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GRANULOCYTE PROTEINASES AS MEDIATORS OF UNSPECIFIC PROTEOLYSIS IN INFLAMMATION: A REVIEW

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Abstract

In severe inflammatory response, various blood and tissue cells, including polymorphonuclear granulocytes, release lysosomal proteinases extracellularly and into the circulation. Such enzymes, as well as normally intracellular oxidizing agents produced during phagocytosis, enhance the inflammatory response by degrading connective tissue structures, membrane constituents and soluble proteins by proteolysis or oxidation. We first used polymorphonuclear elastase (E) as a marker of such release reactions. The liberated proteinase competes with susceptible substrates, including α_1-proteinase inhibitor (α_1PI) and α_2-macroglobulin, and is eliminated finally as inactive enzyme-inhibitor complexes by the reticulo-endothelial system. Using an enzyme-linked immunosorbent assay, we determined the plasma levels of E-α_1PI following major abdominal surgery, multiple trauma and pancreatogenic shock. Whereas the operative trauma was followed by up to 3-fold increase of the E-α_1PI, postoperative septicemia was associated with a 10- to 20-fold increase. The increase of E-α_1PI and a

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concomitant decrease of plasma factors, such as antithrombin III, clotting factor XIII and α_2-macroglobulin, were correlated. Multiple trauma causes a substantial increase of E-α_1PI up to 14 hours after accident. The released elastase seems to correlate with the severity of injury, but assessing the relationship to consumption of plasma factors is complicated by concomitant transfusions. In acute pancreatitis, peaks of E-α_1PI coincide with a massive consumption of antithrombin III and α_2-macroglobulin during shock.

Introduction

Severe injury or infection triggers an inflammatory response, including the activation of (a) such humoral systems as clotting, fibrinolysis, complement and kallikrein-kinin cascades, and (b) cellular systems, especially phagocytes, mast cells and lymphocytes, but also stress hormone producing cells. The humoral factors are often potent stimulators of the inflammatory cells, and conversely effectors from these cells may activate some humoral systems. Similarly, the repair mechanisms resulting from inflammation in their aggregate establish multiple relationships between the various parts of the organism (1).

Lysosomal proteinases

We focus on the potential pathological role of lysosomal proteinases released extracellularly during inflammation. Phagocytes such as polymorphonuclear granulocytes and macrophages contain many lysosomes with a powerful hydrolytic and proteolytic potential (2). Normally, the lysosomal enzymes, along with oxidizing agents produced when phagocytosis is triggered, serve two main purposes (3): (a) intracellular

protein catabolism including the degradation of intra- and extracellular endogenous substances, and (b) the degradation of phagocytized viruses and bacteria.

In summary, lysosomal proteinases normally fulfill their physiological function inside the cell in the phagolysosomes, but under certain conditions, major escape of lysosomal factors from the cell occurs (3,19). Only small amounts leak out during normal phagocytosis, higher amounts escape during frustrated phagocytosis, when the phagocyte is unable to take up a larger structural element such as a piece of vascular plasma membrane or cartilage. Disintegration of phagocytes caused by endogenous or exogenous endotoxins, possibly in combination with complement lysis, releases most or all lysosomal or phagolysosomal constituents (Figure 1).

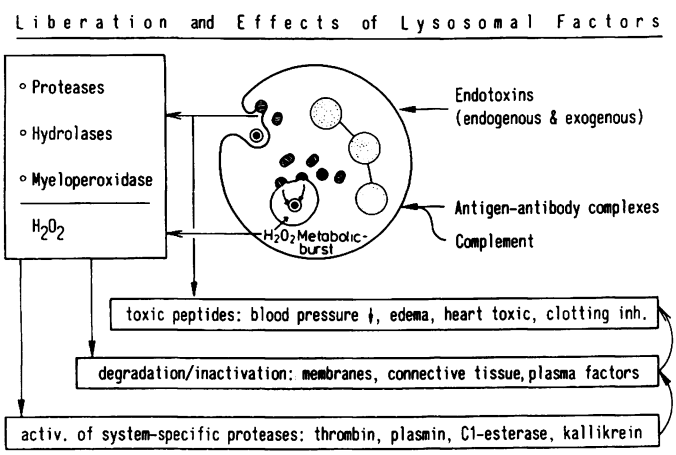

Figure 1. Liberation and effects of lysosomal factors during inflammation. Degradation products present in the phagolysosomes (*e.g.*, toxic peptides) are liberated during cell stimulation or disintegration. Released lysosomal or phagolysosonal constituents degrade or inactivate native structural and humoral factors. System-specific proteinases activate the blood system cascades. In this way, activated proteinases nay also degrade humoral factors, and therefore, toxic polypeptides may be generated as well.

Released extracellularly, lysosomal proteinases may enhance inflammation in two major ways (Figure 2): (a) selective proteolysis activates proenzymes, cofactors or both, and may generate biologically potent peptides such as kinins and anaphylatoxins. The formed proteinases are eliminated by interaction with specific inhibitor proteins, as is true for the *specific* consumption of factors participating in the clotting, fibrinolysis, complement and kallikrein-kinin cascades (called blood systems here); (b) unspecific proteolysis inactivates soluble factors such as antithrombin III, or digests structural elements (4-8). Degradation induced *unspecific* consumption may produce toxic peptides as fibrin-fibrinogen degradation products which inhibit clotting (9).

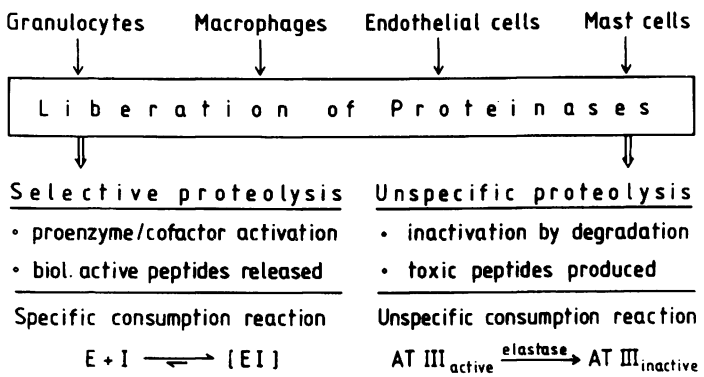

Figure 2. Reaction pathways caused by lysosomal proteinases if liberated from various body cells. E = enzyme; I = inhibitor; [EI] = enzyme-inhibitor complex; AT III = antithrombin III.

Clinical organ failure precipitated by severe inflammation or multiple injury affects primarily lungs, liver and kidneys. These organs are rich in endothelial cells, mast cells, fibroblasts, and macrophages, which contain many lysosomes. Above all, polymorphonuclear granulocytes may accumulate rapidly in the lungs during the inflammation. Hence, the relationships

between the sequence of organ failures and the lysosomal content of different organs merits consideration.

Among known lysosomal proteinases, the neutral proteinases of neutrophil polymorphonuclear granulocytes, elastase and cathepsin G are of special interest. They are stored in the lysosomes in fully active form, like the acid thiol and aspartate proteinases. The most striking feature of both elastase and the chymotrypsin-like cathepsin G is non-specificity (3,10). Both enzymes degrade numerous humoral factors, including proteinase inhibitors (4,11), as well as structural elements, such as elastin (6) and collagen type III and IV (8), under physiological conditions (Figure 3). The digestive potential of lysosomes is demonstrated by the daily synthesis of over 1 g of neutral proteinases in man.

Substrates of PMN Granulocyte Neutral Proteinases

Proteinase*	Biological substrates
Elastase	· elastin, collagen III & IV, proteoglycans, FN+
	· clotting & fibrinolysis factors
	· complement factors & immunoglobulins
	· proteinase inhibitors (AT III, α_2 PI, C1 INA; ITI)
	· transport proteins (transferrin, prealbumin)
Cathepsin G	· collagen II & I, proteoglycans, fibronectin
	· clotting & complement factors
Collagenase	· collagen I & II & III

* >1 g daily turnover

\+ fibronectin (R E S function)

Figure 3. Natural substrates of neutral proteinases from polymorphonuclear (PMN) granulocytes. AT III = antithrombin III; α_2PI = α_2-plasmin inhibitor; C1 INA = C1-inactivator; ITI = inter-α-trypsin inhibitor.

Plasma proteinase inhibitors

Functional aspects. Within the cell, lysosomal proteinases are kept under control first by their localization in membrane coated organelles, and second by proteinase inhibitors in the cytosol (12). Lysosomal proteinases escaping the cell face potent antagonists, such as the proteinase inhibitor proteins (13). α_2-Macroglobulin (α_2M) effectively inhibits serine proteinases, as well as thiol (cysteine), aspartate and metallo proteinases. The high molecular weight of α_2M normally restricts its function to the vascular bed. α_1-Proteinase inhibitor (α_1PI, formerly called α_1-antitrypsin), the major antagonist of lysosomal neutrophil elastase, is present in high concentration in blood, but occurs also in interstitial fluid and mucous secretions. α_1-Antichymotrypsin (α_1AC), a rapidly responding acute phase reactant, reaching up to 6-fold concentration in response to inflammation, is a potent inhibitor of lysosomal neutrophil cathepsin G and mast cell chymase. The plasma concentrations of all other inhibitors are clearly lower. Still, the proteinase inhibitor proteins represent approximately 60% of the plasma proteins other than albumin and immunoglobulins.

The interactions between proteinase inhibitors and their target enzymes in plasma are sketched in Figure 4. Excessive enzyme activation may be checked by three main inhibitors: antithrombin III (AT III) regulates clotting, α_2-plasmin inhibitor (α_2PI) fibrinolysis, and Cl-inactivator (Cl INA) both the classical complement pathway and the intrinsic coagulation cascade. This last function results from inhibition of plasma kallikrein, and Hageman factor or the 28,000 dalton Hageman factor fragment.

The existence in plasma of complexes between α_2-macroglobulin and kallikrein or plasmin suggests an involvement in the regulation of enzyme cascades in certain pathological conditions.

However, the predominant role of α_2M seems to be prevention of unspecific proteolysis by inhibiting all types of lysosomal proteinases including neutrophil elastase, cathepsin G, the thiol proteinases cathepsin B, H, L, the aspartate proteinase cathepsin D and metallo enzymes such as collagenase. α_2M

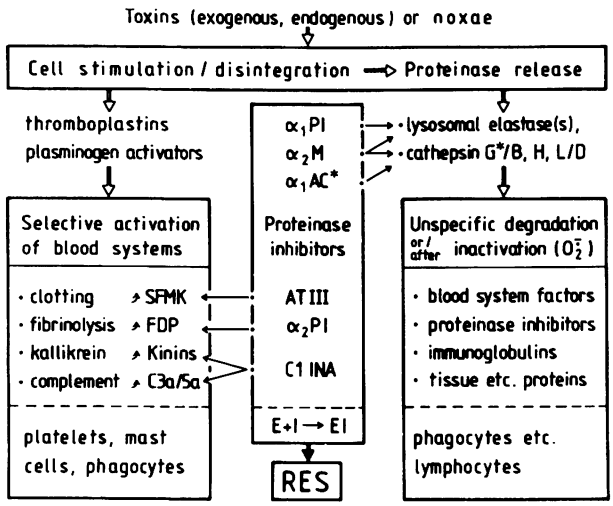

Figure 4. Activation and consumption reactions caused by proteinases released during cell stimulation or disintegration: System-specific proteinases (*e.g.*, thrombokinases and plasminogen activators) trigger activation of blood systems, whereby biologically highly active polypeptides are formed, *e.g.*, SFMK (soluble fibrin monomer complexes), FDP (fibrin/ogen degradation products), kinins, and anaphylatoxins (C 3a and C 5a; left part). Unspecific degradation or inactivation of plasma and tissue factors is caused by lysosomal proteinases and/or oxidizing agents (right part). In both cases, liberated polypeptides may stimulate suitable cellular systems. Finally, complex formation of activated or liberated enzymes (E) occurs with the proteinase inhibitors (I), *e.g.*, α_1PI (α_1-proteinase inhibitor), α_2M (α_2-macroglobulin), α_1AC (α_1-antichymotrypsin), AT III (antithrombin III), α_2PI (α_2-plasmin inhibitor), and C1 INA (C1-inactivator). The enzyme-inhibitor complexes [EI] are eliminated by phagocytes of the reticulo-endothelial system (RES; central part).

is also the most potent inhibitor of pancreatic trypsins which can activate enzyme cascades in blood and release kinins by selective proteolysis, or can unspecifically degrade various factors and structural elements while releasing toxic peptides. Despite this broad inhibitory specificity, α_2M is not an acute phase reactant in man. α_1PI strongly inhibits neutrophil elastase as well as pancreatic elastase, chymotrypsin and bacterial elastases. α_1AC inhibits pancreatic chymotrypsin, neutrophil cathepsin G and mast cell chymase (13).

Inhibition of proteinases prevents formation of vasoactive or toxic peptides, such as kinins or anaphylatoxins, as much as stimulation by proteolytic products of cellular systems, such as thrombin-triggered platelet aggregation or anaphylatoxin-induced chemotaxis of granulocytes.

In essence, plasma proteinase inhibitors form equimolar complexes with their targets by which catalytic activity is irreversibly blocked. α_2M is an exception to this rule, as it can bind two enzyme molecules, and the resulting complex can still cleave polypeptides below about 10,000 daltons (14,15). However, the enzyme-inhibitor complexes formed are rapidly cleared by the reticulo-endothelial system (16).

Consumption of proteinase inhibitors by lysosomal factors. Reduction of the proteinase inhibiting potential by unspecific proteolysis is a striking pathological effect of lysosomal proteinases. Antithrombin III (4), for example, is rapidly inactivated by catalytic amounts of neutrophil elastase. The same is true for α_2-plasmin inhibitor and C1-inactivator (11), and similarly, α_1-proteinase inhibitor is proteolytically inactivated by the lysosomal thiol proteinase cathepsin B, a metallo enzyme from macrophages, and by a bacterial elastase (13,17). Moreover, oxidation of the methionine residue in the enzyme-reactive site of α_1PI greatly reduces the affinity to

neutrophil elastase (18). Oxidizing agents as superoxide, hydroxyl radicals and hydrogen peroxide, are produced in large amounts in the phagolysosomes to facilitate intracellular protein breakdown, and these substances may be released along with lysosomal enzymes in response to the same stimuli (3).

Thus, injury or infection can induce consumption of proteinase inhibitor proteins in three ways: (a) complex formation with proteinases, (b) inactivation by proteolytic degradation, and (c) inactivation by oxidative denaturation. The last mechanism deserves special interest in connection with oxidative effects on α_1PI on neutrophil elastase binding (Figure 5).

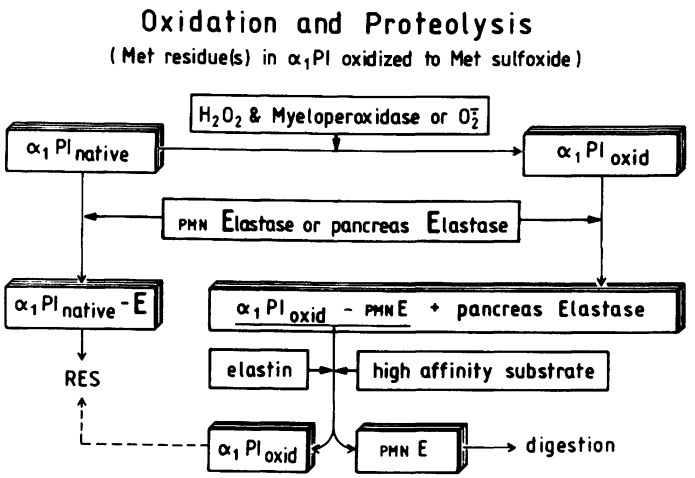

Figure 5. Affinity of native and oxidized α_1-proteinase inhibitor (α_1PI) to polymorphonuclear (PMN) elastase (E) and pancreatic elastase (E), respectively. The oxidized α_1PI reacts much more slowly (approximately 2000 times) with PMN elastase than native $_1$PI. In addition, the elastase complex with oxidized α_1PI is dissociated by substrates with high affinity to PMN elastase, thus again liberating the active enzyme. Oxidized α_1PI does not react with pancreatic elastase.

Clinical studies

Assay of liberated neutrophil elastase. Liberated neutrophil elastase is found in blood primarily in the form of the elastase-α_1-proteinase inhibitor (E-α_1PI) complex. A small amount of neutrophil elastase may be bound to α_2-macroglobulin, but the E-α_2M complex is much more rapidly eliminated from the circulation than the E-α_1PI complex ($t_{\frac{1}{2}}$ ~10' vs. 1 h). Consequently, assay of E-α_2M complex in plasma requires extreme analytical sensitivity (16), but this requirement may not apply in other body fluids, such as synovial fluid, where E-α_2M complexes may clear slowly (20). We assay E-α_1PI in clinical studies by an enzyme-linked immunoassay (Figure 6).

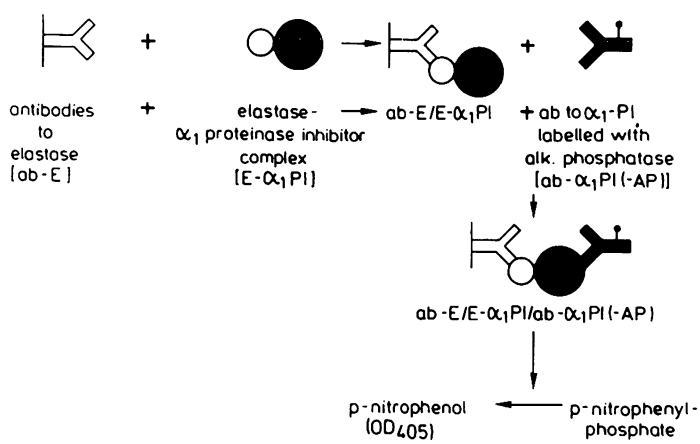

Figure 6. Reaction scheme of the solid phase enzyme-linked immunoassay used for detecting the complex of polymorphonuclear (PMN) elastase (E) with α_1-proteinase inhibitor (α_1PI) in biological samples. AP = alkaline phosphatase.

The assay is performed by (a) incubating standards (*i.e.*, the complex produced *in vitro*) or unknowns for 1 h in polystyrene

tubes coated with sheep antibodies to elastase, (b) washing, (c) incubating the tubes containing the fixed complex of elastase with α_1-proteinase inhibitor for 1 h with alkaline phosphatase labeled rabbit-antibodies to α_1-proteinase inhibitor, (d) washing, and (e) determination of solid phase fixed alkaline phosphatase activity with p-nitrophenylphosphate as the substrate. The absorbance at 405 nm is measured and found to be linearily correlated with the concentration of the elastase-α_1-proteinase inhibitor complex in the sample.

The detection limit of the assay was 0.25 ng of complexed elastase in the test specimen. Normal plasma reacted linearly in this assay with sample volumes from 1 to 80 µl. Recovery of the complex produced *in vitro* and added to different plasma specimens was excellent. Within-run variation (CV) with pooled plasma was 4 to 8%, between-run variation (CV) ranged from 3 to 8%. The normal range in citrated plasma was 98 $\pm$ 26 µg/l (mean $\pm$ s.d., n = 43).

Elastase release induced by major surgery and septicemia. In a prospective study, plasma E-α_1PI complexes were estimated repetitively in over 120 cases of major abdominal surgery followed by either uncomplicated recovery or by septicemia (21). Among the latter, 30 cases met accepted criteria of sepsis: unequivocal site of infection with positive culture; body temperature > 38.5° C; leukocytes > 15,000 or < 5,000/mm^3; platelets < 100,000/mm^3 or platelet drops > 30% below the preoperative value; positive blood culture. Of these patients, fourteen survived (group B) while sixteen succumbed to septicemia (group C); eleven controls (group A) recovered without complications.

Plasma levels of E-α_1PI are below 100 ng/ml in healthy individuals or preoperative patients. The operation causes up to a 3-fold increase (Figure 7). Retrospectively, we noted that the preoperative mean value of group C was already elevated,

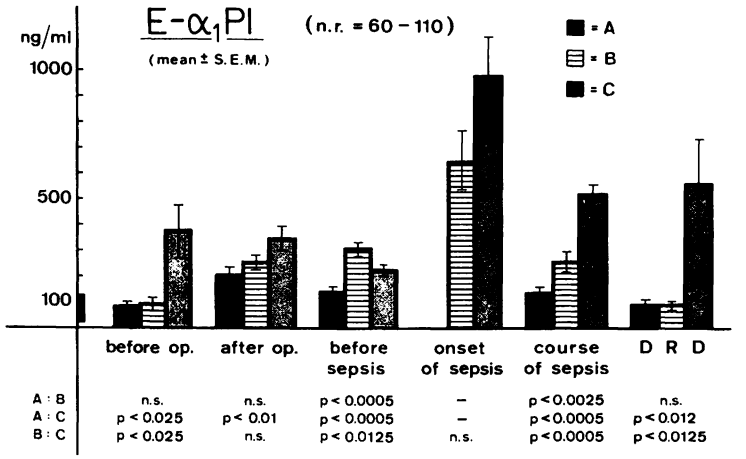

Figure 7. Mean plasma levels of elastase-α_1-proteinase inhibitor complex (E-α_1PI) in groups of patients subjected to major abdominal surgery: Group A patients (n = 11) being without postoperative infection; Group B patients (n = 14) surviving postoperative septicemia; Group C patients (n = 16) dying due to septicemia. The E-α_1PI levels are given as mean values (± SEM) for the day before operation, the day after operation, as well as for the postoperative phase before sepsis, at onset of sepsis, and during septicemia. Last determinations were done on day of discharge (D) for Group A, on day of recovery (R) for Group B, and before death (D) for Group C. nr = normal range; ns = not significant; p = significance.

possibly due to increased concentrations in 6 patients infected before operation. Surgical removal of the infection focuses may have caused the slight mean decrease following operation. Contrasting with group A, groups B and C maintained moderately elevated E-α_1PI levels for several days after operation. When septicemia was diagnosed clinically, E-α_1PI levels were increased manyfold in both groups B and C, with individual peaks as high as 2,500 ng/ml. With persisting septicemia, E-α_1PI remained high until death (group C), while recovery was accompanied by a decrease of E-α_1PI to the normal range.

Elastase release and plasma factor consumption in septicemia. Other indicators of inflammation were assayed in parallel with E-α_1PI. Concentrations of antithrombin III, the most important inhibitor of the clotting cascade, were inversely related to E-α_1PI (Figure 8). Particularly at onset and during the course of septicemia, antithrombin III reached clini-

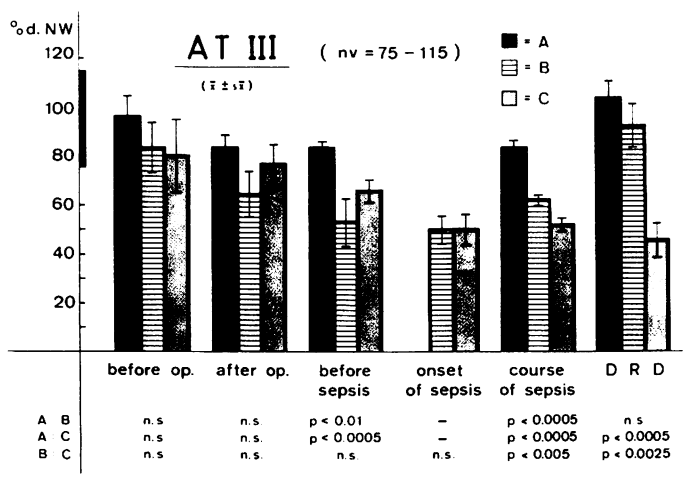

Figure 8. Mean plasma levels of the inhibitory activity of antithrombin III (AT III) in groups of patients subjected to major abdominal surgery. For details, see legend to Figure 7. nv = normal value; % d. NW = percent of normal value (reference plasma = 100%).

cally critical concentrations posing the risk of hypercoagulopathy or disseminated intravascular coagulation. Similarly lowered concentrations were observed for α_2-macroglobulin and for coagulation enzyme factor XIII (Figure 9).

Severe consumption of α_2-macroglobulin along with the carrier subunit of factor XIII (which is easily susceptible to degra-

dation by lysosomal elastase) and the enzymatically active subunit (which is primarily consumed during clotting) evidences the imbalance among various blood enzyme cascades during septicemia. The concomitant elevation of E-α_1PI levels suggest the causal role of lysosomal proteinases released into circulation and there consuming proteinase inhibitor proteins.

Plasma levels, elevated (↑) or decreased (↓):
(↑↑) highly signif. (↑) signif. (n) normal

Parameter		Sepsis	Prefinal	Survival
E-α_1 PI complex	c	↑↑	↑↑	n
Antithrombin III	a	↓↓	↓↓	n
Factor XIII	a	↓↓	↓↓	n
α_2-Macroglobulin	a	↓↓	↓↓	n
	c	↓↓	↓↓	n-↓
C-reactive protein	c	↑↑	↑↑	n
α_1-Proteinase inhibitor	a	n-↓	n-↓	n-↓
α_2-Plasmin inhibitor	a	n	n	n
α_1-Antichymotrypsin	c	↑	n	n
C1 Inactivator	a	n-↓	n	n-↓
	c	n	n	n

[a] activity assay [c] concentration assay

Figure 9. Correlations between mean plasma levels of elastase-α_1-proteinase inhibitor complex (E-α_1PI) and other plasma factors in patients suffering from septicemia after major abdominal surgery. Significantly elevated plasma levels of E-α_1PI and the unspecific acute phase reactant C-reactive protein are inversely correlated to a high statistically significant consumption of antithrombin III, factor XIII and α_2-macroglobulin during sepsis and prefinal; all protein levels normalized in patients who survived the infection. The other given proteinase inhibitors, which are also acute phase reactants, showed normal or only slightly increased plasma levels.

Remarkably, α_1-proteinase inhibitor, α_2-plasmin inhibitor, α_1-antichymotrypsin, and C1-inactivator were not measurably changed at the onset or during the course of septicemia (Figure 9). In contrast to antithrombin III and α_2-macroglobulin, these inhibitors are known to be acute phase reactants, and their synthesis can be expected to be considerably increased during postoperative response or in infection. Thus, it can be hypothesized that the organism compensates for the consumption of the inhibitory proteins by complex formation with target enzymes, or by other inactivation. Under the given conditions, the operative trauma presented the first inflammatory stimulus in non-infected patients, allowing enough time for a full acute phase response of plasma proteinase inhibitors to occur. C-reactive protein (CRP), an acute phase reactant without specific inhibitor activity, showed highest plasma concentrations up to three days after operation, and no further increase during septicemia. Thus, CRP did not allow discrimination between group A and B or C patients at onset of the infection and between group B and C patients in the course of septicema (Figure 10). We believe that E-α_1PI more specifically reflects both onset and severity of the postoperative infection than CRP.

Assay methods for proteinase inhibitors deserve special comment. Jointly using a functional inhibition assay, and an immunological concentration assay (assaying active inhibitor, inactive inhibitor and the enzyme-inhibitor complex together) allows calculation of the actual consumption of inhibitors (Figure 11). Unless this information is desired, a functional inhibition assay is the method of choice. Only for α_2-macroglobulin did both assay principles produce similar results (Figure 9), probably because α_2-M-proteinase complexes are rapidly eliminated from the circulation.

Pancreatogenic shock, and acute inflammation in general. In a second trial, nine cases of acute pancreatitis were studied.

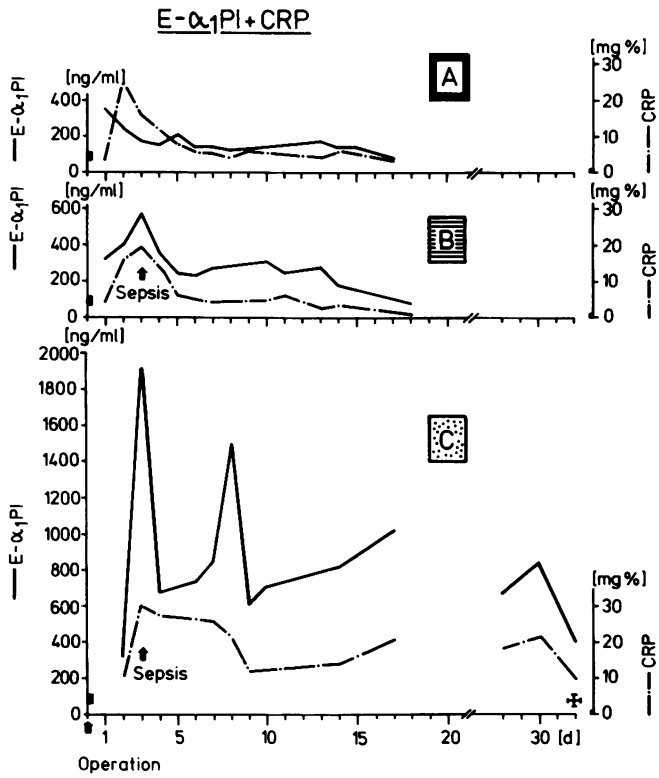

Figure 10. Comparison of the plasma levels of acute phase reactant C-reactive protein (CRP) and elastase-α_1-proteinase inhibitor complex (E-α_1PI) of three patients subjected to major abdominal surgery: Patient A being without postoperative infection; Patient B surviving postoperative septicemia; Patient C dying due to septicemia. Last determination was done on day of discharge for Patient A, on day of recovery for Patient B and before death for Patient C.

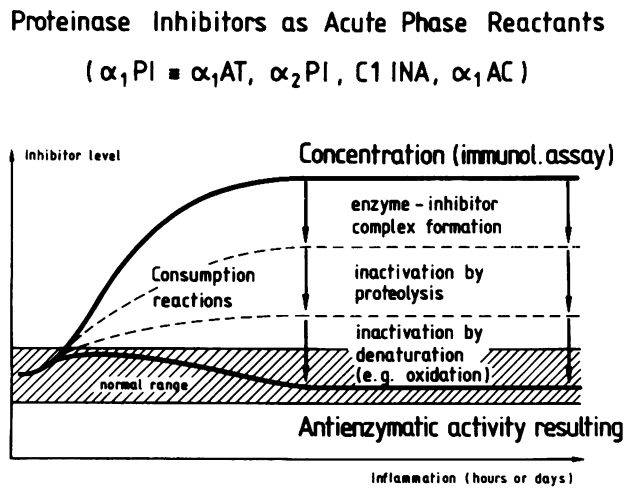

Figure 11. Reasons for the discrepancy between measureable protein concentration and anti-enzymatic activity of plasma proteinase inhibitors responding as acute phase reactants during inflammation. Consumption by complex formation and other inactivation processes may be compensated by increased production, resulting in an inhibitory activity close to the normal range. See legend of Figure 4 for abbreviations.

In these patients, plasma E-α_1PI was highly elevated, particularly in the shock phase (Figure 12). As in septicemia, plasma E-α_1PI was inversely correlated with α_2-macroglobulin and antithrombin III, *i.e.*, the release of neutrophil elastase was again accompanied by concomitant consumption of proteinase inhibitors and effects on the blood enzyme cascades.

Our biochemical and clinical data accord with the following hypothesis: In acute inflammation, such as septicemia and pancreatogenic shock, the liberated neutrophil elastase corresponds to the severity of the inflammatory response as well as the clinical condition (for clinical data, see H. Duswald (1982) and H. Kortmann (1984), Habilitation theses, Medical Faculty of the University of Munich; they will be published in detail elsewhere). Imbalance of the physiological equili-

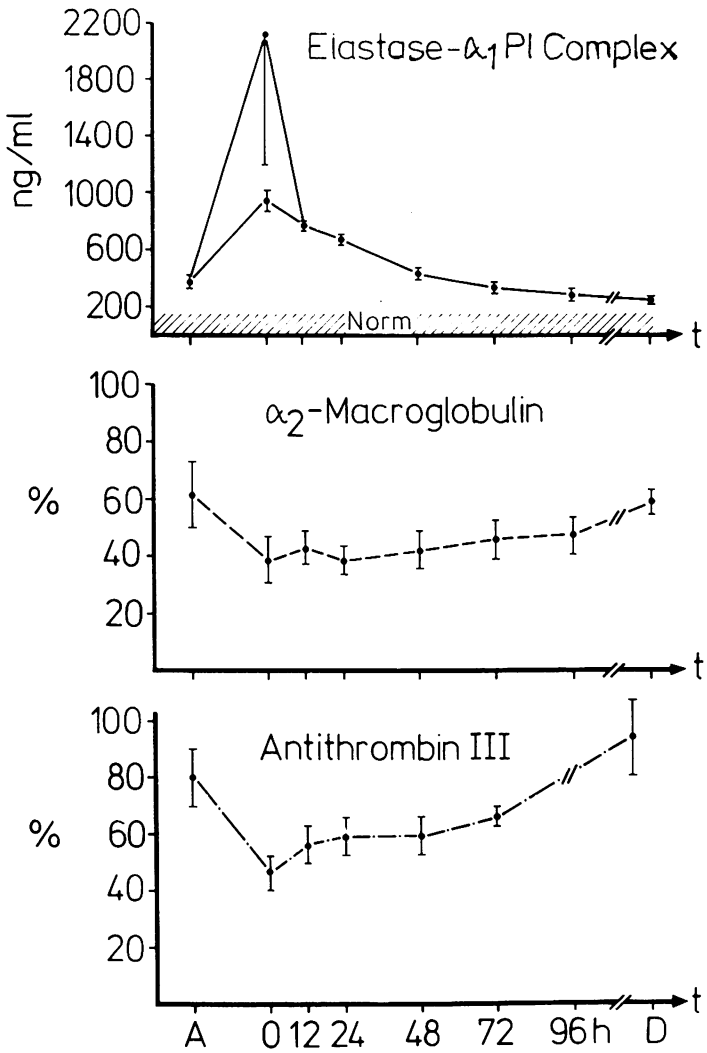

Figure 12. Mean plasma levels of the E-α_1PI complex as well as of AT III and α_2M in 9 patients suffering from pancreatogenic shock. In the mean value of the lower E-α_1PI curve, a patient is excluded which showed 60-fold increase in E-α_1PI complex in the shock phase. A = diagnosis of acute pancreatitis; O = pancreatogenic shock phase followed by recovery (abscissa in hours); D = discharge. The levels of α_2M and AT III are related to pool plasma as standard (100%, *cf.* left ordinates). E-α_1PI levels are given in ng/ml.

brium between endogenous proteinases and their inhibitors may be a major reason for the underlying pathological mechanisms. Our view is supported by animal studies (22), in which early application of potent exogenous proteinase inhibitors diminished the consumption of various plasma factors including antithrombin III and factor XIII induced by endotoxemia.

Multiple trauma and blood transfusions. An interesting pattern of plasma E-α_1PI was observed in patients with multiple injuries. To quantify tissue destruction and blood loss in accidents, an injury scale was developed, and as shown in Figure 13, plasma E-α_1PI correlated with the degree of injury so expressed. From 8 to 14 hours after accident, moderately injured patients had mean E-α_1PI levels 5-fold normal. Patients with more severe injuries showed 10-fold mean increases while the most heavily injured patients showed peak E-α_1PI levels 20-fold above the norm. In all three groups, a slow but continuous decrease of the E-α_1PI levels towards normal values occurred during recovery. On the other hand, we could not demonstrate a correlation between liberated lysosomal elastase and the consumption of prothrombin, plasminogen, α_2-plasmin inhibitor, α_2-macroglobulin and antithrombin III for multiple trauma patients. Extensive substitution of blood in these cases may explain this inconsistency with our other findings, as the plasma factors in question are quite stable during blood preservation. On the other hand, a continuous release of elastase from neutrophils was observed with storage (Figure 14). Thus, although appreciable E-α_1PI is administered by transfusion, detailed investigation showed that exogenous E-α_1PI did not significantly alter the endogenous level (results not shown), due to the faster clearance of E-α_1PI from circulation ($t_{1/2}$ about 1 h) than that of transfused plasma factors ($t_{1/2}$ of several days normally). Further investigations have to show whether a relationship between neutrophil elastase release and consumption of blood

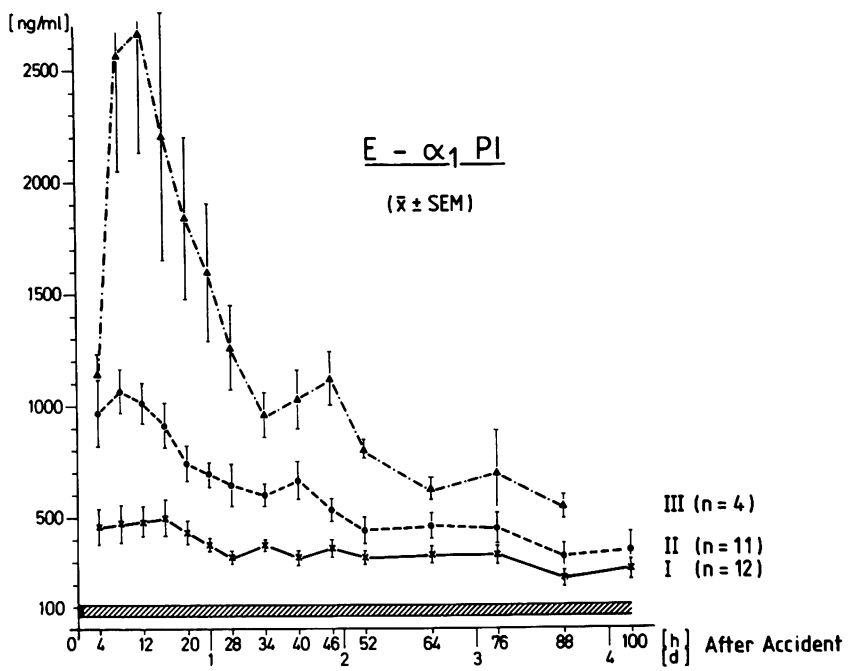

Figure 13. Mean plasma levels of the elastase-α_1-proteinase inhibitor complex (E-α_1PI) in 27 patients after multiple traumas. On the basis of a hospital internal scale (HIS), reflecting the severity of injury, patients were allied to 3 groups: I (n = 12), moderately wounded (HIS: 6.3 $\pm$ 0.6); II (n = 11), severely injured (HIS: 10.0 $\pm$ 1.0); III (n = 4), most heavily injured (HIS: 15.3 $\pm$ 1.0). The normal range of E-α_1PI (60 - 110 ng/ml) is also indicated.

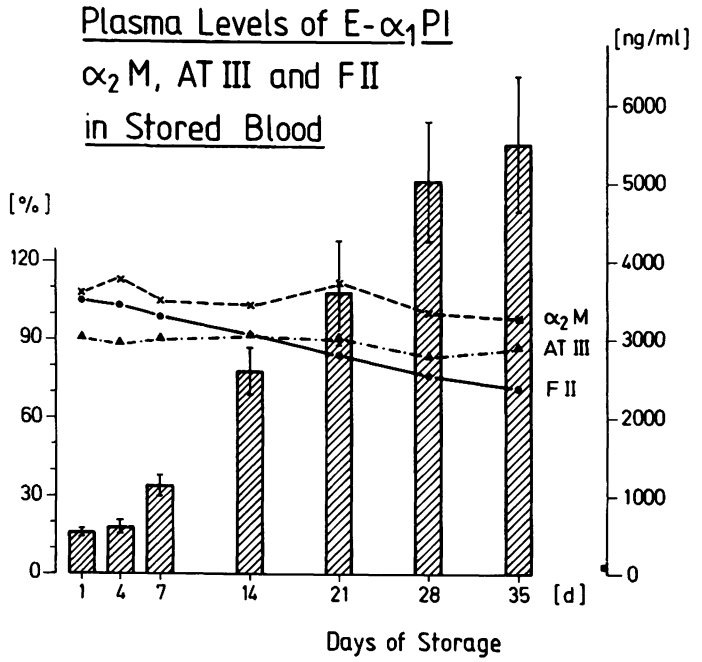

Figure 14. Mean plasma levels of elastase-α_1-proteinase inhibitor complex (E-α_1PI), α_2-macroglobulin (α_2M), antithrombin III (AT III) and prothrombin (F II) in stored blood samples (n = 11) as a function of storage time (in days). The levels of α_2M, AT III and F II are related to pool plasma as standard (100%, *cf.* left ordinate). E-α_1PI levels are given in ng/ml (*cf.* right ordinate).

components is measureable only in effluents and lavage fluids as well as lymph and venous blood samples from the wounded or inflamed area in patients receiving large blood transfusions. Nevertheless, the high E-α_1PI levels in severely injured patients indicate the strong inflammatory response of the organism.

Other clinical studies. The possible diagnostic significance of plasma neutrophil elastase was studied further in gestosis (23) and in rheumatoid arthritis (20,23) (Figure 15). Combined with clinical information, plasma E-α_1PI seems to allow

PMN Elastase as a Marker of the Inflammatory Response

Range of application	Meaning of elevated E -α_1 PI levels
Postoperative infections	Diagnosis, course, prognosis (sepsis)
Multiple trauma	Severity, course, complications (sepsis)
Pregnancy: gestosis	Early diagnosis of ARDS
Rheumatoid arthritis	Differentiation: inflammatory / non-inflammatory
Pleural effusion	Differentiation: inflammatory / malignant
Hemodialysis	Suitability / effect of dialyzer membranes
Myelocytic leukemia	Refined classification: normal / defective enzyme equipment of leukemic cells

Figure 15. Polymorphonuclear (PMN) elastase as a marker of the inflammatory response under various pathological conditions. See text for literature references.

early diagnosis of adult respiratory distress syndrome (ARDS) and differentiation of an inflammatory from a non-inflammatory state. The latter seems to be true also for pathological events leading to pleural effusions (23). Further, E-α_1PI is a sensitive marker for the stimulatory effect of foreign surfaces on neutrophils during hemodialysis (23,24).

Finally, investigations on the lysosomal enzyme make-up of leukemic cells and the release of elastase in myelocytic leukemia patients under certain clinical and therapeutic conditions represents yet another application not directly related to inflammation (21,25).

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