Molecular Aspects of Inflammation

Edited by
H. Sies, L. Flohé and G. Zimmer

With 124 Figures

Contents

Proteolysis-Induced Pathomechanisms in Acute Inflammation and Related Therapeutic Approaches

M. Jochum[1], W. Machleidt[2], and H. Fritz[1]

1 Introduction

1.1 Aspects of Proteolytic Pathomechanisms in Inflammation

Proteolysis-induced pathomechanisms seem to play a major role in the primary response of the organism to inflammatory stimuli such as tissue destruction due to multiple trauma and major surgery or invasive microbes and endotoxins in sepsis. Physiologically, the inflammation response is directed towards inactivating and eliminating the deleterious agents and to initiate the process of repair and healing. Yet, the activation of the complex interacting cellular and humoral defence mechanisms necessary for this purpose carries with it also the risk of damaging healthy tissue, thus perpetuating the inflammatory process. In this respect, the lysosomal serine proteinase elastase and the cysteine proteinase cathepsin B of the primary inflammatory cells, polymorphonuclear (PMN) granulocytes, and monocytes/macrophages are supposed to be potent effectors of proteolytic tissue damage if they are discharged extracellularly in high amounts during activation of the phagocytes. Moreover, proteinases of humoral origin (plasma kallikrein, thrombin, plasmin, complement esterases) and protein split products generated by their proteolytic action (fibrinopeptides and fibrin monomers, fibronectin peptides, complement-derived factors such as C3a, C4a, and C5a, etc.) have been proven to be of major importance as strong stimulators of the primary defence cells.

In addition to lysosomal proteinases, highly reactive oxygen species produced by the respiratory burst are also extracellularly liberated during phagocytosis. By this means, vital structural elements (basal membranes, cell receptors, fibronectin, elastin, collagens, proteoglycans, etc.) as well as humoral factors including plasma proteins in close vicinity to the phagocytizing cells may be impaired unless the lysosomal proteolytic enzymes and oxidants are inactivated by their physiological regulators, the proteinase inhibitors (α_1-proteinase inhibitor, α_2-macroglobulin, cysteine proteinase inhibitors) and antioxidants (superoxide dismutase, catalase, glutathione redox system, ceruloplasmin).

Yet, due to an overstressed phagocytic activity of PMN granulocytes and monocytes/macrophages during severe inflammation, the main antagonist of PMN elastase,

[1] Department of Clinical Chemistry and Clinical Biochemistry, Surgical Clinic City of the University of Munich, Nußbaumstraße 20, D-8000 Munich 2, FRG
[2] Institute of Physiological Chemistry, Physical Biochemistry and Cell Biology of the University of Munich, Goethestraße 33, D-8000 Munich 2, FRG
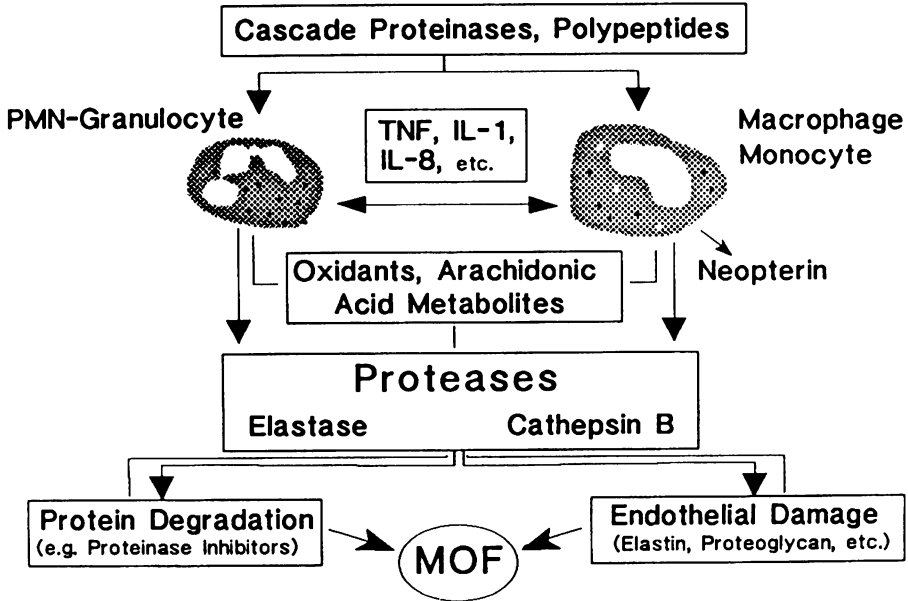
Fig. 1. Schematic depiction of humoral and cellular proteolytic pathomechanisms involved in the development of multiple organ failure (MOF). Destructive proteases (elastase, cathepsin B) and other cell constituents are released after activation of the phagocytes by blood cascade proteinases (plasma kallikrein, factor XIIa, thrombin, etc.) and polypeptides (fibrinogen and fibronectin split products, complement factors C3a, C4a, and C5a, etc.). For detailled explanation, see text

the α_1-proteinase inhibitor (α_1PI), is highly susceptible not only to proteolytic cleavage by cysteine and metallo proteinases released from the mononuclear cells, but also to oxidative denaturation of the reactive inhibitory site in the molecule. This enables an unrestricted local digestive activity by PMN elastase combined also with fatal consequences for the hemostasis system. As shown by several authors, PMN elastase can easily degrade and inactivate the principal inhibitors (antithrombin III, α_2-plasmin inhibitor, C1-inactivator) of proteinases of the blood cascade systems (clotting, fibrinolysis, complement), thus allowing the maintenance of life-threatening consumption of hemostasis factors and the additional production of potent stimulators of the phagocytes as mentioned above. Moreover, the proteolytic inactivation of the plasma inhibitors may be greatly enhanced by their oxidative denaturation in the surroundings of phagocytizing cells.

The interrelationship of cellular and humoral proteolytic pathomechanisms eventually contributing to the development of multiple organ failure (MOF) in the course of severe acute inflammation is depicted in a simplified version in Fig. 1. A comprehensive review of literature on this topic is given by Jochum (1988), Jochum and Fritz (1989), Assfalg-Machleidt et al. (1990), and Jochum and Fritz (1990).

1.2 Rationale for Therapeutic Use of Proteinase Inhibitors

To obtain fundamental information about the extent proteases may participate in the initiation and perpetuation of inflammatory processes, the following indications – as a modification of the Koch-Dale criteria – have to be proven in clinical and/or experimental studies:

1. The release of the lysosomal proteinases (e. g., PMN elastase, cathepsin B) and the activation of proteolytic blood cascade enzymes have to be verified in correlation to the severity of the inflammation.
2. The consumption of proteinase inhibitors and other plasma factors susceptible to proteolytic degradation should coincide with the occurrence of proteolytic activity.
3. Specific split products of functional proteins generated by the proteolytic action of lysosomal proteinases have to be shown in correlation to the extracellular release of these enzymes.
4. The use of specific exogenous proteinase inhibitors as therapeutic tools should prevent or at least reduce to some extent severe signs of inflammation.

In the following, characteristic results obtained from several clinical and experimental studies dealing with multiple trauma and/or septicemia are given. They demonstrate clearly the involvement of proteolysis-induced pathomechanisms in acute inflammation necessitating proteinase inhibition as a convenient therapeutic approach.

2 Methodology

Serially drawn citrated plasma, bronchoalveolar lavage fluid (BALF), and peritonitis exudate samples were used to quantify proteinases, inhibitors, and plasma protein split products.

Measurements of the lysosomal serine proteinase PMN elastase were carried out with a commercially available, highly specific two-site sandwich enzyme immunoassay kit (E. Merck, Darmstadt, Germany), which detects elastase only as an inactive complex with α_1PI (Neumann and Jochum 1984). Since, in contrast to plasma, BALF and peritonitis exudate samples may contain proteolytically active elastase in addition to the complex, parts of these specimens were incubated also with a surplus of α_1PI in vitro and reassayed for an increase in elastase-α_1PI complex as a measure of enzymatic elastase activity in vivo. The cysteine proteinase cathepsin B activity was quantified using a fluorometric assay as described by Assfalg-Machleidt et al. (1988). Caseinolytic activity of local body fluids due to elastase and/or cathepsin B was detected using resorufin-labeled casein (Boehringer, Mannheim, Germany) as a substrate. The method is outlined in detail by the manufacturer (Boehringer).

The coagulation proteinases, plasma kallikrein and thrombin, were estimated by their cleavage activity on the chromogenic peptide substrates S-2302 (Kabi, Stockholm, Sweden) and Chromozym TH (Boehringer, Mannheim, Germany), respectively, after the turnover of the proenzymes to the active proteinases.

The inhibitory activities of antithrombin III and α_1PI were measured with commercially available test systems (Coatest Antithrombin, Kabi, Stockholm, Sweden; α_1-Antitrypsin Farbtest, Boehringer, Mannheim, Germany) applying chromogenic peptide substrates for thrombin (S-2238) and trypsin (BAPA), respectively. The antigen levels of the inhibitors were quantified with commercial radial immunodiffusion plates (Nor-Partigen plates, Behringwerke, Marburg, Germany).

Opsonic activity, antigen levels, and split products of the opsonins IgG and complement factor C3 were determined as outlined in Billing et al. (1990, 1991) and Machleidt et al. (1991).

An elastase-specific split product of the Aα-chain of fibrinogen, called fibrino-elastase-peptide (FEP; equivalent to the Aα 1-21 peptide described by Weitz et al. 1986), was quantified with a two-step competitive immunoassay just recently developed in our laboratory (Gippner-Steppert 1991).

Clinical methods, treatment of patients, and experimental therapeutic studies are extensively described in the original publications cited in the following sections.

3 Results and Discussion

3.1 Proteolysis-Induced Pathomechanisms in Humans

3.1.1 Multiple Trauma

In an extended exploration 100 severely injured patients fulfilling previously defined study criteria were enrolled in a prospective study (directed by Drs. Nast-Kolb and Waydhas, Surgical Clinic City, University of Munich) on inflammatory mediators and multiorgan failure (MOF) associated with polytraumatic events (Nast-Kolb et al. 1990; Waydhas et al. 1991). Collection of blood samples and recording of clinical data were started within 30 minutes after arrival of the patients in the emergency department (about 1 h after the accident) and continued on a 6 h basis up to 48 h. Subsequently, the monitoring interval was extended to 24 h for a period of 14 days. Thereafter the clinical course was recorded until either transfer to a general ward or death of the patient.

Retrospectively, the patients could be assigned to three different groups: 16 of them died due to MOF between 3 and 28 days (mean survival time: 16 days) after the traumatic incident (group I), 47 patients survived the development of organ failure (group II), and 37 patients recovered from the accident without evident signs of organ dysfunctions (group III).

The extracellular release of PMN elastase and monocyte/macrophage-derived cathepsin B into plasma of the patients in the three outcome groups is depicted in Fig. 2. PMN elastase was elevated in all groups significantly above normal (upper range 120 ng/ml) already within 1 to 2 h after trauma and showed an additional increase up to the 12th posttraumatic hour. The differences between mean plasma levels in patients with (groups I and II) and without organ failure (group III) were highly significant ($p < 0.01$) throughout the whole observation period. Moreover, patients dying due to organ failure (group I) and those who survived organ dysfunctions (group II)
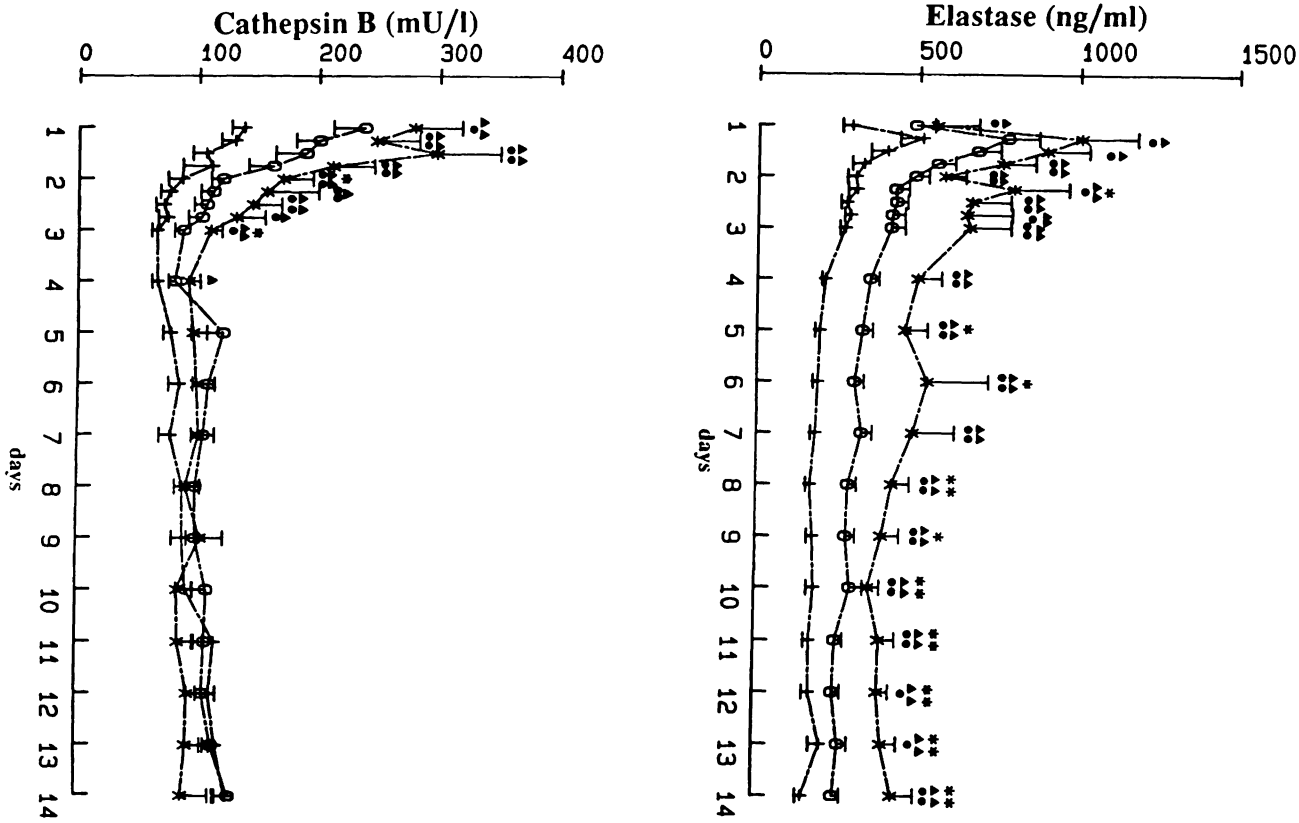
Fig. 2. Mean values (± SEM) of PMN elastase and cathepsin B in plasma of multiply injured patients assigned to three outcome groups: Group I (n = 16): nonsurvivors with multiple organ failure * --- *; group II (n = 47): survivors with reversible organ failure o --- o; group III (n = 37): survivors without organ failure + --- +. * $p < 0.05$, ** $p < 0.01$ group I vs group II; • $p < 0.05$, •• $p < 0.01$ group I vs group III; ▲ $p < 0.05$, ▲▲ $p < 0.01$ group II vs group III

were also significantly ($p < 0.05$) differentiated according to their mean PMN elastase plasma concentrations from the third posttraumatic day onwards. Cathepsin B plasma levels reached their maxima at least 6 h before complexed PMN elastase (Fig. 2). This early cathepsin B activity in circulating blood proved to be a sensitive and specific predictor of subsequent developing organ failure. Yet, it did not discriminate between lethal (group I) and reversible (group II) dysfunctions at that time. In contrast to PMN elastase, cathepsin B activity returned to only slightly elevated levels within 3 days in all patients (Fig. 2), thus not allowing a further differentiation of the three outcome groups. From these data the conclusion can be drawn, that the early release of lysosomal phagocyte proteinases after a severe traumatic event contributes to the occurrence of anticipated organ failure, and that at least the ongoing discharge of PMN elastase may be involved in the exacerbation of the clinical situation. The latter seems to be confirmed by the appearance of the elastase-induced split product FEP of fibrinogen which is correlated to the continuous release of elastase and the development of lethal multiple organ failure as outlined in Fig. 3 for a severely injured patient.

Fig. 3. Plasma levels of PMN elastase (in complex with α_1-proteinase inhibitor) and fibrino-elastase-peptide (FEP) in a polytraumatized patient with lethal multiple organ failure. *Resp. F* Respiratory failure; *DIC* disseminated intravascular coagulation; *Ren. F.* renal failure; *ARDS* acute respiratory distress syndrome; *Hep. F.* hepatic failure
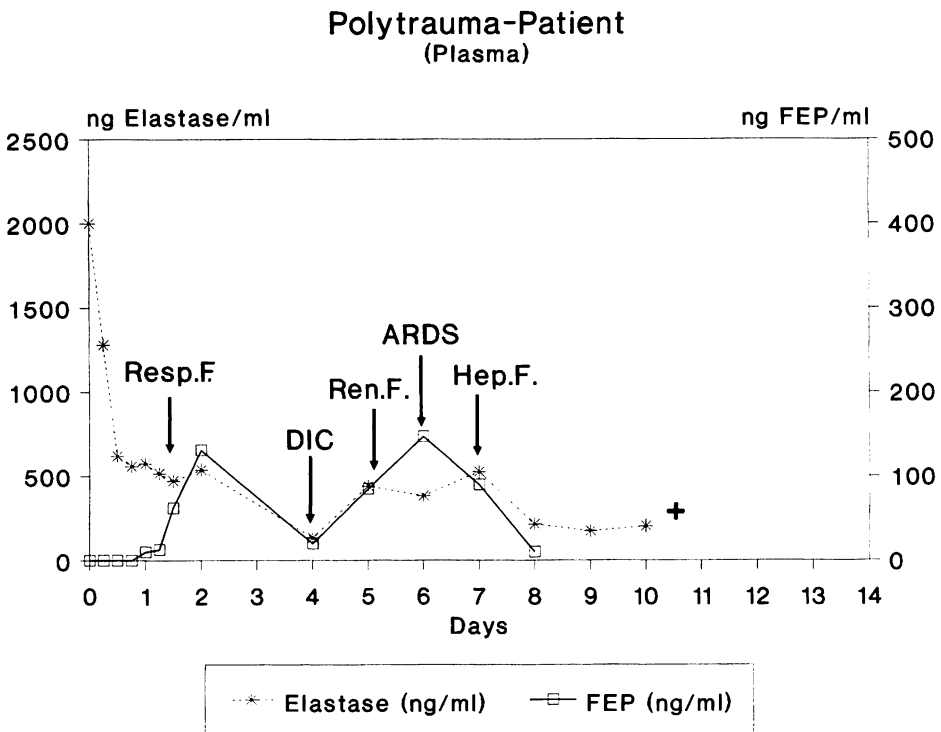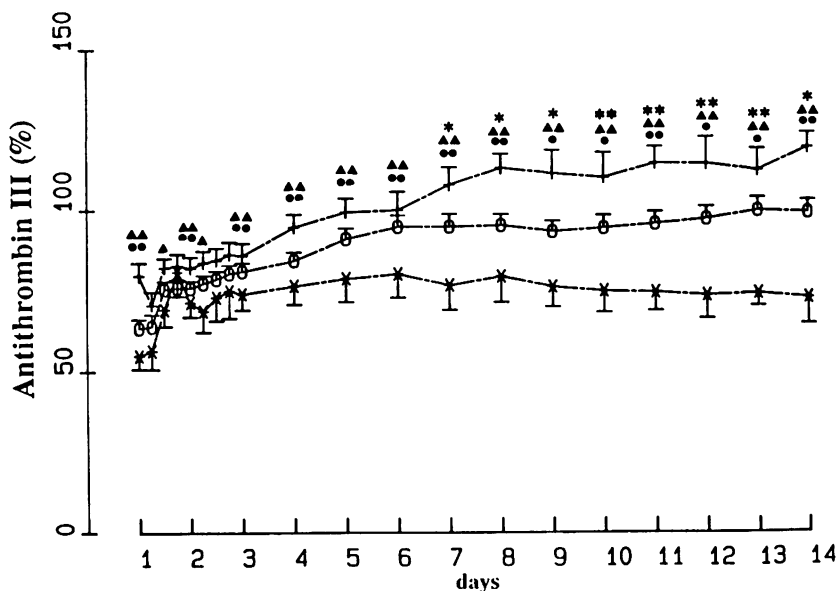
Fig. 4. Mean values (± SEM) of antithrombin III inhibitory activity in plasma of multiply injured patients assigned to three outcome groups. For further details, see Fig. 2

Interestingly, the main clotting inhibitor, antithrombin III (AT III), showed a significantly lower inhibitory activity ($p < 0.01$) in plasma of group I and II patients compared to group III during the whole observation period and in nonsurvivors (group I) compared to survivors with organ failure (group II) from the first posttraumatic week onwards (Fig. 4). Similar results were obtained concerning the rapid turnover of the coagulation proenzyme, prothrombin (data not shown) to thrombin, the essential target proteinase of AT III. Thus, plasma levels of AT III and prothrombin 60% below normal in the early posttraumatic phase were highly associated with the later appearance of severe organ dysfunctions, indicating that in addition to the release of lysosomal proteinases an overwhelming activation of the humoral proteolytic cascade systems is also conducive to the perpetuation of the posttraumatic inflammatory process.

In another clinical study on multiply injured patients (Sturm 1991) we were especially interested in local proteolysis-induced mechanisms which might be involved in the pathogenesis of the most severe lung dysfunction, the acute respiratory distress syndrome (ARDS). Daily drawn BALF (method described in Obertacke et al. 1991) samples allowed us to confirm a significantly increased local discharge of the phagocyte proteinases, elastase and cathepsin B, in subjects at high risk of developing ARDS (Jochum 1991). Although the α_1PI antigen levels in all BALF specimens of the traumatized patients were far above the normal values of healthy volunteers, these amounts were apparently insufficient in most cases to completely inhibit the PMN elastase released in the local epithelial milieu of the alveoli. Therefore, the obviously deficient inhibitory capacity of α_1PI may have been caused by proteolytic and oxidative denaturation as well. Applying special assay systems, both ways of α_1PI destruc-
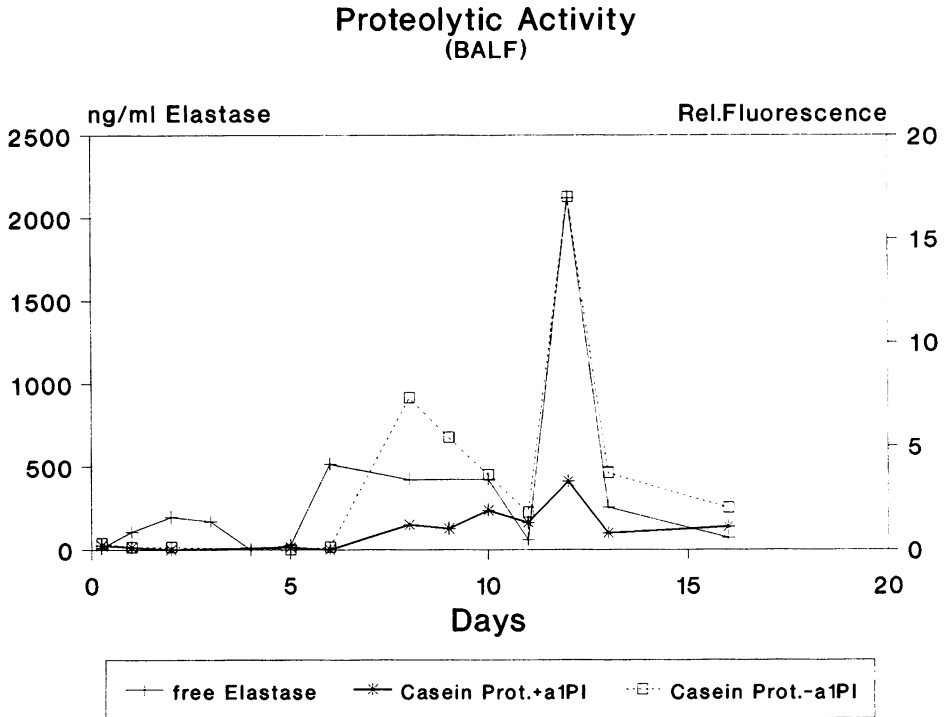
Proteolytic Activity
(BALF)

Fig. 5. Proteolytic activity (degradation of resorufin-casein with and without addition of α_1-proteinase inhibitor) and free PMN elastase in serial bronchoalveolar lavage fluids (BALF) of a severely traumatized patient. Acute respiratory distress syndrome and multiple organ failure persisted from the 5th to 12th posttraumatic day

tion were demonstrated by Schraufstatter et al. (1984), at least in some individual BALF samples of patients with manifest ARDS. Although other researchers could not detect elastase activity against high molecular weight protein substrates in BALF samples of ARDS patients (Idell et al. 1985; Wewers et al. 1988), we were recently able to show caseinolytic activity in a variety of BALF samples (kindly given to us by Dr. Obertacke, Surgical Clinic, University of Essen) from trauma patients with severe lung dysfunctions (Machleidt et al. 1991). In vitro incubation of these specimens with α_1PI abolished nearly completely this proteolytic activity (Fig. 5) and gave rise to an additional increase in the elastase-α_1PI complex, indicating that the enzymatic activity present in the alveolar environment was mostly due to PMN elastase.

Moreover, the destructive potency of elastase in vivo could be directly documented by the proof of augmented generation of FEP in close correlation to the rising amount of the extracellularly discharged PMN enzyme in BALF samples of patients with aggrevating respiratory failure (Fig. 6). Hence, the aggressive components of inflammatory cells, especially the lysosomal proteinases, may indeed contribute to the occurrence of lung dysfunction in severely traumatized patients.
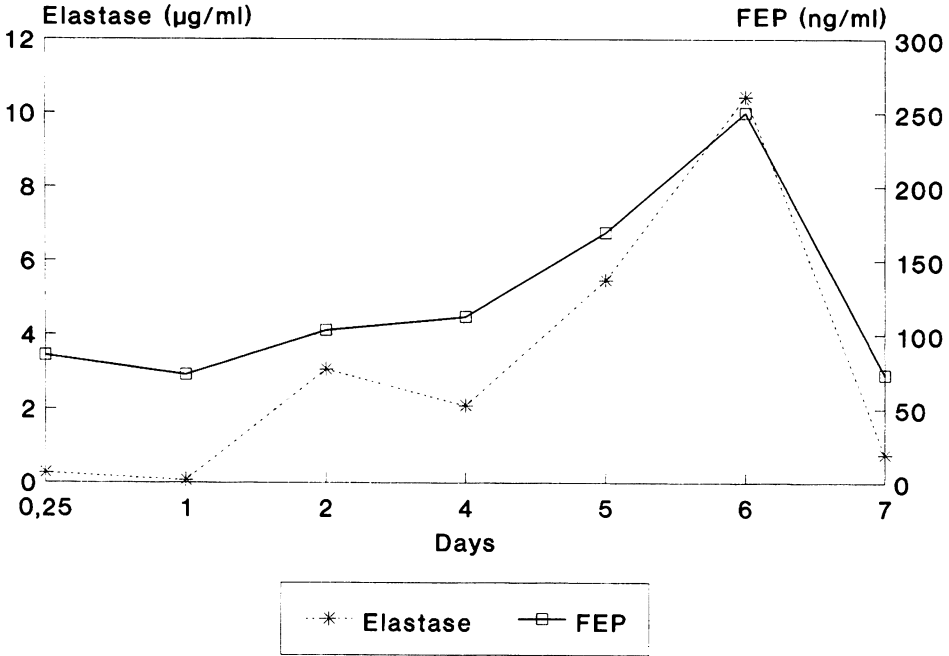
Polytrauma-Patient
(BALF)

Fig. 6. Total amount of PMN elastase (measured as complex with α_1-proteinase inhibitor) and fibrino-elastase-peptide (FEP) in serial bronchoalveolar lavage fluids (BALF) of a severely traumatized patient with reversible respiratory dysfunction from the 2nd to the 7th posttraumatic day

3.1.2 Sepsis and Peritonitis

In several prospective clinical studies on patients suffering from bacterial infections after major surgical treatments, we could demonstrate an increasing release of PMN elastase into the circulation in accordance with the worsening of the inflammatory reaction (Duswald et al. 1985; Inthorn and Jochum 1988; Jochum et al. 1990) Patients without postoperative infections showed only moderate, transiently elevated plasma levels of complexed elastase (up to three times that of normal) after operation, whereas onset and course of sepsis were characterized by markedly increased concentrations, up to 20-fold in individual cases. In patients with persisting septicemia, the elastase plasma levels remained significantly elevated until death, while a recovery phase was accompanied by an obvious return to normal values. When complexed elastase increased in plasma, a prominent reduction of the humoral blood cascade proenzymes, prekallikrein and prothrombin, as well as of the AT III inhibitory activity was measurable. This indicates that the proteolytic mechanisms in this pathological situation are quite similar to those seen in the course of developing posttraumatic organ failure. In addition, we could demonstrate in some pilot measurements that
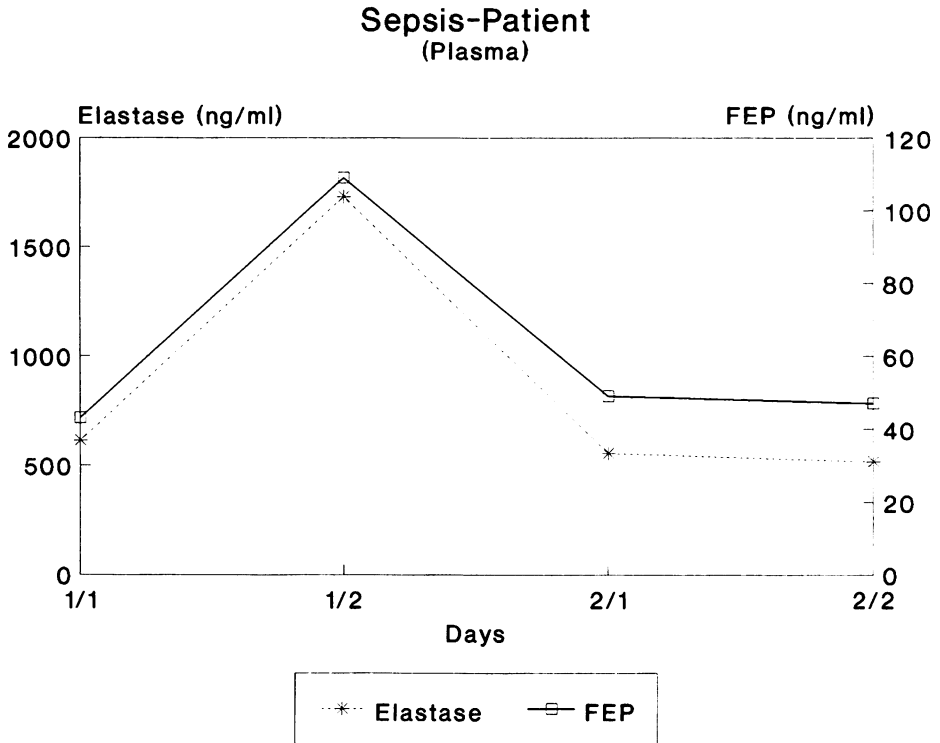
Sepsis-Patient
(Plasma)

Fig. 7. Plasma levels of PMN elastase (in complex with α_1-proteinase inhibitor) and fibrino-elastase-peptide (FEP) of a patient with lethal outcome 2 days after diagnosis of sepsis due to bifurcation malacia of the trachea.

during a septic shock phase, the monocyte/macrophage-derived cathepsin B was released in a manner similar to the PMN elastase, thus intensifying the extracellular proteolytic capacity.

Just recently we were able to verify such a destructive proteolytic potency in a still ongoing prospective study (clinically directed by Dr. Inthorn, Surgical Clinic Groß-hadern, University of Munich) on septic patients. To be enrolled in this investigation, patients had to fulfill all four previously defined septic criteria (septic focus and/or positive blood culture; body temperature > 38.5 °C; leukocytes > 15 000/µl or < 5000/µl; platelets < 100 000/µl or drop of platelets > 20% within 24 h), indicating a severe inflammatory situation. Under these circumstances the generation of the elastase-induced fibrinogen split product FEP was highly correlated to the plasma level of PMN elastase as shown in Fig. 7 for a patient who died 2 days after diagnosis of sepsis. Moreover, the unspecific proteolytic degradation of AT III, which is also highly susceptible to PMN elastase cleavage (Jochum et al. 1981; Jordan et al. 1989), could be determined by an indirect method comparing the amount of AT III antigen with its inhibitory activity as depicted in Fig. 8 for a patient suffering from eventually lethal sepsis. Besides the fact that AT III was already highly consumed throughout the
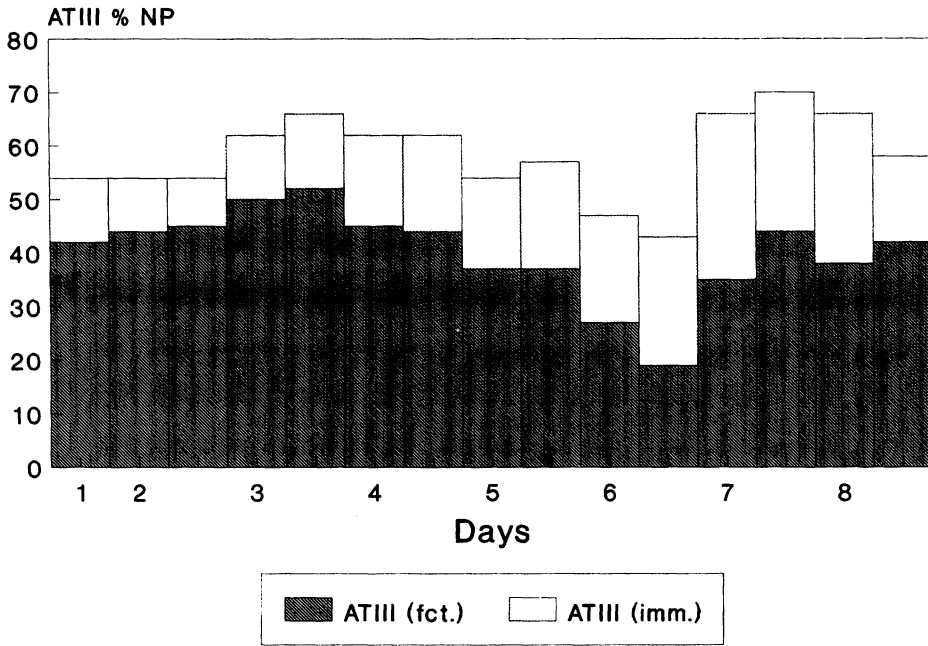
Sepsis-Patient
(Plasma)

Fig. 8. Comparison of antithrombin III antigen (AT III) levels (*imm*) and inhibitory activity (*fct.*) in plasma samples of a patient with lethal sepsis due to stomach cancer. *NP* % of a norm plasma pool

whole sepsis period of 8 days, the clearly lower inhibitory activity indicates that part of the in antigen level may be due either to complexes of the inhibitor with clotting enzymes or to proteolytically degraded molecules. Since the latter have an only slightly lower molecular weight and nearly the same half-life as the intact molecules (Jordan et al. 1989), they cannot be distinguished from the native AT III when quantified by radial immunodiffusion. In our patient, however, we could definitely rule out the possibility that AT III-proteinase complexes contributed significantly to the higher antigen levels, because due to the very short half-life of only a few minutes, concentrations of AT III-thrombin complexes amounted to, at most, 0.01% of the overall AT III (data not shown in detail). Thus, elastase-inactivated AT III molecules should be present with high probability in the pathological situation of severe sepsis. This assumption is further confirmed by results of Jordan et al. (1989) which demonstrate that even catalytic amounts of heparin react with elastase in a way considerably accelerating the rate of AT III inactivation by this enzyme. Since heparin exists not only as a minor component on vascular endothelial cells, but is also used as a therapeutic drug in our patients, the positive regulation of hemostasis by heparin may be significantly counteracted by the adverse effect on AT III in the case of a high release

of elastase from PMN granulocytes adhering to the endothelial layer of the blood vessel walls.

Diffuse peritonitis is often the source of a systemic spreading of local infectious complications, eventually leading to sepsis and multiorgan failure. In a previous work we were able to demonstrate that the impairment of opsonization in the peritoneal cavity allows the survival of huge numbers of bacteria despite the presence of intact phagocytes (Billing et al. 1988). Since the proteolytic breakdown of the main opsonins IgG and complement factor C3 in peritonitis exudates correlated well with the extracellularly released lysosomal proteinases elastase and cathepsin B in the local body fluids, we assumed that these enzymes are the principal causes of the deficiency in opsonic capacity. To assure this supposition, isolated IgG was incubated in vitro either with PMN elastase and cathepsin B or (in its isothiocyanate-labeled form = FITC IgG) with a cell-free, purulent peritonitis exudate (kindly supplied by Dr. Billing, Surgical Clinic Großhadern, University of Munich). The proteolytic degradation was followed by gel chromatography of the split products exhibiting the same type of IgG cleavage pattern under all conditions (Billing et al. 1991; Machleidt et al. 1991). Using resorufin-labeled casein as a substrate, similar results were obtained substantiating proteolytic elastase activity despite the presence of an up to 40-fold molar sur-
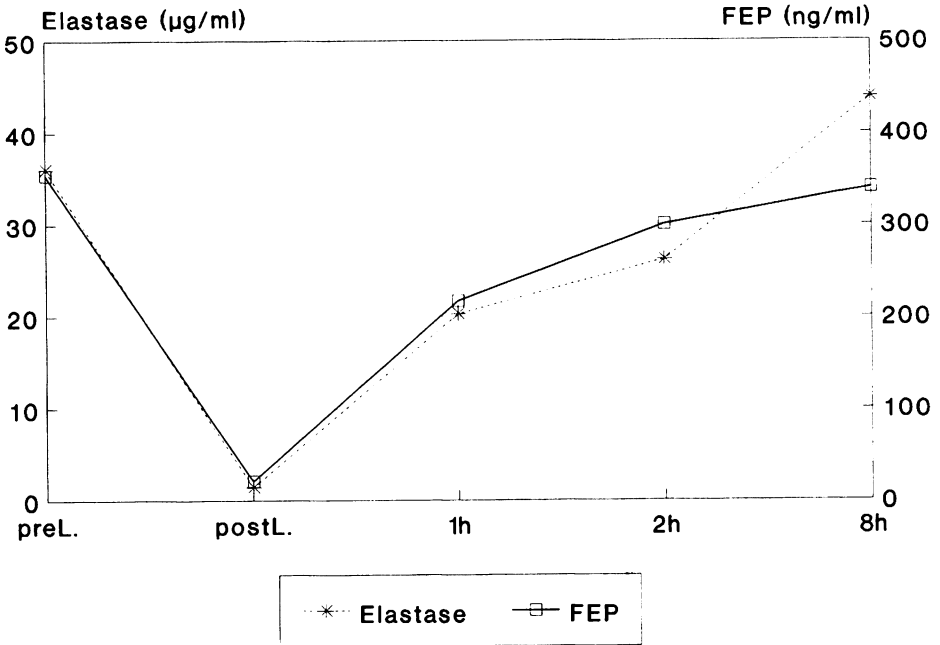
Peritonitis-Patient
(Exudate)

Fig. 9. PMN elastase (in complex with α_1-proteinase inhibitor) and fibrino-elastase-peptide (FEP) in exudate samples taken before (*preL.*) and immediately after (*postL.*) lavage of the abdomen of a peritonitis patient with Ringer lactate solution and in drainage fluids collected between 0 to 1 h, 1 to 2, and 2 to 8 h after lavage

plus of α_1PI antigen in peritonitis exudates (data not shown) as already discussed for BALF samples in trauma patients. In addition to the in vivo degradation of IgG and C3, we have successfully proved the generation of FEP in peritonitis exudates just recently. As depicted in Fig. 9, high amounts of complexed PMN elastase coincided with highly elevated FEP in the specimen drawn before surgical treatment of the abdomen of a patient with severe peritonitis. After rinsing the peritoneal cavity with 10 l of Ringer lactate solution both parameters decreased nearly to zero. Yet, as can be seen from the abdominal drainage fluids collected between 0 and 1, 1 and 2, and 2 and 8 h after operation, the release of PMN elastase started again, inducing also the production of FEP (Fig. 9). These observations may be taken as an indication of a still ongoing inflammatory reaction in this patient.

Summarizing the data obtained in our clinical studies on patients suffering from multiple trauma and/or septicemia, we could clearly show that excessive local (α_1PI) or systemic (AT III) consumption of proteinase inhibitors concomitant to the release of lysosomal phagocyte proteinases and the activation of proteolytic blood cascade enzymes during severe inflammatory reactions is a most critical event which may contribute to the propagation of (multiple) organ damage. Therefore, supplementation of the body's inhibitor potential by exogenous proteinase inhibitors – isolated from human material or produced by gene technology (Fritz et al. 1991) – seems to be a most promising therapeutic approach.

3.2 Proteinase Inhibition as a Suitable Therapeutic Approach in Acute Inflammation

3.2.1 Inhibitors of PMN Elastase or Thrombin in Experimental Endotoxin Shock

In a preliminary, controlled study on sepsis in young pigs the prophylactic application of the relatively specific recombinant elastase inhibitor, eglin c (originally isolated from the leech hirudo medicinalis), caused a significant reduction in the consumption of antithrombin III and other plasma proteins as well as in the formation of interstitial pulmonary edema (Jochum et al. 1987). As assessed by measurement of arterial blood pressure and total protein concentration in plasma, Siebeck et al. (1989a) demonstrated in a more extended study that eglin c can also reduce the overall capillary leakage induced by the infusion of live *E. coli* in pigs. Moreover, in a further controlled investigation, Siebeck et al. (1989b) and Hoffmann et al. (1990) could show that besides eglin c the thrombin-specific inhibitor hirudin – another recombinant inhibitor also formerly isolated from the medical leech – significantly improved endotoxin shock syndromes in minipigs. Fibrinogen consumption, formation of fibrin monomers, the occurrence of pulmonary vasoconstriction, and the release of PMN constituents were clearly lower in endotoxemic animals treated with hirudin as compared to those without continuous intravenous inhibitor infusion. Interestingly, the supplementation of a purified antithrombin III-heparin complex in another randomized porcine endotoxin shock model had only a slight, insignificant positive effect on the endotoxin-induced mortality and oxygen saturation in arterial blood (as an indication of pulmonary function), although the consumption of fibrinogen and the formation of soluble fibrin monomers were clearly prevented in the drug-treated animals

(Spannagl et al. 1991). Furthermore, prothrombin consumption was similar in the treatment and placebo groups, suggesting that local thrombin generation via factor Xa and binding of both enzymes to cell membrane receptors (e.g., thrombin to thrombomodulin) may protect them from inhibition by the AT III-heparin complex. On the other hand, the applied amount of the AT III-heparin complex may not have been sufficient to inhibit the activation of contact phase enzymes (plasma kallikrein, factor XIIa) which are also supposed to be potent stimulators of PMN granulocytes (Wachtfogel et al. 1983; 1985), thus maintaining the inflammatory process via the release of phagocyte proteinases and reactive oxygen metabolites. Moreover, since after an initial rise (up to about 130%) the AT III activity decreased during further drug application, while the antigen level still increased, the heparin in the complex may have also facilitated the inactivation of AT III by released PMN elastase (Jordan et al. 1989), thereby lowering the inhibitory capacity under an otherwise presumably effective threshold level.

The requirement of high levels of AT III inhibitory activity in the circulation is also confirmed by results of Emerson et al. (1989) concerning the efficacy of antithrombin III supplementation in several animal models (rat, sheep, baboon) of fulminant *E. coli* endotoxemia or bacteremia. Only very high dosage (up to threefold that of normal) and prophylactic administration of AT III prevented organ damage and increased permanent survival in the experimental animals. Interestingly, the combined application of AT III and α_1PI showed a significant synergistic improvement of the pulmonary function compared to the single drug treatment in the endotoxemic sheep model. This indicates again that a complex interaction of lysosomal and humoral blood cascade proteinases contribute to the perpetuation of a septic-like inflammation.

3.2.2 Antithrombin III Supplementation in Clinical Sepsis

From the therapeutic animal experiments and the apparent lack of sufficient efficacy of AT III supplementation in clinical studies (Blauhut et al. 1985; Vinazzer 1987), we draw the conclusion that only AT III levels well above the normal value may be able to improve organ dysfunctions in clinical sepsis. Therefore, we conducted a study (clinically directed by Dr. Inthorn, Surgical Clinic Großhadern) on septic patients with the aim to increase the AT III inhibitory activity above 120% that of normal. To achieve this, AT III concentrates were i.v. infused twice daily over 21 days according to a modified regimen originally described by Blauhut et al. (1985). Blood samples were taken twice daily throughout the whole observation period. Preliminary data and a detailled outline of the performance of the still ongoing study have been published (Jochum et al. 1991). Here, only the most important results will be presented. Up to now, 15 patients, each fulfilling the above-mentioned septic criteria, could be enrolled in the control and therapy groups. With our application scheme the AT III activity in the treatment group was elevated to mean plasma levels slightly below 120% during the first 9 days followed by an increase above 120% thereafter, whereas in the control group levels between 60% (early phase) and 80% (later phase) were measurable (Fig. 10). Although all AT III-treated patient received nearly the same amount of the inhibitor concentrate (between 8000 and 4000 U/day), those individuals who survived the septic events showed clearly higher AT III levels (up to a mean of 135% during
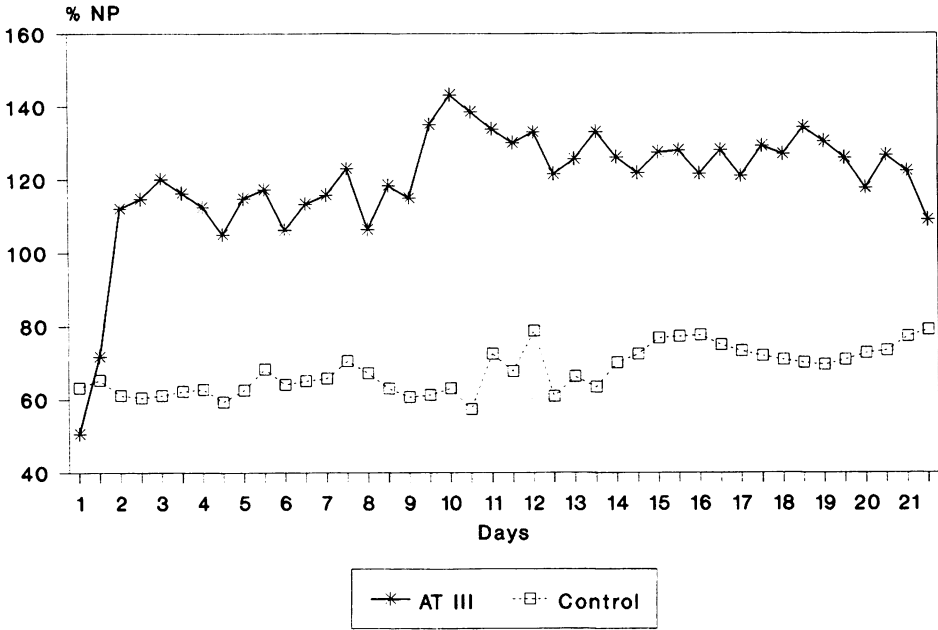
Fig. 10. Mean values of antithrombin III inhibitory activity in plasma of septic patients with (AT III; n = 15) or without (control; n = 15) antithrombin III supplementation
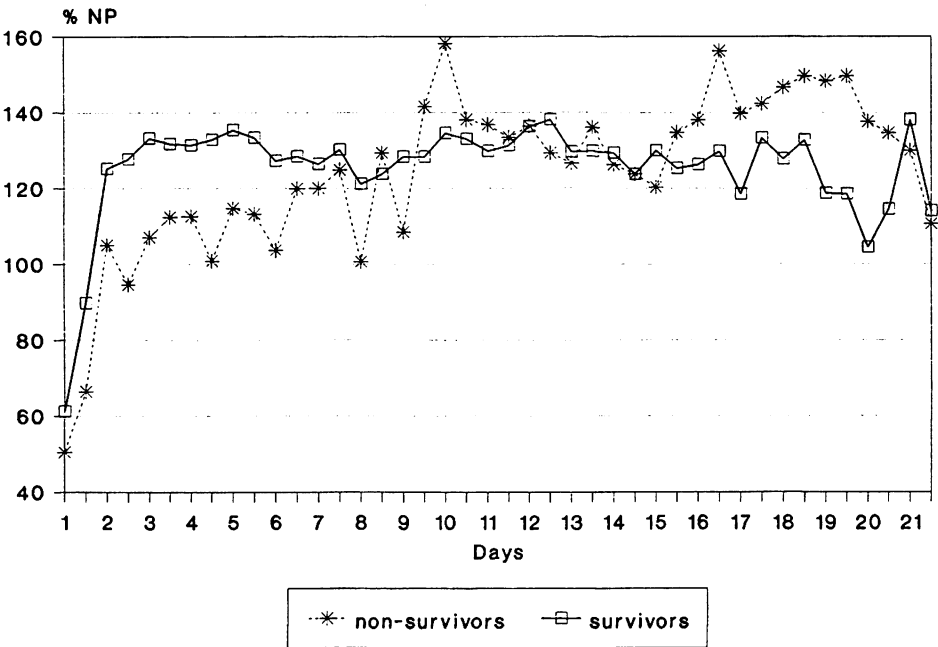

Fig. 11. Mean values of antithrombin III inhibitory activity in plasma of survivors (n = 6) and nonsurvivors (n = 9) of the AT III-treatment group
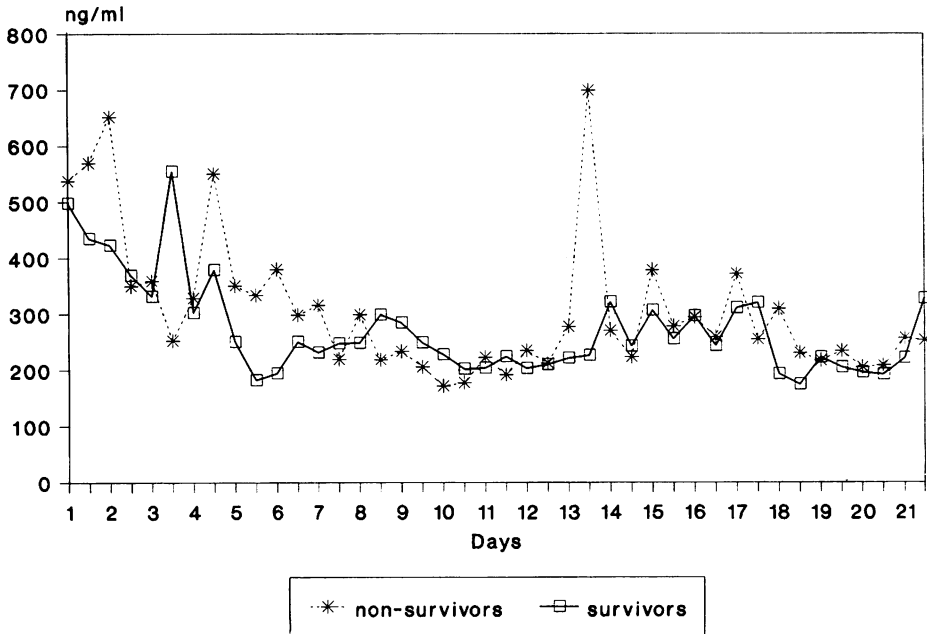
Fig. 12. Mean values of PMN elastase (in complex with α_1-proteinase inhibitor) in plasma of survivors (n = 6) and nonsurvivors (n = 9) of the AT III-treatment group

the first 5 days after sepsis diagnosis; Fig. 11) than patients who died despite AT III supplementation (mean AT III activity between 100% and 115% in the early septic phase). Thus, immediate AT III substitution in sufficiently high amounts after early diagnosis of a septic episode appears to be of great importance in improving the survival of the patients.

Probably due to a too late onset of the inhibition therapy and the application of still insufficiently high AT III dosages in some of our patients, the overall mortality could be reduced only from 87% in the control group to 60% in the AT III-treated collective. This diminution in lethality was statistically insignificant, yet a clear improvement of organ functions – especially lung, liver and kidney – in the treated patients as well as a further deterioration in the control group became evident. Mean plasma levels of complexed elastase were elevated up to sixfold that of normal upon admission and decreased gradually to about threefold in both groups until the end of the observation period. Although there were no statistically significant differences, a slight trend to lower plasma elastase levels appeared in the AT III group. Similar minute distinctions were seen in AT III-treated patients who survived compared to those who died (Fig. 12). Plasma prekallikrein and prothrombin levels were highly reduced up to 30 and 50%, respectively, upon admission and showed a more or less pronounced change to higher values (40 and 80% that of normal) later on without obvious differences in the placebo and treatment groups. Interestingly, however, prekallikrein levels rose steadily up to 50% in AT III-treated survivors, whereas in the non-
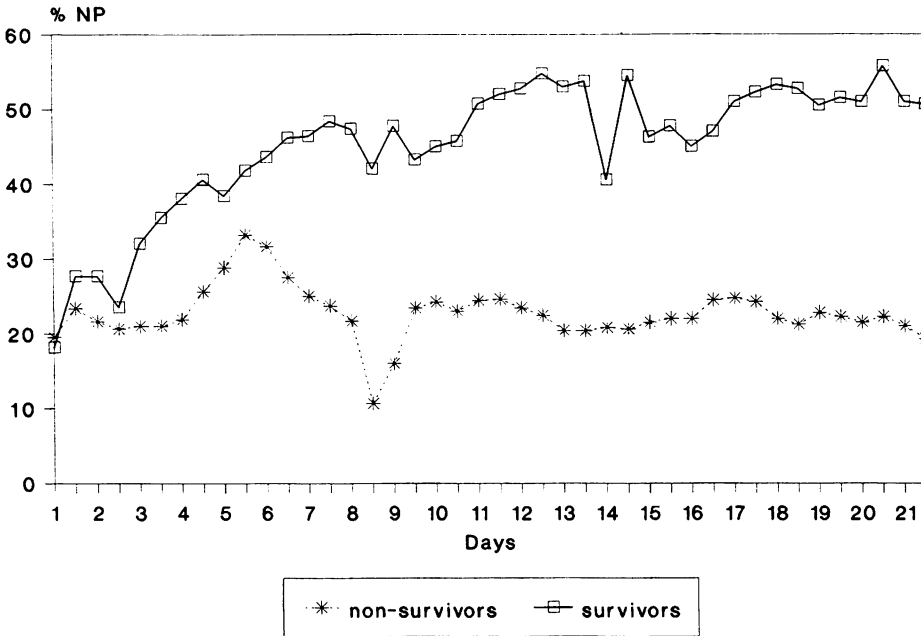
Fig. 13. Mean values of plasma prekallikrein (*PKK*) in plasma of survivors (n = 6) and nonsurvivors (n = 9) of the AT III-treatment group

survivors a further transient decrease up to 20% occurred (Fig. 13). Comparable behavior was observed for prothrombin which reached 80% of normal already 10 days after onset of sepsis in the AT III-treated survivors, while in the moribund patients plasma prothrombin levels as low as 60% were measured at that time. Thus, the rise in AT III inhibitory activity to nearly 140% in the early septic phase may have been beneficial enough to preserve clotting proenzymes from excessive activation throughout a septic period.

4 Conclusion

The given data, derived from clinical and experimental studies on acute inflammation, unequivocally indicate that proteolytic pathomechanisms play an important role in the onset and perpetuation of inflammatory processes like trauma- and sepsis-induced multiple organ dysfunctions. Therefore, the plasma levels of the regulatory proteinase inhibitors have to be elevated by suitable supplementation and kept well above the normal values to achieve significant improvement of the clinical situation. As the natural sources for the isolation of proteinase inhibitors from human material are very limited, the design of highly effective inhibitory proteins, on the basis of human inhibitor molecules by molecular modeling and their production by recombi-

nant DNA technology, is the most promising approach at present to obtain the quantities necessary for proteinase inhibition therapy in the future (Fritz et al. 1991).

References

Assfalg-Machleidt I, Jochum M, Nast-Kolb D, Siebeck M, Billing A, Joka Th, Rothe G, Valet G, Zauner R, Scheuber HP, Machleidt W (1990) Cathepsin B – indicator for the release of lysosomal cysteine proteinases in severe trauma and inflammation. Biol Chem Hoppe-Seyler 371(Suppl):211–222

Billing A, Fröhlich D, Jochum M, Kortmann H (1988) Impaired phagocytosis in peritonitis exudate secondary to complement consumption. Surg Res Comm 3:335–345

Billing A, Fröhlich D, Jochum M, Kortmann H (1990) Deficient phagocytosis in abdominal sepsis: the influence of intraperitoneal substitution of opsonins – first results. Surg Res Comm 9:297–302

Billing A, Fröhlich D, Assfalg-Machleidt I, Machleidt W, Jochum M (1991) Proteolysis of defensive proteins in peritonitis exudate: pathobiochemic aspects and therapeutic approach. Biomed Biochim Acta 50 (in press)

Blauhut B, Kramer H, Vinazzer H, Bergmann H (1985) Substitution of antithrombin III in shock and DIC. A randomized study. Thromb Res 39:81–89

Duswald K-H, Jochum M, Schramm W, Fritz H (1985) Released granulocytic elastase: an indicator of pathobiochemical alterations in septicemia after abdominal surgery. Surgery 98:892–898

Emerson TE, Fournel MA, Redens TB, Taylor FB (1989) Efficacy of antithrombin III supplementation in animal models of fulminant *Escherichia coli* endotoxemia or bacteremia. Am J Med 87(Suppl. 3B):27–33

Fritz H, Collins J, Jochum M (1991) Proteinase inhibitor candidates for therapy of enzyme-inhibitor imbalances. In: Grassi C, Travis J, Casali L, Luisetti M (eds) Current concepts in the biochemistry of pulmonary emphysema. Springer, Berlin Heidelberg New York, Bi & Gi, Verona Publishers, London (in press)

Gippner-Steppert C (1991) Entwicklung eines spezifischen Testsystems für den Nachweis der Bildung eines proteolytischen Spaltproduktes des Fibrinogens durch lysosomale PMN-Elastase sowie Untersuchungen am Miniplasminogen, einem Elastase-spezifischen Spaltprodukt des Plasminogens. Dissertation, Fakultät für Chemie, Biologie und Geowissenschaften der Technischen Universität München

Hoffmann H, Siebeck M, Spannagl M, Weipert J, Geiger R, Jochum M, Fritz H (1990) Effect of recombinant hirudin, a specific inhibitor of thrombin, on endotoxin-induced intravascular coagulation and acute lung injury in pigs. Am Rev Respir Dis 142:782–788

Idell S, Kucich U, Fein A, Kueppers F, James HL, Walsch PN, Weinbaum G, Colman RW, Cohen AB (1985) Neutrophil elastase-releasing factors in bronchoalveolar lavage from patients with adult respiratory distress syndrome. Am Rev Respir Dis 132:1098–1105

Inthorn D, Jochum M (1988) Auswirkungen chirurgischer Infektionen auf die Stimulierbarkeit zur Chemilumineszenz von Granulozyten und die Freisetzung granulozytärer Elastase. In: Häring R (Hrsg) Risiko in der Chirurgie. Analyse und Kalkulation. Walter de Gruyter, Berlin, pp 219–224

Jochum M (1988) Lysosomale Faktoren aus polymorphkernigen Granulozyten: Pathobiochemische, diagnostische und therapeutische Aspekte. Habilitationsschrift an der Medizinischen Fakultät der Ludwig-Maximilians-Universität München

Jochum M (1991) Specific proteins of inflammatory cells and α_1-proteinase inhibitor in alveolar epithelial lining fluid of polytraumatized patients: do they indicate posttraumatic lung failure? In: Sturm JA (ed) Posttraumatic acute respiratory distress syndrome. Springer, Berlin Heidelberg New York, pp 193–211

Jochum M, Fritz H (1989) Pathobiochemical mechanisms in inflammation. In: Faist E, Ninnemann JL, Green DR (eds) Immune consequences of trauma, shock and sepsis. Springer, Berlin Heidelberg New York, pp 165–172

Jochum M, Fritz H (1990) Elastase and its inhibitors in intensive care medicine. Biomed Prog 3:55–59

Jochum M, Lander S, Heimburger N, Fritz H (1981) Effect of human granulocytic elastase on isolated human antithrombin III. Hoppe Seyler's Z Physiol Chem 362:103–112

Jochum M, Welter HF, Siebeck M, Fritz H (1987) Proteinase inhibitor therapy of severe inflammation in pigs. First results with eglin, a potent inhibitor of granulocyte elastase and cathepsin G. In: Taylor JC, Mittmann C (eds) Pulmonary emphysema and proteolysis. Academic Press, Orlando, pp 85–90

Jochum M, Assfalg-Machleidt I, Inthorn D, Nast-Kolb D, Waydhas Ch, Fritz H (1990) Leukozytäre Proteinasen und Hämostasestörung bei der Sepsis. In: Matthias R, Tilsner V (Hrsg) XXXII. Hamburger Symposium über Blutgerinnung: Infektion, Entzündung und Blutgerinnung. Editiones Roche, Basel, pp 241–254

Jochum M, Inthorn D, Nast-Kolb D, Fritz H (1991) AT III – ein neues therapeutisches Konzept bei der Behandlung der Sepsis und beim Organversagen? In: Henschel WF (Hrsg) Blut, Blutkomponenten und Blutersatzstoffe in der Intensivmedizin. Bericht über das 10. Bremer Interdisziplinäre Intensivtherapie-Colloquium. W. Zuckschwerdt-Verlag, München pp 46–58

Jordan RE, Nelson RM, Kilpatrick J, Newgren JO, Esmon PC, Fournel MA (1989) Antithrombin inactivation by neutrophil elastase requires heparin. Am J Med 87 (Suppl. 3B):19–22

Machleidt W, Assfalg-Machleidt I, Billing A, Fröhlich D, Joka Th, Nast-Kolb D (1991) The role of lysosomal cysteine proteinases as markers of macrophage activation and as non-specific mediators of inflammation. In: Proc 2nd Int. Congr on the Immune consequences of trauma, shock, and sepsis. Springer, Berlin Heidelberg New York (in press)

Nast-Kolb D, Waydhas Ch, Jochum M, Spannagl M, Duswald KH, Schweiberer L (1990) Günstigster Zeitpunkt für die Versorgung von Femurschaftfrakturen beim Polytrauma? Chirurg 61:259–265

Neumann S, Jochum M (1984) Elastase-α_1-proteinase inhibitor complex. In: Bergmeyer HU, Bergmeyer J, Graßl M (eds) Methods of enzymatic analysis, 3rd edn, vol 5. Verlag Chemie, Weinheim, pp 184–195

Obertacke U, Joka Th, Reuter M, Schmit-Neuerburg KP (1991) Bronchoalveolar lavage. In: Sturm, JA (ed) Adult respiratory distress syndrome. Springer, Berlin Heidelberg New York, pp 17–21

Schraufstatter I, Revak SD, Cochrane CG (1984) Biochemical factors in pulmonary inflammatory disease. Fed Proc 43:2807–2810

Siebeck M, Hoffmann H, Jochum M, Fritz H (1989a) Inhibition of proteinases with recombinant eglin c during experimental *Escherichia coli* septicemia in the pig. Eur Surg Res 21:11–17

Siebeck M, Hoffmann H, Weipert J, Spannagl M (1989b) Therapeutic effects of the combination of two proteinase inhibitors in endotoxin shock of the pig. In: Schlag G, Redl H (eds) Progress in clinical and biological research, vol 308. Second Vienna Shock Forum. Alan R. Liss, New York, pp 937–943

Spannagl M, Hoffmann H, Siebeck M, Weipert J, Schwartz HP, Schramm W (1991) A purified antithrombin III-heparin complex as a potent inhibitor of thrombin in porcine endotoxin shock. Thromb Res 61:1–10

Sturm JA (ed) (1991) Adult respiratory distress syndrome. An aspect of multiple organ failure. Results of a prospective clinical study. Springer, Berlin Heidelberg New York

Vinazzer H (1987) Clinical use of antithrombin III concentrates. Vox Sang 53:193–198

Wachtfogel YT, Kucich U, James HL, Scott CF, Schapira M, Zimmerman M, Cohen A, Colman RW (1983) Human plasma kallikrein releases neutrophil elastase during blood coagulation. J Clin Invest 72:1672–1677

Wachtfogel YT, Pixley RA, Kucich U, Abrams W, Weinbaum G, Schapira M, Colman RW (1985) Purified plasma factor XIIa aggregates human neutrophils and releases elastase. Circulation 70(Suppl. II):352

Waydhas Ch, Nast-Kolb D, Jochum M, Trupka A, Lenk S, Fritz H, Duswald KH, Schweiberer L (1991) Inflammatory mediators, infection, sepsis, and multiorgan failure after severe trauma. Arch Surg (in press)

Weitz JI, Landmann SL, Crowley KA, Birken S, Morgan FJ (1986) Development of an assay for in vivo human neutrophil elastase activity. J Clin Invest 78:155–162

Wewers MD, Herzyk DJ, Gadek JE (1988) Alveolar fluid neutrophil elastase activity in the adult respiratory distress syndrome is complexed to alpha-2-macroglobulin. J Clin Invest 82:1260–1264