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Clotting and Other Plasma Factors in Experimental Endotoxemia: Inhibition of Degradation by Exogenous Proteinase Inhibitors

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Abstract. Endotoxemia in dogs was induced by a slow intravenous infusion of *E.* coli endotoxin for 2 h. Thereby, a significant decrease was observed in the plasma levels of several clotting, fibrinolysis and complement factors. The changes were studied over an experimental period of 14 h and checked for statistical significance by three-way analysis of variance. Application of the broad-spectrum proteinase inhibitor aprotinin (Trasylol®) from bovine organs clearly lowered the endotoxin-induced decline of the plasma proteins studied. By intravenous application of a specific granulocytic proteinase inhibitor (Bowman-Birk inhibitor from soybeans), the endotoxin-induced reduction of the plasma proteins was prevented in a similar manner. It can be concluded that at least some of the pathobiochemical mechanisms observed in clotting, fibrinolysis and complement systems during endotoxemia are not only caused by a severe consumption reaction but also by unspecific proteolytic degradation due to neutral granulocytic proteinases.

Introduction

Characteristic changes in the clotting, fibrinolysis and complement systems (summarized as 'blood systems') during both gram-negative sepsis in man or experimental endotoxin shock in other mammalian species are usually considered to be an indication of disseminated intravascular coagu-

lation (DIC) [1, 15, 16, 18, 19, 26, 28]. There are, however, divergent views concerning the trigger mechanism of endotoxin-induced DIC [26, 28, 29]. Direct activation of the coagulation system by endotoxin does not play any major role since the amounts of endotoxin, highly active in vivo, only cause minor changes in the coagulation system in vitro [28]. More likely, the release of several activators from leukocytes [20, 22, 24, 25, 30, 35], platelets [29, 30] and endothelial cells [11, 26, 34] leads to specific activation of the above-mentioned blood systems resulting in severe consumption (DIC) of the involved proteins. Besides this drastic activation followed by elimination of the plasma proteins via complex formation with inhibitors (in the case of the blood system proteinases) or specific degradation (e.g. of factor V and VIII), an unspecific proteolytic degradation of plasma proteins by granulocytic proteinases is also discussed [8–10, 38]. Only recently it has been demonstrated that both human and dog granulocytes contain large amounts of neutral proteinases such as elastase and cathepsin G, which are probably released by stimulation with endotoxin [2, 8, 10, 40]. The activities of clotting factors either in their isolated form or in plasma are strongly reduced by digestion with elastase or cathepsin G [10, 39]. Even in vivo the reduction of such factors could be due to degradation by these proteinases under certain pathological conditions [9].

The in vitro interactions of human granulocytic elastase and cathepsin G with proteinase inhibitors from animal, plant and microbial tissues have been thoroughly investigated [37]. Interactions under in vivo conditions could also be demonstrated indirectly by application of an elastase-cathepsin G inhibitor (Bowman-Birk inhibitor, BBI) in the course of experimental gram-negative sepsis in dogs [38]. Inhibitor medication (BBI infusion) prevented the rapid fall in activity of the fibrin-stabilizing factor, F XIII. The authors suggested that the decrease in F XIII activity is caused by direct nonspecific proteolysis rather than by the action of thrombin throughout severe DIC.

The purpose of this investigation was: (i) to establish an endotoxemia model by studying the concentration patterns of several clotting (prothrombin, antithrombin III, F XIII), fibrinolysis (plasminogen, antiplasmin) and complement (C3) factors after slow intravenous endotoxin infusion in dogs; (ii) to study the therapeutical effect of a relatively specific inhibitor of granulocytic elastase and cathepsin G (BBI) on the concentration pattern of the above-mentioned proteins, and (iii) to distinguish between consumption of plasma proteins by DIC and/or by nonspecific proteolytic degradation due to granulocytic proteinases. To achieve this purpose we applied both BBI

directed against granulocytic proteinases and the broad spectrum proteinase inhibitor aprotinin, the effective agent in the drug Trasylol®. Aprotinin should be able to prevent primarily the activation of the clotting and fibrinolysis systems (and thus indirectly also the complement system) by inhibition of plasma kallikrein and of plasmin.

Materials and Methods

Endotoxin and Proteinase Inhibitors Used

Endotoxin of *E. coli* 026: K 60 (B) H 11 (H 311b) was prepared by extraction with trichloroacetic acid and heated phenol according to *O'Neill and Todd* [33].

The elastase-cathepsin G inhibitor from soybeans (type: Bowman-Birk) was isolated from a raw extract ('trypsin inhibitor from soybeans', Serva No. 37340) by gel filtration on Sephadex G-75 (Pharmacia, Uppsala) with 2% acetic acid as eluant. The inhibitor-containing fractions were sampled, freeze-dried, and redissolved in an isotonic saline solution; the pH value was adjusted to 7.4 by 2 *N* NaOH [43].

The trypsin-kallikrein inhibitor aprotinin from bovine organs, trade name Trasylol® (6,660 kallikrein inhibitor units/mg), was obtained from Bayer AG, Wuppertal-Elberfeld.

Experimental Procedure

Animals. 12 mongrel dogs of either sex served to establish an endotoxemia model. 6 animals were randomly allocated to the control (placebo) group and another 6 dogs to the endotoxemia group. 4 dogs were employed for the BBI medication group, 6 dogs for the aprotinin group only to observe trends of the inhibitor therapy.

Experiments. Anesthesia was induced by intravenous application of 30 mg/kg body weight of pentobarbital (Nembutal®). Small doses of this drug were given further on as bolus injections during the experimental period. For laryngeal catheterization we used a cuffed endotracheal tube. For catheterization of the descending aorta a nylon catheter was inserted via the femoral artery. The inferior vena cava was catheterized via the femoral vein. Mean aortic blood pressure (p_A) and heart rate were monitored continuously using a Siemens oscillograph recorder and a Statham element.

Endotoxin Application. Endotoxemia was induced by continuous infusion of 2 mg/kg body weight of *E. coli* endotoxin, dissolved in isotonic saline solution, into the inferior vena cava over a 2-hour period. Control animals were subjected to the same experimental procedure except that they received a sterile isotonic saline solution (placebo) instead of endotoxin solution.

Sampling Procedure. The first data (mean aortic blood pressure, blood samples) were collected immediately after onset of anesthesia and just before application of endotoxin. Treatment with the endotoxin lasted 2 h. At this time and 6, 10 and 14 h after starting the experiment additional data were recorded. Each time two 4.5-ml blood samples from the

inferior vena cava were withdrawn into plastic syringes containing 0.5 ml of sodium citrate (2.2 g/100 ml distilled water). If the blood samples were not analyzed immediately after withdrawal, they were stored at −70 °C until assayed. Throughout the experiment all animals received a constant infusion of an isotonic saline solution (40 ml/h). The withdrawn blood volume did not exceed 50 ml/dog over the experimental period of 14 h.

Inhibitor Application. In inhibitor-medicated dogs endotoxin and inhibitor infusion were started simultaneously, but the inhibitor application was continued over the 14-hour experimental period. Each of these animals received per kilogram body weight, 10–25 inhibitor units (3–8 mg) of BBI or 40,000 or 80,000 kallikrein inhibitor units of Trasylol.

Special Methods

Blood Cells. Leukocyte counting was performed in a cell counter (Coulter Counter, Mod. B) while platelets were determined using a counting chamber (Neubauer Zählkammer). Whole blood hemoglobin and hematocrit values were measured using a hemoglobinometer (Counter Electronics Ltd.) and microhematocrit capillaries.

Amidolytic Activities of Clotting and Fibrinolysis Factors. The amidolytic activities of individual factors were determined using specific chromogenic peptide substrates. Chromozym TH (Tos-Gly-Pro-Arg-pNA) for prothrombin determination was from Boehringer, Mannheim; S–2238 (H-D-Phe-Pip-Arg-pNA) for antithrombin III determination and S–2251 (H-D-Val-Leu-Lys-pNA) for plasminogen and antiplasmin determination were donated by Deutsche Kabi, Munich, and Kabi Diagnostica, Stockholm, Sweden. The commercial companies' instructions were modified as indicated in table I.

Biological Activity of Clotting Factor F XIII. The biological activity of the fibrin-stabilizing factor (F XIII) was measured with a marketable test system (Faktor XIII-Schnelltest, Behringwerke Marburg, No. 0TXS 10) according to the modification of *Bohn and Haupt* [5]. By applying very small dilution steps, this semiquantitative test was refined to a nearly quantitative method. As the concentrations of F XIII varied significantly between individual animals, the activity changes throughout the experiment are given as percentages of the individual starting value.

Immunochemical Determination of Complement Factor C3. Complement factor C3 was quantified by modifying the single radial immunodiffusion technique of *Mancini* et al. [27] as follows: 3 ml of 1% agarose was mixed with 150 µl antiserum of goat anti-dog C3 (Nordic, Tilburg, The Netherlands) and the mixture poured into transparent boxes from Desaga, Heidelberg. Precipitation was achieved in each case by applying 10 µl of 1:8 diluted dog plasma into suitable wells. Internal standardization was done by setting up a dilution series (1:8, 1:16, 1:32) of each dog's starting plasma. The diameters of the precipitation rings were squared and plotted against the concentration in percentages (100%, 50%, 25%). From these graphs, the changes in the C3 amount in percent were determined for each animal throughout the experiment.

Table I. Conditions for the photometric measurements of various clotting and fibrinolysis factors with chromogenic substrates

	Buffer		Dog plasma	Activation		Incubation		Chromogenic substrate
	µl	pH	µl	by	µl	min	°C	µl
Prothrombin	1,025	8.6	30	cephalin[1]	100			125
	(0.05 *M* Tris, 0.1 *M* NaCl, 0.01 *M* $CaCl_2$, 10 µg Trasylol/ml)		(diluted 1:20 with 0.9% NaCl)	RVV[2]	50 (= 6 µg)	5	25	(chromozym TH, 1.5 *M*)
Antithrombin III	370	8.4	30	thrombin	100			300
	(0.05 *M* Tris, 7.5 m*M* EDTA, 3 IU heparin/ml)		(diluted 1:100 with buffer)	(45 nkat/25 ml)		5	25	(S-2238, 0.75 *M*; 1 mg polybrene[3]/ml)
Plasminogen	300	7.4	20	Kabikinase	40			840
	(0.05 *M* Tris, 0.12 *M* NaCl)		(diluted 1:5 with buffer)	(= streptokinase)	(8,000 IU)	20	37	(S-2251, 3 m*M*)
Antiplasmin	380	7.4	20	plasmin	400			200
	(0.05 *M* Tris, 0.12 *M* NaCl)		(undiluted)	(22 nkat/10 ml buffer)		1	25	(S-2251, 3.5 m*M*)

IU = Inhibitor units.
[1] PTT reagent from Boehringer, Mannheim.
[2] Russel's viper venom from Sigma, Munich.
[3] From EGA-Chemie, Steinheim.

Statistical Evaluations

Three-Way Analysis of Variance. To study the established endotoxemia model, the statistical evaluation of the differences between the control (placebo) group (n = 6) and the endotoxemia group (n = 6) was done by a three-way analysis of variance without replications [7]. The random factor 'animals' was considered to be nested within the treatment groups since the animals could only be observed under endotoxin or placebo, respectively. Treatment (endotoxin or placebo) and time were used in the analytical model as fixed factors. Therefore different effects could be analyzed: the effects of treatment and time and the interaction between time and treatment. The existence of a significant interaction between time and treatment was interpreted as different time courses in the placebo group and the endotoxemia group. The level of significance was established at $p \leq 0.05$. For the different parameters the values obtained just before the application of endotoxin or placebo served as 100% values.

Student's t Test. Statistical evaluation of the results obtained from endotoxemia animals with *inhibitor-treatment* (n = 4 for BBI and n = 6 for aprotinin) and the corresponding endotoxin-treated dogs (n = 6) was performed by Student's t test for independent observations. Considering that the tests were not independent we used the t tests only to observe trends of the inhibitor therapy. We considered p values ≤ 0.05 to be appropriate for interpretation.

Results

Endotoxemia Model

Aortic Blood Pressure. At the end of the endotoxin infusion period the mean aortic blood pressure was reduced from 18.11 ± 0.88 to 13.86 ± 1.04 kPa (mean ± SEM). The blood pressure usually remained constant at this level until termination of the experiment. This difference turned out, however, to be not statistically significant (table II).

Hematological Data. The blood *hemoglobin* and *hematocrit* values in the control group did not differ significantly (table II) from those of the endotoxemia group, although there was a moderate hemoconcentration in the animals of the latter group as described by other authors, too [1]. Endotoxin infusion caused a substantial and rapid decrease of circulating *platelets* (starting value: $\bar{x}$ = 251,700 ± 22,923 cells/μl; 2-hour value: $\bar{x}$ = 98,233 ± 18,396 cells/μl). From the end of endotoxin infusion until the end of the experiment only a small increase in cell count ($\bar{x}$ = 145,060 ± 15,226 cells/μl) was observed (fig. 1). Similarly, a marked decline occurred in the numbers of circulating *leukocytes* ranging from approximately 9,000 to 4,000 cells/μl during the endotoxin-infusion period (fig. 2). From the 6th

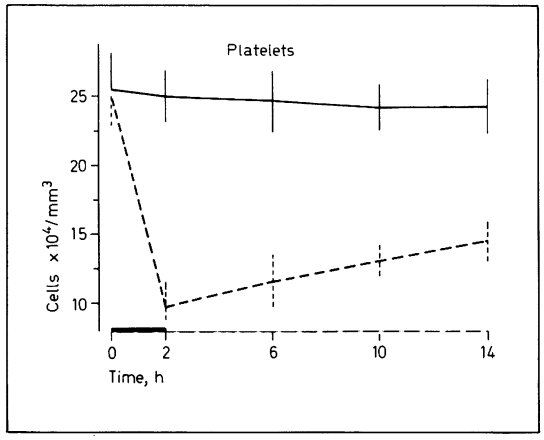

Fig. 1. Changes in the platelet counts during the acute phase of experimental endotoxemia in dogs (mean values ± SEM). —— = Control group (n = 6); – – – = endotoxin group (n = 6); thick line at the abscissa = endotoxin infusion period.

Table II. Three-way analysis of variance in an endotoxemia model with dogs (control group n = 6, endotoxin group n = 6)

Parameter	p values for differences in		
	groups	time points	time courses between groups
Leukocytes	0.006	0.001	0.001
Thrombocytes	0.001	0.001	0.001
Factor XIII	0.001	0.001	0.001
Prothrombin	0.003	0.001	0.001
Antithrombin III	0.05	0.001	0.004
Plasminogen	0.003	0.001	0.05
Antiplasmin	0.002	0.001	0.004
Complement C3	0.001	0.001	0.003
Aortic blood pressure	0.22 (n.s.)	0.001	0.368 (n.s.)
Hemoglobin	0.207 (n.s.)	0.960 (n.s.)	0.043
Hematocrit	0.111 (n.s.)	0.123 (n.s.)	0.043

The significances (p values) are indicated for the differences between the control and the endotoxin group as well as for different behavior of both groups at each time point and during the whole time course of experimental treatment.
n.s. = Not significant.

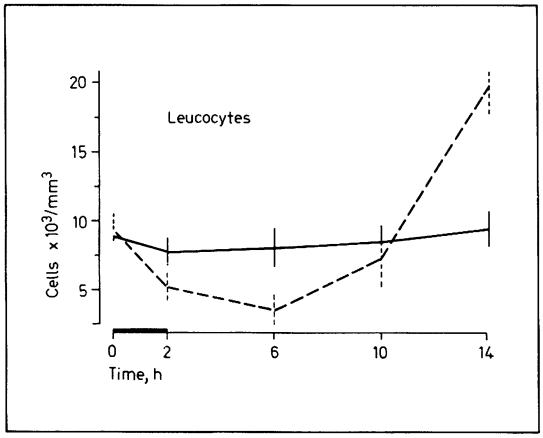

Fig. 2. Changes in the leukocyte counts during the acute phase of experimental endotoxemia in dogs (mean values ± SEM). —— = Control group (n = 6); – – – = endotoxin group (n = 6); thick line at the abscissa = endotoxin infusion period.

until the 14th h, however, a strong leukocytosis developed leading to leukocyte counts ($\bar{x}$ = 20,000 ± 2,790 cells/µl) being far higher than before endotoxin application.

Clotting Parameters. In the animals receiving endotoxin the plasma levels of *prothrombin* and *antithrombin III* (fig. 3) declined linearly up to the 6th h and remained nearly constant at these levels (14-hour value for prothrombin: $\bar{x}$ = 34.2 ± 8.5%; for antithrombin III: $\bar{x}$ = 62.8 ± 10 %. As indicated in table II, these values were reduced most significantly for prothrombin but less so for antithrombin III. The biological activity of *F XIII* (fig. 3) was strongly affected by the endotoxin infusion decreasing linearly to $\bar{x}$ = 39.2 ± 4.7% at the end of the observation period.

Fibrinolysis Parameters. Plasminogen levels (fig. 3) fell markedly during endotoxin infusion resulting after 14 h in a mean value of $\bar{x}$ = 47.4 ± 7.9%. The antiplasmin levels (fig. 3), determined as 'immediate' antiplasmin activity (a_2-antiplasmin, [41]), nearly paralleled the decrease in plasminogen concentration to $\bar{x}$ = 48.6 ± 7.8% at the 14th h.

Complement Factor C3. A significant drop in the plasma concentration of C3 to $\bar{x}$ = 56.4 ± 9.6% of the starting value was already observed during

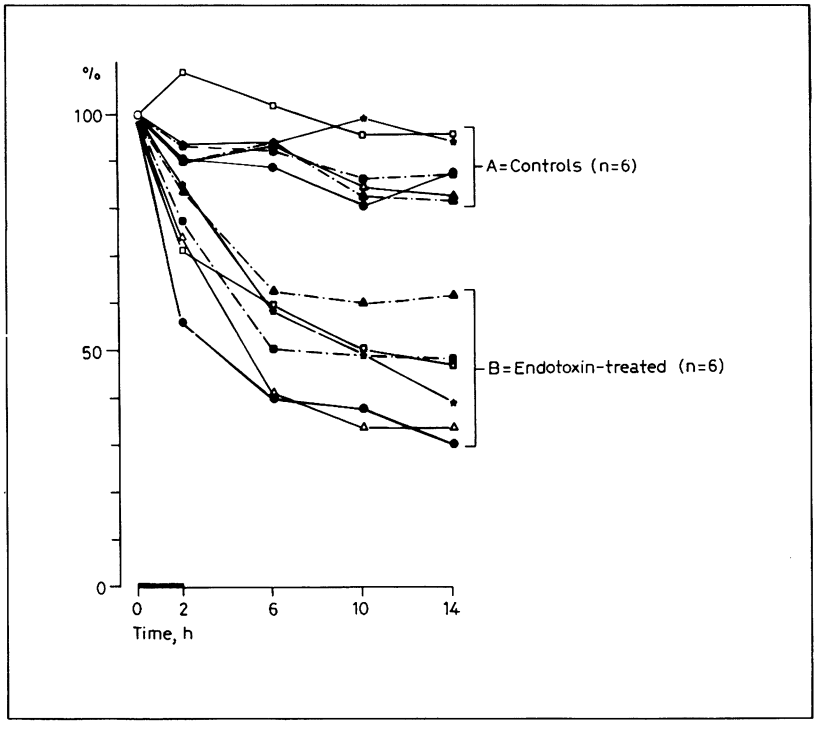

Fig. 3. Changes in the plasma levels of various plasma factors during the acute phase of experimental endotoxemia in dogs (standard deviations are indicated in table III). Thick line at the abscissa = endotoxin infusion period. ● = Complement C3; ★ = factor XIII; △ = prothrombin; ▲ = antithrombin III; □ = plasminogen; ■ = antiplasmin. For further details see text.

the endotoxin application period. At the end of the experiment (14th h) the concentration had finally reached $\bar{x}$ = 30.8 ± 2.8% (fig. 3). In comparison, the control animals showed only a small decrease in their C3 level ($\bar{x}$ = 86.0 ± 4.9%) up to the 14th experimental hour.

Inhibitor-Treated Animals

Hematological Data. The blood *hemoglobin* and *hematocrit* values of the animals subjected to inhibitor therapy showed no significant changes compared to those of the endotoxemia dogs.

As already demonstrated in figure 1, there was a dramatic decline of circulating *platelets* during the endotoxin infusion period, followed there-

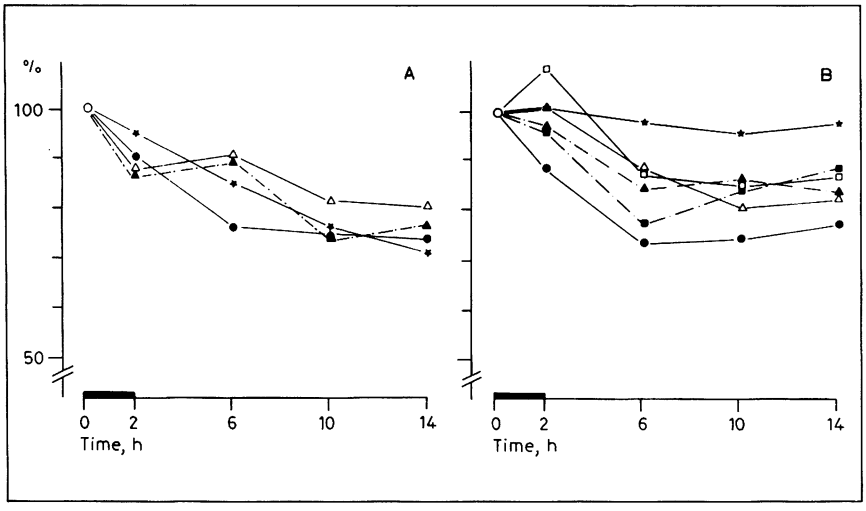

Fig. 4. Changes in the plasma levels of various plasma factors during the acute phase of inhibitor-treated experimental endotoxemia in dogs (standard deviations are indicated in table III). **A** Aprotinin-treated group (n = 6). **B** Bowman-Birk inhibitor-treated group (n = 4). Thick line at the abscissa = endotoxin infusion period. For definition of the symbols see figure 3. For further details see text.

after by a modest increase in cell count. It seems that this endotoxin-induced fluctuation could not be influenced by either BBI or aprotinin application. Similarly, dogs subjected to BBI or aprotinin treatment showed a fluctuation in leukocyte numbers comparable to that of the endotoxemia animals. So, both the endotoxin-induced decline and increase of leukocyte numbers is not influenced by the exogenously administered proteinase inhibitors.

Factors of the Clotting, Fibrinolysis and Complement Systems. Figure 4 summarizes the patterns of the chosen parameters as obtained under various inhibitor applications. During therapy with the *elastase-cathepsin G-inhibitor BBI,* the endotoxin-induced decrease of the measured plasma factor levels (fig. 3B) was reduced after 14 h ($p \leq 0.05$) as shown in figure 4B. The corresponding mean values and their standard deviations are compiled in table III. Remarkably, the positive influence of the inhibitor medication was independent of the applied dose (3–8 mg/kg body weight) suggesting that even the lowest inhibitor dosage used (3 mg) was capable of preventing

Table III. Changes in the plasma levels of various plasma factors during the acute phase of experimental endotoxemia in dogs and under inhibitor medication

Parameter	Test time h	Control group (n = 6)	Endotoxin group (n = 6)	BBI group (n = 4)	Aprotinin group (n = 6)
Factor XIII	0	100	100	100	100
	2	94.0 ± 4.0	88.0 ± 3.0	101.5 ± 1.5	95.2 ± 5.2
	6	94.2 ± 4.7	63.2 ± 4.2	98.3 ± 1.8	85.2 ± 4.4
	10	99.2 ± 1.3	51.0 ± 4.0	96.5 ± 2.1	76.7 ± 7.2
	14	94.5 ± 3.0	41.2 ± 4.3	98.3 ± 1.8	71.7 ± 7.9
Prothrombin	0	100	100	100	100
	2	90.0 ± 3.0	78.0 ± 8.5	101.8 ± 2.7	88.2 ± 7.1
	6	93.7 ± 2.7	48.0 ± 11.5	88.5 ± 4.7	91.0 ± 1.8
	10	85.0 ± 3.5	40.0 ± 9.0	81.5 ± 6.2	82.3 ± 6.4
	14	82.7 ± 5.7	40.0 ± 9.0	83.0 ± 6.6	81.2 ± 4.2
Antithrombin III	0	100	100	100	100
	2	90.0 ± 3.7	87.0 ± 7.0	97.8 ± 5.8	86.8 ± 8.6
	6	93.8 ± 2.4	67.0 ± 8.0	85.3 ± 7.9	89.7 ± 3.9
	10	83.0 ± 2.7	65.0 ± 8.0	87.0 ± 5.8	74.5 ± 11.0
	14	82.0 ± 3.2	67.0 ± 9.0	84.0 ± 8.5	77.2 ± 6.5
Plasminogen	0	100	100	100	n.d.
	2	109.0 ± 2.8	78.0 ± 13.0	109.3 ± 3.8	
	6	102.0 ± 3.0	64.0 ± 11.0	87.3 ± 6.9	
	10	96.0 ± 3.7	57.0 ± 9.3	86.0 ± 11.3	
	14	96.0 ± 4.3	50.0 ± 7.0	87.5 ± 6.5	
Antiplasmin	0	100	100	100	n.d.
	2	93.5 ± 3.7	80.7 ± 7.5	97.0 ± 6.4	
	6	92.5 ± 2.7	55.5 ± 8.3	78.3 ± 9.9	
	10	86.2 ± 5.5	52.5 ± 5.7	85.3 ± 6.7	
	14	87.5 ± 4.0	52.5 ± 7.5	89.0 ± 8.4	
Complement C3	0	100	100	100	100
	2	90.7 ± 4.3	62.5 ± 10.0	89.3 ± 10.1	90.6 ± 7.9
	6	88.3 ± 5.0	47.0 ± 8.8	74.3 ± 4.9	76.6 ± 4.4
	10	80.7 ± 6.0	41.3 ± 5.7	75.3 ± 9.8	75.2 ± 6.1
	14	88.0 ± 4.0	33.7 ± 3.7	78.0 ± 9.3	74.4 ± 8.0

The data of each group at different test times are indicated as percentages of the individual starting values (mean values ± SEM). The endotoxin infusion (2 mg/kg body weight) was performed for the first 2 h, the inhibitor infusion (per kg body weight 3 or 8 mg BBI and 40,000 or 80,000 kallikrein inhibitor units of aprotinin, respectively) for the whole observation period (14 h).

The statistical significance was evaluated by the three-way analysis of variance ($p \leq 0.05/0.001$ for the differences between the control and the endotoxin group) and by Student's t test ($p \leq 0.05/0.01$ for the differences between the endotoxin group and each inhibitor-medicated group). For further details, see text.

n.d. = Not done because the aprotinin present in the plasma acts as a strong plasmin inhibitor.

the endotoxin-induced degradation of the plasma proteins. In addition, the broad spectrum proteinase inhibitor *aprotinin* also significantly prevented the endotoxin-induced decline of the coagulation and complement proteins (fig. 4A). In this case the fibrinolysis factors (plasminogen and antiplasmin) could not be assayed with the chromogenic substrate because aprotinin present in the plasma samples acts as a strong plasmin inhibitor.

Discussion

In recent years, numerous reports have been published dealing with endotoxin-induced changes of hematological and biochemical parameters in various mammalian species. Due to different sensitivities of the diverse species to endotoxin [14, 21] as well as to different experimental approaches [1, 32] individual results can be compared only with strong reservations. Therefore, we took advantage of an experimental procedure enabling us to study some of the most interesting factors simultaneously with the intention of establishing a statistically verifiable model of endotoxemia.

Indeed, the statistical evaluation displayed the differences in the levels of the various parameters as being significant ($p \leq 0.05$) for the control and endotoxemia group, the test times and the time course. This clearly shows the validity of our experimental model as a convenient model of endotoxemia. Obviously, the substantial alterations in blood cell counts and in plasma protein levels are characteristic for endotoxin-induced DIC, especially the decrease in blood platelets and the activation and consumption of clotting and fibrinolysis factors [1, 3, 16, 28, 30]. Moreover, a significant loss of factor C3 of the complement system could be observed in the early hours of the experimental period. Although the relationship between the complement and the coagulation-fibrinolysis systems is only partly defined as yet [6, 16, 31], involvement of the complement systems seems to be a major requirement for the full expression of progressive DIC in endotoxemia. Activation of the complement system via the alternative pathway, that means via C3, should be due not only to direct endotoxin action [17] but also to proteolysis by plasmin liberated during endotoxemia [3, 13, 14, 42]. In addition, plasmin has been shown to activate complement factor C1 [36] thus causing complement consumption through the classical pathway. The given assumptions are in agreement with recent observations of *Aasen* et al. [4], indicating complement activation via both pathways in the course of canine endotoxin shock.

It should be emphasized, however, that besides activation of complement in connection with DIC, also a direct activation of factor C3 by granulocytic elastase may occur [23]. In fact, *Aasen and Ohlsson* [2] recently demonstrated release of granulocytic elastase in canine endotoxin shock, regularly accompanied by a marked decrease in the concentration of factor C3.

Although DIC certainly arises in the course of septicemia and acute myeloic leukemia [8, 10], there is also strong evidence of a direct proteolysis of coagulation factors by granulocytic proteinases. For example, *Egbring* et al. [8] and *Egbring and Havemann* [10] were able to demonstrate that subunit A (the active transglutaminase) and subunit S (the carrier protein) of F XIII were consumed to a comparable degree throughout the above-mentioned diseases. The reduction of both subunits, however, is not consistent with the usual consumption of F XIII during blood clotting, since subunit A and F XIII activity disappear completely after coagulation of plasma, whereas subunit S remains unchanged. As the elastase-like granulocytic proteinase (ELP) caused a similar reduction of the activity of both F XIII subunits in plasma, the authors suggested that unspecific proteolysis by ELP is involved to an appreciable degree in patients with septicemia and acute leukemia.

The results of our experimental approach are an indirect but clear indication that besides DIC unspecific proteolysis has to be considered as an important factor responsible for the severe consumption of C3, F XIII and other plasma proteins as well. This conclusion is based on the observation that upon medication with BBI, a relative specific inhibitor of granulocytic elastase and cathepsin G, the decrease of the plasma protein levels was prevented highly significantly, though not totally (fig. 4B). In this respect the F XIII level is of special interest. Upon BBI infusion the endotoxin-induced fall of F XIII activity was completely prevented (fig. 4B), whereas upon application with aprotinin, which is supposed to inhibit preferable plasmin and plasma kallikrein, a considerable reduction of the F XIII clotting activity was maintained (fig. 4A). This indicates that F XIII inactivation is rather due to unspecific proteolysis than to DIC, thus confirming results of more recent studies dealing with gram-negative sepsis in dogs [38].

The residual consumption of the other plasma factors during BBI medication (fig. 4B) should reflect, at least to some extent, their DIC-induced elimination. However, quantitation of the degree of consumption due to either unspecific proteolysis or DIC is not yet feasible. On one hand, our knowledge on the interrelations between both mechanisms as well as on the

inhibition spectrum of both inhibitors is still incomplete. On the other hand, aprotinin was applied in an unusually high dosage (corresponding to 2.8 and 5.6 $\times$ 10^6 kallikrein inhibitor units within 14 h in a 70-kg patient) so that at least partial inhibition of elastase and cathepsin G by this drug cannot be excluded; a specific affinity of aprotinin to both proteinases has been recently reported [12]. That means, in the aprotinin-treated animals the degree of reduction of the plasma factors (fig. 4A) probably does not only reflect the prevention of DIC-induced consumption, but also the effect of other properties of this drug [12] not yet quantifiable in our experimental model.

The present study indicates clearly that during inflammatory processes, such as septicemia or endotoxemia, the potential of the plasma proteinase inhibitors may be considerably overstressed, thus leading to severe degradation of the blood system proteins. Obviously, the liberated granulocytic proteinases can degrade individual factors in a pathophysiologically relevant extent already in the presence of an excess of the plasmatic antiproteinases. Results of in vitro investigations from *Egbring* et al. [8] confirm this view. The authors showed that elastase-like and chymotrypsin-like proteinases are able to degrade several coagulation factors in plasma although the antiproteinase capacity of α_1-antitrypsin, α_2-macroglobulin and α_1-antichymotrypsin has not been exhausted. As shown for the first time in our endotoxemia model, application of a suitable inhibitor of leukocytic proteinase (BBI) can significantly prevent unspecific degradation of plasma proteins, thus maintaining the physiological balance of the natural defence mechanisms. However, it is just as important to minimize simultaneously DIC induced by system-specific proteinases throughout the inflammatory diseases. For the latter purpose aprotinin (Trasylol®), already used in medical therapy, and/or the thrombin-specific inhibitor hirudin are potential candidates, whereas BBI or inhibitors with similar properties might be applied in future to prevent unspecific proteolysis.

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