Activated protein C is a plasma anticoagulant. For activated protein C to function as an anticoagulant, it must form a complex with protein S. Protein S anticoagulant activity is neutralized by formation of a reversible complex with C4b binding protein (C4bBP). C4bBP is an acute-phase plasma protein. When C4bBP levels increase, mass action forces the level of free protein S to decrease, giving rise to an acquired functional protein S deficiency. It has been proposed that these elevated C4bBP levels and the resultant acquired deficiency of protein S that occurs in inflammation could contribute to a hypercoagulable state. An experimental model to test this hypothesis was suggested by our previous studies that demonstrated that inhibition of protein C activation rendered baboons hypercoagulable in response to sublethal Escherichia coli infusion (J Clin Invest 79:918, 1987). We have extended these studies to examine the effect of inhibition of protein S activity with C4bBP in the host (baboon) response to infusion of sublethal concentrations of E. coli organisms. Five sets of animals were studied: (1) those challenged with sublethal concentrations of E. coli alone (0.4 × 10^9/kg); (2) those supplemented only with C4bBP (20 mg/kg); (3) those challenged with the same level of E. coli but supplemented with C4bBP (20 mg/kg); (4) those challenged with sublethal E. coli and supplemented with C4bBP (20 mg/kg) and sufficient protein S (2.3 mg/kg) to fill the protein S binding sites on C4bBP; and (5) those challenged with lethal concentrations of E. coli. Sublethal E. coli infusion (group 1 animals) caused only an acute-phase response with no consumption of fibrinogen, detectable organ damage, or detectable tumor necrosis factor (TNF) in the plasma. C4bBP infusion (group 2 animals) resulted in no significant physiologic changes, no detectable plasma TNF, and little change in fibrinogen level. The group 3 animals, receiving both sublethal E. coli and C4bBP, exhibited rapid consumption of fibrinogen, systemic organ damage, and detectable circulating TNF ultimately leading to death. The overall response of this group was very similar to the response of the group 5 animals receiving an LD50 dose of E. coli. The group 4 animals, which were treated exactly as above except that C4bBP was supplemented with a slight excess of protein S, responded essentially like those that received sublethal E. coli alone. These studies suggest that the elevation of C4bBP during an inflammatory response can contribute to fibrinogen consumption and vascular damage. This vascular damage may be associated with enhanced elaboration of cytokines like TNF. Protein S supplementation appears to be effective in preventing both of these responses.

PROTEIN S AND PROTEIN C are two vitamin K-dependent plasma proteins involved in a natural anticoagulant pathway. Protein C is a precursor of the anticoagulant protease, activated protein C.1 Protein C activation is catalyzed rapidly by a complex between thrombin and the endothelial cell surface protein, thrombomodulin.2 Activated protein C functions as an anticoagulant through the selective inactivation of factors Va and VIIIa.3,4 Protein S functions by enhancing the cell surface anticoagulant activity of activated protein C.4 This enhancement appears to be accomplished both by increasing the affinity of the enzyme for the cell surface,5,6 and by blocking the capacity of factor Xa to protect factor Va from inactivation by activated protein C.6 In plasma, protein S circulates in at least two forms. Approximately 40% of protein S is free and 60% is complexed to the complement regulatory protein, C4b binding protein (C4bBP).7 Only the free form retains protein S anticoagulant activity.11

Deficiencies of either protein C or protein S are associated with an increased thrombotic tendency. For instance, homozygous protein C deficiency is responsible for purpura fulminans in some infants, which can lead to death if untreated.12,13 Familial protein S deficiency can be divided into at least two separate classes. One class results from decreased protein S antigen,14,15 and the other results from normal protein S antigen, but increased C4bBP-protein S complex with a concomitant decrease in free protein S.15 In addition to the inherited deficiencies, several lines of evidence support the concept that the protein C anticoagulant pathway is downregulated in a variety of disease states, especially those associated with inflammation. For instance, exposure of endothelial cells to endotoxin,16 interleukin-1 (IL-1),21 and tumor necrosis factor (TNF)22-24 inhibits thrombomodulin activity on the surface of endothelial cells. In addition, protein C levels also decrease during disseminated intravascular coagulation (DIC),25 further impairing the function of the anticoagulant pathway. Of particular interest to the present study, acquired deficiencies of protein S have been observed in inflammatory disease states such as systemic lupus erythematosus (SLE)26 and DIC.27 It has been proposed that the acquired deficiency of protein S in inflammation is due to increased plasma levels of the acute-phase protein, C4bBP, and that this may contribute to coagulation abnormalities associated with inflammation by reducing the level of free protein S.26-29 While there is an association between increased C4bBP levels and either thrombosis or DIC, no direct studies have demonstrated that elevation of C4bBP levels alone can contribute to a hypercoagulable state.

Previous studies have shown that inhibition of protein C activation rendered baboons considerably more hypercoagulable in response to sublethal Escherichia coli infusion.30 These studies suggest that protein C, and perhaps other

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components of the protein C anticoagulant pathway, play a role in a protective host response to \( E. \) coli. We have used this model to investigate the influence of increasing C4bBP levels, and hence decreasing free protein S levels, on the response to sublethal \( E. \) coli.

**MATERIALS AND METHODS**

**Reagents.** Cephalin for the clotting assays, polyethylene glycol 8000, and QAE Sephadex A-50 were purchased from Sigma Chemical Company (St Louis, MO). Afii-Gel 10 and Enzymo beads were purchased from BioRad (Richmond, CA). Ketamine HCl was purchased from Aveco (Ft Dodge, IA). Na pentobarbital was purchased from Anthony Product Co (Arcadia, CA).

Infusion studies. For these infusion studies, type \( B \) \( E. \) coli organisms were isolated from a stool specimen at Children's Memorial Hospital, Oklahoma City. They were stored in the lyophilized state at 4°C after growth in tryptic soybean agar and reconstituted and characterized as described by Hinshaw et al.

Experiments were performed on 19 juvenile baboons (\( Papiso \) cynocephalus cynocephalus), each with a hematocrit exceeding 36% and free from tuberculosis. Baboons were fasted overnight before each experiment and administered 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. Animals were intubated orally and allowed to breathe spontaneously. The femoral artery and vein were cannulated aseptically and used for measuring arterial pressure and obtaining blood samples, respectively. The percutaneous catheter in the cephalic vein was used for all infusions as described previously. Each baboon was placed on its side in the supine position and then allowed to breathe spontaneously. The femoral artery and vein were cannulated aseptically and used for measuring arterial pressure and obtaining blood samples, respectively. The percutaneous catheter in the cephalic vein was used for all infusions as described previously.

Mean systemic arterial pressure (MSAP) and heart rate were monitored with a stethoscope pressure transducer and Hewlett Packard (Avondale, PA) recorder. Rectal temperature was measured with a Telethermometer (Yellow Springs Instrument Co, Yellow Springs, OH). The above measurements were made, and blood samples were collected at \( T - 30, 0, +60, +120, +180, +240, +360 \) minutes. \( T - 0 \) designated the point at which the infusion of \( E. \) coli was started. Not more than 7 mL/kg of the estimated total blood volume (70 mL/kg) was withdrawn over the 6-hour monitoring period. The blood collected at the times indicated above included: 1 mL anticoagulated with ethylenediaminetetraacetic acid for complete blood count, hematocrit, platelet and differential counts; 2.0 mL anticoagulated with 3.8% sodium citrate for fibrinogen; 1.0 mL in trisyl thorium for fibrin degradation products, 1.0 mL of clotted blood for blood urea nitrogen, creatinine, and TNF by 1.929 cell cytotoxicity. Two samples of 1.0 mL each were taken at \( T - 0 \) and \( T + 120 \) minutes for colony counts.

For analysis of the clearance of the C4bBP from the circulation, the protein was radioiodinated using Enzymo beads (BioRad) according to the manufacturer's instructions. After desalting and dialysis, \( 125 \)I-C4bBP (0.79 mg/mL, 5.7 mL, 20,000 cpm/mL) was infused into the baboon 60 minutes before the \( E. \) coli infusion. Fractions of 0.5 mL whole blood were collected at \( T - 60, 0, +60, +120, +180, +240, +360 \) minutes and at 24 hours and counted in a gamma counter (Nuclear Enterprises, 1600; San Carlos, CA).

**Statistical analysis.** Data were analyzed using Fisher's exact test to determine significant differences (\( P < 0.05 \)) in means at significant groups at given times. The analysis of variance and the multicom-parison Duncan's test were used to determine significant differences between means at time 0 and subsequent times within groups.

**Protein preparation.** Activated protein C was prepared from protein C\( 6 \)\( \) isolated from human plasma. The monoclonal antibodies (MoAbs) used in this study were selected and isolated essentially as described previously. Bovine factor Xa was inactivated with dansyl-Glu-Gly-Arg-chloromethylketone to yield DEGR-Xa as described previously.

Because of the large amounts of human C4bBP and protein S that were required for the in vivo experiments presented in this study, and the relative difficulty in obtaining large amounts of human plasma, it was necessary to design two separate isolation procedures. The first isolation procedure was large scale and provided most of the protein S and a relatively low yield of C4bBP. The second method provided a rapid isolation of large quantities of C4bBP. The first method involved QAE adsorption and elution of the plasma before adsorption with a battery of MoAbs. This method allowed isolation of both the free protein S and the C4bBP-protein S complex. Protein C and other vitamin K-dependent clotting factors could also be isolated from the concentrate. All procedures were performed at room temperature. The QAE eluate was prepared by diluting 30 L of human plasma 1:1 with 0.02 mol/L Tris-HCl, pH 7.5, 10 mmol/L benzamidine HCl, 2 U/mL heparin. The diluted plasma was adsorbed for 1 hour with 30 g of preswollen QAE sephadex (1 g/L starting plasma swollen in 0.1 mol/L NaCl, 0.02 mol/L Tris-HCl, pH 7.5). The QAE was allowed to settle for 30 minutes, the supernatant was decanted, and the QAE was packed into a 10-cm diameter column, washed with 2 L of 0.15 mol/L NaCl in the above buffer, and eluted with 0.5 mol/L NaCl. This eluate concentrated the proteins to about 800 mL and was bright green in color. Protein S was isolated from the eluate by chromatography on 100 mL of an immobilized MoAb to protein S, designated HPS2 as described previously. This procedure yielded approximately 20 mg of protein S of which 80% was uncleaved as judged by electrophoresis. The cleaved material migrated in the same position as protein S that had been inactivated with thrombin. The C4bBP and the C4bBP-protein S complex were still present in the breakthrough from the HPS2 column. These proteins were isolated with an antibody to C4bBP, designated BP45. Two hundred milliliters of immobilized BP45 (immobilized at a final concentration of 5 mg/mL BP45 on Afii-gel 10) was mixed in batch with the HPS2-adsorbed, QAE Sephadex eluate. The BP45 column was washed overnight with 4 L of 0.25 mol/L NaCl, 10 mmol/L benzamidine HCl, 0.02 mol/L Tris HCl, pH 7.5, then with 200 mL of 0.1 mol/L NaCl with 0.02 mol/L Tris-HCl, pH 7.5, and then eluted with 80% ethylene glycol in 1 mmol/L 3-[N-Morpholino]propanesulfonic acid (MOPS), pH 7.5. The eluate was applied to a heparin agarose column (5 x 8 cm, 2 mg/mL heparin immobilized by the cyanogen bromide [CNBr] method onto Bio Gel A-15M medium mesh). C4bBP binds to the heparin agarose column. Protein S, which dissociates from the C4bBP under these conditions, does not bind to the column and these fractions were processed as described below. The heparin column was washed with 100 mL of 80% ethylene glycol in the MOPS buffer to ensure complete dissociation of the protein S and then with 100 mL of 0.1 mol/L NaCl, 0.01 mol/L MOPS, pH 7.5. The C4bBP was eluted with 1 mol/L NaCl, 0.01 mol/L MOPS, pH 7.5. This procedure yielded approximately 100 to 200 mg of C4bBP from 30 L of plasma, and the protein had no detectable contaminants when analyzed by sodium dodecyl SO4 gel electrophoresis either before or after disulfide bond reduction.

The protein S found in the breakthrough from the heparin agarose column was dialyzed into 0.1 mol/L NaCl, 0.02 mol/L Tris-HCl, pH 7.5, and applied to a column of immobilized MoAb,
designated S163. This MoAb is a calcium-dependent antibody. Therefore, the sample was made 2 mmol/L in calcium chloride immediately before application. The S163 column (2.5 x 10 cm) was washed with 100 mL of 0.1 mol/L NaCl, 0.02 mol/L Tris-HCl, pH 7.5, in 2 mmol/L CaCl₂, and the column was eluted with the same buffer except that the CaCl₂ was replaced with 2 mmol/L EDTA. This procedure usually yielded approximately 5 to 10 mg of protein S that was about 80% uncleaved.

The second method involved polyethylene glycol 8000 precipitation and gave the highest yield procedure for C4bBP. Fresh frozen plasma (3 L) was melted at 37°C, and made 10 mmol/L in benzamidine HCl. The plasma was then chilled to 4°C, and polyethylene glycol 8000 was added to 6% wt/vol. Stirring was allowed to continue for 1 hour. The C4bBP contained in the precipitate was collected by centrifugation at 5,000g for 30 minutes and resuspended in 1 L of 0.3 mol/L NaCl, 0.02 Tris, 10 mmol/L benzamidine HCl, pH 7.5, for 1 hour at room temperature. Residual particulate matter was removed by centrifugation as described above. This material was adsorbed to 200 mL of BP45 resin as a batch procedure for 1 hour at room temperature. The resin was allowed to settle and packed into a 2.5 x 60 cm column. The column was washed and eluted as described above. The protein S from the C4bBP-protein S complex was also isolated as described above. The yields from this procedure were approximately 150 mg of C4bBP and 5 mg of protein S.

For these studies, C4bBP and protein S were isolated by both methods, and preparations derived from both procedures were used in each experimental setting with indistinguishable results.

RESULTS

Before examining the influence of C4bBP on the response to E coli in the baboon, it was important to demonstrate that C4bBP could inhibit protein S function in baboon plasma. Inhibition of protein S anticoagulant function would be manifested by a loss of the ability of activated protein C to function as a plasma anticoagulant. As anticipated, when the C4bBP concentration in baboon plasma was increased, the anticoagulant response to activated protein C was decreased (Fig 1). C4bBP addition to plasma had no effect on the clotting time in the absence of added activated protein C.

In studying the effects of exogenous C4bBP on the response of the baboon to sublethal E coli, we examined five groups of animals. They included animals infused with sublethal E coli alone (group 1); C4bBP alone (group 2); sublethal E coli plus C4bBP (group 3); sublethal E coli plus C4bBP saturated with protein S (group 4); and LD₅₀ E coli alone (group 5). Comparisons of the number of organisms infected and survival times of members of the five groups are presented in Table 1. All animals in the sublethal E coli plus C4bBP and in the LD₅₀ E coli groups died in 27 hours or less, while animals in the other three groups were permanent survivors (>168 hours). Thus, C4bBP supplementation exacerbates the response to sublethal E coli. This deleterious effect, however, is largely prevented by supplementation with protein S.

The influence of C4bBP on fibrinogen consumption and TNF formation in response to sublethal E coli is examined in Fig 2. Figure 2A shows that infusions of sublethal E coli alone and C4bBP alone induced no change in these parameters, whereas coinfusion of C4bBP with sublethal E coli (Fig 2B) was followed by a consumption of fibrinogen, appearance of TNF in plasma, and death (Table 1). In contrast, supplementation of the C4bBP with protein S largely prevented the fibrinogen consumption and TNF elaboration in response to sublethal E coli (Fig 2C). The supplementation also prevented death (Table 1). It should be noted that coinfusion of C4bBP with sublethal E coli produced similar responses to those observed with LD₅₀ E coli with respect to fibrinogen consumption, plasma TNF levels, and survival (Fig 2D, Table 1).

From these studies, the question arises as to whether the C4bBP functions solely by enhancing the coagulation response. To approach this problem, we used active site blocked factor Xa (DEGR-Xa). This protein has no clotting activity, but instead serves as an anticoagulant, presumably by complexing with factor Va and competing for the binding of activated factor X. As a result, it blocks prothrombin activation. Figure 3 shows the effect of coinfusion of DEGR-Xa with LD₅₀ E coli on the plasma levels of fibrinogen, TNF, and survival. It shows that while the coagulant response as monitored by fibrinogen consump-
tion was completely inhibited by DEGR-Xa, the TNF response and lethal effects of LD50 E coli were not affected.

Table 2 compares changes in physiologic responses to sublethal E coli alone (group 1) versus sublethal E coli plus C4bBP (group 3). The decrease of fibrinogen levels was accompanied by a reciprocal increase in fibrin degradation products (FDP) from white cells reached a nadir at 120 to 180 minutes in the sublethal E coli + C4bBP group, whereas sublethal E coli alone caused no significant changes in either of these parameters. The blood urea nitrogen and creatinine levels almost doubled by 360 minutes in the sublethal E coli + C4bBP group, whereas sublethal E coli alone caused no significant changes in either of these parameters. The MSAP remained stable except for a transient fall at T + 120 minutes in the sublethal E coli + C4bBP group. The white cell and platelet counts decreased in both groups. The white cells reached a nadir at 120 minutes followed by a return toward normal. The platelet response was different between the group 1 and 3 animals. Those administered only sublethal E coli exhibited a small but progressive decrease in platelets over the first 6 hours, while those administered both C4bBP and a sublethal E coli infusion decreased their platelet number much more dramatically.

Postmortem examinations were conducted on all baboons. Tissues were removed for analysis within minutes after death, thereby avoiding postmortem autolytic changes. Kidneys (Fig 4) and adrenals removed from baboons receiving C4bBP plus sublethal E coli (group 3) showed...
Table 2. Summary Comparing the Systemic Arterial Pressure, the Sympathomimetic/Inflammatory, the Coagulant/Fibrinolytic, and the Cell Injury Responses to Sublethal E coli Alone (N = 4) and to Sublethal E coli Plus C4bBP (N = 4)

<table>
<thead>
<tr>
<th>Time (min)</th>
<th>T = 0</th>
<th>T = 60</th>
<th>T = 120</th>
<th>T = 180</th>
<th>T = 240</th>
<th>T = 360</th>
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<td>MSAP (mm Hg)</td>
<td></td>
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<tr>
<td>E coli</td>
<td>105 ± 5.0</td>
<td>115 ± 6.0</td>
<td>97 ± 10.0</td>
<td>99 ± 8.6</td>
<td>96 ± 12.6</td>
<td>100 ± 4.2</td>
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<td>E coli + C4bBP</td>
<td>104 ± 3.3</td>
<td>89 ± 8.6</td>
<td>70 ± 6.8</td>
<td>89 ± 6.3</td>
<td>104 ± 3.2</td>
<td>102 ± 3.4</td>
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<tr>
<td>Heart rate (B/min)</td>
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<tr>
<td>E coli</td>
<td>124 ± 8.8</td>
<td>154 ± 16.0</td>
<td>176 ± 21.1</td>
<td>182 ± 15.7</td>
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<td>175 ± 15.5</td>
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<td>E coli + C4bBP</td>
<td>147 ± 23.9</td>
<td>192 ± 10.3</td>
<td>220 ± 9.1</td>
<td>212 ± 16.0</td>
<td>212 ± 13.5</td>
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<td>Resp. rate (R/min)</td>
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<tr>
<td>E coli</td>
<td>33 ± 3.3</td>
<td>43 ± 8.8</td>
<td>41 ± 4.4</td>
<td>45 ± 5.0</td>
<td>43 ± 3.3</td>
<td>36 ± 3.3</td>
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<td>46 ± 9.9</td>
<td>58 ± 3.1</td>
<td>53 ± 3.8</td>
<td>65 ± 6.5</td>
<td>62 ± 4.8</td>
<td>62 ± 6.3</td>
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<td>Temp (°C)</td>
<td>37.0 ± 0</td>
<td>36.5 ± 0.07</td>
<td>36.8 ± 0.43</td>
<td>37.2 ± 0.59</td>
<td>37.2 ± 0.71</td>
<td>37.7 ± 0.58</td>
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<td>E coli</td>
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<td>37.0 ± 0.23</td>
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<tr>
<td>WBC (×10⁹/µL)</td>
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<td>E coli</td>
<td>7.7 ± 2.0</td>
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<td>2.0 ± 0.70</td>
<td>3.9 ± 2.0</td>
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<td>E coli + C4bBP</td>
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<td>Fibrinogen (%)</td>
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<tr>
<td>E coli</td>
<td>100 ± 0</td>
<td>100 ± 0</td>
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<td>89 ± 7.2</td>
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<td>Platelets (×10³/µL)</td>
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<tr>
<td>E coli</td>
<td>276 ± 37</td>
<td>264 ± 40</td>
<td>236 ± 41</td>
<td>212 ± 49</td>
<td>201 ± 45</td>
<td>184 ± 54</td>
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<tr>
<td>E coli + C4bBP</td>
<td>173 ± 52</td>
<td>121 ± 20</td>
<td>117 ± 20</td>
<td>93 ± 26</td>
<td>112 ± 6</td>
<td>39 ± 13</td>
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<tr>
<td>FDP (µg/dL)</td>
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<tr>
<td>E coli</td>
<td>10 ± 0</td>
<td>—</td>
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<td>—</td>
</tr>
<tr>
<td>E coli + C4bBP</td>
<td>10 ± 0</td>
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<tr>
<td>BUN (mg/dl)</td>
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<td>E coli</td>
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<td>17 ± 2.3</td>
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<tr>
<td>E coli + C4bBP</td>
<td>29</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>39 ± 6.6</td>
</tr>
<tr>
<td>CR (mg/dL)</td>
<td></td>
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<tr>
<td>E coli</td>
<td>0.46 ± 0.03</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>0.56 ± 0.06</td>
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<tr>
<td>E coli + C4bBP</td>
<td>0.50</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1.42 ± 0.43</td>
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Summary comparing the systemic arterial pressure, the sympathomimetic/inflammatory, the coagulant/fibrinolytic and cell injury responses to sublethal E coli alone (N = 4) with those to sublethal E. coli + C4bBP (N = 4).

Abbreviations: MSAP, mean systemic arterial pressure; FDP, fibrin degradation products; BUN, blood urea nitrogen; CR, creatinine.

Fig 4. Photomicrographs of kidney sections from animals receiving sublethal E coli and C4bBP demonstrate necrosis and microvascular damage. A photomicrograph magnified 10 times of an eosin stained section of kidney from an animal (group 3) that received sublethal E coli and C4bBP and the tissues were processed immediately following death. Arrows indicate microvascular thrombosis involving the capillary loops of the renal glomeruli.
evidence of wide spread microvascular thrombosis with extensive infarction and hemorrhage. Neither thrombosis nor hemorrhage was detected in these organs of those animals receiving sublethal E coli alone (group 1) or sublethal plus C4bBP saturated with protein S (group 4) killed after 7 days.

The other findings were as follows. Lungs: alveolar capillary congestion, edema, intravascular fibrin thrombi and aggregation of neutrophils. Liver: severe vascular congestion and accumulation of neutrophils in vascular spaces. Adrenals: severe cortical congestion, hemorrhage, necrosis, and neutrophil accumulation. Kidneys: cortical necrosis, hemorrhage, vascular congestion, and fibrin thrombi. Spleen: severe lymphoid follicular necrosis, medullary congestion, and intrafollicular hemorrhage. Organs not examined after sublethal plus C4bBP saturated with protein S included in animals receiving C4bBP alone, sublethal E coli alone, or C4bBP saturated with protein S coinflused with sublethal E coli. Because these animals, however, were examined after 168 hours (7 days), direct comparison cannot be made with the results obtained from those animals that received either C4bBP plus sublethal E coli or LD100 E coli. The blood chemistry profiles of these two groups can be compared. Those animals that died with histologic evidence of microvascular thrombosis had the corresponding abnormalities of blood chemistry, including elevated levels of TNF. Those animals that survived and whose tissue histology was found to be normal at the time of killing (day 7) had relatively minor changes in their blood chemistry profiles.

**DISCUSSION**

Elevation of C4bBP levels in vivo converts a non-lethal acute-phase response to a sublethal concentration of E coli into a lethal shock response that includes DIC and organ damage. This finding demonstrates that increased levels of plasma C4bBP can influence the response to an inflammatory stimulus. The in vitro studies of the concentration-dependent effect of C4bBP on activated protein C anticoagulant activity are consistent with the concept that human C4bBP reduces the activity of free protein S in baboon plasma. The toxic contributions of elevated C4bBP to the response to E coli appear to be mediated through its capacity to bind protein S because these toxic effects could be reversed by protein S addition. These studies raise the possibility that protein S might be of therapeutic value in some inflammatory disease states where C4bBP levels are elevated.

These observations extend the previous studies that demonstrated that activated protein C could protect animals from the lethal effects of E coli. In these studies, TNF appearance in plasma was usually suppressed, but this suppression has proven variable from animal to animal, preventing definitive conclusions. In the studies reported here we observed that elevation of the C4bBP level is associated not only with DIC in response to sublethal E coli, but also with lethality and the appearance of TNF in the plasma. This finding suggests that there is a link between protein S function and cytokine responses.

The above studies would be consistent with the hypothesis that coagulation, and fibrinogen consumption in particular, might augment the inflammatory response. That this is not the case is illustrated by the studies in which DEGR-Xa was used as an anticoagulant and coinflused with lethal E coli. The coagulant response as measured by fibrinogen consumption was completely inhibited. The appearance of TNF in plasma and lethal effects of LD100 E coli, however, were not inhibited. Thus, these studies suggest that the protein C system (proteins C and S) attenuates the inflammatory response by some means other than blocking fibrin formation.

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