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

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Summary

We investigated whether the third component of complement (C3) is involved in the pathophysiology of endotoxic 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 O_2 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-

Portions of this work have appeared in abstract form (1992 *Clin Res* 40 153A, 1993 *Clin Res* 41 243; and 1993 *Crit. Care Med.* 21 S285)

cytic ingestion (6), stimulate human mononuclear cells to secrete TNF and IL-1 (7, 8), promote neutrophil chemotaxis, aggregation, degranulation, and generation of superoxide anions radicals (9–12), and increase vascular permeability (13, 14). In addition, a number of studies have demonstrated that the complement system is activated in patients with gram-negative sepsis, and that the degree of activation is related to the severity of shock and death (15–18). Thus, there is a great deal of evidence that complement may participate in endotoxic shock and multiple organ failure.

However, studies in experimental animals have yielded conflicting data as to whether the complement system contributes to the pathophysiology of endotoxic shock and multiple organ failure (19–30). These studies have generally been performed in animals that have been pharmacologically depleted of complement using cobra venom factor (CoVF)[1] (24–27, 29, 31), in animals that have a genetically determined deficiency of a complement component (20–23), or in animals that have been treated with antibody against phlogistic cleavage products of individual complement components (28, 30). In some studies, the complement system has been shown to protect the host from the lethal effects of endotoxin (20–22). In others, it has been shown to mediate some of the hemodynamic and hematologic consequences of endotoxin challenge (23, 24, 28, 30, 31). In still others, it has been shown to play little, if any, role at all (20, 25–27). The conflicting results may be the consequence of the different methods used to interfere with the complement system, the different components that are depleted or deficient, the different species studied, and/or the different parameters of endotoxin shock and multiple organ failure that have been examined. In addition, in those studies in which animals have been depleted of C3 by injection of CoVF, the results may be confounded by the fact that CoVF-induced activation and depletion of C3 and C5-9 is incomplete and transient and may itself mimic the effects of endotoxin (19, 23, 25).

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 also 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 endotoxic 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 thermodilution pulmonary arterial catheters using previously described techniques (35) and included mean arterial pressure (MAP, mm Hg), heart rate (HR/min) central venous pressure (CVP, mm Hg), pulmonary capillary wedge 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 pool scans using conventional techniques (35). Hemodynamic data

[1] *Abbreviations used in this paper:* A-a O_2, 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 O_2, kPa). End-diastolic volume index (EDVI) and end-systolic volume index (ESVI, ml/kg) were calculated from catheter measurements and simultaneously obtained radionuclide 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 (pO_2, kPa), and carbon dioxide (pCO_2, 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 (10^9/liter), hemoglobin, and platelet count (10^9/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 (μmol/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 (10^9/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 anorexic. 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).

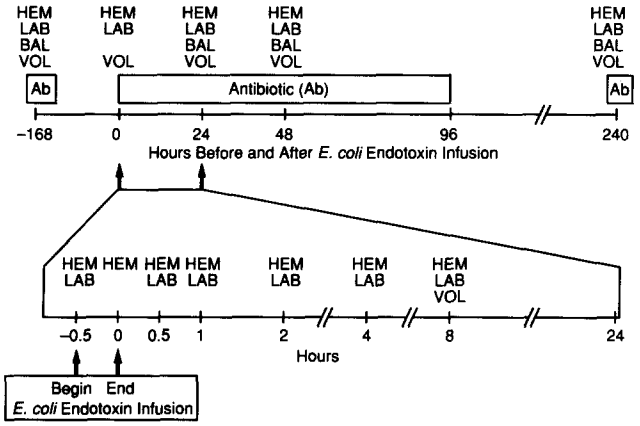

Figure 1. Sequence of evaluations and therapies during this study. (*HEM*) Hemodynamic evaluation, including radionuclide heart scan, and measurements obtained from pulmonary (thermodilution) and femoral arterial catheters; (*LAB*) laboratory evaluation including C5, endotoxin and TNF levels, routine 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.

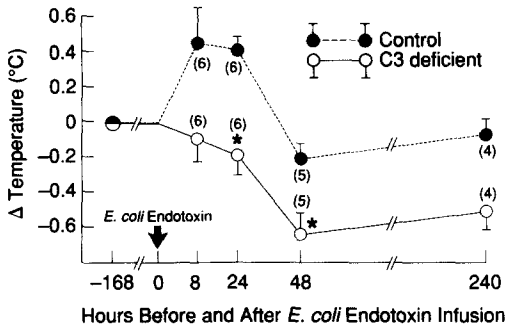

Figure 2. Serial (mean ± SEM) changes in temperature in C3-deficient animals and controls. In parentheses are shown number of survivors at each time point. (*)$P = 0.049$, comparing C3-deficient animals and controls at comparable time points.

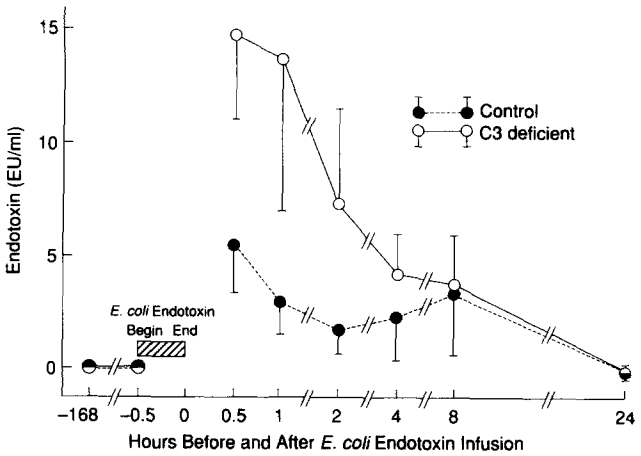

Figure 3. Serial (mean ± SEM) endotoxin concentrations. $P = 0.05$ comparing peak endotoxin levels in C3-deficient animals and controls.

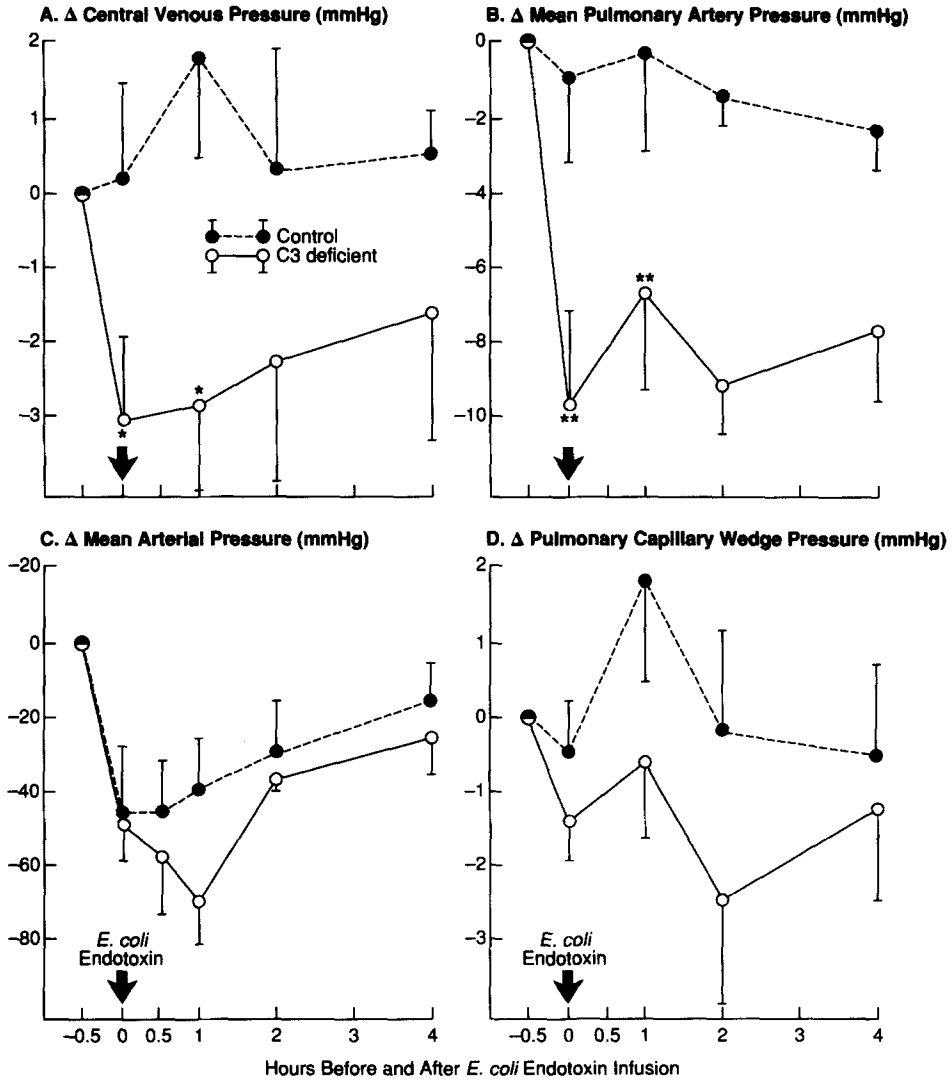

Figure 4. Serial (mean ± SEM) acute changes in hemodynamic values. (*)$P = 0.01$, (**)$P = 0.02$, comparing C3-deficient animals and controls at comparable time points.

Endotoxemia Levels

C3-deficient animals had higher mean peak levels of endotoxin compared with controls (P = 0.05, 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 pO_2 after 2 h (1.2 ± 0.7 vs 3.1 ± 0.4 kPa [mean ± SEM], P = 0.03), and after 4 h (0.9 ± 0.5 vs 2.7 ± 0.4 kPa, P = 0.03), and less of a decrease in mean pCO_2 after 4 h (−0.9 ± 0.1 vs −1.7 ± 0.3 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 = 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 O_2 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 = NS), except C3-deficient animals had significantly greater increases in ESVI (2.0 ± 0.5 vs 0.4 ± 0.3 ml/kg^{-1} [mean ± SEM], P = 0.009), less of a decrease in mean pCO_2 (−0.5 ± 0.1 vs −0.9 ± 0.1 kPa, P = 0.03), and less of a decrease in mean pO_2 at 24 h (−1.1 ± 0.7 vs 0.8 ± 0.8 kPa, P = 0.03). In survivors, by day 10, all hemodynamic variables returned to mean baseline values (P = 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, Table 1). Also during this time period, C3-deficient animals and controls alike had increases

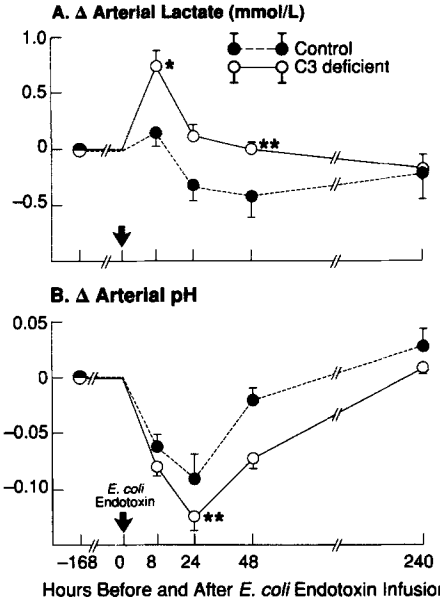

Figure 5. Serial (mean ± SEM) changes in arterial lactate and pH. (*)P = 0.03, (**)P <0.008, comparing C3-deficient animals and controls at comparable time points.

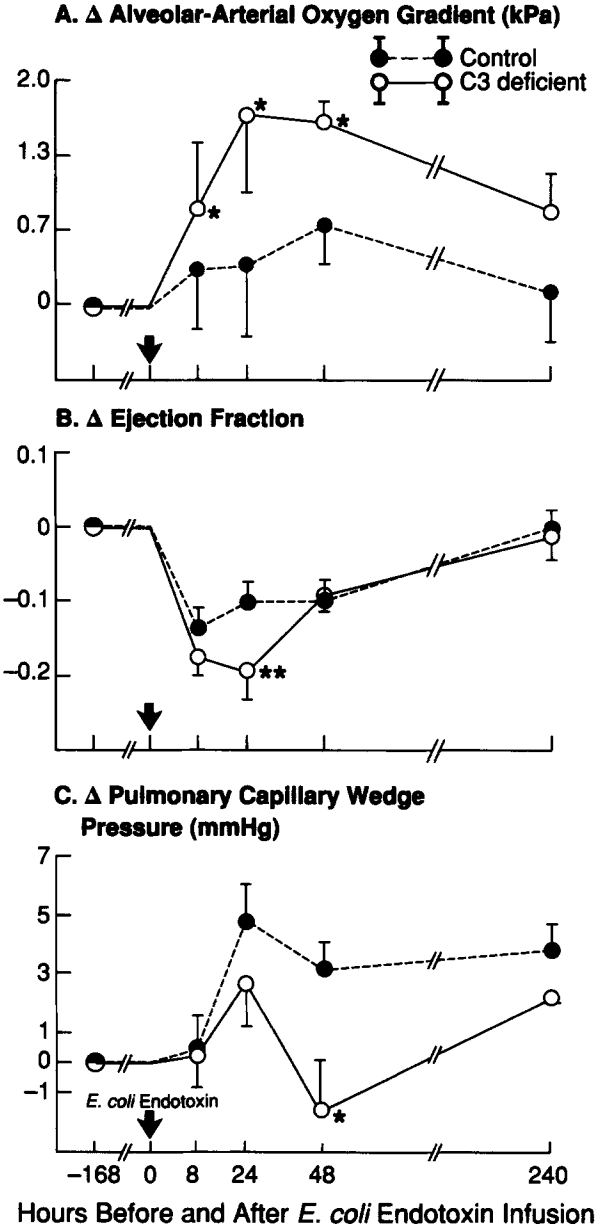

Figure 6. Serial (mean ± SEM) changes in A-a O_2 gradient, ejection fraction, and PCWP. (*)P = 0.04, (**)P = 0.009, comparing C3-deficient animals and controls at comparable time points.

Table 1. *Serial Geometric Mean BAL Cells and Protein before and after E. coli Endotoxin Infusion*

	Total cells (10^4/ml)		Neutrophils		Neutorphils (10^4/ml)		Protein (μg/100λ)	
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 significant decreases early (0.5 h, both $P < 0.003$, Table 3) and late (24 and 48 h, both $P < 0.009$, data not shown) in platelet count. At 2 and 4 h after *E. coli* endotoxin infusion, C3-deficient animals and controls alike, had significant increases (both, $P < 0.04$) in prothrombin time and partial thromboplastin time, but no significant changes (P = NS) in fibrin split products throughout (data not shown). C3-deficient animals had greater decreases in (mean ± SEM) fibrinogen at 0.5 and 1 h ($P = 0.03$) after *E. coli* endotoxin infusion compared to controls. In survivors, by day 10, all laboratory variables returned to mean baseline values (data not shown, P = NS). The other serial mean routine chemistries and complete blood count values were similar (data not shown) in C3-deficient animals and controls (P = NS).

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

	AST		ALT	
Time	C3 deficient	Control	C3 deficient	Control
h		*U/liter*		
−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.

Table 3. *Serial Geometric Mean White Blood Cell and Platelet Counts before and after E. coli Endotoxin Infusion*

Time	White Blood Cell (10^9/liter)		Platelets (10^9/liter)	
	C3 deficient	Control	C3 deficient	Control
h				
−0.5	10.8 ⁄ 1.10	13.2 ⁄ 1.10	365 ⁄ 1.12	265 ⁄ 1.37
0.5	3.2 ⁄ 1.21	3.1 ⁄ 1.15	196 ⁄ 1.16	167 ⁄ 1.20
1	3.2 ⁄ 1.07	3.3 ⁄ 1.16	243 ⁄ 1.26	153 ⁄ 1.28
2	4.9 ⁄ 1.16	3.4 ⁄ 1.13	238 ⁄ 1.11	197 ⁄ 1.24
4	4.9 ⁄ 1.23	6.5 ⁄ 1.22	228 ⁄ 1.14	225 ⁄ 1.25

the endotoxin. The greater degree of endotoxemia was associated with a more pronounced acute (within hours) drop in intravascular filling pressures as indicated by lower CVP, MAP, and PCWP, findings consistent with a diffuse vascular leak. In C3-deficient animals, this worse vascular leak may have led to decreased tissue perfusion, more severe lactic acidosis, and multiple organ failure, i.e., more pronounced cardiac (decreased LVEF fraction), pulmonary (increased A-a O_2 gradient), and hepatic damage (elevated serum transaminases) than in their littermate controls.

To preserve this valuable colony of C3-deficient canines, animals were challenged with a dose of *E. coli* endotoxin that was previously shown to produce physiologic changes but not lead to death in normal canines (34). With regard to survival, previous studies of small animals with genetically determined complement deficiencies have established the protective role of C4 (22) and of later components of the complement system, C5 and C6 (20, 21), in endotoxin-induced lethality. Thus, these studies and the present investigation strongly suggest that an intact complement system is necessary to prevent endotoxin-induced shock, organ failure, and death.

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

Time	C5*	
	C3 deficient	Control
h		
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, lipoxygenate 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-

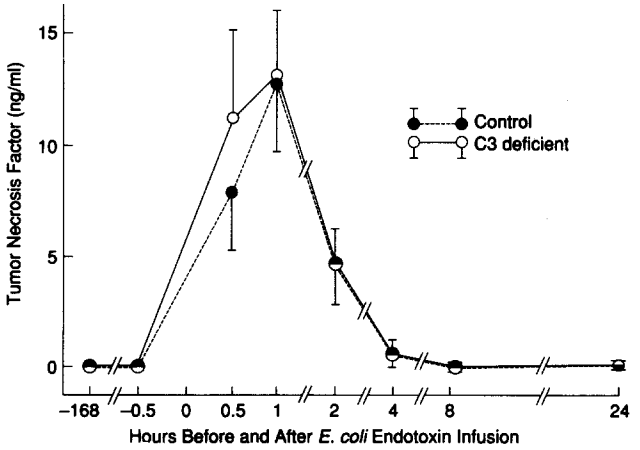

Figure 7. Serial (mean ± SEM) TNF concentration, comparing C3-deficient animals and controls. At 0.5, 1, and 2 h after *E. coli* endotoxin, C3-deficient animals and controls alike, had similar (P = NS) significant increases in circulating TNF levels (both P = 0.0001).

sponse to endotoxin challenge compared with controls. 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 phagocyte (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) CoVF-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.

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

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