Protein Degradation in Health and Disease

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Proteinase inhibitors in severe inflammatory processes (septic shock and experimental endotoxaemia): biochemical, pathophysiological and therapeutic aspects

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Abstract

Plasma levels of antithrombin III, α2-macroglobulin and inter-α-trypsin inhibitor, as well as those of various clotting, complement and other plasma factors, were significantly decreased in 18 patients suffering from hyperdynamic septic shock. A similar statistically significant reduction of the concentrations of several plasma factors (prothrombin and antithrombin III, plasminogen and α2-plasmin inhibitor, complement factor C3 and clotting factor XIII) was observed in experimental endotoxaemia. In this model the reduction in the plasma levels of these factors was considerably diminished by the intravenous injection of a granulocytic elastase–cathepsin G inhibitor of lower molecular weight from soybeans. The results of both studies indicate that consumption of plasma factors in the course of Gram-negative sepsis proceeds not only via the classical routes (by activation of the clotting, fibrinolytic and complement cascades by system-specific proteinases such as thrombokinase or the plasminogen activator) but also to an appreciable degree by unspecific degradation of plasma factors by neutral proteinases such as elastase and cathepsin G. The endotoxin-induced release of both sorts of proteinases, the system-specific ones and the unspecific lysosomal proteinases from leucocytes and other cells, is likely to be mainly responsible for the consumption of antithrombin III and α2-macroglobulin via complex formation (followed by elimination of the complexes) and the increased turnover of the inter-α-trypsin inhibitor as observed in the clinical study.

The therapeutic use of an exogenous elastase–cathepsin G inhibitor in the experimental model was stimulated by the observation that human mucous secretions contain an acid-stable inhibitor of the neutral granulocytic proteinases, called HUSI-I or antileucoproteinase. This inhibitor protects mucous membranes

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and soluble proteins against proteolytic attack by leucocytic proteinases released in the course of a local inflammatory response. Preliminary results indicate that HUS1-I, which is produced by the epithelial cells of mucous membranes, does not belong to any known structural type of acid-stable proteinase inhibitor. The search for other candidates suitable for medication in humans led to the discovery of a potent elastase–cathepsin G inhibitor, called eglin, in the leech *Hirudo medicinalis*. This acid-stable inhibitor with a molecular weight close to 8100 has an unusual structural property in that the structure of the molecule is not stabilized by any disulphide bridge.

The course of a disease like septicaemia or septic shock is often complicated by severe pathobiochemical processes in the circulation. These are, for example, disseminated intravascular coagulation (DIC) caused by activation of the clotting and fibrinolytic cascades, and anaphylactic responses induced by activation of the complement system (Hamilton et al 1978, Müller-Berghaus et al 1976, Garner et al 1974, McCabe 1973). These activation reactions may be triggered by endotoxins — that is, lipopolysaccharides from Gram-negative bacteria (Jeljaszewicz & Waldström 1978, Urbaschek et al 1975). Endotoxins can damage biological membranes and thus induce the release of constituents, so-called mediators of inflammation, including lysosomal enzymes, from various body cells (Weissmann 1974, Urbaschek et al 1975, Myrvold 1976, Movat 1979). These enzymes normally exhibit their physiological function, namely degrading phagocytosed material, inside the cell (Klebanoff & Clark 1978). If released into the circulation, however, they may enhance the inflammatory response by several routes.

System-specific proteinases such as the plasminogen activator and thrombokinase activate the blood systems (see Fig. 1) by proenzyme → enzyme conversion — that is, by specific proteolytic cleavages. These enzymes are responsible, therefore, for the ‘classical’ or specific consumption of factors of these systems, including the inhibitors of clotting (AT III), kallikrein (C1 INA), fibrinolysis (α2PI) and complement (C1 INA) factors (cf. Fig. 2 and Table 1). Elimination of both the proteinases and inhibitors of the blood systems also proceeds specifically by the formation of enzyme–inhibitor complexes that are phagocytosed by cells of the reticuloendothelial system (RES) (Ohlsson 1974, Ohlsson & Laurell 1976, Ohlsson 1978).

More recently it became evident from studies *in vitro* and *in vivo* that endotoxin-induced consumption of plasma proteins might also be due to a significant degree to unspecific degradation by leucocytic proteinases, especially an elastase (cf. Figs. 1 and 2) (Schmidt et al 1974, Haschen 1975, Egbring et al 1977, Egbring & Havemann 1978, Schiessler et al 1978a, Aasen
Proteinases released from cells can cause:

<table>
<thead>
<tr>
<th>Activation</th>
<th>Degradation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Blood systems such as: clotting, fibrinolysis, complement, kallikrein</td>
<td>Blood system factors, other blood proteins, proteinase inhibitors</td>
</tr>
</tbody>
</table>

Specific consumption

Unspecific consumption

Fig. 1. Effects of proteinases released from blood and tissue cells during inflammation on blood systems and plasma factors.

& Ohlsson 1978). In such unspecific consumption reactions — which, in contrast to the specific consumption, are not limited to the factors of the blood systems (cf. Fig. 1) — the biological activity of the plasma proteins is irreversibly destroyed by proteolytic degradation. The liberated leucocytic proteinases are eliminated, however, in a specific manner by the formation of complexes with proteinase inhibitors such as $\alpha_2$M, $\alpha_1$A and $\alpha_1$AC (see Fig. 2 and Table 2) and the phagocytosis of the complexes by the RES (Ohlsson & Laurell 1976, Debanne et al 1976).

Consumption reactions

Specific activation

Unspecific degradation

Blood systems

Blood proteins

Plasma proteinase inhibitors

e.g. AT III $\alpha_2$PI, C1 INA

e.g. $\alpha_1$A, $\alpha_1$AC, $\alpha_2$M

Elimination (RES)

Fig. 2. Consumption of plasma factors during inflammation. System-specific proteinases such as thrombokinase activate the blood system factors which are inhibited thereafter by complex formation with antithrombin III (AT III), $\alpha_2$-plasmin inhibitor ($\alpha_2$PI) and C1 inactivator (C1 INA), respectively. Proteinases liberated from blood and tissue cells like granulocytic (PMN) elastase can degrade plasma factors unspecifically before being inhibited by complex formation with $\alpha_1$-antitrypsin ($\alpha_1$A), $\alpha_1$-antichymotrypsin ($\alpha_1$AC) and $\alpha_2$-macroglobulin ($\alpha_2$M), respectively. The enzyme–inhibitor complexes are eliminated from the circulation by cells of the reticuloendothelial system (RES).
TABLE 1

Plasma proteinase inhibitors of blood system factors (cf. Fig. 1). Theoretically highest possible plasma concentrations of these factors are given in parentheses (in μmol/l). For literature references see Heimburger 1975, Harpel & Rosenberg 1975, Collen et al 1979

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Abbreviation</th>
<th>Concentration (μmol/l)</th>
<th>Factors primarily inhibited in vivo</th>
</tr>
</thead>
<tbody>
<tr>
<td>Antithrombin III</td>
<td>AT III</td>
<td>4.0</td>
<td>Thrombin (1)a, factor Xa² + plasminb, + kallikreinb</td>
</tr>
<tr>
<td>α₂-Plasmin inhibitor</td>
<td>α₂PI</td>
<td>0.9</td>
<td>Plasmin/ogen (2)</td>
</tr>
<tr>
<td>CI inactivator</td>
<td>CI INA</td>
<td>2.4</td>
<td>CI₅, CI₇ (&lt;1), kallikrein (~1)</td>
</tr>
</tbody>
</table>

a Reacting with AT III slowly without heparin but rapidly with heparin.
b Only in the presence of heparin after consumption of α₂PI or CI INA (Highsmith & Rosenberg 1974, Venneröd et al 1976), respectively.

The factual basis of this concept is that the early phase of experimental septic shock is characterized by the release of cell constituents including proteinases from leucocytes, platelets, macrophages, mast cells and endothelial cells of the vessel walls, especially those of the lung capillaries (Weissmann 1974, Urbaschek et al 1975, Myrvold 1976, Starkey 1977, Vane & Ferreira 1978, Aasen & Ohlsson 1978, Movat 1979). Because of the presence

TABLE 2

Plasma inhibitors directed primarily against lysosomal proteinases of various body cells. For literature references see Heimburger 1975, Starkey & Barrett 1977 (α₂M), Travis et al 1978 (α₁A, α₁AC), Ohlsson 1978 (α₁A, α₁AC), Woolley et al 1976 (β₁Cl)

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Abbreviation</th>
<th>Concentration (μmol/l)</th>
<th>Strong inhibition of (in vitro, probably also in vivo):</th>
</tr>
</thead>
<tbody>
<tr>
<td>α₂-Macroglobulin</td>
<td>α₂M</td>
<td>3.6</td>
<td>Neutral and acidic proteinases² (complex elimination τ½ ~ 10 min)</td>
</tr>
<tr>
<td>α₁-Antitrypsin (α₁-Protease inhibitor)</td>
<td>α₁A</td>
<td>52</td>
<td>Neutral proteinases from leucocytesb, pancreas and other tissues</td>
</tr>
<tr>
<td>α₁-Antichymotrypsin</td>
<td>α₁AC</td>
<td>6.4</td>
<td>Chymotrypsin, cathepsin G</td>
</tr>
<tr>
<td>β₁-Collagenase inhibitor</td>
<td>β₁Cl</td>
<td>~0.4c</td>
<td>True collagenases (metallo enzymes)</td>
</tr>
<tr>
<td>Inter-α-trypsin inhibitor²</td>
<td>ITI</td>
<td>2.8</td>
<td>Trypsin, chymotrypsin, acrosin</td>
</tr>
</tbody>
</table>

a e.g. granulocyte neutral proteinase (formerly collagenase, Ohlsson 1980), cathepsin G, elastases, collagenases, trypsin, chymotrypsin, plasma kallikrein; cathepsin B and D, etc.
b e.g. elastase and neutral proteinase (cf. a).
c Estimated value according to Woolley et al 1976.
d The biological antagonists are not yet known, see text.
of potent proteinase inhibitors, however, direct measurement of proteinase activities in plasma or serum is not feasible. We have focused our efforts, therefore, on indirect indications such as the consumption of plasma proteinase inhibitors, the turnover of selected plasma proteins, and the concentrations of plasma factors expected to be either significantly elevated or diminished during septicaemia or septic shock. In another approach we tested the therapeutic effect of an exogenous inhibitor of neutral leucocytic proteinases on the plasma levels of various plasma proteins including proteinase inhibitors during experimental endotoxaemia. The results obtained stimulated us to continue the search for potent inhibitors of leucocytic proteinases as possible therapeutic agents preventing unspecific consumption of plasma factors during septicaemia or septic shock.

HYPERDYNAMIC SEPTIC SHOCK: A CONTROLLED CLINICAL TRIAL

Criteria and methods

Criteria. The patients suffering from hyperdynamic septic shock \((n=18,\ \text{age 17–70 years})\) had to fulfil all of the following criteria: body temperature \(>38.5\ ^\circ\text{C}\), leucocytes \(>15\ 000\ \text{or}\ <5\ 000/\text{mm}^3\), platelets \(<130\ 000/\text{mm}^3\), positive blood culture (twice), positive evidence of endotoxin in serum (at least twice), cardiac index \(>6\ \text{l/min/m}^2\ \text{body surface}\), total peripheral resistance of the vessel system \(<600\ \text{dyn \times s/cm}^5\), mean arterial blood pressure \(80–90\ \text{mmHg (10.7–12.0 kPa)}\), all clinical signs of septic shock (Witte 1979).

Therapy. The following therapeutic measures were taken: surgical cleansing of the septic focus, volume substitution with human albumin under control of the pulmonary capillary wedge pressure, continuous low dose heparin therapy \((200–400\ \text{U/h})\), the infusion of 100-400 \(\mu\text{g/min dopamine to maintain an urine output of at least 80–100 ml/h, administration of 30 mg/kg body weight of methylprednisolone every 6 h up to 48 h (to prevent acute respiratory distress syndrome), medication with antibiotics according to the sensitivity patterns of the microorganisms, and parenteral nutrition as well as mechanical ventilatory assistance (Witte 1979).}

Methods. All parameters were followed up for four days after the patients fulfilled all the above criteria of hyperdynamic septic shock. Haemodynamic measurements were made and blood samples were taken at the beginning and 6, 12, 18, 24, 36, 48, 72 and 96 h thereafter. Further experimental details are given in the academic thesis of J. Witte (1979) and will be published
elsewhere. Of the various haematological, haemodynamic and biochemical
data followed up during the observation period, only those relevant to the
topic of this paper are reported here.

*Activation of blood systems and concentration pattern of plasma factors*

**Acute-phase proteins.** Inflammatory processes in the organism are
accompanied by increased serum or plasma levels of the acute-phase proteins.
Indeed, the serum concentrations of *C-reactive protein* and *α1-glycoprotein*
were significantly elevated during the septic shock phase (Table 3). Probably
because of competition with consumption (cf. below), the mean plasma level
of *fibrinogen*, another acute-phase protein, was in the upper part of the
standard range rather than above it (Table 3). The high production rate of
these acute-phase proteins strongly indicates that the synthetic capacity of the
organism, especially of the liver, for the various plasma proteins was not yet
restricted during the hyperdynamic septic shock phase.

**Clotting and fibrinolysis.** Permanent activation of the clotting cascade by
system-specific proteinases throughout the observation period is indicated by
the highly significantly increased plasma concentration of *fibrinopeptide A*
(Table 3), which is cleaved from fibrinogen by the specific action of thrombin
(Gaffney 1977, Schramm et al 1980). Similarly, activation of plasmin and
thus of fibrinolysis is responsible for the elevated plasma levels of *fibrin(ogen)*
*split products* (FSP, cf. Table 3) (Skansberg et al 1974). In this case, however,
a tendency to normalization was clearly visible towards the end of the
observation period.

**Complement.** Complement activation during the shock phase proceeded
primarily via the alternative pathway. This may be deduced from the clearly
lower plasma level of complement factor *C3* than of *C4* (Table 3). This result
is in agreement with our present knowledge of the activation mechanism of
the complement cascade in endotoxaemia (Johnson et al 1976, Spragg &
1980). Of special interest in this respect is the observation that the
granulocytic elastase is capable of cleaving C3 in such a way that direct
activation of the complement cascade via the alternative route could indeed
result (Johnson et al 1976, Aasen et al 1980). On the other hand, there is
growing evidence that endotoxins can trigger the release of elastase from

Liberated elastase could also be responsible, at least partly, for the highly
TABLE 3

Plasma or serum levels of various biochemical parameters in septic shock. Standard values (norm) are given both as mean value and range (in parentheses). The values given at the beginning of the shock phase (0 h) and after 96 h are mean values with the corresponding standard deviations (± SEM). $P$, statistical significance (Student $t$-test)

<table>
<thead>
<tr>
<th>Measured parameter</th>
<th>Norm</th>
<th>0 h</th>
<th>96 h</th>
<th>$P$</th>
</tr>
</thead>
<tbody>
<tr>
<td>C-reactive protein (mg/dl)</td>
<td>&lt;1.2</td>
<td>13.5 ± 1.3</td>
<td>10.4 ± 1.6</td>
<td>$\leq 0.001^a$</td>
</tr>
<tr>
<td>$\alpha_1$-Glycoprotein (mg/dl)</td>
<td>90(55-140)</td>
<td>139.3 ± 7.9$^b$</td>
<td>132.9 ± 15.2$^b$</td>
<td>$\leq 0.001^a$</td>
</tr>
<tr>
<td>Fibrinogen (mg/dl)</td>
<td>180-380</td>
<td>359.7 ± 23.4</td>
<td>293.5 ± 17.4</td>
<td>$&gt;0.05^a$</td>
</tr>
<tr>
<td>Fibrinopeptide A (ng/ml)</td>
<td>&lt;3.0</td>
<td>13.1 ± 2.7</td>
<td>18.1 ± 4.5</td>
<td>$\leq 0.001^a$</td>
</tr>
<tr>
<td>Factor XIII (mg/dl)</td>
<td>-2</td>
<td>46.1 ± 4.9$^b$</td>
<td>52.9 ± 4.8$^b$</td>
<td>$\leq 0.001^a$</td>
</tr>
<tr>
<td>FSP$^{c}$ (μg/ml)</td>
<td>&lt;10.0</td>
<td>21.4 ± 4.1</td>
<td>10.7 ± 1.7</td>
<td>$\leq 0.05^d$</td>
</tr>
<tr>
<td>Complement C3 (mg/dl)</td>
<td>82(55-120)</td>
<td>70.1 ± 4.3$^b$</td>
<td>65.3 ± 6.7$^b$</td>
<td>$\leq 0.01^a$</td>
</tr>
<tr>
<td>Complement C4 (mg/dl)</td>
<td>30(20-50)</td>
<td>82.2 ± 11.2$^b$</td>
<td>74.5 ± 11.2$^b$</td>
<td>$&gt;0.05^a$</td>
</tr>
</tbody>
</table>

$^a$For both 0 and 96 h. $^b$Expressed as % of the norm. $^c$Fibrin(ogen) split products. $^d$For the 0 h value only.

significant ($P \leq 0.001$) consumption of factor XIII (45–55% reduction in the plasma concentration) throughout the shock phase. This view is supported by the results of animal studies showing that therapeutic administration of an elastase–cathepsin G inhibitor in experimental sepsicaemia can prevent factor XIII consumption to a large degree (Schiessler et al 1978a).

**Plasma proteinase inhibitors**

Marked consumption or turnover of plasma proteinase inhibitors during the septic shock phase reflects extensive activation of the blood systems as well as the liberation of considerable amounts of proteinases from various body cells, especially the granulocytes, in our patients.

**Antithrombin III** (AT III) is the most important inhibitor for maintaining the homeostasis of the clotting system. To fulfil its physiological or pathophysiological function, namely regulation of the activity of factor Xa and thrombin to prevent thrombosis, AT III levels within or close to the standard range are necessary; the risk of thrombosis increases considerably with decreasing AT III plasma levels (Harpel & Rosenberg 1975, Seegers 1978, Collen et al 1979).

In our patients the AT III concentration was already reduced to 50% of the standard mean value at the beginning of the observation period (Fig. 3). This indicates major consumption of AT III by the formation of complexes with the activated clotting factors. With the heparin medication necessary to prevent thrombosis or disseminated intravascular coagulation, the consumption of AT III is even further intensified, since the heparin-AT III
complex also reacts with other proteinases such as factor VIIa, IXa, XIa, XIIa and plasma kallikrein (Collen et al 1979). Most remarkably, in the patients surviving the shock phase \((n = 15)\) the AT III level increased continuously whereas it decreased dramatically in those patients who died \((n = 3)\) during the shock phase (close to the end of the observation period, cf. Fig. 3).

**α2-Macroglobulin.** The permanently low level of α2-macroglobulin \((α2M)\) throughout the septic shock phase is especially striking (Fig. 4). Obviously, considerable amounts of neutral and acidic proteinases (cf. Table 2) are continuously liberated into the circulation from the various body cells, leading to major consumption of this most important proteinase-eliminating vehicle (Collen et al 1979, Ohlsson 1978, Starkey & Barrett 1977, Ohlsson & Laurell 1976, Harpel & Rosenberg 1975).

Considering the proteolytic potential which might be liberated by endotoxin stimulation from polymorphonuclear (PMN) leucocytes alone, for example approximately 1 g of both elastase and neutral proteinase (formerly collagenase) and 0.3 g of cathepsin G per day (Laurell 1975, Ohlsson 1980), the protective role of α2M against unspecific proteolysis cannot be sufficiently emphasized. In fact, α2M seems to be chiefly responsible for the inhibition (cathepsin G, neutral proteinase) and elimination (including elastase, which is primarily inhibited by α1-antitrypsin but probably transferred to α2M for this purpose) of these granulocytic proteinases (Ohlsson 1978, Ohlsson & Laurell 1976).
**PROTEINASE INHIBITORS IN INFLAMMATION**

**Fig. 4.** Plasma levels of $\alpha_2$-macroglobulin ($\alpha_2$M) and serum levels of native inter-$\alpha$-trypsin inhibitor (ITI$_{160\,000}$) and acid-stable ITI$_{160\,000}$-derived ITI$_{30\,000}$ during hyperdynamic septic shock. The curves represent mean values $\bar{x}$ of $n = 18$ ($\alpha_2$M) or $n = 10$ (ITI) patients; standard deviations (± SEM) of the mean values $\bar{x}$ are indicated for each test point. Ordinate, the percentage of the standard level of $\alpha_2$M (estimated with pooled plasma from normal individuals) and the trypsin inhibitory activity of ITI (substrate BzArgNHNp) per ml serum. Abscissa, observation period. $\alpha_2$M was estimated by Mancini's radial immunodiffusion technique. ITI$_{160\,000}$ and ITI$_{30\,000}$ were determined according to Hochstrasser et al (1977a) by measuring the trypsin inhibitory activity of the supernatant of an acidified serum sample (ITI$_{30\,000}$) and of the trypsin-treated precipitate after trypsin denaturation (ITI$_{160\,000}$).

**Inter-$\alpha$-trypsin inhibitor.** Recent observations indicate increased turnover of the inter-$\alpha$-trypsin inhibitor (ITI) during septicaemia (Hochstrasser et al 1977b). Indeed, the level of native ITI (ITI$_{160\,000}$) was significantly reduced throughout the observation period whereas the concentration of the ITI-derived acid-stable inhibitor (ITI$_{30\,000}$) was significantly increased (Fig. 4). Studies *in vitro* showed that of the various proteinases tested, granulocytic elastase (and cathepsin G; K. Hochstrasser, personal communication) are capable of liberating ITI$_{30\,000}$ most rapidly from ITI$_{160\,000}$ (Dietl et al 1979). This might mean that granulocytic proteinases are also responsible for the high turnover of ITI in septicaemia or septic shock.

Though the biological function of ITI$_{160\,000}$ or ITI$_{30\,000}$ is not yet clear, identification of the inhibitory active domain(s) as Kunitz- or aprotinin-type inhibitors is remarkable (Wachter & Hochstrasser 1979).
Conclusions and outlook

The following aspects of the clinical study should be especially emphasized.

(i) The plasma or serum levels of the measured parameters had already reached pathological values when the first clinical signs of septic shock were established. This means that the search for other biochemical parameters suitable for identifying the onset of septicaemia or septic shock has to be continued.

(ii) Consumption of plasma factors in hyperdynamic septic shock and probably also in Gram-negative septicaemia is due to the proteolytic attack of both system-specific and unspecific proteinases liberated from leucocytes and other body cells. The large consumption of the protective potential of the plasma inhibitors by the formation of complexes with these proteinases, followed by rapid elimination of the complexes, is especially striking.

(iii) The possibility of reducing the consumption of plasma factors by administering suitable proteinase inhibitors should be envisaged. In our opinion such inhibitors should primarily prevent the consumption of $\alpha_2$M, AT III and $\alpha_2$-plasmin inhibitor ($\alpha_2$PI). Application at an early phase of septicaemia seems to be most promising. Whereas a potent plasmin inhibitor (aprotinin, the effective agent in the drugs Trasyloc®, Antagosan® or Iniprol®) is already available for medical use, in the other two cases only substitution with AT III (Schramm 1977) or $\alpha_2$M concentrates is possible at present.

THE ACID-STABLE ELASTASE-CATHEPSIN G INHIBITOR OF HUMAN MUCOUS SECRETIONS

Inhibitory properties

Our search for inhibitors suitable for therapeutic use in humans was stimulated by the finding that the acid-stable trypsin-chymotrypsin inhibitor from human seminal plasma (HUSI-I) also has strong affinity for granulocytic elastase and cathepsin G (Schiessler et al 1976a). For the corresponding enzyme–inhibitor complexes, $K_i$ values close to $1 \times 10^{-9}$ mol/l (human granulocytic elastase, bovine chymotrypsin and trypsin) and $5 \times 10^{-8}$ mol/l (human granulocytic cathepsin G) were found.

Biochemical and structural features

HUSI-I isolated in our laboratory from human seminal plasma consists of
several multiple forms having the same inhibitory characteristics and similar molecular weights of approximately 11 000 (Schiessler et al 1976b). The heterogeneity of HUSI-I preparations seems to be due partly to proteolytic degradation in seminal plasma but also to allelomorphism. This polymorphism complicates both the purification of HUSI-I to homogeneity and the determination of the amino acid sequence. Basically, the HUSI-I molecule consists of a single polypeptide chain of about 100 amino acid residues which is cross-linked by six disulphide bridges. Preliminary results give the amino acid sequence shown below for positions 1–24 and 54–99 of HUSI-I form D (cf. Schiessler et al 1978b). In these sequenced parts of the HUSI-I molecule a structural homology to either a Kazal-type or Kunitz-type inhibitor cannot be recognized. This is surprising, in that inhibitors of similar molecular size and inhibitory properties found so far in animals and men are built up of either Kazal- or Kunitz-type domains (cf. below).

\[
\begin{align*}
1 & \quad 10 \\
Tyr-Val-Asn-Thr-Pro-Asn-Pro-Arg-Asp-Arg- & \\
& \quad 20 \\
Lys-Pro-Gly-Lys-Cys-Pro-Val-Thr-Tyr-Gly- & \\
& \quad 30 \\
\text{Gln-Cys-Leu-Met-} & \quad \text{and} & \\
& \quad 60 \\
& -Met-Leu-Asn-Pro-Pro-Asn-Phe- & \\
& \quad 70 \\
Cys-Glu-Met-Asp-Gly-Gln-Cys-Lys-Arg-Asp- & \\
& \quad 80 \\
& \quad 90 \\
Ser-Lys-Val-Glu-Pro-Val-Ala-Cys,Asp,Arg, & \\
& \quad 100 \\
Gly,Pro,Lys,Lys-Cys-Thr-Thr-Gly-Ser & \\
& \quad 110 
\end{align*}
\]

The occurrence of a new structural inhibitor type, perhaps exclusively in humans, would be especially interesting in relation to the evolution of protein proteinase inhibitors. In addition, this would be an indication that HUSI-I had developed primarily for the inhibition of granulocytic elastase and cathepsin G. This assumption is supported by considering the functional possibilities of HUSI-I in relation to its distribution within the organism.
Inhibitors with HUSI-I-like biochemical and/or immunological properties were found also in cervical mucus, bronchial fluid, nasal secretion and tears (Schiessler 1976, Schiessler et al 1977a, 1978b). Clearly, HUSI-I occurs in human mucous fluids open to invading organisms and thus often exposed to large numbers of leucocytes during local inflammatory processes. The concentration of HUSI-I in these mucous fluids is normally far higher (up to 10 times) than the levels of the transudated plasma inhibitors $\alpha_1$A and $\alpha_1$AC. Whereas the ITI-derived acid-stable inhibitor ITI$_{30,000}$ (cf. above) was also found in bronchial fluid (K. Hochstrasser, personal communication 1979), $\alpha_2$M is not usually present in human mucous secretions (Table 4). The major difference between HUSI-I and the transudated plasma inhibitors concerns its site of production: HUSI-I is synthesized locally by the epithelial cells of the corresponding organs and is thus directly secreted into the mucous fluids (Schiessler et al 1978b, Tegner & Ohlsson 1977, Schill et al 1978).

From the local production, the relatively high level in mucous fluids, and the inhibitory spectrum of HUSI-I (cf. Table 5), it seems very likely to us that HUSI-I has an important defensive function. It presumably protects soluble proteins (such as immunoglobulins) and the mucous membranes against degradation by leucocytic proteinases liberated during local inflammation and thus helps the organism to prevent the inflammatory response from intensifying. Complexes of HUSI-I with leucocytic and/or bacterial proteinases could indeed be demonstrated in mucous fluids during

**TABLE 4**

Inhibitors in human mucous secretions

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Mol. wt. (approx.)</th>
<th>Inhibition of leucocytic:</th>
<th>Elastase</th>
<th>Cathepsin G</th>
<th>Neutral proteinase$^a$</th>
</tr>
</thead>
<tbody>
<tr>
<td>$\alpha_1$AT</td>
<td>50 000</td>
<td>+ +</td>
<td>(+)</td>
<td>+</td>
<td></td>
</tr>
<tr>
<td>$\alpha_1$AC</td>
<td>70 000</td>
<td>-</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
<tr>
<td>ITI$_{30,000}$</td>
<td>30 000</td>
<td>-</td>
<td>-</td>
<td>?</td>
<td></td>
</tr>
<tr>
<td>($\alpha_2$M)$^b$</td>
<td>725 000</td>
<td>-</td>
<td>++</td>
<td>++</td>
<td></td>
</tr>
<tr>
<td>Locally produced</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HUSI-I</td>
<td>11 000</td>
<td>+ +</td>
<td>+</td>
<td>-</td>
<td></td>
</tr>
</tbody>
</table>

$^a$Formerly collagenase (Ohlsson 1980).
$^b$Only occasionally found during severe inflammation.

+ + , very strong; + , strong; (+), weak; - , no inhibition.
TABLE 5

Acid-stable inhibitors of granulocytic elastase and cathepsin G from various sources. For literature references see text

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Source</th>
<th>Mol. wt. (approx.)</th>
<th>Inhibition of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Elastase&lt;sup&gt;a&lt;/sup&gt;</td>
</tr>
<tr>
<td>HUSI-I</td>
<td>Human mucous secretions</td>
<td>11 000</td>
<td>++</td>
</tr>
<tr>
<td>DSI</td>
<td>Dog submandibular glands</td>
<td>13 000</td>
<td>++</td>
</tr>
<tr>
<td>AA</td>
<td>Soybeans</td>
<td>8000</td>
<td>++</td>
</tr>
<tr>
<td>LBI</td>
<td>Lima beans</td>
<td>9000</td>
<td>+</td>
</tr>
<tr>
<td>Elastatinal</td>
<td><em>Actinomycetes</em></td>
<td>500</td>
<td>(+)&lt;sup&gt;c&lt;/sup&gt;</td>
</tr>
<tr>
<td>Elasin</td>
<td><em>Streptomyces</em></td>
<td>400</td>
<td>+&lt;sup&gt;d&lt;/sup&gt;</td>
</tr>
<tr>
<td>Chymostatin</td>
<td><em>Actinomycetes</em></td>
<td>600</td>
<td>(–)</td>
</tr>
<tr>
<td>Aprotinin</td>
<td>Bovine organs</td>
<td>6500</td>
<td>(+)</td>
</tr>
</tbody>
</table>

Ø not detected; ++, very strong; +, strong; (+), weak; (–), very weak; –, no inhibition.

<sup>a</sup>Human.  <sup>b</sup>Bovine.  <sup>c</sup>Pancreatic: +  <sup>d</sup>Pancreatic:(+)  

Inflammation; even free proteolytic activity may appear when the inhibitors are totally consumed (Schiessler et al. 1978b, Ohlsson 1978, Krumme et al. 1977). Clearly, HUSI-I is a functional substitute for α<sub>2</sub>-macroglobulin, normally inaccessible to mucous fluids. Because of the limited amount of HUSI-I available from natural sources — it has so far been found only in humans — we cannot envisage its therapeutic use for some time yet.

INHIBITORS FROM OTHER SOURCES AS POTENTIAL THERAPEUTIC AGENTS

Inhibitors of granulocytic elastase and/or cathepsin G were found in various natural sources; some of them are listed in Table 5.

Inhibitor from dog submandibular glands

The dog submandibular inhibitor (DSI) is present in these glands in exceptionally high concentration. It is a secretory protein consisting of a single polypeptide chain cross-linked by six disulphide bridges (Fritz et al. 1971). The DSI molecule is composed of two independent inhibitory active domains (Fig. 5) both of which are structurally homologous to the so-called Kazal-type inhibitors (Hochstrasser et al. 1975) — the pancreatic secretory trypsin inhibitor (PSTI), the seminal acrosin inhibitor (Tschescie et al. 1976) and the ovomucoids and ovoinhibitors from egg white (Laskowski Jr et al.
The N-terminal domain of DSI contains the trypsin-directed reactive site (Arg-Leu) whereas the chymotrypsin- or elastase-reactive centre (Met-Asp) is located in the C-terminal domain. This structure implies that ternary complexes (e.g. a trypsin–DSI–elastase complex) may be formed.

The double-headed DSI molecule probably developed from a Kazal-type domain (as represented for example by the single-headed PSTI) by gene duplication and several suitable mutations. The driving force for the expansion of the inhibition spectrum could have been the need for the formation of enzymes with new biological functions in the course of evolution. In this respect it is remarkable that the DSI seems to be especially adapted to the food requirements of canines, as it also inhibits pronase, *Aspergillus oryzae* proteinase and subtilisin very effectively (Fritz et al 1971).

In contrast to DSI, HUSI-I does not inhibit bacterial and mould proteinases.

**Inhibitors from soybeans and lima beans**

Other inhibitors which interact strongly with human granulocytic elastase and cathepsin G are inhibitors AA from soybeans and LBI from lima beans (Schiessler et al 1977b). These inhibitors are structurally homologous to each other. They consist of two domains with independent reactive sites against trypsin and chymotrypsin or elastase (Fig. 6), so that ternary complexes may
be formed (Ikenaka et al. 1974). Both are readily available in amounts sufficient for therapeutic studies. However, because agglutinins are present in the plant extracts, they have to be thoroughly purified.

**Aprotinin**

The basic trypsin-kallikrein inhibitor from bovine organs, aprotinin, used as an affinity adsorbent for the purification of granulocytic elastase and cathepsin G (Baugh & Travis 1976, Travis et al. 1978), turned out to be an inhibitor of these enzymes, too, but with relatively low affinity (Lestienne & Bieth 1978, Starkey 1977). Whether inhibition of the granulocytic proteinases plays a role in its therapeutic effectiveness, which is generally assumed to be due to the inhibition of plasmin and/or plasma kallikrein, has still to be investigated.

**Microbial peptides**

Recently inhibitors with more restricted specificity and considerably lower molecular weight but weaker affinity to the granulocytic proteinases have been isolated from bacteria (Table 5) (Umezawa 1976, Feinstein 1978, Ohno et al. 1978). They are potential candidates for studies designed to clarify the pathophysiological role of individual granulocytic proteinases in severe inflammatory processes.
Inhibitors from the leech Hirudo medicinalis

Protein proteinase inhibitors isolated so far from extracts of the leech Hirudo medicinalis are listed in Table 6. Hirudin, the thrombin-specific inhibitor, turned out to be the effective principle of the leech, widely used formerly in medical therapy for 'blood dilution'. The biochemical, pharmacological and clotting-inhibitory properties of hirudin have been studied in detail (Markwardt 1963, Badgy et al 1976); its amino acid sequence has been elucidated recently (Petersen et al 1976). In our opinion hirudin is a most promising candidate for therapeutic application in consumption coagulopathy, especially in acquired or hereditary AT III deficiency.

TABLE 6
Proteinase inhibitors of the leech Hirudo medicinalis (for further details see text)

<table>
<thead>
<tr>
<th>Inhibitor</th>
<th>Mol. wt. (approx.)</th>
<th>Inhibition of:</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Trypsin&lt;sup&gt;a&lt;/sup&gt;</td>
<td>Plasmin&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
<tr>
<td>Hirudin</td>
<td>7000</td>
<td>-</td>
</tr>
<tr>
<td>Bdellin A</td>
<td>6000</td>
<td>+ +</td>
</tr>
<tr>
<td>Bdellin B</td>
<td>4000</td>
<td>+ +</td>
</tr>
<tr>
<td>Eglin</td>
<td>8100</td>
<td>(+)</td>
</tr>
</tbody>
</table>

+ + , very strong; + , strong; (+) , weak; - , no inhibition.
<sup>a</sup>Bovine.  <sup>b</sup>Porcine, human.  <sup>c</sup>Boar, human.  <sup>d</sup>Bovine, human.  <sup>f</sup>Human (elastase and cathepsin G).

The eglins. In the course of isolation of the bdellins from H. medicinalis (these are strong inhibitors of plasmin or sperm acrosin, cf. Table 6) an antichymotrypsin activity was separated. A more detailed investigation revealed that this was due to inhibitors of another type, the eglins (Seemüller et al 1977). They turned out to be especially strong inhibitors of human granulocytic elastase and cathepsin G, with $K_i$ values of the corresponding complexes close to $1.5 \times 10^{-10}$ mol/l (elastase) or $2.5 \times 10^{-10}$ mol/l (cathepsin G). In addition, subtilisin is also strongly inhibited by the eglins ($K_i$ approx. $1.2 \times 10^{-10}$ mol/l). The eglins are a mixture of isoinhibitors with similar biochemical and inhibitory properties.

The amino acid sequence shown below was elucidated very recently for eglin c.

It is most surprising that the eglins are extremely resistant to denaturation and proteolytic degradation, despite the lack of any disulphide bond which
could stabilize the structure of the molecule. This new structural type of protein proteinase inhibitor seems to be especially suitable, therefore, for therapeutic use in both local and generalized inflammation. In this respect it is remarkable that the anti-inflammatory ('anti-phlogistic') effect of leech extracts has long been known; it could be at least partly due to the antiproteolytic effect of the eglin.

It is evident from these results that quite different structural approaches (cf. HUSI-I, inhibitor AA and eglin c) may lead to a reactive site conformation which fits perfectly into the active site of the same enzyme (e.g. of granulocytic elastase).

INHIBITOR THERAPY IN EXPERIMENTAL ENDOTOXAEMIA

The endotoxaemia model

Experimental procedure. Endotoxaemia was induced in six anaesthetized dogs by continuous infusion of Escherichia coli endotoxin, 2 mg/kg body weight, into the inferior vena cava over a period of two hours. The six control dogs were subjected to the same procedure except that isotonic saline was infused instead of the endotoxin solution. The data monitored or collected just before endotoxin (or isotonic saline) application served as 100% values for each parameter. All values obtained during the 14-hour experiment were expressed as percentages of these starting values. Further clearly defined experimental conditions allowing a statistical treatment of the results by the three factorial classification of variance will be published elsewhere (M.
To determine the plasma levels of clotting and fibrinolysis factors we used amidolytic assays with chromogenic substrates: TosGlyProArgNHNp (Boehringer) for prothrombin, D-PhePipArgNHNp (S-2238 KABI) for AT III, and D-ValLeuLysNHNp (S-2251 KABI) for plasminogen and α2PI. The biological activity of factor XIII was assayed according to the procedure 'Faktor XIII Schnelltest' from Behringwerke Marburg/Germany, while complement factor C3 was quantified by the radial immunodiffusion technique.

**Haematological data.** In contrast to the control group the endotoxin-treated dogs showed a rapid and substantial decrease in circulating platelets and leucocytes (Figs. 7 and 8). In addition, a strong leucocytosis developed in the endotoxin group, producing leucocyte counts far higher than before endotoxin administration (Fig. 8).

**Plasma factors.** Compared to the control group the levels of the selected plasma factors decreased substantially in the endotoxin-treated dogs up to six hours (AT III, α2PI, prothrombin, complement C3) or 14 hours (plasminogen, factor XIII) from the start of the experiment (Fig. 9). This was

![Fig. 7. Behaviour pattern of circulating platelets in experimental endotoxaemia. The curves represent mean values ± of the control (full line, n = 6) and endotoxin groups (dashed line, n = 6); the standard deviations, ± SEM, are indicated for each test point. Ordinate, number of platelets/μl plasma. Abscissa, observation period. The endotoxin infusion period is indicated as a thick line on the abscissa.](image-url)
FIG. 8. Behaviour pattern of circulating leucocytes in experimental endotoxaemia. The curves represent mean values \( \bar{x} \) of the control (full line, \( n = 6 \)) and endotoxin groups (dashed line, \( n = 6 \)); the standard deviations, \( \pm \text{SEM} \), are indicated for each test point. Ordinate, number of leucocytes/\( \mu l \) plasma. Abscissa, observation period. The endotoxin infusion period is indicated as a thick line on the abscissa.

FIG. 9. Plasma levels of selected plasma factors during experimental endotoxaemia (left) and under inhibitor medication (right). The curves represent mean values \( \bar{x} \) of the control groups A (\( n = 6 \)), the endotoxaemia group B (\( n = 6 \)) and the inhibitor-medicated endotoxaemia group (right panel, \( n = 4 \)); standard deviations are given elsewhere (Witte 1979). Ordinate, the percentage of the starting level (cf. text). Abscissa, experimental period (the endotoxin infusion period is indicated by the thick line). For further details see text.
also true for the fibrinogen concentration (80% of the starting value at the end of the experiment, not shown in the figure). The parallel decrease in the physiological antagonists prothrombin/AT III and plasminogen/α2PI is especially remarkable.

Establishment of the model. The statistical evaluation revealed that the differences in the levels of the plasma factors and blood cell counts were statistically significant for the control and endotoxaemia group ($P \leq 0.05/0.001$), the various test times ($P \leq 0.04/0.001$), and the time course ($P \leq 0.05/0.001$). This result clearly shows the validity of this experimental system as a model for endotoxaemia. In fact, the substantial alterations observed in blood cell counts and the levels of the selected plasma proteins are characteristic of endotoxin-induced DIC (McCabe 1973, Garner et al 1974, Urbaschek et al 1975, Müller-Berghaus et al 1976, Aasen et al 1978a, 1978b, 1980).

Influence of inhibitor medication

Experimental procedure. In a first approach the elastase–cathepsin G inhibitor AA from soybeans (cf. Table 5), described originally by Bowman and Birk (Birk 1976), was applied in the endotoxaemia model in the dog. The inhibitor was isolated from a commercially available soybean extract (‘trypsin inhibitor from soybeans’ from Serva, Heidelberg, no. 37 340) by repeated gel filtration chromatography on Sephadex G-75 in 2% acetic acid solution (H. Schiessler, personal communication 1978). In the inhibitor medication group (four dogs) infusions of endotoxin (cf. above) and inhibitor were started simultaneously but the inhibitor infusion was continued over the total experimental period (14 h). Each of the four animals received 3–8 mg/kg body weight of the purified inhibitor with a specific trypsin inhibitory activity (substrate BzArgNHNp) close to 3.2 IU/mg. Further experimental details will be published elsewhere (M. Jochum, J. Witte, H. Schiessler, G. Ruckdeschel & H. Fritz, in preparation).

Results and outlook. The inhibitor medication influenced the behaviour pattern of neither the circulating platelets nor the leucocytes when compared to the endotoxin group. However, the endotoxin-induced decrease in plasma levels of the selected factors was significantly ($P \leq 0.05/0.01$, Student t-test) reduced by inhibitor treatment (Fig. 9); even the lowest dosage of inhibitor used (3 mg/kg body weight) was effective. As may be deduced from the inhibition spectrum of inhibitor AA, degradation of the plasma factors in the
course of endotoxaemia is due chiefly to unspecific proteolysis rather than to activation of the blood systems by system-specific proteinases, though quantitation of the degree of consumption due to unspecific proteolysis is not yet feasible.

The results of both the clinical and experimental study indicate clearly that in generalized inflammatory processes such as septicaemia or septic shock the regulatory inhibitor system of the organism may be overstressed. As shown for the first time in the endotoxaemia trial, a suitable inhibitor can significantly prevent unspecific degradation of plasma factors by leucocytic (and other?) proteinases and thus help the natural defence mechanisms to maintain the physiological balance. In view of the key function of $\alpha_2$M in the rapid elimination of proteinases from the circulation, protection against early $\alpha_2$M consumption could be the underlying explanation for the effectiveness of such an exogenous inhibitor.

Just as important, it seems to us, is to minimize hypercoagulability and hyperfibrinolysis or DIC induced simultaneously by system-specific proteinases. For this purpose aprotinin, a strong plasmin inhibitor already used in medical therapy, and the thrombin-specific inhibitor hirudin (cf. Table 6) are potential candidates, the latter especially if AT III is consumed to such an extent that heparin medication is of reduced effectiveness. As the systemic application of suitable elastase–cathepsin G inhibitors and hirudin in humans is not possible until toxicological, pharmacological and clinical trials have been successfully made, substitution with AT III (Schramm 1977, Schramm et al 1978) and $\alpha_2$M concentrates is the method of choice at present.

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References

Aasen AO, Ohlsson K, Larsbraaten M, Amundsen E 1978b Changes in plasminogen levels,
Hochstrasser K, Niebl J, Feuth H, Lempart K 1977b Über Abbaumprodukte des Inter-α-trypsin-
inhibitors im Serum, I. Der Inter-α-trypsininhbinhibitor als Prekursor des säurestabilen Ser-
um-Trypsin-Plasmin-Inhibitors. Klin Wochenschr 55:337-342
Ikenaka T, Odani S, Koide T 1974 Chemical structure and inhibitory activities of soybean pro-
posium V) p 325-343
Jeljaszewicz J, Waldström T (eds) 1978 Bacterial toxins and cell membranes. Academic Press,
London
Johnson U, Ohlsson K, Olsson I 1976 Effects of granulocyte neutral proteases on complement
Klebanoff SJ, Clark RA (eds) 1978 The neutrophil: function and clinical disorders. North-
Holland, Amsterdam
mucus — selected properties in view of their clinical relevance. In: Insler V, Bettendorf G
evolution, multiple domains and hypervariability of reactive sites. In: Choh Hao Li (ed) Ver-
Laurell C-B 1975 Relation between structure and biologic function of the protease inhibitors
(22nd Colloquium) p 3-12
Lestienne P, Bieth JG 1978 The inhibition of human leukocyte elastase by basic pancreatic trypsin
inhibitor. Arch Biochem Biophys 190:358-360
Markwardt F (ed) 1963 Blutgerinnungshemmende Wirkstoffe aus blutsaugenden Tieren. VEB
Gustav Fischer, Jena
McCabe WR 1973 Serum complement levels in bacteremia due to gram-negative organisms. N
Engl J Med 288:21-23
Movat HZ (ed) 1979 Inflammatory reactions. Curr Top Pathol 68
Müller-Berghaus G, Bohn E, Höbel W 1976 Activation of intravascular coagulation by endo-
Ohlsson K 1974 Interaction between endogenous proteases and plasma protease inhibitors in
V) p 96-105
Ohlsson K 1978 Interaction of granulocyte neutral proteases with α1-antitrypsin, α2-
macroglobulin and α1-antichymotrypsin. In: Havemann K, Janoff A (eds) Neutral pro-
teases of human polymorphonuclear leukocytes. Urban & Schwarzenberg, Baltimore/ Munich, p 167-177
Collagenase in normal and pathological connective tissues. Wiley, Chichester, p 209-222
Ohlsson K, Laurell C-B 1976 The disappearance of enzyme-inhibitor complexes from the circu-
Ohno H, Saheki T, Awaya J, Nakagawa A, Ōmura S 1978 Isolation and characterization of
elasin, a new human granulocyte elastase inhibitor produced by a strain of Streptomyces. J
Antibiot Tokyo 31:1116-1123
Petersen TE, Roberts HR, Sottrup-Jensen L, Magnusson S 1976 Primary structure of hirudin,
a thrombin-specific inhibitor. In: Peeters H (ed) Protides of the biological fluids. Pergamon,
Oxford (23rd colloquium) p 145-149
Schissler H 1976 Säurestabile Proteinase-Inhibitoren aus menschlichem Sperma und ihre Ziel-
Schissler H, Arnhold M, Ohlsson K, Fritz H 1976a Inhibitors of acrosin and granulocyte protein-


logical fluids. Pergamon, Oxford (23rd Colloquium) p 255-266
Wächter E, Hochstrasser K 1979 Kunitz-type proteinase inhibitors derived by limited proteolysis of the inter-ß-trypsin inhibitor, III: Sequence of the two Kunitz-type domains inside the native inter-ß-trypsin inhibitor, its biological aspects and also of its cleavage products. Hoppe Seyler’s Z Physiol Chem 360:1305-1311
Witte J 1979 Endotoxinämie und hyperdynamer septischer Schock: Pathobiochemie ausgewählter Gerinnungs- und anderer Plasma-Proteinparameter. Academic Thesis, Medical Faculty of the University of Munich