

The Contact System Contributes to Hypotension but Not Disseminated Intravascular Coagulation in Lethal Bacteremia

In Vivo Use of a Monoclonal Anti-Factor XII Antibody to Block Contact Activation in Baboons

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Abstract

The hypotension and disseminated intravascular coagulation (DIC) in bacteremia is thought to be mediated by the combined actions of cytokines, prostaglandins, and complement. The contact system, via the release of bradykinin and the activation of Factor XI, has been postulated to be contributing to the observed hypotension and DIC. Using a mAb to Factor XII (C6B7), we blocked the activation of the contact system in an established experimental baboon model in which *Escherichia coli* was infused to produce lethal bacteremia with hypotension. The untreated group ($n = 5$) displayed contact activation, manifested by a significant decrease in high molecular weight kininogen (HK) and a significant increase in α_2 macroglobulin-kallikrein complexes (α_2 M-Kal). The C6B7-treated group ($n = 5$) showed an inactivation of Factor XII and the changes in HK and α_2 M-Kal complexes were prevented. Both groups developed DIC manifested by a decrease in platelet, fibrinogen, and Factor V levels. The untreated group developed irreversible hypotension. The treated group experienced an initial hypotension that was reversed and extended the life of the animals. This study suggests that irreversible hypotension correlates with prolonged activation of the contact system, and specific antibody therapy can modulate both the pathophysiological and biochemical changes. (*J. Clin. Invest.* 1993, 91:61-68.) Key words: septic shock • bradykinin • prekallikrein • high molecular weight kininogen • α_2 -macroglobulin

Introduction

Activation of the kallikrein-kinin system concomitant with hypotension has been demonstrated to occur in humans (1, 2) and in a lethal baboon model (3) during gram-negative bacteremia. The consequences of contact activation include the generation of kallikrein, which releases bradykinin from high molecular weight kininogen (HK),¹ and the generation of Factor

XIa. Bradykinin is a potent endogenous vasodilator, which may contribute to the hypotension observed in septicemia. Contact system generation of Factor XIa, and thus the intrinsic coagulation cascade, could theoretically contribute to the disseminated intravascular coagulation associated with septicemia.

Activation of Factor XII is the initial step in the formation of kallikrein and Factor XIa where HK participates as a cofactor and the plasma inhibitors, C1-inhibitor and α_2 macroglobulin (α_2 M), limit the activation (4, 5). Measurement of the zymogens, cofactors, altered inhibitors, and enzyme-inhibitor complexes in clinical samples have documented that changes of these proteins occur in sepsis (6-8).

mAbs are potentially powerful therapeutic tools because of their antigen specificity and their ability to be produced in large homogeneous amounts. A mAb to endotoxin has been shown to attenuate sepsis in humans (9). In baboon studies, mAbs to tissue necrosis factor (TNF) and tissue factor (TF) significantly increased the survival of the treated animals (10, 11).

In this study, we demonstrate that an antifactor XII mAb (mAb C6B7) blocks the activation of Factor XII and subsequently the activation of the remaining contact system in vivo in a lethal hypotensive bacteremic baboon model. Treatment with the mAb attenuated a secondary phase of the observed hypotension, did not affect disseminated intravascular coagulation (DIC), and significantly extended the survival time of the animals.

Methods

Monoclonal antibody. Anti-Factor XII light chain antibody mAb C6B7 was produced by the hybridoma technique in mice using Factor XII fragment (Factor XII_f, β -Factor XII_a) as the antigen (12). The subtyped IgG₁ kappa antibody was affinity purified from mouse ascites under sterile conditions on a Protein-A Sepharose CL-4B resin, dialyzed against 0.9% NaCl, formulated to 5 mg/ml, and packaged in aliquots of 20 ml (100 mg). Antibody specificity was confirmed by Western blot and ELISA technique. A sterility test of an aliquot indicated no bacteria to be present. Limulus lysate assay detected insignificant levels (0.035 EU/ml) of bacterial endotoxin.

Proteins. Human plasma HK was purified by a published method (13). Purified HK was radiolabeled with ¹²⁵I by the iodogen method (14, 15) in a buffer of 0.02 M Tris, 0.5 M NaCl, pH 8.0. The specific activity of the labeled HK was 4 mCi/mg.

In vitro baboon plasma experiments. Human ¹²⁵I-HK was added to normal baboon plasma and dextran sulfate (500 kD; Sigma Chemical Co., St. Louis, MO) was added to activate the contact system at 37°C. In a 1-ml Eppendorf polypropylene centrifuge tube, 90 μ l of baboon plasma was incubated with different combinations of labeled human HK, 40 μ g/ml dextran sulfate, and 1 μ M mAb C6B7 and 0.15 M NaCl to achieve a final volume of 100 μ l. At the indicated times, a 2- μ l aliquot was removed and added to SDS-PAGE sample buffer containing 2% SDS and 50 mM dithiothreitol. SDS-PAGE of the plasma sam-

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1. Abbreviations used in this paper: α_2 M, α_2 macroglobulin; α_2 M-Kal, α_2 M-kallikrein complex; DIC, disseminated intravascular coagulation; HK, high molecular weight kininogen; MSAP, mean systemic arterial pressure; PK, prekallikrein; TF, tissue factor; TNF, tissue necrosis factor.

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ples was performed on an electrophoresis apparatus (Mini Protean II; Bio-Rad Laboratories, Richmond, CA) using slab gels (8 × 7.5 × 0.15 cm, 12% acrylamide running gel, 4% stacking gel). After electrophoresis, the gels were dried on a vacuum dryer and exposed to x-ray film (Cronex-4; Dupont Photo Products Div., X-Ray, Burbank, CA) in a cassette containing screens (Quanta III; DuPont Co.) for the indicated times at -40°C. Laser densitometry analysis was performed using a scanning densitometer (model GS300; Hoefer Scientific Instruments, San Francisco, CA) and data system (model GS360; Hoefer Scientific Instruments). The data is presented as percent intact labeled HK, which is the relative percentage of the total scanned bands for each lane.

Assays. Fibrinogen and platelet count were performed according to standardized methods (16). Factor XII, HK, and Factor V concentrations were determined by coagulant assays as described (17-19). Prekallikrein (PK) and Factor XI were measured using their respective amidolytic assays (20, 21). α_2 M-kallikrein (α_2 M-Kal) complex was measured by an ELISA method previously described (3, 8), using the $t = 0$ min sample for each untreated animal and the $t = -30$ min sample for each treated animal to generate the standard curve. An assayed pooled normal human plasma (George King Bio-Medical, Inc., Overland Park, KS) was used as the primary standard for Factor XII, Factor XI, PK, HK, and Factor V assays. The *in vivo* concentration of murine monoclonal mAb C6B7 was determined using a mouse IgG kit (FLUROTEC; Baxter Healthcare Corp., Pandex Division,) using purified mAb C6B7 as the standard.

Production of septicemia in baboons. The baboon-handling procedures and *Escherichia coli* (type B) preparation were performed using the methodology described in previous publications (3, 10, 11, 22-24). A mixed breed of *Papio c. cynocephalus*/*Papio c. anubis* baboons were purchased from a breeding colony maintained by the University of Oklahoma Health Sciences Center Animal Facility at the Oklahoma City Zoo. The animals weighed 4-16 kg and were tuberculosis free. The blood leukocyte concentrations were $5-7 \times 10^3$ cells/ μ l, and the hematocrits exceeded 36%. They were observed for a minimum of 10 d to assure adequate equilibration before experimentation. In the mAb-treated group ($n = 5$), mAb C6B7 was first infused over 10 to 30 min and was calculated to achieve a concentration of 1 to 2 μ M. In the untreated lethal group ($n = 5$) and mAb-treated lethal group, *E. coli* at a concentration of 40×10^9 organisms/kg were infused over a 2-h period. All animals were observed for 10 h from the start of the experiment. Gentamicin was given at 9 mg/kg *i.v.* at 120 min for 75 min and then at 4.5 mg/kg at 360 and 540 min for 30 min. Gentamicin (4.5 mg/kg) was then given intramuscularly at the end of the experiment and twice daily for 3 d to survivors. Baboons that recovered from shock were observed daily and medically treated as appropriate. Surviving animals were euthanized with sodium pentobarbital after a minimum of 7 d.

Physiological monitoring. Mean systemic arterial pressure (MSAP) and heart rate were monitored with a transducer (model P2306; Statham, San Juan, Puerto Rico) pressure gauge. Values obtained were recorded using a strip recorder (7796A; Hewlett Packard Co., Analytical Products Group, Palo Alto, CA). Rectal temperature was measured with a thermometer (model Telethermometer; Yellow Springs Instrument Co., Yellow Springs, OH). Respiration rates were recorded.

Blood sampling and processing. Blood samples for analysis were obtained from a cannulated femoral vein into polypropylene tubes. Additional samples were taken at $t = -30$ min for the mAb-treated group (before antibody infusion) to compare with the $t = 0$ min value to determine if the antibody infusion had any effects on the variables measured. Additional samples were obtained at the indicated time points (see data). Not more than 10% of the baboon's estimated total blood volume (70 ml/kg) was withdrawn over the 10-h monitoring period. The blood sampled at each drawing included 1 ml anticoagulated with EDTA for determination of hematocrit, white blood cell count, differential counts, and platelet counts; and 2 ml anticoagulated with 3.8% sodium citrate for measurement of Factor XII, Factor XI,

PK, HK, α_2 M-Kal, fibrinogen, and Factor V. Citrated samples were centrifuged to separate cellular material and the resulting plasma was aliquoted with a plastic pipette and stored frozen in polypropylene tubes at -70°C until the time of analysis.

Statistical analysis. A nonparametric statistical analysis of the data was performed (25, 26) on a microcomputer using GB-Stat software (Version 2.0; Dynamic Microsystems, Inc., Silver Spring, MD). The $t = 0$ min (before *E. coli* infusion) was used as the baseline (100%) value for both groups except for Factor XII in the treated group where $t = -30$ was the baseline. The data obtained for Factor XII, Factor V, fibrinogen, Factor XI, PK, and HK were normalized. Each independent assayed value at each time point was compared with the mean of the pooled raw baseline values of each assay for each group. The mean \pm SEM of the normalized values is expressed as a percentage of normalized baseline value (100%). Within each group (Table I), each assay was statistically analyzed using Wilcoxon's rank-sum test. A difference was considered significant, using a two-tailed P , at $P < 0.05$ (*) and highly significant at $P < 0.01$. Each assay was analyzed for significance between groups (see Figs. 4-7), using Wilcoxon's rank-sum/Mann-Whitney U test. A difference was considered significant, using a two-tailed P , at $P < 0.025$ and highly significant at $P < 0.01$. The survival curves were compared for significant differences between the treated and untreated group by using the likelihood ratio test for the proportional hazard model where a two-tailed P value ≤ 0.05 is significantly different.

Table I. Wilcoxon's Rank Comparisons Group Data Compared With Baseline*

	Time				
	60	120	180	240	360
	min				
Lethal ($n = 4-5$)					
Heart rate	‡	‡	‡	‡	—
MSAP	—	‡	‡	‡	—
Factor V	—	‡	‡	‡	‡
Platelets	—	—	—	—	—
Fibrinogen	—	—	‡	‡	‡
XII	—	—	—	—	—
PK	—	—	—	—	—
XI	—	—	—	—	—
HK	—	—	—	‡	‡
α_2 M-Kal	‡	‡	‡	‡	‡
mAb-lethal ($n = 4-5$)					
Heart rate	‡	‡	‡	‡	‡
MSAP	—	‡	‡	—	—
Factor V	—	—	‡	‡	‡
Platelets	—	‡	nd	nd	‡
Fibrinogen	—	‡	‡	‡	‡
XII	↓	↓	↓	↓	↓
PK	—	—	—	—	—
XI	—	—	—	—	—
HK	—	—	—	—	—
α_2 M-Kal	—	—	—	—	—

* Baseline values for both groups are the 0-min value before *E. coli* injection and after mAb treatment for the treated group. Two-tailed P . ‡ $P < 0.05$; § $P < 0.01$; —, Not significant; nd, not determined due to insufficient data; ↓, not determined, value depressed in the presence of mAb.

Results

In vitro studies examining the inhibition of the contact system using a mAb to Factor XII. A murine anti-human Factor XII mAb (mAb C6B7) was produced against purified Factor XII fragment (Factor XII_f or β -Factor XII_a) composed of the 28-kD catalytic unit of activated Factor XII and a small 2-kD portion of the heavy chain disulfide linked to the light chain. The antibody epitope was localized to the catalytic light chain region by its ability to react on a Western blot with nonreduced Factor XII_f. mAb C6B7 was examined first *in vitro* to characterize its ability to block activation of the contact system. First, the ability of the mAb to block the intrinsic coagulant system was assessed by measuring its effect on Factor XII coagulant activity (Fig. 1). Normal human plasma containing endogenous Factor XII was incubated with increasing concentrations of mAb C6B7, and its ability to correct the clotting time of Factor XII-deficient plasma was assayed (Fig. 1, squares). Using mAb concentrations of $\geq 1 \mu\text{M}$ resulted in 92–95% inhibition of coagulant activity. These concentrations are in excess of the human plasma concentration of Factor XII ($0.35 \mu\text{M}$) present in the incubation mixture. Nonspecific murine IgG resulted in no inhibition (not shown). When similar concentrations of antibody were mixed with plasma obtained from a normal baboon, the maximum inhibition of Factor XII coagulant activity obtained was 60–65% (Fig. 1, circles).

Next, the ability of mAb C6B7 to block the cleavage of HK in dextran sulfate-activated baboon plasma was studied (Fig. 2). The cleavage of kininogen is a sensitive assay to indicate the activation of Factor XII and the entire contact system, since HK cleavage depends on activation of PK to kallikrein by Factor XII_a. Kallikrein cleavage of HK results in release of bradykinin and a two-chain molecule linked by a single disulfide bridge, which is separated by reduction, into an NH₂-terminal

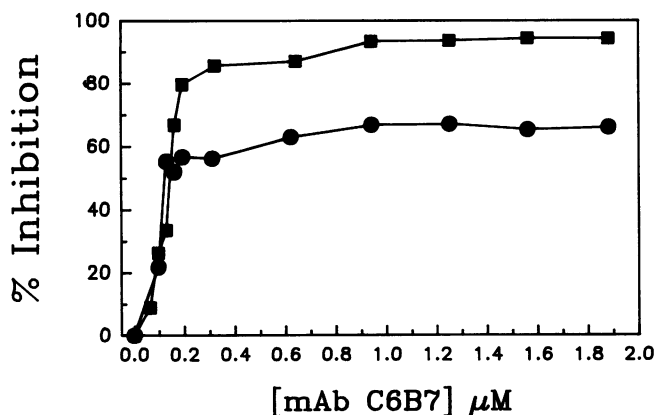


Figure 1. Inhibition of the coagulant activity of Factor XII by mAb C6B7. Antibody or saline ($6 \mu\text{l}$) was added to plasma ($94 \mu\text{l}$) to achieve the indicated final concentrations of antibody solutions. The mixtures were allowed to incubate for 15 min at room temperature. Aliquots were tested ($10 \mu\text{l}$ human; $2 \mu\text{l}$ baboon) for the correction of clotting times. The unit value was determined for each sample by comparing the clotting time with a standard curve generated by dilutions of the original plasma without antibody. The percent inhibition was compared with the $0 \mu\text{M}$ mAb C6B7 value. The values are the mean of triplicates. Human plasma at $0.35 \mu\text{M}$ Factor XII, (■); baboon plasma at $2.54 \mu\text{M}$ Factor XII (●).

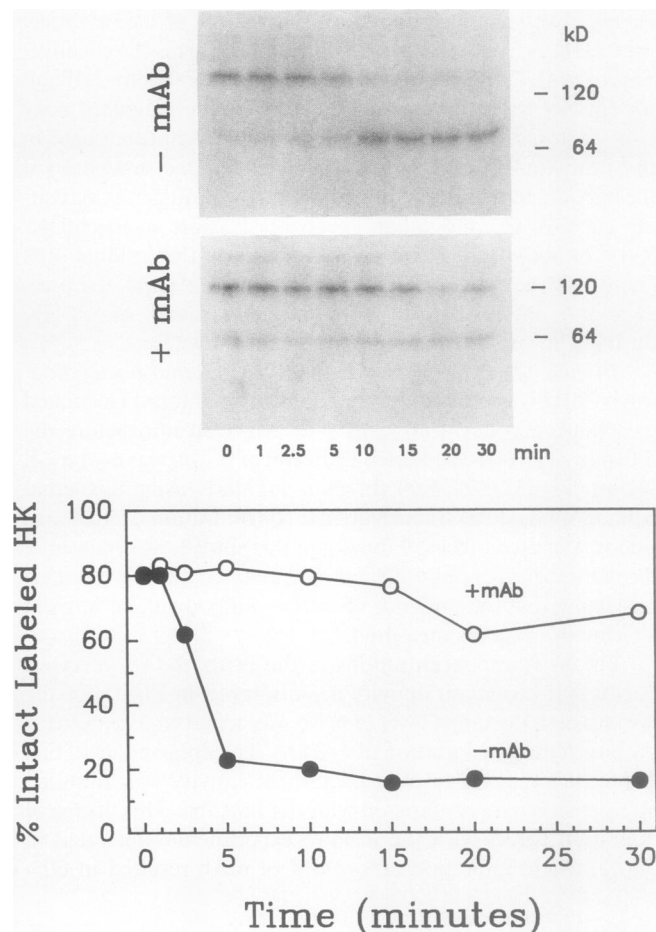


Figure 2. Inhibition of ^{125}I -HK Cleavage by mAb C6B7 in dextran sulfate-activated baboon plasma. $5 \mu\text{l}$ of ^{125}I -HK and $3 \mu\text{l}$ mAb (final $1 \mu\text{M}$) or saline was added to $90 \mu\text{l}$ of normal baboon plasma. The mixtures were incubated for 5 min at room temperature. Dextran sulfate was added ($2 \mu\text{l}$, final $40 \mu\text{g}/\text{ml}$) to activate the contact system. At the indicated times, an aliquot was removed and added to sample buffer containing SDS. Samples were electrophoresed; the gels were dried and exposed to an x-ray film for 72 h and then scanned as described in Methods. Upper gel and (●), HK cleavage pattern in the absence of antibody. Lower gel and (◻), HK cleavage in the presence of $1 \mu\text{M}$ mAb C6B7.

64-kD heavy chain and a COOH-terminal 56-kD intermediate light chain. Further proteolysis results in a relatively stable 45-kD light chain. The preparation of HK used was $\sim 80\%$ intact (120 kD) at 0 min (Fig. 2). Plasma was kept at 90% of its original concentration to allow the expression of inhibitors and other regulatory proteins to control the reaction (4). In the absence of an activating agent, no detectable changes of HK within the 30-min incubation period were observed (not shown). In the presence of dextran sulfate, 80% of the intact HK was cleaved within 10 min (Fig. 2, top), producing primarily radiolabeled HK heavy chain (64 kD), indicating that the contact system has been activated. More heavy chain of HK is observed in these studies due to the preferential labeling of the heavy chain with ^{125}I (the heavy chain contains 14 tyrosines whereas the light chain contains 2 tyrosines). When mAb C6B7 at $1 \mu\text{M}$ is added to this same plasma, the rate of HK

cleavage was significantly reduced, with 18% of the originally intact HK cleaved at 30 min. This result confirms the effectiveness of mAb C6B7 in baboon plasma to inhibit Factor XII and the contact system, as was demonstrated in the coagulant assay (Fig. 1, closed circles). Although, at the concentrations used in this study, mAb C6B7 reduced the rate of contact activation in the baboon plasma, it did not completely eliminate the activating effect of dextran sulfate, probably because of the higher levels or activity of Factor XII present and the residual 40% Factor XII detected (see Fig. 1). In human plasma, the presence of 1 μ M mAb C6B7 prevented all cleavage of 125 I-HK during a 90-min incubation period (not shown).

In vivo effects of mAb to Factor XII in lethal bacteremia. mAb C6B7 was infused into baboons in an amount calculated to achieve a concentration of 1 to 2 μ M 30 min before the infusion of *E. coli* was started. Infusion of *E. coli* was over a 2-h period (Fig. 3, thick bar). In a similar study using this lethal baboon model (10) where a greater concentration of sham antibody was used (15 vs. 9 mg/kg in this study), no changes in the pathological or physiological responses were observed, demonstrating that the presence of mouse antibody alone was not altering the systems measured.

The *in vivo* concentrations of the mAb and its effect on Factor XII coagulant activity are displayed in Fig. 3. In the lower panel, the target level of mAb was achieved to produce a prophylactic concentration of 1–2 μ M. The upper panel of Fig. 3 indicates that the level of Factor XII activity was inhibited 60% (open circles) compared with the baseline. This degree of inhibition agrees with the *in vitro* experiments illustrated in Fig. 1, where inhibition at 1–2 μ M of mAb resulted in 60%

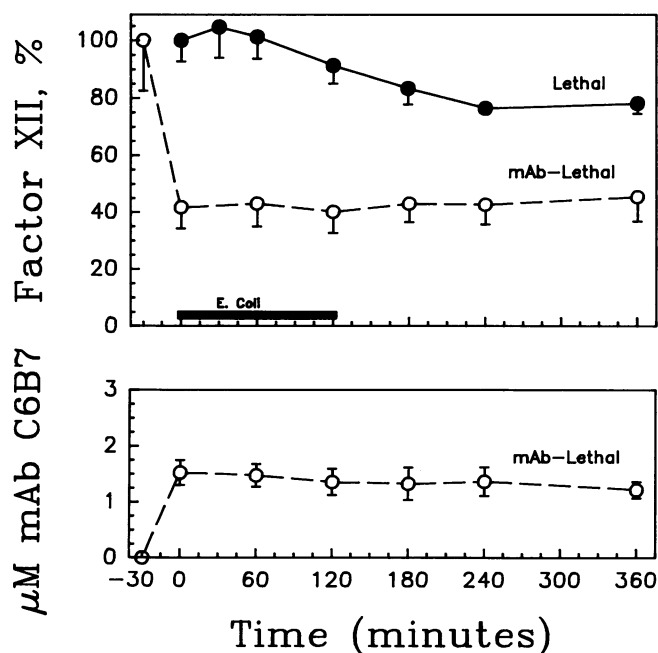


Figure 3. Factor XII and antibody levels measured in lethal baboon group (●) and mAb-treated lethal group (○). The percent of baseline value of Factor XII during the time course of the experiment (top). The thick line represents the time period of *E. coli* infusion. The concentration of mAb C6B7 found in aliquots of plasma at each time point (bottom).

inhibition of the Factor XII coagulant activity. The clearance of mAb C6B7 was slow, allowing protective levels of antibodies ($> 1 \mu$ M) to remain circulating against contact activation over the time of the study (Fig. 3, top and bottom open circles). In the untreated group, no significant change from baseline was observed over the 360-min period (Table I). Although not significantly different from initial values, Factor XII concentrations declined by 10 to 20% after 120 min, indicating that some limited consumption of Factor XII had occurred.

The hematocrit values (mean \pm SD) of the untreated (42.0 \pm 1.2) and treated (42.6 \pm 1.1) groups did not change significantly over the 6-h period, indicating that the animals were not dehydrated, which could result in a spurious change in the assayed levels of proteins due to hemoconcentration. Tachycardia was similarly observed in both groups upon infusion of *E. coli*, increasing significantly after 60 min and remaining at a plateau value over the 6-h experimental period.

The values of Factor V, fibrinogen, and platelets dramatically declined at 1 to 2 h (Fig. 4), indicating the onset of DIC in both groups. There was no measurable difference between the mAb-treated animals and the untreated animals, indicating that DIC was not inhibited by the antibody inactivation of Factor XII.

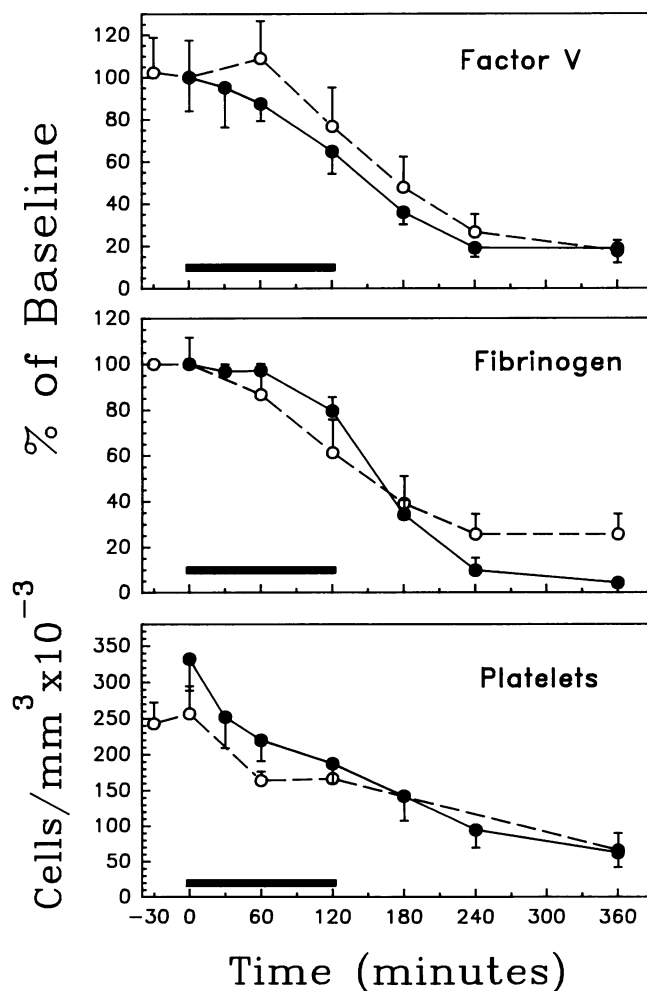


Figure 4. Factor V, fibrinogen, and platelet levels of lethal (●) and mAb-lethal (○) groups.

Although the mean PK value declined by 10 to 20% after 120 min in the untreated group and by less in the antibody-treated group (Fig. 5), the values were not significantly different from the baseline levels (Table I). PK values were not found to be significantly different between the groups. In the untreated group, the mean value of Factor XI activity increased after 120 min, peaking at 200% at 360 min, although this increase was not statistically different from initial values (Table I). In the antibody-treated group, Factor XI levels remained stable throughout the experiment with a slight decline at 360 min. Comparison of the untreated and treated groups indicated a significant difference between the Factor XI levels at 360 min (Fig. 5).

In contrast to the relatively stable levels of contact factor zymogens, there was a marked decline in the levels of HK within 30 min in the untreated group, a 40% decrease in the mean baseline value (Fig. 6), which reached significance at 240 and 360 min (Table I). The mAb to Factor XII prevented the decrease in HK concentration (Fig. 6) where the level remained relatively stable throughout the 360 min. There was a significant difference in the values between the groups at 360 min, indicating that the presence of the antibody had an effect on preserving the HK concentrations.

In the untreated group, the α_2 M-Kal complexes increased progressively and showed a highly significant difference from baseline (Table I). In contrast, the mAb treatment prevented the increase in α_2 M-Kal complex concentrations, indicating that the system was not activated to the extent of our detection limits. Comparisons between the two groups showed highly significant differences in complex formation at 60 min and thereafter (Fig. 6).

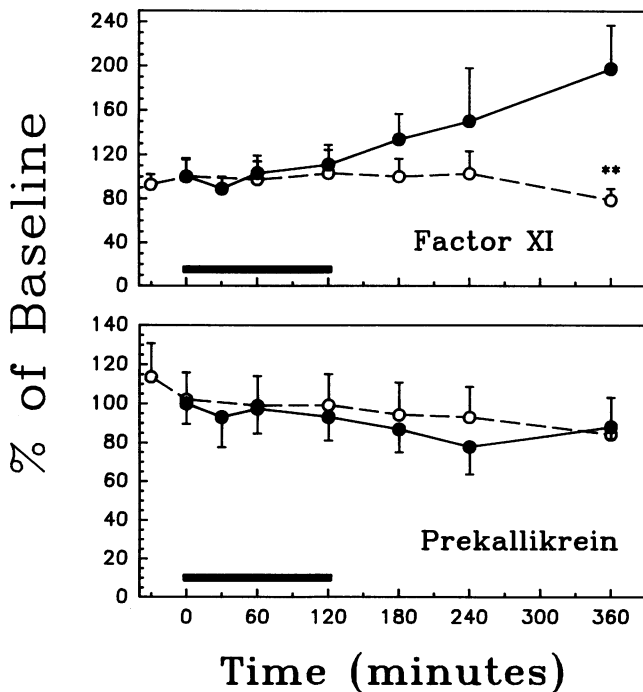


Figure 5. Factor XI and PK levels of lethal (●) and mAb-lethal (○) groups. The differences between lethal and mAb-lethal groups, determined by Wilcoxon/Mann-Whitney *U* test, are highly significant at $**P < 0.01$.

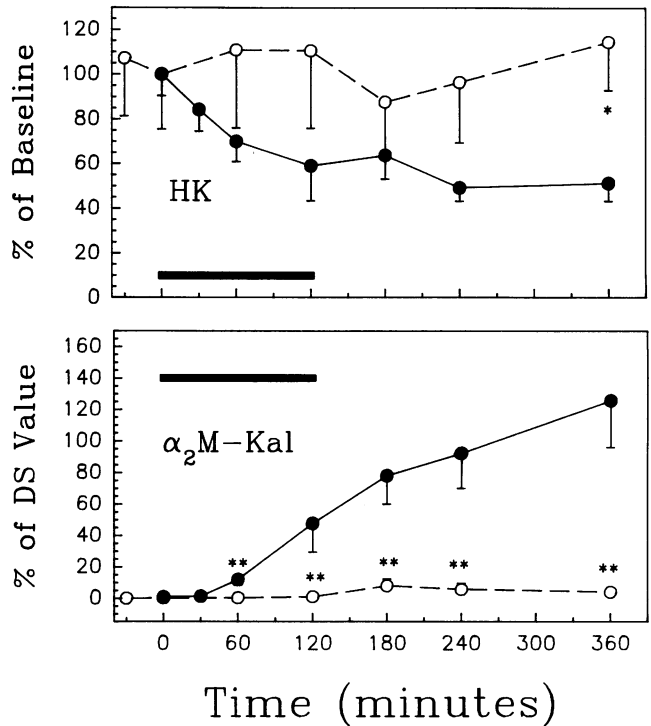


Figure 6. HK and α_2 M-Kal complexes of lethal (●) and mAb-lethal (○) groups. Wilcoxon/Mann-Whitney *U* test, $*P < 0.025$, $**P < 0.01$.

A dramatic significant decline of MSAP was observed in the treated group and similarly in the untreated group between 60 and 120 min (Fig. 7 and Table I). The inability of the antibody to prevent this pressure fall indicates that the initial decline in pressure observed was not related to contact system activation. It should be noted that there was an additional secondary decline in pressure after 120 min in the untreated group until death. This secondary decline in the untreated lethal group is confirmed by data obtained from lethal control baboons used in other studies (unpublished observations) where MSAP was measured at additional times after 360 min (Fig. 7,

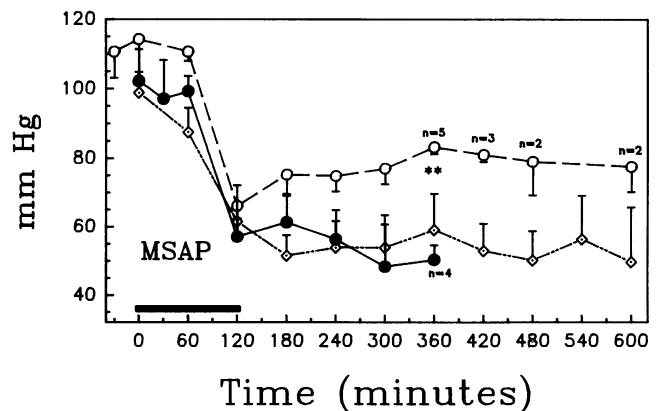


Figure 7. MSAP of lethal (●) and mAb-lethal (○) groups. Wilcoxon/Mann-Whitney *U* test between groups in this study (●, ○), $**P < 0.01$. (◇ · · · ◇) represents data of longer surviving lethal controls from other experiments (unpublished observations), $n = 5$.

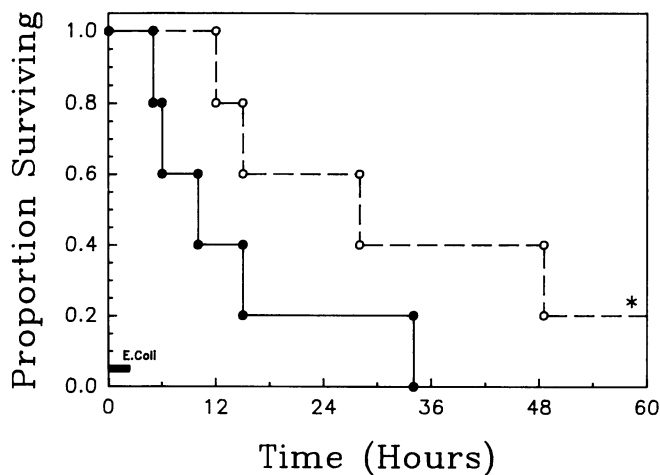


Figure 8. Kaplan–Meier survival curve of lethal (●) and mAb-lethal (○) animals. For both groups, $n = 5$. *One animal survived.

diamonds). The protracted decline in pressure was abrogated in the mAb-treated group and their pressure levels improved as the experiment progressed (Fig. 7). There was a statistical difference between the two groups at 360 min (Fig. 7), indicating that the presence of the antibody had an effect on the secondary pressure decline.

Fig. 8 is a Kaplan–Meier survival curve for both groups. Of the five untreated animals, four survived for 6 h, but only one was alive at 15 h. All animals had died by 34 h with an approximate half survival time of 10 h. In the treated group, all five animals survived beyond 12 h, three over 15 h, with an approximate half survival time of 27 h. One protected animal survived the lethal infusion of *E. coli* for over 120 h, an unusual occurrence for this model. Comparison of the survival curves using the likelihood ratio test indicated a significant difference in survival time of the treated animals compared with the untreated animals.

Gross and histopathological studies in both groups indicated that the lungs had alveolar capillary congestion, edema, intravascular fibrin thrombi, and aggregation of neutrophils similar to changes occurring in adult respiratory distress syndrome. The livers, adrenals, and spleens all demonstrated fibrin thrombi, hemorrhage, neutrophil accumulation, and necrosis consistent with tissue hypoxia and DIC. The kidneys exhibited central necrosis and vascular congestion with limited hemorrhage and fibrin thrombi.

Discussion

A previous baboon study using lethal and nonlethal concentrations of *E. coli* to produce hypotension suggested that irreversible hypotension correlated with the prolonged activation of the contact system (3). Significant changes in the contact system, including the rise in α_2 M–Kal complexes and a decrease in functional HK, occurred in the lethal but not in the nonlethal model. Determination of the concentrations of HK and α_2 M–Kal complexes were found to be the most sensitive indicators of contact activation, but whether triggering the contact system proteases was a cause of or a result of hemodynamic and coagulant changes is not known.

To investigate the relationship of the contact system with hypotension and to explore the role of the contact system in DIC, we used a neutralizing mAb directed to the seminal protein, Factor XII. We tested the hypothesis that a blockade of the contact system in the lethal model could modulate the pathophysiological response by preventing biochemical changes in the contact system and in the coagulation system. A mAb to the light chain region of Factor XII (mAb C6B7) was used to inhibit the contact system. In vitro, it was found to impede the coagulant activity of Factor XII (Fig. 1) and the cleavage of HK (Fig. 2). In vitro studies indicated that the 1–2 μ M concentrations of mAb obtained in the plasma of the treated animals did not completely block both of these activities of baboon Factor XII but inhibited 60% of the coagulant activity and extended the time for 50% cleavage of HK to > 30 min. In the group of animals treated with mAb, the Factor XII levels reflected similar levels of contact activation impediment (Fig. 3). The in vivo activation of the contact system may not be comparable to kaolin and dextran sulfate, which are strong activators of the contact system and were used in the in vitro studies of Figs. 1 and 2. The identity of the in vivo activator is currently not known.

Treatment with the antibody significantly prolonged the lifetime of the animals (Fig. 8). Although four of the five animals ultimately died of DIC, a fifth animal recovered, but showed signs of DIC.

Despite incomplete inhibition of Factor XII coagulant activity, we observed activation of the contact system in the antibody-treated group to be decreased compared with the control group as evident by the minimal concentrations of α_2 M–Kal complexes formed and the almost complete preservation of HK activity (Figs. 5 and 6).

An interesting finding was that activation of the contact system seems to increase the Factor XI coagulant activity two-fold (Fig. 5). We speculate that the cause of this activity may be due to either the increased synthesis of Factor XI or the release of Factor XI from the liver. Normal Factor XI is present at a 40-nM concentration in humans. On the basis of a functional assay, it is about the same concentration in baboons. In examination of one normal baboon where TNF was injected without endotoxin, a serial increase in the coagulant activity of Factor XI was observed (not shown). Additionally, we have observed increases in the values of Factor XI in humans given TNF for cancer therapy (not shown). Thus we tentatively ascribe the increase in Factor XI to an effect of TNF on its release or synthesis.

The treatment with the antibody did not modulate the initial blood pressure response. In both groups of animals, the initial MSAP declined during the first 2 h of the experiment (Fig. 7). In the untreated group, contact activation occurred during this period, as shown by the fall in HK concentration and the rise in α_2 M–Kal complexes (Fig. 6). Similar changes were not observed in the treated group. We cannot exclude the possibility that some degree of Factor XII activation occurred at the antibody concentrations used (as suggested by Fig. 1), however, the formation of α_2 M–Kal complexes were barely or not detectable (Fig. 6), indicating significant blockage of the contact system. These observations suggest that although the contact system was activated in the untreated group initially, there is an apparently more potent mediator affecting the MSAP. In previous examinations of the lethal baboon model

(24), TNF and LPS levels peaked and then declined in the first 2 h of the experiment. Levels of IL-1 β and IL-6 were observed, starting at 1 h. In an additional study (10), anti-TNF α antibody infused 30 min after initiating the infusion of the *E. coli* endotoxin largely eliminated the initial decline observed during the 1- and 2-h period, indicating that TNF and the subsequent cytokine release may be the contributing factor in the dramatic decline of MSAP during the second hour.

In our study, a more important effect of the antibody treatment was on the secondary irreversible hypotension that occurs after 2 h (Fig. 7). We conclude that the sustained decline in MSAP after the initial fall of MSAP may be attributed at least in part to bradykinin release due to activation of the contact system.

Although the contact system in the treated animals was blocked, the changes in the Factor V, fibrinogen, and platelet counts were similar (Fig. 4), and both treated and untreated animals had gross and microscopic changes, including thrombi typical of DIC. We conclude that activation of the contact system may not be the most important initiator of DIC and that this role may be played by TF (11). However, because of the incomplete inhibition of Factor XII, it is possible that 40% of the normal levels of this protein, although insufficient for kallikrein activation, may suffice for activation of Factor XI. We suggest that the role of the contact system in septic shock relates to maintaining the irreversible phase of hypotension.

These conclusions are in agreement with reports in both lethal and nonlethal baboon models, which suggest that tissue factor and the protein C system may play a role in regulating the DIC (11, 22). The regulation of the systemic blood pressure is less clear. Anti-TNF antibody prevented the initial decline in MSAP but failed to attenuate the secondary decline in MSAP and DIC (10). Anti-TF antibody attenuated DIC and the secondary MSAP decline and protected the animals from death (11). This similar attenuation of the secondary blood pressure decline suggests that inhibition of both TF and Factor XII expression may affect some common factor controlling this stage of the bacteremia. An attenuation of DIC and of both the initial and sustained decline in pressure was also observed when activated protein C was infused as an *in vivo* anticoagulant (22). These investigators were not able to account for the effects of activated protein C on the hypotension. One possibility is that activated protein C downregulates the contact system by inactivating one of its components and thus prevents the hypotension, but this hypothesis has not yet been tested. In contrast to the effect of activated protein C, when C4b-binding protein was used to block protein C activation, the decline in fibrinogen and platelet count were accelerated, but little effect on the MSAP was noted (27). The addition of DEGR-Xa, which prevents Factor Xa from binding to cells, effectively blocked the decline in fibrinogen levels but did not affect the decline in MSAP nor prevent the death of the animals (28).

The findings of the current experiments are in agreement with the concept that the morbidity and mortality of this model of gram-negative septic shock results from synergism of more than one pathogenic pathway, each participating during different stages of the syndrome. If several mediators, acting synergistically, were responsible for the irreversible hypotension, inhibition of any one might alter the hypotensive response. If contact system activation was a critical factor in the secondary lethal hypotension observed, then intervention after the initial

decline of the blood pressure with inhibitors of the contact system may attenuate the secondary lethal shock and allow antibiotic therapy the necessary time to contain bacterial proliferation. This study suggests that therapy of septic shock and DIC may require a combination of anticoagulants to control the DIC concurrent with an inhibitor of the contact system to prevent the action or generation of bradykinin. Such combined therapy might aid in reducing the mortality rate from septic shock.

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