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Activation of the Contact System in Lethal Hypotensive Bacteremia in a Baboon Model

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The hypotension in septicemia is believed to be mediated by the combined action of many mediators including cytokines, prostaglandins, and complement components. To evaluate the contribution of the contact/kinin-forming system to hypotension, the authors used an established experimental baboon model of bacteremia in which two concentrations of Escherichia Coli (E. coli) ***were used to produce lethal and nonlethal hypotension. The lethal group (n = 5) developed irreversible hypotension that significantly correlated with the decline in levels of high molecular weight kininogen (HK) and an increase in $\alpha_2$macroglobulin-kallikrein complexes (α2M-kal). The nonlethal group (n = 9) experienced reversible hypotension, a less striking decline in HK, and only slight elevation in α2M-kal. No significant changes were found in levels of factor XII, prekallikrein, and factor XI in either group. A significant change in the contact system, which reflects the fatal outcome, is the rise in α2M-kal. This study suggests that irreversible hypotension correlates with prolonged activation of the contact system. (Am J Pathol 1992, 140:897–906)***

Gram-negative septicemia continues to be a significant clinical problem despite the use of antibiotics. The interaction of microbial agents with host systems results in a wide array of pathophysiologic syndromes, from well-localized foci of infection to disseminated bacteremia with shock. Although bacteremic shock is incompletely understood on a molecular and biochemical basis, bacterial endotoxin, which is released from the cell wall of gram-negative organisms, has been implicated as the major initiating factor. Although tumor necrosis factor (TNF) released from monocytes is a necessary mediator in the primate host response to *E. coli*, it is not sufficient to account for all of the defensive and/or detrimental host responses observed.[1,2] Interleukins, particularly interleukin-1,[3] prostaglandins,[4] and complement proteins[5,6,7] participate in the host response to endotoxin as well.

A mortality rate of 57% occurs in patients having hypotension accompanying gram-negative bacteremia.[8] Although disseminated intravascular coagulation is often observed during bacteremic shock,[9] it is the decreased blood pressure that best correlates with increased mortality. The irreversible hypotension that characterizes these fatal cases results in tissue hypoxia and multiple-organ failure.[10] The hypotension is attributed to low systemic vascular resistance and the cardiac output is usually noted to be increased as a compensatory response in most cases.[11] Since bradykinin is one of the most potent endogenous vasodilators,[12] it may play a major role in the shock encountered in gram-negative bacteremia.

Bradykinin is released from plasma high molecular weight kininogen (HK) by plasma kallikrein.[13] In turn, plasma kallikrein is generated by the interaction of three proteins in the plasma contact activation system; factor XII, prekallikrein, and the procofactor, high molecular weight kininogen. Each of these proteins is converted to the active enzyme or cofactor by limited proteolysis, with the autoactivation of factor XII initiating the reactions leading to the liberation of bradykinin. The amount of kallikrein and factor XIIa generated is tightly regulated by the presence of the plasma protease inhibitors. Although the serine proteinase inhibitor (SERPIN), C1̄-inhibitor

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(C1INH), is the major inhibitor for both these proteases at 37°C,[14,15] alpha$_2$-macroglobulin (α2M) is also a potent inhibitor of kallikrein[15] and indeed becomes the major inhibitor when C$\bar{1}$-inhibitor is low as in hereditary angioedema.[16] A reaction between factor XI, high molecular weight kininogen and activated factor XII generates factor XIa which, in turn converts factor IX to IXa and activates the intrinsic coagulation cascade.

Plasma kallikrein not only can trigger the inflammatory response by release of bradykinin but also mediates other inflammatory responses. It has been shown to stimulate neutrophils to release lysosomal granule constituents,[17] such as elastase. Kallikrein may convert prourokinase to urokinase to activate the fibrinolytic system.[18] Activated factor XII, acting on the first component of complement, C$\bar{1}$, activates the classical pathway of the complement system.[19] Gram-negative organisms contain endotoxin that may activate factor XII directly[20,21] or, alternatively cellular bacterial proteases released may also activate the contact system during the course of the infection.[22] The contact system is an attractive candidate as one of the contributors to the hypotension which often accompanies gram-negative bacteremia. Studies of the human sepsis syndrome have provided considerable evidence for involvement of the contact system in the pathophysiology of hypotension shock associated with bacteremia.[16,23–34]

In this report we study the contact system in a well-characterized primate model in which irreversible hypotension associated with experimental bacteremia results in death. We assessed levels of all of the contact system proteins and the formation of active plasma kallikrein by measuring $\alpha_2$macroglobulin-kallikrein (α2M-kal) complexes. Finally, we modified the concentration of injected bacteria to produce a model of bacteremia that was nonlethal. We compared and contrasted the nonlethal model with moderate reversible hypotension to the lethal model that exhibited profound, prolonged, irreversible hypotension to elucidate physiologic and biochemical differences between simple septicemia and septic shock.

Methods

Production of Septicemia in Baboons

The baboon handling and procedures were performed using the methodology described in previous publications.[35–37]

E. coli (Type B) were isolated from a stool specimen at Children's Memorial Hospital, Oklahoma City and were stored in the lyophilized state at 4°C. The bacteria were reconstituted before use and characterized as described by Hinshaw et al.[38]

A mixed breed of *Papio c. cynocephalus/Papio c. anubis* baboons were purchased from a breeding colony maintained at the University of Oklahoma Health Sciences Center Animal Facility at the Oklahoma City Zoo. The animals weighed 6–17 kg and were tuberculosis-free. The blood-leukocyte concentrations were 5 to 7 $\times$ 10^3 cells/mm^3, and hematocrits exceeded 36%. They were observed for a minimum of 10 days to assure adequate equilibration before experimentation. Baboons that recovered from shock were observed daily and medically treated as appropriate. Surviving animals were euthanized after a minimum of 7 days with sodium pentobarbital.

The baboons were fasted overnight before the study and immobilized the morning of the experiment with ketamine (14 mg/kg IM). Sodium pentobarbital was then administered in the cephalic vein through a percutaneous catheter to maintain a light level of anesthesia (2 mg/kg every 20 to 40 min). They were orally intubated and positioned on their right side on a heating pad. A femoral vein was exposed aseptically and cannulated in one hind limb for sampling blood. The percutaneous catheter was used to infuse the *E. coli* organisms and other agents. In the lethal group (n = 5) *E. coli* at a concentration of 40 $\times$ 10^9 organisms per kg were infused over a 2-hour period. In the nonlethal group (n = 9), *E. coli* at a concentration which was tenfold less, 4.0 $\times$ 10^9 organisms per kg, were infused over a 2-hour period. All animals were observed for 10 hours from the start of the experiment. Gentamicin was given at 9 mg/kg intravenously at 120 minutes for 75 minutes and then at 4.5 mg/kg at 360 and 540 minutes for 30 minutes. Gentamicin (4.5 mg/kg) was then given intramuscularly at the end of the experiment and twice daily for 3 days to survivors.

Physiologic Monitoring

Mean systemic arterial pressure (MSAP) and heart rate were monitored with a transducer (Statham P2306, Puerto Rico) pressure gauge. Values obtained were recorded using a strip recorder (Hewlett Packard 7796A). Rectal temperature was measured with a 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. Samples were taken at t = 0 (before *E. coli* infusion, to obtain baseline (100%) values, and at

indicated time points (see data). Not more than 10% of the baboon's estimated total blood volume (70 ml/kg) was withdrawn over the 10–12 hour monitoring period. The blood sampled at each drawing included 1 ml anticoagulated with EDTA for determination of hematocrit, white blood cell count (WBC), and differential counts; and 2 ml anticoagulated with 3.8% sodium citrate for measurement of factor XII, factor XI, prekallikrein (PK), high molecular weight kininogen (HK), $\alpha_2$macroglobulin-kallikrein complex (α2M-Kal) and other plasma proteins. Citrated samples were centrifuged to separate cellular material and the resulting plasma was aliquoted and frozen at −70°C until the time of analysis.

Assays

WBC was performed according to a standardized method. Factor XII and HK concentrations were determined by coagulant assays described in the respective previous publications.[39,40] PK and factor XI were measured using their respective amidolytic assays.[41,42] An assayed pooled normal human plasma (PNP, George King Biomedicals, Inc., Overland Park, KS) was used as the primary standard for factor XII, factor XI, PK, and HK assays. For each assayed time point, factor XII, factor XI, PK, and HK concentration of each baboon were normalized (see below). α2M-kal complex was measured by an ELISA method described in a recent publication.[16] A standard curve for the determination of the percentage of maximum activation in the α2M-kal complex assay was performed for each baboon using plasma obtained from the t = o point. This was performed by activating the t = o plasma at 0°C in the presence of 20 μg/ml dextran sulfate, as described for the human standard curve in Kaufman et al.[16] A typical concentration-dependent response curve is illustrated in Figure 1. The percentage of maximum dextran sulfate activation (=100), a relative value, is used since the absolute concentration (μM) of baboon α2M, prekallikrein or α2M-kal complex as measured by ELISA is not known. Maximal activation is not equated with complete activation of prekallikrein to kallikrein since the presence of inhibitors limit the generation of kallikrein using this method.[43] Thus, concentrations of α2M-kal greater than 100 are possible with inhibitor detection. Standard curves for the assay were generated by plotting the absorption values of the standards against the log 1/dilution of the DS-activated plasma. The plot was fitted to a third-order polynomial equation by an iterated procedure using Sigma Plot (Jandel Scientific, CA). The polynomial equation was then used to interpolate the value of the diluted unknown sample, and the value was corrected for dilution.

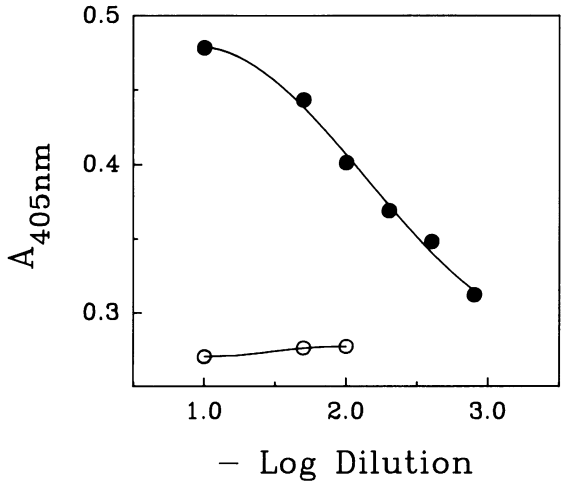

Figure 1. *Response curve of dextran sulfate activated baboon plasma. Plasma of the t-o timepoint was activated with 20 μg/ml dextran sulfate at 0°C for 10 minutes. ELISA response of serial dilution of DS activated plasma (●), or non-activated plasma (○).*

Statistical Analysis

A nonparametric statistical analysis of the data was performed[44] on a microcomputer using GB-Stat software (Version 2.0, Dynamic Microsystems, Inc, Silver Spring, MD). A normalized mean ± SEM of values of the assays for each group (lethal and nonlethal) for each timepoint was determined by comparing to a mean value of the raw baseline values at 0 minutes using all 14 animals from both groups. The normalized values were expressed as a percent of the normal pooled mean ± SEM and plotted. Within each group (Table 1), 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 $P < 0.01$ (**). Between groups (Figures 2–4), each assay was analyzed 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 $P < 0.01$ (**). Correlations were calculated using Spearman's rank correlation and tested using Spearman's test for correlation (two-tail *P* values).

Results

Two groups of baboons were examined for markers of contact system activation, a lethal group consisting of five baboons, and a nonlethal group consisting of nine baboons. The lethal model of bacteremic shock in the baboon has been described in detail in previous investigations.[35–37] Infusion of lethal concentrations of *E.coli* (40×10^9 organisms/kg) over a 2-hour period resulted in a four-

Table 1. *Wilcoxon's Rank Comparisons: Baboon Lethal and Nonlethal*

Enzyme/Time (min)	30	60	120	180	240	360
Lethal (n = 4–5)						
Heart rate	–	*	*	*	*	–
Respiration	–	–	–	–	*	–
MSAP	–	–	*	*	*	*
WBC	–	–	*	–	–	–
XII	–	–	–	–	–	–
XI	–	–	–	–	–	–
PK	–	–	–	–	–	–
HK	–	–	–	–	*	*
α2M-Kal	–	*	*	*	*	*
Nonlethal (n = 7–9)						
Heart rate	*	*	**	**	*	*
Respiration	–	–	–	–	–	–
MSAP	*	–	**	–	–	–
WBC	*	**	**	*	*	*
XII	–	–	–	–	–	–
XI	–	–	–	–	*	–
PK	–	–	–	–	*	*
HK	–	–	*	*	*	*
α2M-Kal	–	–	–	*	–	–

– Not significant.
* $P < 0.05$.
** $P < 0.01$.
Group data, compared with initial conditions (0 min). Two-tailed *P*.

stage response over a 18–32 hour period, terminating in death of all animals. The time of death for the five baboons receiving lethal concentrations of *E. coli* were 6, 10, 5, 15, and 34 hours, respectively. Gross and histopathologic studies 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, WBC accumulation, and necrosis consistent with tissue hypoxia and DIC. The kidneys exhibited central necrosis and vascular congestion with limited hemorrhage and fibrin thrombi. In contrast, the surviving nine baboons receiving nonlethal concentrations of *E. coli* were euthanized and examined pathologically at 7 days, and showed all organs to be histologically normal.

The hematocrit values (mean ± SD) of the lethal (42.4 ± 1.1) and nonlethal (41.4 ± 1.2) groups did not change significantly over the 6-hour time period, indicating that the animals were not dehydrated which could result in a spurious change in the assayed protein levels due to hemoconcentration.

Lethal Group

After a lethal infusion of *E. coli*, the heart rate increased significantly from baseline at 60 minutes and the rate remained high at 360 minutes (Figure 2, Table 1). The respiration rate increased within the first 60 minutes and remained high with a significant difference at 60–240 minutes from the baseline levels. MSAP (Figure 3, Table 1) showed the expected decline after 60 minutes (half-way into the *E. coli* infusion) and became significantly different from baseline values at 120 minutes. After 120 minutes, when the infusion of *E. coli* was terminated, the MSAP continued to decline until death. WBC declined within 30 minutes and remained low beyond 120 minutes (Figure 2, Table 1).

No significant changes from baseline were observed for the values of factor XII, factor XI and prekallikrein in the lethal group (Table 1). Although not statistically significant, factor XII and prekallikrein concentrations declined by 10–20% of baseline after 120 minutes (Figure 4). Factor XI levels increased after 120 minutes (Figure 4) but this trend was not significantly different from baseline values (Figure 4, Table 1).

In contrast to the stable levels of contact factor zymogens, there was a decline in the levels of HK within 30 minutes (40% drop in the mean value of baseline, Figure 3) which reached significance at 240 and 360 minutes (Table 1). The decline in HK values was temporally related to a decline in MSAP, suggesting a possible relation between bradykinin release and blood pressure regulation. A decline in HK correlated with a decline in MSAP with a Spearman R value of 0.929, giving a *P* value (two-tail) of 0.0025. As a reflection of kallikrein activation, α2M-kal complex formation showed a dramatic, significant increase in concentration beginning at 60 minutes. The increase in α2M-kal complexes correlated inversely with a

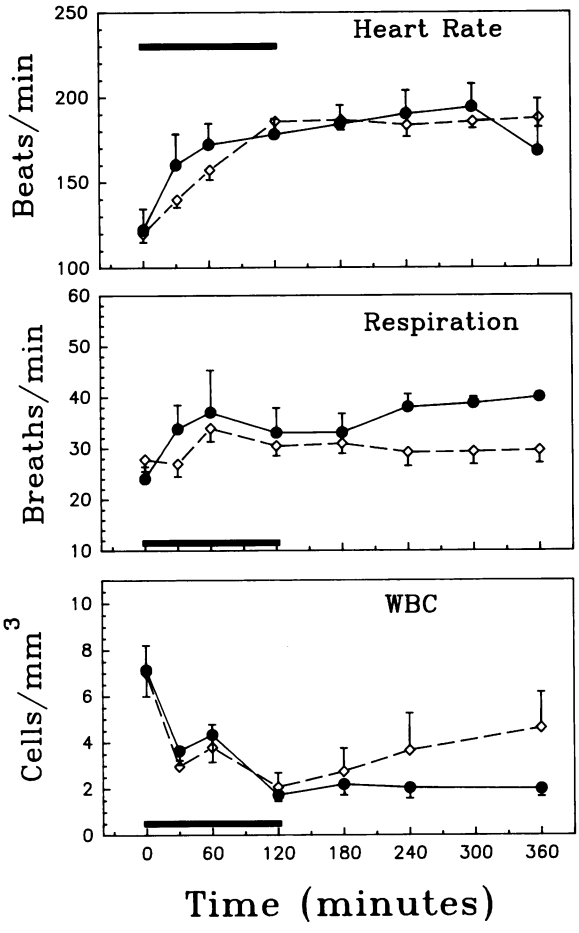

Figure 2. *Heart rate, respiration, and white blood cell count (WBC) of lethal (●) and nonlethal group (◇). Solid bar is the time period of* E. coli *infusion. The symbols and bars are the mean ± SEM.*

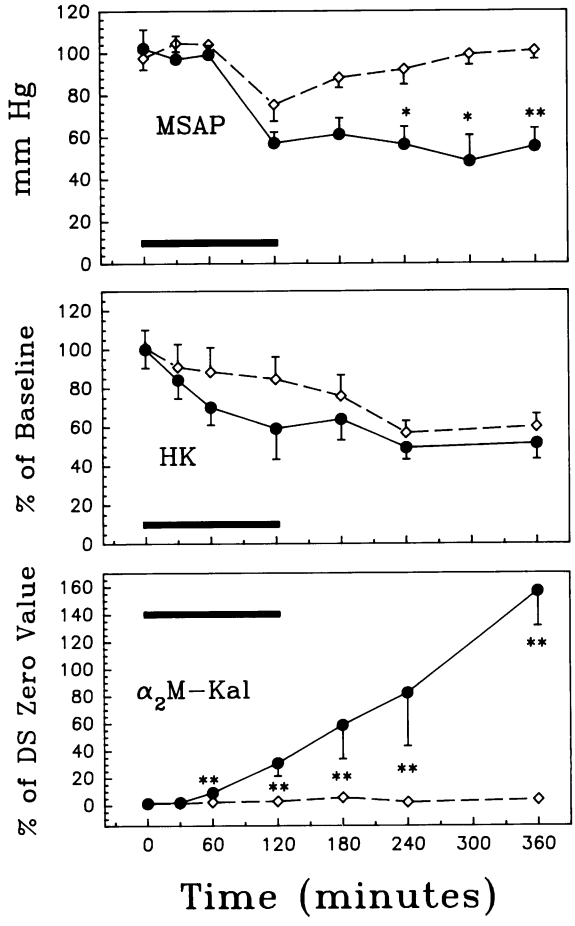

Figure 3. *Mean systemic arterial pressure (MSAP), and levels of high molecular weight kininogen (HK) and α2M-Kal complexes of lethal (●) and nonlethal (◇) group. The differences between lethal and nonlethal groups, determined by Mann-Whitney U test are significant *P < 0.025, and highly significant **P < 0.01.*

decline in MSAP with a Spearman R value of −0.929 and a two-tailed *P* value of 0.0025.

Nonlethal Group

Similar to what is observed in the lethal group, the heart rate in the nonlethal group increased significantly by 30 minutes and remained significantly different from baseline values throughout the study period of 360 minutes (Table 1, Figure 2), indicating a sympathomimetic response in the nonlethal animals to *E. coli* infusion. The respiration rate mean value increased between 30 and 60 minutes, then returned to a normal rate at 120 minutes, when *E. coli* infusion was terminated. However, this trend was not significantly different from baseline (Table 1). No change in temperature was noted over the entire experimental period (not shown). There was a 20% decline in MSAP between 30 and 60 minutes with a return to baseline after 120 minutes (Figure 3). The number of WBC declined significantly within 30 minutes, similar to the decline observed for the lethal group, and reached a significant nadir at 120 minutes before returning to normal after 360 minutes (Figure 2).

No observable or significant differences were found in the factor XII levels at any of the time points (Figure 4, Table 1). Factor XI and prekallikrein concentrations remained stable throughout the testing period (Figure 4). Since the variance in factor XI and prekallikrein values was small, a significant difference from baseline was found at 240 minutes for both, and at 360 minutes for prekallikrein.

The HK levels showed a slower rate of decline from baseline levels (Figure 3), reaching a nadir at 240 minutes (Table 1) with a 20% decline and returning to baseline levels beyond 360 minutes (not shown). A small but significant increase in α2M-kal concentration was ob-

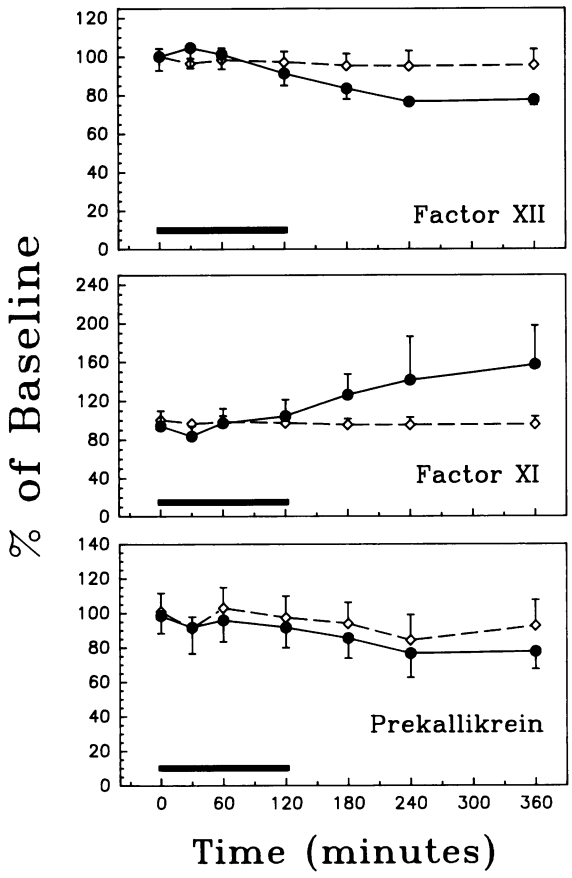

Figure 4. *Factor XII, factor XI and prekallikrein levels of lethal (●) and nonlethal (◇) groups.*

served only at 180 minutes (Figure 3, Table 1). No significant correlations were found between MSAP values and HK levels or α2M-kal levels.

Lethal Versus Nonlethal Groups

Since there was a similar increase in heart rate and respiration and a decline in WBC, no significant differences were found in these variables between the two groups (Figure 2). MSAP was significantly different between the two models during the period of 240 to 360 minutes (Figure 3). This difference reflects the fact that the individuals in the nonlethal group were recovering from a modest decline in MSAP, while in the lethal model the MSAP remained profoundly depressed.

Factor XII values showed no difference between the groups except at 30 minutes (Figure 4). This statistical difference is most likely attributed to random chance since the variance value of the nonlethals was small at this timepoint. No differences were found in the comparisons of prekallikrein and factor XI (Figure 4). No significance was found between levels of HK as this protein declined in a parallel fashion in both models. The differences between lethal and nonlethal in the values of α2M-kal complexes were highly significant beginning at 60 minutes (Figure 3) due to a dramatic increase in complex formation in the lethal group.

Discussion

We examined the contact system proteins to ascertain whether contact activation correlated with the occurrence of hypotension. The profile that might be expected with contact system activation can include any or all of the following: a decrease in the levels of factor XII, PK, or HK with an increase in α2M-kal complexes. When activation of the contact system occurs, only a small percentage of the total concentration of zymogens need be activated (1–5%) to cleave their substrates in a catalytic manner.[19,45] The activation of the zymogens, factor XII and prekallikrein, is limited by the presence of their respective plasma protease inhibitors. We would not expect a dramatic drop in the levels of these proteins unless there was a severe, continuous activation of the contact system, since biosynthesis at a normal or increased rate tends to replete the inhibitors.

The changes in the coagulant activity of activated HK are more difficult to predict. HK is known to circulate complexed to prekallikrein, factor XI, and in the free form. HK is also a substrate for kallikrein. As kallikrein cleaves HK, bradykinin is released from the procofactor. This cleaved, kinin-free kininogen (HKa) has unchanged activity by a coagulant assay but binds more readily to negatively charged surfaces *in vitro*.[46] The HKa light chain is then cleaved by other proteases, such as factor XIa,[45] plasmin[47] or elastase[48] to inactivate this cofactor function. Loss of HK activity requires the initial cleavage by kallikrein which then facilitates the proteolytic inactivation of the HKa.[45] Therefore, we would expect some decline in HK coagulant activity if the contact system is active.

Blood levels of α2M-kal complexes, under normal conditions, are undetectable.[16] With the generation of kallikrein, due to contact activation, increasing concentrations of α2M-kal complexes should be found. α2M-kal complex formation is only one reflection of kallikrein generation, since the other major inhibitor of kallikrein is C1̄-inhibitor which also consumes the active kallikrein. One advantage of measuring α2M-kal complexes in contrast to C1̄-inhibitor-kallikrein complexes is that the former may take longer to clear from circulation.[49] A tentative explanation for the slower clearance rate might be that at high concentration of α2M-kal complexes, an impaired clearance capacity of the mononuclear phagocyte system may occur resulting in an increased accumulation of α2M-enzyme complexes in the plasma.[50] We therefore

might observe an accumulation of α2M-kal complexes over the 6-hour period of the experiment since α2M is regulating a number of active enzymes participating in other systems, such as thrombin, plasmin, and elastase. In addition, if C1̄-inhibitor is consumed, then α2M becomes a significant regulator of the contact system, by inhibition of kallikrein. This observation also supports findings that correlations of inactive C1̄-inhibitor with lower levels of factor XII or kallikrein could not be detected in sepsis patients or HAE patients, probably due to low amounts of enzyme activated and rapid clearance of the complexes.[50–53]

As anticipated, only modest changes could be measured in either the nonlethal or lethal group for factor XII, prekallikrein, or factor XI values. These findings confirm that measuring the levels of the contact zymogens may not be a sensitive indicator of contact activation in the baboon model. The most striking change noted for contact factors was in HK, which demonstrated significant decreases at 240 and 360 minutes (Figure 3), which could indicate kinin release in both lethal and nonlethal groups. In the lethal group, significant changes in HK levels were evident within the first 120 minutes. The loss of HK activity correlated with MSAP decline ($R = 0.93$). Thus, the contact system was activated with the subsequent release of bradykinin which likely contributes to the further decline of the MSAP. This conclusion is supported by the early (60 min) increase of α2M-kal complexes (Figure 3, Table 1) indicating kallikrein formation, which again demonstrated a significant inverse correlation of α2M-kal complexes with MSAP ($R = 0.93$).

In the nonlethal group, a significant decrease in HK begins later than 120 minutes and was less profound than that of the lethal group. Significant levels of α2M-kal complexes were not found in the nonlethal group. Most likely this finding is due to the greater contribution by C1̄-inhibitor in regulating the contact system in the nonlethal group where a more limited activation of the contact system may occur without a marked decline of C1̄-inhibitor levels.[52] No correlation was found between MSAP and either HK decline or α2M-kal complex formation in the nonlethal group.

Hypotension frequently occurs as a complication in patients with bacteremia. The pathophysiology of hypotension associated with bacteremia is not yet understood, but increased vascular permeability and arteriolar vasodilation are an important mechanism. Vasodilation may be initiated by the release of bacterial cell wall components: endotoxin, from gram-negative organisms, and peptidoglycan, from gram-positive pathogens. These complex molecules activate the contact and complement systems with release, respectively, of the nonapeptide bradykinin and the anaphylatoxins, C3a and C5a. Endotoxin also activates monocytes to produce cytokines that include IL-1 and TNF, which also contribute to increased vasodilation and capillary permeability. Whole bacteria or endotoxin when infused was found by some investigators to activate factor XII or prekallikrein directly.[21,22] Alternatively, the presence of bacteria in the blood can stimulate neutrophils and/or monocytes causing release of granular proteolytic enzymes or active oxygen radicals, which in turn may perturb endothelial cells and expose components of the subendothelium. These changes can create a reactive environment (i.e., provide an activating surface and/or a protected area from serpins) which favors activating the contact system beginning with factor XII.

The SERPIN, C1̄-inhibitor is the primary regulatory inhibitor of activated factor XII, kallikrein (the other kallikrein inhibitor is α_2-macroglobulin) and the complement protease, C1̄.[14,15,54–56] The activation of factor XII and the contact system can thus be potentiated by the reduction in levels of C1̄-inhibitor due to complex formation with proteases or due to the proteolytic cleavage of the SERPIN.[52] The lowering of C1̄-inhibitor levels may facilitate contact activation allowing for bradykinin-mediated hypotension, as observed for the cases of patients with hereditary angioedema.[57] In previous studies, detailed interpretation of the levels of contact system proteins participating in bacteremic shock has not been possible owing either to constraints of experimental design or the fact that a limited number of components of the kallikrein-kinin system were measured. The value of the baboon models used in this study is the ability to compare several samples to the initial value, allowing an assessment of the temporal sequence of the changes.

This information together with information obtained in earlier baboon studies[35–37] suggests the following hypothesis. On exposure of monocytes to increasing quantities of endotoxin, TNF, and IL-1 are released. These cytokines act on the vascular endothelium and smooth muscle, causing vasodilation and the decline in MSAP observed during the 60–120 minute interval. During the first 60 minutes, these cytokines also act on neutrophils causing margination and granular release, which results in endothelial cell damage exposing putative contact-activating surfaces, such as elastin, collagen, and basement membrane. During this first 60 minutes, limited but sustained contact and complement activation is occurring, with bradykinin release contributing to vasodilation. The contribution of bradykinin to the initial decline in MSAP is masked by the more dramatic effects of cytokines on endothelial cells to stimulate formation of the vasodilators PGI_2 and nitrous oxide. The contact and complement system during the first 60-minute interval is highly regulated by the presence of C1̄-inhibitor. Formation of α2M-kal complexes during this interval is not significant, since C1̄-inhibitor predominates. However, C1̄-

inhibitor is consumed by regulating proteases[52] or by bacterial protease cleavage,[22] resulting in lower concentrations of functional C1̄-inhibitor, subsequently allowing an increase in the participation of α2M in regulating the activity of kallikrein after 60 minutes. At 120 minutes, or the end of bacterial infusion, endotoxin levels decline along with the levels of TNF and IL-1, no longer modulating endothelial cell function. At this time, C1̄-inhibitor levels would be low and the damaged endothelium exposes activating surfaces, which sustains contact activation and bradykinin release. Continued release of bradykinin probably sustains and enhances the decline in MSAP after 120 minutes, allowing for irreversible end organ damage and eventual death. This mechanism is supported by the nonlethal model where the contact system is less active, i.e., the decline in HK is less intense and α2M-kal complexes are not usually detectable. MSAP declines during the 60–120 minute time period, as a result of cytokine or other influencing factors. However, due to a lack of sustained contact activation and bradykinin release, the MSAP was able to return to baseline after 120 minutes.

Additional support for the contribution of the contact system to the secondary MSAP in this lethal baboon model comes from recent studies in which a monoclonal antibody against factor XII was used to block activation of the contact system.[58] Inhibiting the contact system resulted in no effect on the initial MSAP decline nor the coagulopathy observed in this model. The secondary MSAP decline was reversed, with values returning toward normal during the 6 hours of this study.

Further support for this mechanism is suggested in a rat hypotensive model using the bradykinin antagonist, NPC567.[59,60] In this model, lipopolysaccharide (LPS) infusion over 10 minutes causes an initial decline in blood pressure reaching a nadir at 1 hour. A second decline in blood pressure is observed after 2.5 hours and continues until death. Infusion of the bradykinin antagonist inhibited the initial blood pressure decline by 60%, and completely blocked the secondary hypotensive effect and extended the lifetime of the animals with a 50% decrease in the mortality of the treated animals.

Therapy directed against specific mediators such as bradykinin or inhibitors of the contact system enzymes, which can abort a continuing decline in MSAP during bacteremia and allow continued tissue perfusion may aid in preventing extensive organ damage; however, the uncorrected DIC may also serve as a contributing factor to mortality. Inhibition of mediator formation or their effects in combination with an agent to control DIC, may allow the administered antibiotics time to contain the bacterial proliferation, thus decreasing the high mortality rate for this disease.

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