The Third Component of Complement Protects against *Escherichia coli* Endotoxin–induced Shock and Multiple Organ Failure

By Zenaide M. N. Quezado,* William D. Hoffman,* Jerry A. Winkelstein,§ Ido Yatsiv,‖ Cezar A. Koev,* Linda C. Cork,§ Ronald J. Elin,‡ Peter Q. Eichacker,* and Charles Natanson*

Summary

We investigated whether the third component of complement (C3) is involved in the pathophysiology of endotoxemic shock, and if it is involved, whether it plays a protective role or whether it mediates shock and multiple organ failure. In a prospective, controlled investigation, six Brittany spaniels that were homozygous for a genetically determined deficiency of C3 (C3 deficient, <0.003% of normal serum C3 levels) and six heterozygous littermates (controls, =50% of mean normal serum C3 level) were given 2 mg/kg of reconstituted *Escherichia coli* 026:B6 acetone powder as a source of endotoxin, intravenously. All animals were given similar fluid and prophylactic antibiotic therapy, and had serial hemodynamic variables obtained. After *E. coli* endotoxin infusion, C3-deficient animals had higher peak levels of endotoxin and less of a rise in temperature than controls (P <0.05). During the first 4 h after *E. coli* endotoxin infusion, C3-deficient animals had significantly greater decreases in mean central venous pressure and mean pulmonary artery pressure than controls (P <0.02). During the first 48 h after *E. coli* endotoxin infusion, C3-deficient animals had significantly greater decreases in mean arterial pH, left ventricular ejection fraction, and mean pulmonary capillary wedge pressure, and greater increases in mean arterial lactate, arterial-alveolar O2 gradient, and transaminases (aspartate aminotransferase and alanine aminotransferase) than controls, (all P <0.05). After *E. coli* endotoxin infusion, C3-deficient animals compared to controls had significantly less of a decrease in mean C5 levels (P <0.01), but similar (P = NS) increases in circulating tumor necrosis factor levels, bronchoalveolar lavage neutrophils, and protein, and similar (P = NS) decreases in blood leukocytes and platelets. Two of six C3-deficient animals and two of six controls died. In summary, after intravenous infusion of *E. coli* endotoxin, canines with C3 deficiency have decreased endotoxin clearance and worse *E. coli* endotoxin–induced shock and organ damage. Thus, the third component of the complement system plays a beneficial role in the host defense against *E. coli* endotoxic shock.

Approximately 400,000 patients develop sepsis each year in the United States. Of these, 50% develop septic shock and multiple organ damage, which is associated with a mortality rate of 50–70% (1). In the pathogenesis of sepsis, endotoxin interacts with a number of endogenous mediators such as complement and clotting systems, bradykinin, arachidonic acid, TNF, and a variety of other cytokines (2). Each of these mediators has the potential to participate in the pathophysiology of endotoxic shock and organ damage. However, it is unknown what the relative contribution of each is to the pathogenesis of endotoxic shock and whether that contribution is beneficial or detrimental to the host.

Endotoxin is a potent activator of the complement system via either the classical or alternative pathways (3–5), can generate phlogistic cleavage products of C3 (C3a and C3b) and C5 (C5a), and can assemble the membrane attack complex (C5b-9) (6). In turn, these activated components of complement have the potential to opsonize particles for phago-
The third component of complement plays a critical role in the action of the complement system. Not only do the cleavage products of C3 (C3a and C3b) have direct inflammatory and defensive functions, but one of them (C3b) is a component of the enzymes that activate C5-C9 through the classical and alternative pathways. Therefore, C3 is critical in the generation of the inflammatory and defensive reactions of the complement system.

The current studies were performed in dogs with a genetically determined complete deficiency of C3 (32). Homozygous C3-deficient animals have <0.003% of normal amount of C3 and markedly decreased serum opsonic, chemotactic, and hemolytic activities (33). Furthermore, in animals of this size, it is technically possible to monitor serial hemodynamic, cardiovascular, pulmonary, hepatic, and renal functions, and administer fluid therapy, as is done in humans subjects. Thus, the C3-deficient dog offers a unique opportunity to determine whether C3 plays a significant role, in vivo, in the pathogenesis of endotoxin-induced shock and organ failure, and if so, whether this role is beneficial or detrimental.

Materials and Methods

Experimental Subjects

Six adult C3-deficient (homozygotes) Brittany spaniels with a genetically determined complete deficiency of C3 (32, 33) and six littermate controls (heterozygotes) were used in this investigation. Heterozygous animals have a normal complement system function and are clinically asymptomatic. Because heterozygotes come from the same line-bred colony and are genetically closely related to homozygotes, they were used as controls. Animals were studied in pairs composed of one C3-deficient animal and one control. Sex, weight, and age were closely matched.

Endotoxin Preparation

Escherichia coli acetone powder, strain ATCC 12795, serotype number 026:B6 (Sigma Chemical Co., St. Louis, MO) was utilized as the source of endotoxin and is referred to as E. coli endotoxin (34). The product was provided as a powder, without any viable cells. Using sterile techniques and pyrogen-free instruments and glassware, the E. coli endotoxin powder was reconstituted with 0.9% saline 30 min before intravenous administration.

Experimental Protocol

Fig. 1 shows the treatments given and evaluations obtained during this study. Hemodynamic and laboratory evaluations were performed in awake animals. On each study day listed in Fig. 1, using local anesthesia (lidocaine 1%), animals had an 8 Fr introducer sheath percutaneously placed into the external jugular vein, and a 20-Ga single lumen catheter into the femoral vein, which were removed each day after completion of laboratory and hemodynamic studies. Ceftriaxone (Roche, Nutley, NJ), 100 mg/kg i.v., was given prophylactically each day in which venous or arterial lines were placed, and for five consecutive days after endotoxin infusion. All blood cultures from the times specified in Fig. 1 were negative for bacterial pathogens in C3-deficient animals and controls. Ringer’s solution (10 ml/kg/h body weight [bw]) was given continuously for 8 h after endotoxin infusion. Animals had unrestricted access to food and water throughout the study except for 12 h before endotoxin infusion.

Endotoxin Infusion. 2 mg/kg of E. coli acetone powder as source of endotoxin was administered intravenously to each dog in a total volume of 100 ml of saline over 30 min. The 2-mg/kg dose was used because, in previous experiments with normal canines, this dose produced the cardiovascular, pulmonary, and hematologic abnormalities of endotoxin shock with minimal mortality (34). It was necessary to use a nonlethal dose of endotoxin in order to preserve the valuable colony of C3-deficient Brittany spaniels.

Hemodynamic Measurements. Values were obtained from femoral arterial and balloon flotation thermocoupling pulmonary arterial catheters using previously described techniques (35) and included mean arterial pressure (MAP, mm Hg), heart rate (HR/min) central venous pressure, pulmonary arterial pressure (PCWP, mm Hg), mean pulmonary arterial pressure (mm Hg), and cardiac output (ml/min). To determine left ventricular ejection fraction (LVEF), we performed radionuclide-gated blood pools scans using conventional techniques (35). Hemodynamic data

---

1 Abbreviations used in this paper: A-a O2, alveolar-arterial oxygen gradient; ALT, alanine aminotransferase; AST, aspartate aminotransferase; BAL, bronchoalveolar lavage; CoVF, cobra venom factor; CVP, central venous pressure; ESVI, end-systolic volume index; LVEF, left ventricular ejection fraction; MAP, mean arterial pressure.
were indexed to body weight in kilograms. The following values were calculated according to standard formulas: cardiac index, stroke volume index (SVI), left ventricular stroke work index, systemic vascular resistance index (SVRI), oxygen extraction ratio (ER), and alveolar-arterial oxygen gradient (A-a O2, kPa). End-diastolic volume index (EDVI) and end-systolic volume index (ESVI, ml/kg) were calculated from catheter measurements and simultaneously obtained radiouclide scans using the formulas EDVI = SVI/EF and ESVI = EDVI - SVI. After collection of the first hemodynamic evaluation, two fluid challenges of Ringer's solution, one of 15 ml/kg and another of 45 ml/kg, were given over 30 min, and temperature and all hemodynamic studies were repeated after each fluid challenge, except ejection fraction was repeated only after the second fluid challenge because of time constraints. In addition, arterial pH, arterial and mixed venous partial pressures of oxygen (pO2, kPa), and carbon dioxide (pCO2, kPa) were measured at 37°C with a blood gas system (model 288; Radiometer, Medfield, MA), and blood lactate levels were measured using a glucose-lactate analyzer (YSI, model 2300 STAT, Yellow Springs Instrument Co., Yellow Springs, OH).

Bronchoscopy and Bronchoalveolar Lavage (BAL). On days -7, 1, 2, and 10, after collection of all hemodynamic values (3 h), the animals were anesthetized. After anesthesia was established with ketamine (0.5 mg/kg bw), and muscle relaxation with succinylcholine (1 mg/kg bw), animals were intubated and bronchoscopy and BAL were performed using previously described techniques (36). On day 0, a BAL was not performed to minimize the potential effects of general anesthesia on hemodynamics during the first 48 h after E. coli endotoxin infusion. During bronchoscopy, animals were mechanically ventilated to have a minute volume of 0.35 liter/kg/min with a fractional inspired oxygen concentration of 0.40. After BAL (~30-min duration), animals were allowed to recover from anesthesia and extubated. Subsequent BALs in each animal were performed alternating right and left lungs.

Laboratory Measurements. Levels of C5 were measured by a functional hemolytic assay (37). Total and differential blood white cell count (10⁹/liter), hemoglobin, and platelet count (10⁹/liter) were measured on an automatic analyzer (model STK-S; Coulter Corp., Hialeah, Fl.; MetPath Laboratory, Rockville, MD). Partial thromboplastin time, prothrombin time, fibrinogen (g/liter), and fibrin split products were measured using a fibrometer (BBL, Baltimore, MD; MetPath Laboratory). Serum sodium, potassium, chloride, total carbon dioxide, calcium, phosphorus, glucose, blood urea nitrogen (mmol/liter), creatinine (mmol/liter), uric acid, alanine aminotransferase (ALT, U/liter), aspartate aminotransferase (AST, U/liter), γ-glutamyl transpeptidase, alkaline phosphatase, lactate dehydrogenase (U/liter), total bilirubin, triglycerides, and cholesterol were measured by an automated chemistry analyzer (model AU 500; Olympus, Irving, TX; MetPath Laboratory). Endotoxin concentration (EU/ml) was determined from heparinized plasma, which was diluted, heat treated, and then assayed using a modification of the chromogenic Limulus amebocyte lysate assay (Whittaker M.A. Bioproducts, Walkersville, MD) (38). Serum TNF level (ng/ml) was measured by a cytotoxicity assay using previously described methods and employing WEHI-164 cells (American Type Culture Collection, Rockville, MD) (39). BAL fluid total and differential cell counts (10⁶/liter) were obtained using an electronic cell counter (ZB1; Coulter Corp.), and BAL protein concentration (µg/ml) was measured using the BCA protein assay technique (Pierce, Rockford, IL) (40).

Statistical Methods

Hemodynamic data were analyzed using a four-way analysis of variance (ANOVA) (41). The four factors included group (C3-deficient animals and controls), dog nested within group, time, and fluid. In addition to these four main effects, all two- and three-way interactions with group, time, and fluid were included in the model. The group–time interaction was used to assess the similarity of the hemodynamic time course in the two groups, and this term was statistically decomposed to detect significance at various time points. The analysis revealed that interactions involving fluid were nonsignificant, thus figures show hemodynamic data averaged over fluid loadings. Laboratory studies, including C5, TNF, and BALs, were analyzed with a three-way ANOVA using group, dog, and time effects. Baseline differences were examined with one-way ANOVA. Since there were no significant differences in baseline values among treatment groups, the data are presented as changes from a common origin.

Endotoxin data were analyzed by determining the peak endotoxin response in each dog, and then calculating the difference between C3-deficient and controls done on similar days. One of these differences was excluded as an outlying value (P < 0.001), and the remaining differences were analyzed using a one-sample Wilcoxon test (42).

Animal Care

This protocol was approved by the Animal Care and Use Committee of the Clinical Center of the National Institutes of Health and Johns Hopkins University School of Medicine. All efforts were undertaken to minimize animal pain and suffering.

Results

Clinical Manifestations and Survival

After E. coli endotoxin infusion, all animals had similar signs of endotoxemia, appearing weak, lethargic, and anorectic. During the first 48 h after E. coli endotoxin infusion, C3-deficient animals had significantly less of a rise in temperature than controls (Fig. 2). Two out of six C3-deficient animals and two out of six controls died (Fig. 2).

Figure 1. Sequence of evaluations and therapies during this study. (HEM) Hemodynamic evaluation, including radionuclide heart scan, and measurements obtained from pulmonary (thermodilution) and femoral arterial catheters; (LAB) laboratory evaluation including C5, endotoxin and TNF levels, routine chemistries, quantitative blood cultures, complete blood counts, and coagulation studies; (BAL) bronchoscopy and bronchoalveolar lavage; (VOL) volume infusion of 15 ml/kg and 45 ml/kg of Ringer's solution. At -168, 8, 24, 48, and 240 h, hemodynamic evaluation was obtained before and after each of the two volume infusions.
Figure 2. Serial (mean ± SEM) changes in temperature in C3-deficient animals and controls. In parentheses are shown number of survivors at each time point. (*)P = 0.049, comparing C3-deficient animals and controls at comparable time points.

Figure 3. Serial (mean ± SEM) endotoxin concentrations. P = 0.05 comparing peak endotoxin levels in C3-deficient animals and controls.

Figure 4. Serial (mean ± SEM) acute changes in hemodynamic values. (*)P = 0.01, (**)P = 0.02, comparing C3-deficient animals and controls at comparable time points.
Endotoxemia Levels

C3-deficient animals had higher mean peak levels of endotoxin compared with controls \( (P = 0.05, \text{Fig. 3}) \).

Cardiopulmonary and Metabolic Variables

Early Response to E. coli Endotoxin. For the first 4 h after E. coli endotoxin infusion, both groups had similar significant decreases in mean MAP. However, C3-deficient animals had significantly greater decreases in mean CVP and mean pulmonary artery pressure, and greater decreases in mean PCWP \( (P = 0.07) \) than controls (Fig. 4). C3-deficient animals had significantly less of an increase in pO2 after 2 h \((1.2 \pm 0.7 \text{ kPa}[\text{mean} \pm \text{SEM}], P = 0.03)\), and after 4 h \((0.9 \pm 0.5 \text{ vs } 2.7 \pm 0.4 \text{ kPa}, P = 0.03)\), and less of a decrease in mean pCO2 after 4 h \((-0.9 \pm 0.1 \text{ vs } -1.7 \pm 0.3 \text{ kPa}, P = 0.04)\) compared with controls. During the first 4 h, all other serial hemodynamic values (data not shown) outlined in Materials and Methods were similar between the two groups \( (P = \text{NS}) \).

Late Response to E. coli Endotoxin. During the first 48 h after E. coli endotoxin infusion, C3-deficient animals had significantly greater increases in mean arterial lactate, and greater decreases in mean arterial pH than controls (Fig. 5). During the first 48 h, C3-deficient animals also had significantly greater increases in mean A-a O2 gradient, and greater decreases in mean LVEF and PCWP than controls (Fig. 6). For the first 48 h, the other serial hemodynamic values outlined in Materials and Methods were similar in C3-deficient and control groups \( (P = \text{NS}) \), except C3-deficient animals had significantly greater increases in ESVI \((2.0 \pm 0.5 \text{ vs } 0.4 \pm 0.3 \text{ ml/kg}^{-1}[\text{mean} \pm \text{SEM}], P = 0.009)\), less of a decrease in mean pCO2 \((-0.5 \pm 0.1 \text{ vs } -0.9 \pm 0.1 \text{ kPa}, P = 0.03)\), and less of a decrease in mean pO2 at 24 h \((-1.1 \pm 0.7 \text{ vs } 0.8 \pm 0.8 \text{ kPa}, P = 0.03)\). In survivors, by day 10, all hemodynamic variables returned to mean baseline values \( (P = \text{NS}) \), except C3-deficient animals still had a significantly \( (P < 0.05) \) lower mean CVP (data not shown), and temperature (Fig. 2) compared with controls.

BAL. During the 48 h after E. coli endotoxin infusion, C3-deficient animals and controls had similar \( (P > 0.68) \) significant increases in mean percent and total number of neutrophils in BAL \( (P < 0.006, \text{Table 1}) \). Also during this time period, C3-deficient animals and controls alike had increases...
Table 1. Serial Geometric Mean BAL Cells and Protein before and after E. coli Endotoxin Infusion

| Time | C3 deficient | Control | C3 deficient | Control | C3 deficient | Control | C3 deficient | Control |
|------|--------------|---------|--------------|---------|--------------|---------|--------------|---------|
| h    |              | %       |              |         |              |         |              |         |
| -168 | 9 ± 1.8      | 11 ± 1.5| 3 ± 2        | 3 ± 1   | 0.01 ± 7.8   | 0.02 ± 5.9| 11 ± 1.3    | 20 ± 1.1|
| 24   | 17 ± 1.2     | 11 ± 1.3| 17 ± 6      | 30 ± 11 | 2.0 ± 1.9    | 2.0 ± 2.2| 14 ± 1.7    | 17 ± 2.1|
| 48   | 10 ± 1.1     | 20 ± 1.7| 33 ± 11     | 36 ± 12 | 2.0 ± 2.3    | 4.0 ± 3.4| 30 ± 1.8    | 30 ± 1.5|

in BAL fluid protein that did not reach statistical significance (both P = 0.065, Table 1). The other serial laboratory values determined in BAL fluid outlined in Materials and Methods were also similar in C3-deficient animals and controls throughout (P = NS).

Hepatic, Renal, and Hematologic Changes

During the 48 h after E. coli endotoxin infusion, C3-deficient animals had greater increases in mean AST and ALT compared with controls (Table 2). Although mean creatinine values were within the normal range (71–177 μmol/liter, MetPath Laboratory) throughout the experiment, controls had a significant decrease in mean creatinine at 24 h, which was not seen in C3-deficient animals (data not shown, P = 0.02).

After E. coli endotoxin infusion, C3-deficient animals and controls alike had early (0.5, 1, 2, and 4 h) significant decreases (both P <0.0001, Table 3) and late (24 and 48 h) significant increases (both P <0.02) in white blood cell counts. After E. coli endotoxin infusion, C3-deficient animals and controls alike had early (0.5, 1, 2, and 4 h) significant decreases (both P <0.0001, Table 3) and late (24 and 48 h) significant increases (both P <0.009, data not shown) in platelet count.

Table 2. Serial Geometric Mean Transaminases before and after E. coli Endotoxin Infusion

| Time | C3 deficient | Control | C3 deficient | Control | C3 deficient | Control |
|------|--------------|---------|--------------|---------|--------------|---------|
| h    |              |         |              |         |              |         |
| -168 | 42.3 ± 1.16  | 55.2 ± 2.62| 41.6 ± 1.37  | 62.4 ± 2.95 |
| 24   | 242.4 ± 1.48*| 93.6 ± 1.56| 240.8 ± 1.46*| 79.4 ± 1.23 |
| 48   | 56.5 ± 1.24  | 45.3 ± 1.21| 150.5 ± 1.44*| 69.9 ± 1.22 |

* P <0.04. P value compares C3 deficient and controls at comparable time points.

Complement and TNF Levels

At 1 and 4 h after E. coli endotoxin infusion, C3-deficient animals had significantly less of a decrease in mean C5 levels (P = 0.01) than controls (Table 3). At 48 h, C3-deficient animals had a significantly greater increase in mean C5 levels (P = 0.002) than controls (Table 4). At 0.5, 1, and 2 h after E. coli endotoxin, C3-deficient animals and controls, had similar (P = NS) significant increases in circulating TNF levels (both P = 0.0001, Fig. 7).

Discussion

After E. coli endotoxin challenge, dogs with a genetically determined deficiency of C3 had higher levels of endotoxemia and developed more severe cardiovascular and pulmonary dysfunction, hepatic injury, and lactic acidosis than did their littermate controls. Thus, the results of this study demonstrate that C3 plays a significant role in protecting the host against E. coli-endotoxin-induced shock and organ damage.

After intravenous challenge with E. coli endotoxin, C3-deficient animals had a greater degree of endotoxemia than did control animals. Presumably, the lack of C3 led to ineffective clearance of the endotoxin from the bloodstream because of defective C3b-mediated opsonization and phagocytosis of...
Table 3. Serial Geometric Mean White Blood Cell and Platelet Counts before and after E. coli Endotoxin Infusion

| Time (h) | C3 deficient | Control |
|---------|--------------|---------|
| -0.5    | 10.8 ± 1.10  | 13.2 ± 1.10 |
| 0.5     | 3.2 ± 1.21   | 3.1 ± 1.15  |
| 1       | 3.2 ± 1.07   | 3.3 ± 1.16  |
| 2       | 4.9 ± 1.16   | 3.4 ± 1.13  |
| 4       | 4.9 ± 1.23   | 6.5 ± 1.22  |

Table 4. Serial Mean (%) Changes from Baseline in C5 Levels after E. coli Endotoxin Infusion

| Time (h) | C3 deficient | Control |
|---------|--------------|---------|
| 1       | 88 ± 5       | 76 ± 14†|
| 4       | 95 ± 26      | 77 ± 22†|
| 24      | 90 ± 7       | 99 ± 28  |
| 48      | 218 ± 38†    | 105 ± 41 |

* Percent baseline value.
† P = 0.01.
‡ P = 0.002. P values compare C3 deficient and control at comparable time points.

The mechanism by which C3-deficient animals developed worse shock and organ damage is unknown. It is conceivable that sustained elevated levels of endotoxin led to greater release of potentially harmful endogenous mediators. Of note, levels of TNF, a key mediator of septic and endotoxic shock, were similar despite significantly different endotoxin levels between C3-deficient animals and controls. Although circulating TNF levels may not reflect local or total production of this cytokine, these data suggest that C3-beneficial effects are through mechanisms other than decreases in TNF release into or decreases in TNF clearance from the systemic circulation. Endotoxin also promotes release of prostaglandins, lipooxygenase products, and bradykinin. Consequently, one could postulate that prolonged exposure to high endotoxin levels induced overproduction of these other potentially toxic mediators, leading to more severe organ damage. Alternatively, lack of complement activation could possibly cause some protective mediators not to be released or to be released in smaller quantities.

Interestingly, C3-deficient animals had less of a febrile re-
The degree of febrile response to E. coli endotoxin in heterozygous controls, but not C3-deficient animals, was similar to normal beagles given endotoxin (43). In vitro studies have shown that C5a and C5a des arg induce secretion of IL-1, a recognized endogenous pyrogen, by human mononuclear phagocytes (7, 8). It is possible, therefore, that the lack of a febrile response to endotoxin by C3-deficient animals is related to decreased production of IL-1 and/or other endogenous pyrogens. However, C5a also stimulates secretion of TNF from human mononuclear cells in vitro (8), but in this study, serial TNF levels were similar in C3-deficient animals and controls. Nonetheless, although the mechanism of the decrease febrile response to E. coli endotoxin in C3-deficient animals is unknown, this finding suggests that C3 promotes the febrile response to endotoxin.

The role of the complement system in endotoxin-induced coagulopathy is not fully defined. In canines, (24) CoVIDF-induced complement deficiency ameliorates endotoxin-induced disseminated intravascular coagulation. However, these findings were not confirmed in rabbits with a genetically determined C6 deficiency (25). In the present investigation, after E. coli endotoxin infusion, C3-deficient animals and controls alike developed abnormalities suggestive of disseminated intravascular coagulation including elevations of prothrombin and partial thromboplastin times, and decreases in platelet count, but without increases in fibrin degradation product. The cause of the greater decrease in fibrinogen levels in C3-deficient animals in the first hour after E. coli endotoxin infusion is unknown. Nevertheless, these findings in total suggest that C3 does not appear to play an important role in endotoxin-induced coagulopathy.

The influx of neutrophils into the lungs in C3-deficient animals and littermate controls was remarkably similar, suggesting that C3 is not essential for neutrophil migration. Contrary to in vitro and in vivo studies that have suggested that complement activation plays a role in the lung injury associated with sepsis, the results of this study demonstrate that C3-deficient animals, with less activation of complement (higher C5 levels), had worse lung injury, as manifested by higher A-a gradients (despite lower cardiac filling pressures). Increased leakage of protein into the alveolar space after E. coli endotoxin infusion (which approached statistical significance) was similar in C3-deficient animals and controls. It is possible that other potentially more sensitive measures of lung function (compliance) and injury (histology, wet/dry lung ratio), not obtained in this investigation, might have further elucidated the mechanism of worse pulmonary injury in C3-deficient animals. Nevertheless, in this study, C3 actually protects against endotoxin-induced lung injury, and neutrophil recruitment to the lung does not require C3.

A number of studies in patients have shown that there is an association between the degree of complement activation and the severity of endotoxic shock and mortality (15, 18, 44). The results of the present study suggest that the degree of complement activation in humans is not necessarily causally related to the development of shock and lethality (15, 18). In this study, control animals with more complement activation had less, not more, organ injury (heart, lung, and liver) than C3-deficient animals. Thus, in patients with septic shock, greater complement activation may represent only a marker of more severe endotoxemia and/or disease.

It is important to recognize that this study demonstrates the net effect of C3 in E. coli endotoxic shock. It is still possible that the activation of C3 in E. coli endotoxin shock is both beneficial, by playing an important role in the clearance of endotoxin, and detrimental, by participating in the activation of C5-C9 and generating their inflammatory effects. In support of this are studies in primates with gram-negative bacteremia (28) and rats with endotoxemia (30) demonstrating that treatment of these animals with anti-C5a antibody decreases mortality and attenuates adult respiratory distress syndrome. Thus, it is possible that interfering with the activation of C5-C9, but leaving certain functions of C3 intact, would be beneficial in septic shock. It is also important to recognize that, because in this study, we used E. coli endotoxin reconstituted from an acetone powder as the source of endotoxin, it is possible that components of acetone-treated bacteria other than endotoxin may have played a role in the protection against shock and organ injury afforded by C3.

This study raises important questions about potential therapies for the treatment of septic shock which are aimed at inhibiting and neutralizing the host inflammatory response (45). Although data from a variety of studies suggest that a number of endogenous mediators may play harmful roles in the pathophysiology of septic shock (15-18), it is clear from this and other studies (20-22) that these inflammatory mediators also play a pivotal role in host defense. In human septic shock, new antiinflammatory agents may likely be beneficial by inhibiting the harmful effects of these host mediators. However, blockade of certain components of the inflammatory cascade such as the complement system could also result in less optimal host defense and worsen outcome (46).

The authors thank Dr. Steven M. Banks, for doing the statistical analysis; Gary L. Akin, Donald P. Dolan, Allen T. Hilton, Stephen Richmond, Laura Wilson, and Beth A. Winkelstein for providing technical support during the study; Dr. Victoria Hampshire for giving veterinary care; Scott Groene for measuring TNF levels; and Pat Madara for measuring BAL fluid protein.

Address correspondence to Dr. Zenaide M. N. Quezado, Critical Care Medicine Department, National Institutes of Health, Building 10, Room 7D43, 9000 Rockville Pike, Bethesda, MD 20892.

Received for publication 30 August 1993 and in revised form 12 November 1993.

C3 Deficiency in E. coli Endotoxin–induced Shock/Multiple Organ Failure
References

1. Parrillo, J.E., M.M. Parker, C. Natanson, A.F. Suffredini, R.L. Danner, R.E. Cunnion, and F.P. Ognibelone. 1990. Septic shock in humans. Advances in the understanding of pathogenesis, cardiovascular dysfunction and therapy. Am. Intern. Med. 113: 227.

2. Danner, R.L., A.F. Suffredini, C. Natanson, and J.E. Parrillo. 1989. Microbial toxins: role in the pathogenesis of septic shock and multiple organ failure. In New Horizons: Multiple Organ Failure. F.B. Cerra, editor. Society of Critical Care Medicine. Williams & Wilkins Co., Baltimore. 151–191.

3. Marcus, R.L., H.S. Shin, and H.H. Mayer. 1971. Alternative complement pathway: C3-deactivating activity not due to C4,2a on endotoxic lipopolysaccharide after treatment with guinea pig serum; relation to properdin. Proc. Natl. Acad. Sci. USA. 68:1351.

4. Snyderman, R., and M.C. Pike. 1975. Interaction of complex polysaccharide with the complement system: effect of calcium depletion on terminal component consumption. Infect. Immun. 11:273.

5. Morrison, D.C., and L.F. Kline. 1977. Activation of the classical and properdin pathways of complement by bacterial lipopolysaccharides (LPS). J. Immunol. 118:362.

6. Mergenhagen, S.E., R. Snyderman, H. Gewurz, and H.S. Shin. 1969. Significance of complement to the mechanism of action of endotoxin. Curr. Top. Microbiol. Immunol. 50:37.

7. Okusawa, S., C.A. Dinarello, K.B. Yancey, S. Endres, T.J. Lawley, M.M. Frank, J.F. Burke, and J.A. Gelfand. 1987. C5a induction of interleukin 1. Synergistic effect with endotoxin or interferon gamma. J. Immunol. 139:2635.

8. Okusawa, S., K.B. Yancey, J.W.M. van der Meer, S. Endres, G. Lonnemann, K. Hefer, M.M. Frank, J.F. Burke, C.A. Dinarello, and J.A. Gelfand. 1988. C5a stimulates secretion of tumor necrosis factor from human mononuclear cells in vitro. Comparison with secretion of interleukin 1β and interleukin 1α. J. Exp. Med. 168:443.

9. Sacks, T., C.F. Moldow, P.R. Craddock, and T.K. Bowers. 1978. Oxygen radicals mediate endothelial cell damage by complement-stimulated granulocytes. An in vitro model of immune vascular damage. J. Clin. Invest. 61:161.

10. Craddock, R.P., D.E. Hammerschmidt, J.G. White, A.P. Dalmasso, and H.S. Jacob. 1977. Complement (C5a)-induced granulocyte aggregation in vitro. J. Clin. Invest. 60:260.

11. Tonnensen, M.G., L.A. Smedly, and P.M. Henson. 1984. Neutrophil-endothelial cell interactions. Modulation of neutrophil adherence induced by complement fragments C5a and C5a des arg and formyl-methionyl-leucyl-phenylalanine in vitro. J. Clin. Invest. 74:158.

12. Fehr, J., and H.S. Jacob. 1977. In vitro granulocyte adherence and in vivo margination: two associated complement–dependent functions. Studies based on the acute neutropenia of filtration and leukophoresis. J. Exp. Med. 146:641.

13. Bjork, J., T.E. Hugli, and G. Smedegard. 1985. Microvascular effects of anaphylatoxins C3a and C5a. J. Immunol. 134:1115.

14. Jose, FJ., M.J. Forrest, and T.J. Williams. 1981. Human C5a des arg increases vascular permeability. J. Immunol. 127:2376.

15. Fearn, D.T., S. Ruddy, P.H. Schur, and W.R. McCabe. 1975. Activation of the properdin pathway of complement in patients with gram-negative bacteria. N. Engl. J. Med. 292:937.

16. Sprung, C.L., D.R. Schultz, E. Marcial, P.V. Caralis, M.A. Gelbard, P.L. Arnold, and W.M. Long. 1986. Complement activation in septic shock patients. Crit. Care Med. 14:525.

17. Füst, G., G. Petras, and E. Ujhelyi. 1976. Activation of the complement system during infections due to gram-negative bacteria. Clin. Immunol. Immunopathol. 5:293.

18. Brandtzæg, P., T.E. Molines, and P. Kierulf. 1989. Complement activation and endotoxin levels in systemic meningococcal disease. J. Infect. Dis. 160:58.

19. From, A.H.L., H. Gewurz, R.P. Gruninger, R.J. Pickering, and W.W. Spink. 1970. Complement in endotoxic shock: effect of complement depletion on the early hypotensive phase. Infect. Immun. 2:38.

20. Johnson, K.J., and P. Ward. 1971. Protective function of C6 in rabbits treated with bacterial endotoxin. J. Immunol. 106:1125.

21. Johnson, K.J., and P. Ward. 1972. The requirement for serum complement in the detoxification of bacterial endotoxin. J. Immunol. 108:611.

22. May, J.E., M.A. Kane, and M.M. Frank. 1972. Host defense against bacterial endotoxemia-contribution of the early and late components of complement to detoxification. J. Immunol. 109:893.

23. Kane, M.A., J.E. May, and M.M. Frank. 1973. Interaction of the classical and alternate complement pathway with endotoxin lipopolysaccharide. Effect on platelets and blood coagulation. J. Clin. Invest. 52:370.

24. Garner, R., B.V. Chater, and D.L. Brown. 1974. The role of complement in endotoxic shock and disseminated intravascular coagulation: experimental observations in the dog. Br. J. Haematol. 28:393.

25. Ulevitch, R.J., C.G. Cochrane, P.M. Henson, D.C. Morrison, and W.F. Doe. 1975. Mediation systems in bacterial lipopolysaccharide–induced hypotension and disseminated intravascular coagulation. I. The role of complement. J. Exp. Med. 142:1570.

26. Ulevitch, R.J., and C.G. Cochrane. 1978. Role of complement in lethal lipopolysaccharide-induced hypotensive and coagulative changes. Infect. Immun. 19:204.

27. Ulevitch, R.J., C.G. Cochrane, K. Bangs, C.M. Herman, J.R. Fletcher, and C.L. Rice. 1978. The effect of complement depletion on bacterial lipopolysaccharide (LPS)–induced hemodynamic and hematologic changes in the Rhesus monkey. Am. J. Pathol. 92:227.

28. Stevens, J.H., P. O’Hanley, J.M. Shapiro, F.G. Mihn, P.S. Satoh, J.A. Collins, and T.A. Rafin. 1986. Effects of anti-C5 antibodies on the adult respiratory distress syndrome in septic primates. J. Clin. Invest. 77:1812.

29. Flick, M.R., J.K. Horn, J.M. Hoeffel, and I.M. Goldstein. 1986. Reduction of total hemolytic complement activity with Naïve haje koala venin factor does not prevent endotoxin-induced lung injury in sheep. Am. Rev. Respir. Dis. 133:62.

30. Smedegard, G., L. Cui, and T.E. Hugli. 1989. Endotoxin-induced shock in the rat. A role for C5a. Am. J. Pathol. 135:489.

31. Fink, M.P., H.R. Rothschild, Y.F. Deniz, and S.M. Cohn. 1993. Complement depletion with Naïve haje koala venom factor limits prostaglandin release and improves visceral perfusion in porcine endotoxic shock. J. Trauma. 1989:1076.

32. Winkelstein, J.A., L.C. Cork, D.E. Griffin, J.W. Griffin, R.J. Adams, and D.L. Price. 1981. Genetically determined deficiency of the third component of complement in the dog. Science (Wash. DC). 212:1169.

33. Winkelstein, J.A., J.P. Johnson, A.J. Swift, F. Ferry, R. Yolken, and L.C. Cork. 1982. Genetically determined deficiency of the third component of the complement in the dog: in vitro studies on the complement system and complement-mediated serum activities. J. Immunol. 129:2598.

34. Guerrero, R., F. Velasco, M. Rodriguez, A. Lopez, R. Rojas,
M.A. Álvarez, R. Villalba, V. Rubio, A. Torres, and D. del Castillo. 1993. Endotoxin-induced pulmonary dysfunction is prevented by Cl-esterase inhibitor. J. Clin. Invest. 91:2754.

35. Natanson, C., M.P. Fink, H.K. Ballantyne, T.J. MacVittie, J.J. Conklin, and J.E. Parrillo. 1986. Gram-negative bacteremia produces both severe systolic and diastolic cardiac dysfunction in a canine model that simulates human septic shock. J. Clin. Invest. 78:259.

36. Eichacker, P.Q., W.D. Hoffman, A. Farese, S. Banks, G.C. Kuo, T.J. MacVittie, and C. Natanson. 1991. TNF but not IL-1 in dogs causes lethal lung injury and multiple organ dysfunction similar to human sepsis. J. Appl. Physiol. 71:1979.

37. Shin, H.S., R.J. Pickering, and M.M. Mayer. 1971. The fifth component of the guinea pig complement system. J. Immunol. 106:473.

38. Natanson, C., R.L. Danner, R.J. Elin, J.M. Hosseini, K.W. Peart, S.M. Banks, T.J. MacVittie, and J.E. Parrillo. 1989. The role of endotoxemia in cardiovascular dysfunction and mortality: Escherichia coli and Staphylococcus aureus challenges in a canine model of human septic shock. J. Clin. Invest. 83:243.

39. Eichacker, P.Q., W.D. Hoffman, A. Farese, A.F. Suffredini, Y. Waisman, S.M. Banks, T.C. Mouginis, L. Wilson, R. Rothlein, R.J. Elin, et al. 1993. Leukocyte CD18 monoclonal antibody worsens endotoxemia and cardiovascular injury in canines with septic shock. J. Appl. Physiol. 74:1885.

40. Smith, P.K., R.L. Krohn, G.T. Hermanson, A.K. Mallia, F.H. Gartner, M.D. Provenzano, E.K. Fujimoto, N.M. Goeke, B.J. Oslo, and D.C. Klenk. 1985. Measurement of protein using bicinecholinic acid. Anal. Biochem. 150:76.

41. Scheffe, H. 1959. The Analysis of Variance. John Wiley & Sons, Inc., New York. 90–136.

42. Siegel, S. 1956. Nonparametric Statistics for the Behavioral Sciences. McGraw-Hill, Inc., New York. pp 20–77.

43. Cobb, J.P., C. Natanson, W.D. Hoffman, R.F. Lodato, S. Banks, C.A. Koev, M.A. Solomon, R.J. Elin, J.M. Hosseini, and R.L. Danner. 1992. Nω-amino-L-arginine, an inhibitor of nitric oxide synthase, raises vascular resistance but increases mortality rates in awake canines challenged with endotoxin. J. Exp. Med. 176:1175.

44. Hack, C.E., J.H. Nuijens, R.J.F. Felt-bersma, W.O. Schreuder, A.J.M. Eerenberg-Belmer, J. Paardekooper, W. Bronsveld, and L.G. Thijs. 1989. Elevated plasma levels of the anaphylatoxins C3a and C4a are associated with a fatal outcome in sepsis. Am. J. Med. 86:20.

45. Lowry, S.F. 1993. Anticytokine therapies in sepsis. New Horizons. 1:120.

46. Dinarello, C.A. 1992. Role of interleukin-1 in infectious disease. Immunol. Rev. 127:119.

578 C3 Deficiency in E. coli Endotoxin–induced Shock/Multiple Organ Failure