, IL-10, IL-6, and IL-8. Thus, the administration of C1-inh may have a beneficial but modest effect on the clinical course and outcome of severe sepsis in nonhuman primates. We suggest that activated complement and/or contact system proteases may, at least in part, contribute to the attendant manifestations of septic shock through an augmentation of the cytokine response. The Journal of Immunology, 1998, 160: 475–484.

Septic shock is a clinical syndrome that results from bacterial infection and is characterized by an extensive triggering of multiple endogenous mediators (1). Among the mediators implicated are the complement system and the contact system of intrinsic coagulation, both of which can be activated directly in vitro by cell wall components of Gram-negative and Gram-positive bacteria (2–4). Ample evidence has now accumulated that activation of these plasma cascade systems occurs in human sepsis, notably when complicated by shock and/or adult respiratory distress syndrome (5–12). Activation of the complement and contact systems results in the liberation of biologically active peptides, the anaphylatoxins and bradykinin, respectively, which may induce vasodilation and enhance the permeability of endothelial cells (13, 14). It has been previously shown that the anaphylatoxins in particular C5a and factor XIIa also stimulate neutrophils (13, 15) and induce production of cytokines by mononuclear cells (16–22). Moreover, C5a and the terminal complex of complement, C5b-9, may promote coagulation by evoking tissue factor expression on endothelial cells (23), and assembly of C5b-9 on the surface of platelets has been shown to induce the release of α-granules, the exposure of negatively charged phospholipids, assembly of the prothrombinase complex, and release of vesicles that express prothrombinase activity (24–26). Hence, activation of complement and contact systems may contribute to the coagulant and inflammatory sequelae of sepsis through several mechanisms.

Activation of both the complement and contact system is regulated by C1-esterase inhibitor (C1-inh).3 C1-inh, which belongs to the superfamily of serine-proteinase inhibitors, is the only known inhibitor of Clr andCls, components of the classical pathway of complement (27), as well as the major inhibitor of factor XII and prekallikrein of the contact system (28, 29). Although C1-inh is an acute phase protein, antigenic levels of C1-inh tend to be normal in patients with fatal septic shock, while levels of proteolytically inactivated C1-inh are increased, suggestive of an increased turnover and a relative deficiency of biologically active C1-inh during sepsis (30).

We have previously demonstrated that C1-inh substitution therapy in patients with septic shock may reduce the need for vasopressor medication and attenuate complement and contact activation (31, 32). Moreover, effects of C1-inh have been observed in several animal models of sepsis: C1-inh supplementation abrogated endoxin-induced disseminated intravascular coagulation and

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3 Abbreviations used in this paper: C1-inh, C1-inhibitor; NBLA, normal baboon serum aged; NBS, normal baboon serum; NBS-AHG, normal baboon serum incubated with heat-aggregated IgG; NBP-MA-UK, normal baboon plasma incubated with methylamine and urokinase; PAP, plasmin-α2-antiplasmin complex; TAT, thrombin-antithrombin complex; IPA, tissue-type plasminogen activator; Fischer LSD, Fischer least significant difference.
hypotension in rabbits (33, 34) and pulmonary dysfunction in endotoxemic dogs (35). In this study, we evaluated the effect of i.v. administration of C1-inh on hemodynamic, coagulant, inflammatory, and cell injury responses in an established model of severe septic shock in nonhuman primates. Our results indicate that in this experimental model, exogenous administration of C1-inh may exert beneficial effects, in part through modulation of cytokine release, and support the notion that activation of complement and/or contact system proteases is associated with organ injury and impending lethality.

Materials and Methods

C1-inh preparation

Pasteurized human C1-inh was provided by Behringwerke AG (Marburg, Germany). On SD5-PAGE, this preparation consisted of >95% native C1-inh.

Experimental and infusion procedures

Experiments were performed on 10 juvenile baboons (Papio anubis/Cynocephalus), each with a hematoctrit exceeding 36% and free from tuberculosis. The animal-handling procedures and Escherichia coli (type B) preparation were performed using the methodology described in previous publications (36, 37). Briefly, baboons were fasted overnight before each experiment and given water ad libitum. Each animal was sedated with ketamine hydrochloride (14 mg/kg, intramuscularly) on the morning of the study and anesthetized with sodium pentobarbital (2 mg/kg) via a percutaneous catheter positioned in the cephalic vein. The femoral artery and both femoral veins were cannulated aseptically and used for measuring aortic pressure, obtaining blood samples, infusing live organisms, C1-inh, and for fluid and anesthetic administration as reported elsewhere (36, 37). Gentamicin was given (9 mg/kg) as a 75-min infusion immediately after the E. coli infusion had been stopped, and then (4.5 mg/kg) as a 30-min infusion at 6 and 9 h after the start of the E. coli infusion. Additional gentamicin (4.5 mg/kg) was given as an intramuscular injection at 10 h after the start of the infusion and twice daily for the subsequent 3 days.

Each animal was i.v. challenged with a lethal dose of E. coli (4 × 109 CFU/kg of body weight), given as a 2-h infusion. The time point at which the infusion was started is further indicated as T + 0, a time point of n hours thereafter referred to as T + n h. Time points before the start of the challenge are indicated as T − n h.

Two experimental E. coli groups were studied: one group consisted of five baboons that received an initial dose of 500 U/kg C1-inh (1 U is the maximum of 7 days). Blood samples were collected at given time points for measurement of cytokines, complement activation products, contact system proteins, 1, 2, 3, 4, 6, 8, and 10 h after the start of the infusion was started is further indicated as T1/2.

An additional group of five baboons that received saline according to a similar scheme of administration before and after lethal E. coli infection (control group).

All animals were maintained under anesthesia and monitored for 10 h. They were observed continuously for an additional 36 h and daily for a maximum of 14 days. Blood samples were collected for hematology, clinical chemistry, and C1-inh determinations. Additional blood samples were collected on EDTA/soy bean trypsin inhibitor (final concentrations, 10 mM and 0.1 mg/ml, respectively) before (T + 0), and at 0.5, 1, 2, 3, 4, 6, 8, and 10 h after E. coli challenge for plasma determination of cytokines, complement activation products, contact system proteins, neutrophil degranulation products, and coagulation (anti)fibrinolytic parameters. Baboons surviving for 7 days were considered permanent survivors and were subsequently killed with sodium pentobarbital for necropsy on the eighth day.

Assays

All assays used in this study were developed with mono- or polyclonal Abs raised against human proteins. When human standards had to be used, we established that dilution curves of these standards were parallel to those obtained with baboon plasma samples. Notably, the lower affinity of the Abs for baboon proteins may have led to an underestimation of the baboon proteins.

Antigenic C1-inh. Plasma levels of exogenously administered C1-inh were measured by nephelometry (Behringwerke Nephelometer Analyzer, Behringwerke AG) and expressed as mg/L. Functional C1-inh levels were measured by RIA as described previously (30).

Complement activation products. C3b and C4b were measured in baboon plasma by means of RIA as reported elsewhere (39–41). C3b/C4b was expressed as a percentage of the amount present in normal baboon serum aged (NBA), i.e., normal baboon serum (NBS) incubated for 7 days at 37°C in the presence of 0.02% (w/v) NaN3. Results for C3b/C4b were expressed as a percentage of the amount generated in NBS by incubation with heat-inactivated IgG (NBS-AHG). Levels of the terminal complex of complement (C5b-9) were measured by ELISA according to the instructions of the manufacturer (Behringwerke AG, Marburg, Germany), and were expressed as µg/L with reference to a serially diluted standard of human C5b-9.

Cytokines. Plasma concentrations of TNF-α, IL-6, IL-8, and IL-10 were measured by ELISA as previously described (42–44).

Coagulation and (anti)fibrinolytic parameters. Levels of tissue-type plasminogen activator (t-PA), plasminogen activator inhibitor type 1 (PAI-1), and thrombin-antithrombin III (TAT) complexes were determined by ELISA as described previously (41, 45, 46). Values were expressed as ng/ml. Plasmin-α2-antiplasmin (PAP) complexes were measured by RIA as described (46). PAP complex levels were expressed as percentage of the level present in normal baboon plasma in which a maximal amount of complexes was generated by incubation with an equal volume of urokinase (50 µg/ml) in the presence of 0.2 mol/L methylamine (final concentration) to inactivate α2-macroglobulin, further referred to as NBP-MA-UA.

Measurement of prekallikrein and factor XII in plasma. Plasma prekallikrein and factor XII were determined by a sandwich-type ELISA. Flat-bottom microtiter (96-well) plates (Dynatech, Chantilly, VA) were coated overnight at room temperature with 100 µl of 2.5 µg/ml anti-human prekallikrein mAb K15, or mAb OT-2 against human factor XII, in carbonate buffer, pH 9.5, and blocked for 30 min with 150 µl PBS containing 2% (v/v) cow’s milk. All subsequent incubations were in 100-µl volumes at room temperature, and plates were washed after each incubation with PBS containing 0.05% (w/v) Tween-20. The plates were then incubated for 2 h with baboon plasma samples diluted in 100 µl high performance ELISA (HEP) buffer (CLB, Amsterdam, The Netherlands). Bound prekallikrein and factor XII were determined by subsequent 1-h incubation with HPE buffer containing 1 µg/ml of biotinylated mAbs 13G11 (kindly provided by Dr. R. W. Colman, Temple University, Philadelphia, PA) and F3, respectively, followed by a 1:10,000 dilution of streptavidin-polymerized horseradish peroxidase (polyHRP; CLB) in PBS/2% (v/v) cow’s milk for 30 min. The plates were developed with a solution of 100 µg/ml of 3,5,3’-5’-tetratmethylenedizin (Mercer, Darmstadt, Germany) with 0.003% (v/v) H2O2 in 0.11 mol/L sodium acetate, pH 5.5. The reaction was stopped by the addition of an equal volume of 2 mol/L H2SO4 to the wells. Serial dilutions of normal pooled baboon plasma was used as a standard. Values were expressed as percentage of the amount present before E. coli infusion (T = 0).

Neutrophil degranulation products. Elastase-α1-protease inhibitor complex levels were determined with a RIA that has been described in detail elsewhere (47). Results were expressed as nanograms of elastase per milliliter by reference to a standard curve that consisted of normal baboon plasma to which human neutrophil elastase (Elastin Products Co., Pacific, MO) was added at a final concentration of 2 µg/ml. In this standard, >95% of the elastase is complexed to α1-antitrypsin.

Statistical analysis

Results are expressed as mean ± SEM. Statistical analysis was performed using a commercial statistical package (StatView; Abacus Concepts, Inc., Berkeley, CA). Comparisons between groups during the course of the observation period were performed using repeated measures analysis of variance (ANOVA). Data were analyzed by two-tailed ANOVA to determine the significance of differences in means between groups at given times. Within one group, differences from baseline levels were determined with ANOVA using Fischer’s least significant difference (Fischer LSD). Statistical significance was defined at the 95% confidence level.

Results

Recovery of C1-inh

C1-inh was infused into baboons to achieve a concentration of ~10 times the level observed in normal human plasma (i.e., 270 mg/L) just before the start of the E. coli infusion (T = 0). Figure
The influence of C1-inh on clinical and hematologic response patterns to lethal \textit{E. coli} challenge is summarized in Table II. Infusion of \textit{E. coli} produced a severe hypotensive shock in all baboons, which was unaffected by supplementation with C1-inh. In both control and treatment groups, a significant decline in mean systemic arterial pressure was noted at 2 h, remaining below baseline values during the 10-h observation period. Mean heart rate and temperature were similarly elevated in all animals. \textit{E. coli}-infusion with or without C1-inh induced a prompt fall in leukocyte count to 40\% of control at T + 1 h, a steady decline in platelet count to 30\% of control at T + 10 h, a small rise in hematocrit from T + 6 h and on, and a progressive decrease in fibrinogen levels over the entire observation period. Changes in mean systemic arterial pressure, heart rate, temperature, white blood cell, platelet counts, and hematocrit were not different between the nonsurviving C1-inh-treated animals and the animal surviving treatment (not shown). However, the rate of fibrinogen consumption appeared to be modestly decreased in the surviving C1-inh-supplemented animal (Table II).

To evaluate whether the administration of C1-inh affected biochemical changes related to organ damage, we compared several markers of cell injury at T + 0 and T + 10 h in C1-inh-treated and control groups. Table III shows that levels of blood urea nitrogen, creatinine, uric acid, lactate dehydrogenase (LDH), serum glutamic oxaloacetic transaminase (SGOT), and serum glutamic pyruvate transaminase (SGPT) were increased in all animals at 10 h. The magnitude of the increases, however, tended to be lower in the C1-inh treatment group, especially in the treated baboon that was rescued from lethal challenge, and in the animal surviving for >60 h after C1-inh supplementation. At 10 h postchallenge, these animals exhibited only moderate changes in uric acid, LDH, SGOT, and SGPT, while a pronounced increase in the levels of these parameters was observed in the treated and control animals that died within 14 to 27 h.

Postmortem examinations were conducted in all baboons. Tissues were removed for analysis within minutes after death, thereby avoiding autolytic changes. Kidneys and adrenals removed from all baboons in the control group showed evidence of widespread microvascular thrombosis with extensive infarction and hemorrhage. In each case, alveolar capillary congestion, edema, and aggregation of neutrophils were observed in the lungs. There was severe vascular congestion and accumulation of neutrophils in the vascular spaces of the liver. The spleen showed lymphoid follicular necrosis and medullary congestion. In contrast, in the surviving animal (No. 9) in the C1-inh treatment group, all organs appeared unaffected, while in the treated animal surviving for >60 h (No. 8), the only significant changes were limited to the lungs, which showed moderate edema without signs of thrombosis. In addition, in one nonsurviving C1-inh-treated animal (No. 5), C1-inh treatment protected adrenals and kidneys, in which no pathology was observed, while in another animal (No. 10), the lungs, showing only mild congestion and leukocyte influx, were essentially spared. Thus, less severe damage to various organs was observed in four of five C1-inh-treated animals when compared with controls.

\section*{Effect of high dose C1-inh supplementation on inflammatory response patterns in lethal \textit{E. coli} sepsis}

\subsection*{Complement activation.}

Plasma levels of C4b/c, C3b/c, and C5b-9 were measured to evaluate the effect of C1-inh supplementation on \textit{E. coli}-induced complement activation (Fig. 2, A–C). In the control group, circulating levels of C4b/c and C3b/c continued to rise during the entire observation period, reaching maximal levels of 13.3 \pm 3.1\% and 10.9 \pm 2.8\%, respectively, of fully activated NBS at T + 10 h (Fig. 2, A and B). Administration of high doses of C1-inh almost completely abrogated the appearance of C4b/c (p < 0.0001) in all animals thus treated, indicating efficient inhibition of \textit{Papio C1} by human C1-inh. Moreover, C1-inh markedly attenuated the appearance of C3b/c at all time points (p < 0.01), suggesting that at least part of the C3 activation had occurred via the classical pathway. Plasma levels of the terminal complex of complement, i.e., C5b-9, also rapidly increased upon \textit{E. coli} challenge (Fig. 2C). In control animals, peak levels of 3691 \pm 221 \mu g/L were noted at T + 2 h, remaining elevated until the end of the observation period. C1-inh treatment only modestly affected concentrations of C5b-9 (p = 0.05), and peak levels of 3061 \pm 289 \mu g/L were measured at 2 h after the \textit{E. coli} infusion was started. A uniform response was observed for all activation patterns to lethal \textit{E. coli}.

\begin{figure}
\centering
\includegraphics[width=\textwidth]{figure1.png}
\caption{Recovery of C1-inh in baboons with lethal sepsis. Mean \pm SEM levels of antigenic C1-inh in the treatment group receiving 500 U/kg + 200 U/kg/9 h of human C1-inh.}
\end{figure}
products in the treatment group, and this response appeared unrelated to survival and/or organ damage.

**Fibrinolytic response.** In agreement with previous observations (41, 46), a pronounced activation of (anti)fibrinolysis was observed with lethal E. coli challenge (Fig. 3, A–C). In both control and treatment groups, a protracted increase of t-PA concentrations was noted from T + 1 h and on, to reach a maximum level of 77.8 ± 16.1 and 117.6 ± 15.2 ng/ml at T + 10 h in control and C1-inh treatment groups, respectively (Fig. 3A). Concentrations of circulating PAP complexes, which reflect the generation of plasmin, the key enzyme of the fibrinolytic system, transiently increased upon E. coli infusion, peak levels being 10.3 ± 3.5% and 15.7 ± 4.2% of maximally activated plasma at T + 2 h in controls and treated animals, respectively (Fig. 3B). This difference in time course of t-PA and PAP complexes was not statistically significant between both groups (p > 0.05). In contrast, C1-inh administration markedly attenuated the appearance of PAI into the circulation, especially at and beyond T + 6 h (Fig. 3C; p < 0.0001). In

### Table I. Weight, sex, E. coli dose, and E. coli concentration in blood at the end of the infusion (T + 2 hr), and survival in control and C1-inh treatment groups

<table>
<thead>
<tr>
<th>No.</th>
<th>Weight (kg)</th>
<th>Sex</th>
<th>E. coli Dose (x10^9 CFU/kg)</th>
<th>E. coli Dose in Blood at T + 2 (x10^9 CFU/kg)</th>
<th>Survival (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>7</td>
<td>5.5</td>
<td>F</td>
<td>9.48</td>
<td>15.00</td>
<td>19.5</td>
</tr>
<tr>
<td>11</td>
<td>5.0</td>
<td>F</td>
<td>10.02</td>
<td>3.65</td>
<td>27</td>
</tr>
<tr>
<td>3</td>
<td>6.6</td>
<td>M</td>
<td>7.68</td>
<td>1.72</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>6.1</td>
<td>M</td>
<td>8.16</td>
<td>1.28</td>
<td>15</td>
</tr>
<tr>
<td>7b</td>
<td>6.8</td>
<td>M</td>
<td>8.15</td>
<td>2.22</td>
<td>18.5</td>
</tr>
<tr>
<td>mean ± SEM</td>
<td>6.0 ± 0.3</td>
<td></td>
<td>8.70 ± 0.45</td>
<td>4.77 ± 2.59</td>
<td>19.4 ± 2.0</td>
</tr>
</tbody>
</table>

### C1-inh group

<table>
<thead>
<tr>
<th>No.</th>
<th>Weight (kg)</th>
<th>Sex</th>
<th>E. coli Dose (x10^9 CFU/kg)</th>
<th>E. coli Dose in Blood at T + 2 (x10^9 CFU/kg)</th>
<th>Survival (h)</th>
</tr>
</thead>
<tbody>
<tr>
<td>5</td>
<td>6.4</td>
<td>F</td>
<td>9.00</td>
<td>7.90</td>
<td>14.5</td>
</tr>
<tr>
<td>6</td>
<td>5.0</td>
<td>M</td>
<td>9.72</td>
<td>1.84</td>
<td>17</td>
</tr>
<tr>
<td>8</td>
<td>5.7</td>
<td>F</td>
<td>10.26</td>
<td>4.40</td>
<td>62.5</td>
</tr>
<tr>
<td>9</td>
<td>6.8</td>
<td>F</td>
<td>8.34</td>
<td>3.85</td>
<td>&gt;168</td>
</tr>
<tr>
<td>10</td>
<td>5.9</td>
<td>F</td>
<td>9.92</td>
<td>5.20</td>
<td>25.5</td>
</tr>
<tr>
<td>mean ± SEM</td>
<td>5.9 ± 0.3</td>
<td></td>
<td>9.45 ± 0.35</td>
<td>4.64 ± 0.99</td>
<td>&gt;57.5 ± 28.9</td>
</tr>
</tbody>
</table>

* No significant difference was noted by two-tailed ANOVA.

### Table II. Hemodynamic parameters, vital signs, and hematologic response patterns in baboons after infusion of lethal E. coli alone (n = 5) and after lethal challenge supplemented with C1-inh (n = 5)

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>T + 0</th>
<th>T + 1</th>
<th>T + 2</th>
<th>T + 3</th>
<th>T + 4</th>
<th>T + 5</th>
<th>T + 6</th>
<th>T + 8</th>
<th>T + 10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>MSAP</strong></td>
<td>119.0 ± 6.7</td>
<td>119.0 ± 2.9</td>
<td>119.0 ± 6.7</td>
<td>119.0 ± 2.9</td>
<td>119.0 ± 6.7</td>
<td>119.0 ± 2.9</td>
<td>119.0 ± 6.7</td>
<td>119.0 ± 2.9</td>
<td>119.0 ± 6.7</td>
</tr>
<tr>
<td><strong>HR</strong></td>
<td>113.6 ± 4.1</td>
<td>113.6 ± 4.1</td>
<td>113.6 ± 4.1</td>
<td>113.6 ± 4.1</td>
<td>113.6 ± 4.1</td>
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<td>113.6 ± 4.1</td>
<td>113.6 ± 4.1</td>
</tr>
<tr>
<td><strong>Temp</strong></td>
<td>113.0 ± 11.2</td>
<td>113.0 ± 11.2</td>
<td>113.0 ± 11.2</td>
<td>113.0 ± 11.2</td>
<td>113.0 ± 11.2</td>
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<td>113.0 ± 11.2</td>
</tr>
<tr>
<td><strong>WBC</strong></td>
<td>36.6 ± 0.2</td>
<td>36.6 ± 0.2</td>
<td>36.6 ± 0.2</td>
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<td>36.6 ± 0.2</td>
<td>36.6 ± 0.2</td>
<td>36.6 ± 0.2</td>
</tr>
<tr>
<td><strong>Platelets</strong></td>
<td>35.9 ± 0.3</td>
<td>35.9 ± 0.3</td>
<td>35.9 ± 0.3</td>
<td>35.9 ± 0.3</td>
<td>35.9 ± 0.3</td>
<td>35.9 ± 0.3</td>
<td>35.9 ± 0.3</td>
<td>35.9 ± 0.3</td>
<td>35.9 ± 0.3</td>
</tr>
<tr>
<td><strong>HCT</strong></td>
<td>323.4 ± 26.0</td>
<td>323.4 ± 26.0</td>
<td>323.4 ± 26.0</td>
<td>323.4 ± 26.0</td>
<td>323.4 ± 26.0</td>
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<td>323.4 ± 26.0</td>
<td>323.4 ± 26.0</td>
<td>323.4 ± 26.0</td>
</tr>
<tr>
<td><strong>Fibrinogen</strong></td>
<td>37.2 ± 1.1</td>
<td>37.2 ± 1.1</td>
<td>37.2 ± 1.1</td>
<td>37.2 ± 1.1</td>
<td>37.2 ± 1.1</td>
<td>37.2 ± 1.1</td>
<td>37.2 ± 1.1</td>
<td>37.2 ± 1.1</td>
<td>37.2 ± 1.1</td>
</tr>
</tbody>
</table>

* Values represent mean ± SEM changes in mean systemic arterial pressure (MSAP, in mmHg), heart rate (HR, in beats/min), temperature (°C), white blood cell counts (WBC, in 10^3/mm^3), hematocrit (HCT, in %), and fibrinogen (% of baseline) after start of the infusion (T + 0). Individual fibrinogen levels in the C1-inh treatment group are given. The course of all parameters was not different between both groups by ANOVA-repeated measurements.

*Animals surviving for >7 days.

*p < 0.05 vs baseline by two-tailed ANOVA using Fischer PLSD.
both groups, peak levels of PAI were noted at 8 h postchallenge, and were 5180 ± 6877 and 2379 ± 446 ng/ml in controls and C1-inh-supplemented animals, respectively.

Clotting response. TAT complexes were measured in control and C1-inh groups to determine the extent of thrombin generation (Table IV). Consistent with a pronounced decline in fibrinogen and platelets observed upon lethal *E. coli* administration (Table II), indicating the onset of diffuse intravascular coagulation (DIC), circulating TAT complexes sharply increased from T + 1 h and on, and maximal levels of 2236 ± 314 and 1668 ± 452 ng/ml were measured at 6 h postchallenge in control and treatment groups, respectively. Although the course of TAT complexes was not statistically different between both groups, the magnitude of increase was markedly lower in the surviving C1-inh-treated animal that also showed a retarded fibrinogen consumption (animal No. 9; Tables II and IV).

Cytokine response patterns. The administration of *E. coli* was associated with a transient increase of plasma TNF concentrations (Fig. 4A). Consistent with our previous studies (48, 49), peak TNF levels of 16.3 ± 1.9 ng/ml were observed in control animals at T + 2 h, i.e., at the end of the *E. coli* infusion. Administration of lethal *E. coli* supplemented with C1-inh elicited a similar, but significantly lower TNF response, with peak plasma concentrations of 11.0 ± 1.7 ng/ml (*p* < 0.05). Notably, the lowest TNF peak was observed in the treated animal surviving *E. coli* challenge (No. 9; 4.1 ng/ml at T + 2 h).

C1-inh administration markedly modulated the appearance of IL-10, especially during the later stages of the septic process (Fig. 4B). In excipient control baboons, IL-10 levels sharply increased to reach a maximum of 3564 ± 772 pg/ml 3 h after the start of the experiment and were still elevated at T + 10 h. Upon C1-inh treatment, peak levels of IL-10 were noted at T + 3 h (2, 266 ± 767 pg/ml), to become near baseline values at the end of the observation period (*p* < 0.05). In addition, administration of C1-inh significantly attenuated the sustained release of IL-6 and IL-8 into the circulation, and maximal levels of 279.6 ± 74.9 and 167.4 ± 19.1 ng/ml were noted at 8 and 4 h, respectively, i.e., a two- to fivefold reduction compared with peak control levels (Fig. 4, C and D; *p* < 0.001).

![FIGURE 2. Complement activation after lethal *E. coli* infusion. Mean ± SEM plasma levels of C3b/c (A), C4b/c (B), and C5b-9 (C) in control (○) and C1-inh (○) groups. C3b/c and C4b/c are expressed as percentage of NBA and NBS-AHG, respectively. Differences between both group by ANOVA repeated measurements were significant at *p* < 0.05.](http://www.jimmunol.org/doi/suppl/10.4049/jimmunol.479.0.0001?2)
The activation of complement and contact system proteases in human and animal septic shock has been well documented (1, 5–12, 41, 50, 51). In this study, we aimed to restrict activation of these plasma cascade systems in baboons challenged with lethal E. coli by exogenous administration of C1-inh, to evaluate whether inhibition of these enzymes modulated the pathophysiologic response observed.

Metabolic studies with radiolabeled C1-inh in human volunteers have yielded a fractional catabolic rate of 2.5% of the plasma pool of C1-inh per hour (38). Based on this catabolic rate, we calculated that a continuous infusion of 200 U/kg over 9 h would be necessary to sustain the increase in circulating C1-inh induced by a loading dose of 500 U of C1-inh/kg of body weight. Constant levels after the loading dose were indeed observed (Fig. 1), indicating that the fractional catabolic rate in humans can be used to calculate dosing of C1-inh to baboons.

A rise in activation products of various components of the complement system was observed immediately after starting the E. coli infusion. C1-inh supplementation abrogated the increase in plasma C4b, indicating efficient inhibition of classical pathway activation. By contrast, the appearance of C3b and C5b-9 was incompletely blocked by C1-inh, which indicates that circulating organisms may have directly activated the alternative pathway and/or that only a small percentage of classical pathway zymogens need to be activated to cleave their substrates in a catalytic manner, resulting in less efficient inhibition of activation downstream the cascade. In addition, the more pronounced effect of C1-inh administration on the generation of C3b as compared with C5b-9 generation may also support a bypass mechanism of activation of C5, which is mediated by reactive oxygen species with the formation of a novel terminal complex containing oxidized C5 rather than C5b (52, 53).

Notably, reduced activation of the complement cascade had no effect on the clearance of the infused E. coli bacteria, since circulating numbers of these organisms at 2 h postchallenge were similar in treatment and control groups. Thus, although complete inhibition of complement at the level of C5 may impair bacterial clearance (54), inhibition at the level of C1 apparently does not.

C5a is generally regarded to be the most powerful anaphylatoxin: i.v. administration of purified C5a to animals can induce hypotension (55), and pretreatment with anti-C5a Abs in a primate model of sepsis results in a recovery in mean arterial pressure (56). Moreover, it potently stimulates neutrophils to generate toxic oxygen radicals, to degranulate, and to aggregate (13). Our data show that downstream complement activation was only modestly blocked by C1-inh supplementation; effects of C1-inh are therefore not likely related to inhibition of C5a generation.

Infusion of E. coli was associated with a protracted drop in antigenic levels of factor XII and prekallikrein within 1 h of starting the experiment, which was impeded by supplementation with C1-inh (Fig. 5). Reduced levels of factor XII and prekallikrein in the control group likely reflected activation of the contact system (51). Despite the apparent inhibition of contact system activation in the treatment group, C1-inh administration was unable to prevent the severe hypotension observed upon E. coli infusion. This finding does not agree with a study by Pixley et al. showing that pretreatment with a mAb that inhibits activation of factor XII abrogated the secondary decline in arterial pressure observed in this model (51). Moreover, in further contrast to the data presented here, we demonstrated that blockade of the contact system by use of this anti-factor XII mAb modestly reduced the release of neutrophil elastase and t-PA and inhibited the generation of...

![Figure 3](http://www.jimmunol.org/)

**FIGURE 3.** (Anti-)fibrinolytic parameters in baboons upon lethal E. coli challenge. Mean ± SEM plasma levels of t-PA (A), plasmin-α2-antiplasmin complexes (B), and PAI-1 (C) in control (○) and C1-inh (□) groups. PAP complexes are expressed as percentage of NBP-MAUK. The difference between both groups by ANOVA repeated measurements were significant for PAI-1. NS, not significant.

Contact system proteases. Mean plasma levels of factor XII and prekallikrein declined by ~20 to 25% after 1 h in the untreated control group, which was statistically different from baseline values (Fig. 5, A and B; T + 0 to T + 10 h vs baseline: p < 0.05 by Fischer LSD). In contrast, in the animals receiving C1-inh treatment, factor XII levels remained relatively stable until the end of the observation period and were only significantly reduced at T + 10 h (Fig. 5A). Similarly, plasma prekallikrein did not decline until after 6 h, although this decrease was not statistically different from initial levels (Fig. 5B). Comparison of the untreated and treated groups indicated a significant difference in the 10-h course of factor XII and prekallikrein (p < 0.05).

Neutrophil degranulation. Elastase-α1-antitrypsin complexes were assayed in the plasma of both groups to study the effects of C1-inh administration on E. coli-induced neutrophil degranulation. In control animals, levels of these complexes steeply increased shortly after start of the bacterial challenge, reaching plateau levels from T + 3 h on, with maximal concentrations of 1491 ± 82 ng/ml at T + 10 h. C1-inh treatment neither affected kinetics nor levels of elastase complexes, and the highest concentrations were noted at 6 h (1448 ± 109 ng/ml; not shown).
PAP complexes, suggestive of the involvement of the contact system in neutrophil activation and fibrinolysis (41). The findings described here need to be reconciled with those data as well as with reported hemodynamic effects of C1-inh in septic patients (31, 32) and endotoxemic rabbits (34). In the present study, some degree of factor XII and prekallikrein activation is likely to have occurred at the C1-inh concentrations used (as suggested by slightly reduced factor XII and prekallikrein levels at the end of the observation period; Fig. 5). In addition, although bacteria or their products are likely candidates to have initiated activation of the contact system shortly after start of the challenge, negatively charged surfaces such as cell membranes and extracellular matrix exposed as a consequence of sepsis-induced tissue damage may have contributed to contact system activation during later stages of the septic process. We have previously reported that in vitro, various glycosaminoglycans may alter the relative contribution of serpins to inactivation of contact proteases, and a twofold protection of inhibition of α-factor XIIa and β-factor XIIa by C1-inh could be demonstrated in the presence of dextran sulfate (57). Moreover, activator-bound factor XIIa may not be accessible by C1-inh, whereas it can still be inhibited by Abs (58). The inactivation rate of C1-inh may therefore depend on the identity, localization, and phase (fluid or solid) of the in vivo activator, and parameters such as severity and distribution of organ damage and/or the model of sepsis employed may influence the relative efficacy of C1-inh supplementation. Thus, some activation of the contact system and the generation of bradykinin may have escaped inhibition by C1-inh, but not by anti-factor XII mAb, in this baboon model. We suggest that this may explain observed differences between the effects of C1-inh and anti-factor XII mAb (51).

![Figure 4](http://www.jimmunol.org/)

**FIGURE 4.** Mean ± SEM plasma levels of TNF (A), IL-10 (B), IL-6 (C), and IL-8 (D) in control (●) and C1-inh (○) groups after a lethal dose of live E. coli. Differences between groups were significant (p < 0.05) by ANOVA repeated measurements.

<table>
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<th>T + 2</th>
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<th>T + 4</th>
<th>T + 6</th>
<th>T + 8</th>
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<tr>
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<td>10 ± 5</td>
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<td>223 ± 35</td>
<td>964 ± 918</td>
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<td>2236 ± 314</td>
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<td>240 ± 67</td>
<td>918 ± 267</td>
<td>1554 ± 378</td>
<td>1668 ± 452</td>
<td>1140 ± 270</td>
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<td>390</td>
<td>1097</td>
<td>1146</td>
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*Values represent mean ± SEM changes in thrombin-antithrombin III (TAT) complexes (in ng/ml) after start of the challenge (T + 0). Individual TAT levels are given for the C1-inh treatment group. No significant difference was observed by ANOVA-repeated measurements.

*Surviving C1-inh-treated animals.*
Administration of high-dose C1-inh reduced circulating levels of TNF, IL-6, IL-8, and IL-10 elicited by lethal infusion with *E. coli*. Differences in cytokine release were not due to variations in bacterial challenge, since the number of organisms in the infusion fluid was similar for both treatment and control groups. Rather, our data suggest the possibility of links between activated complement and/or contact proteases and the cytokine response during severe sepsis. In vitro, the anaphylatoxins as well as bradykinin and factor XIIa can stimulate the synthesis and release of early response cytokines such as TNF, IL-1 and IL-6 (16–22). Recently, engagement of monocyte receptors for the fixed C3 fragments iC3b and C3b has been shown to induce IL-1 synthesis or synthesis and secretion, respectively (59, 60). Our results do not allow conclusion concerning the mechanism of reduced cytokine release upon 1-inh administration. However, considering the mild effects of this treatment on the activation of C3 and C5 (see Fig. 2), we favor the explanation that the attenuated cytokine release resulted from reduced generation of contact activation products.

Many symptoms of septic shock can be reproduced by direct infusion of TNF into animals, and anti-TNF Abs reduces LPS-induced mortality and abrogates many of the attendant manifestations of sepsis (61, 62). Among other effects, TNF has been shown to increase endothelial procoagulant activity (63) and induce the secretion of pro- and antiinflammatory cytokines such as IL-10, IL-6, and IL-8 (64–66). We show here that lowest TNF levels were measured in a treated surviving animal displaying retarded fibrinogen consumption and reduced thrombin formation. Survival benefit in this animal may therefore be interpreted to result indirectly from an impediment of systemic TNF release. On the other hand, we have previously reported that the progressive elaboration of IL-6 and IL-8 during the later stages in this model is associated with sepsis-induced tissue damage and death, which may occur despite the absence of circulating immunoreactive TNF (43, 49).

Accordingly, the ability of C1-inh to modulate ongoing production of IL-6 and IL-8 may go beyond a reduction of TNF release, and protective effects on *E. coli*-induced organ injury during C1-inh treatment may be linked to a direct interruption of these protagonists in the proinflammatory merge of cascades. Modulatory effects of C1-inh treatment on the cytokine response may also explain observed effect on PAI-1 release (Fig. 2C). Synthesis and release of PAI-1 from endothelial cells and hepatocytes is induced by IL-1, IL-6, and TNF (67–69). PAI-1 belongs to the serpin family and acts as a pseudosubstrate for t-PA and urokinase-type plasminogen activator, forming inactive t-PA/PAl or urokinase-type plasminogen activator/PAI complexes, respectively (70). However, since t-PA levels were unaffected by C1-inh supplementation, and PAP concentrations peaked before inhibitory effects on PAI became apparent, i.e., not until 4 h postchallenge, the attenuating effects of C1-inh on (anti)fibrinolysis are likely of secondary importance in this *E. coli* model.

Hematologic and biochemical response profiles revealed that the lethal effects of *E. coli* were related to the occurrence of disseminated intravascular coagulation and organ damage. None of the control animals lived beyond 27 h, while in the treatment group, one animal survived the challenge and another lived beyond 48 h. Moreover, pathologic examination revealed less severe damage to various organs in four of the five animals receiving C1-inh, consistent with reduced organ dysfunction during the later stages (Table III). Notably, we administered a twofold lower dose of C1-inh to two additional animals, one of which survived (data not shown). These findings, together with the observed effects on the elaboration of cytokines, support the notion that C1-inh does interfere with reactions that occur in the microenvironment of the plasma/target cell interface and show that C1-inh supplementation has a beneficial, although mild effect on the inflammatory and physiologic sequelae of lethal *E. coli* challenge.

In conclusion, we demonstrate here that administration of C1-inh to baboons suffering from lethal sepsis blocked classical complement activation and reduced the decrease in plasma levels of factor XII and prekallikrein. We suggest that, in this model, activated complement and/or contact system proteases may promote *E. coli*-induced organ injury and lethality, at least in part, by augmenting the cytokine response.

References

10. Hammerschmidt, D. E., L. D. Hudson, L. J. Weaver, P. R. Craddock, and H. S. Jacob. Association of complement activation and elevated plasma C5a with