

Elastase and its Inhibitors in Intensive Care Medicine

Of the numerous diseases afflicting mankind, those rendering their victims suddenly intensive care patients have been, besides cancer, perhaps one of the greatest challenges for biomedical research in the last two decades. Examples of such diseases are the acute respiratory distress syndrome (ARDS) and/or septicemia due to extensive selective operations or accidents. Recent developments have considerably increased our understanding of the etiology of such inflammatory processes and, consequently, enabled the development of chemotherapeutic agents for these and other related ailments¹.

Aspects of Inflammation

The inflammatory response of the organism to various insults (mechanical trauma, operation, infection) is characterized by the activation as well as complex interactions of humoral and cellular systems^{1, 2, 3} (Fig. 1). This includes the turnover of proenzymes of the clotting, fibrinolytic, complement and kallikrein/kinin cascades to active proteinases (thrombin, plasmin, complement esterases, plasma kallikrein etc.) which in turn are able to produce further potent inflammatory mediators like, for example, permeability increasing fibrin monomers and fibrin split products, anaphylactic complement-derived factors C3a, C4a and C5a, or vasoactive kinins, prostaglandins and leukotrienes. Most of these compounds have strong stimulatory effects on the primary inflammatory defense cells, the polymorphonuclear (PMN) granulocytes and monocytes/macrophages. After having been attracted to the inflam-

matory focus by the chemotactic mediators or toxins from bacteria as well as leukocytic products themselves (tumor necrosis factor, interleukins), the main task of the phagocytes is the uptake and inactivation of invasive microorganisms and metabolic or degraded products via the process of phagocytosis (Fig. 2).

During incorporation of such particles into the cell membrane-derived phagosome, this digestion vacuole fuses with lysosomes, i.e. membrane-coated organelles containing various destructive proteinases (elastase, cathepsin G, cathepsin B etc.) and hydrolases. In addition to these digestive enzymes, the phagocytotic process is amplified by reactive oxygen species produced by the respiratory burst and the catalytic action of the lysosomal proteins myeloperoxidase and lactoferrin.

Although these substances are indispensable for an effective intracellular digestion, they bear a tremendous pathogenic potential if released extracellularly

M. Jochum, H. Fritz
Dept. for Clinical Chemistry
and Biochemistry
Surgical Clinics
University Munich/FRG

Despite the already high standard in intensive-care-treatment it is obvious that severe acute inflammatory processes like acute respiratory distress syndrome and septicemia still deserve a further improvement in therapeutic regimens. The well-timed application of potent elastase inhibitors seems to be a promising tool in preventing or at least ameliorating sequelae of massive inflammatory organ failure. Production of such therapeutic drugs by recombinant DNA-techniques may be the method of choice for the near future.

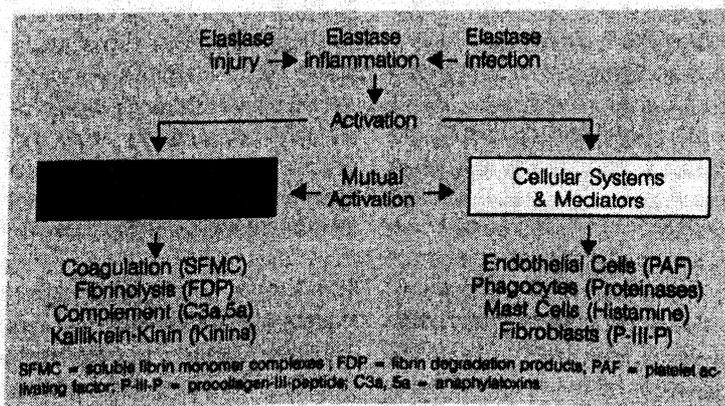
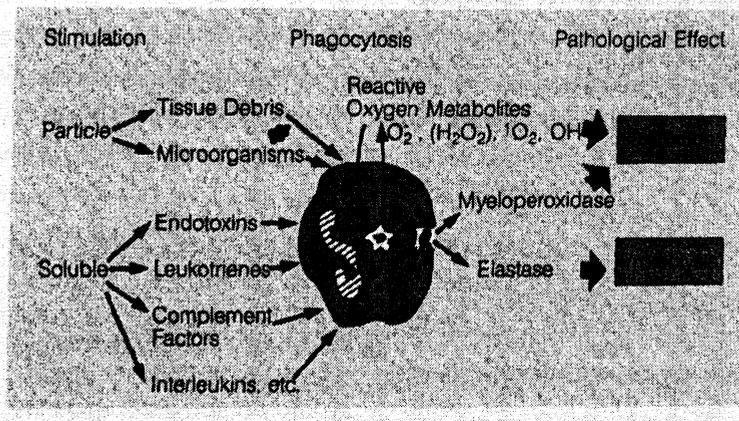


Fig. 1: Schematic depiction of the mutual activation of humoral and cellular systems and the release of potent mediators during acute inflammation

Fig. 2:
Diagram of the events
associated with the
phagocytosis of PMN cells.



in higher amounts before the phagolysomes have been closed. By this means, vital structural elements (basal membranes, cell receptors, fibronectin, elastin, collagen, 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 oxidative phagocyte-derived products are inactivated by physiological regulatory proteinase inhibitors.

Functional aspects

Of the lysosomal proteinases studied so far, the neutral serine proteinase elastase from PMN granulocytes has attracted most interest because of its occurrence in high quantities (3–5 $\mu\text{g}/10^6$ PMN cells) within the granules and its low substrate specificity². The latter enables elastase to degrade intracellularly nearly all species of phagocytized foreign proteins, but also to initiate the breakdown of the body's own vital tissues if released into the extracellular milieu. In this respect

the so-called shock organs are especially endangered, because PMN granulocytes are primarily sequestered and activated in the capillaries of those tissues during severe inflammatory processes. Consequently, we have been able to show a clear correlation between the amount of elastase released extracellularly and the severity of organ failure or septicemia in several clinical studies over the last few years^{4, 5, 6}.

In addition, liberated elastase seems to play also a fatal role with systemic consequences for hemostasis by proteolytic inactivation of the plasma proteinase inhibitors antithrombin III, α_2 -plasmin inhibitor, and C1-inactivator, the principal antagonists of active proteinases of the blood cascade systems^{7, 8, 9}. As shown recently, this proteolytic degradation may be enhanced by oxidative denaturation in the surroundings of phagocytizing cells¹⁰.

Contrasting these pathological effects of high amounts of elastase released extracellularly during severe inflammation, small concentrations of this en-

zyme either membrane-associated or in close vicinity to the phagocyte may also be of physiologic relevance. Secretion of PMN elastase upon chemotactic activation could be a prerequisite for migration of granulocytes through connective tissues¹¹ towards the inflammatory focus, where the cells have to eliminate the deteriorating stimuli. To balance the positive and negative effects of elastase, a delicate control of the enzyme by convenient proteinase inhibitors is obviously necessary.

Elastase inhibitors

Up to date, four different proteinase inhibitors of human origin have been described, which may be involved in the in vivo regulation of PMN elastase in plasma and other body fluids and tissues (Table 1)^{11, 12}. α_1 -Proteinase inhibitor (α_1 PI) plays the major role in this process, not only because of its highest molar concentration in plasma (and presumably also in the extravascular environments), but also due to the rapid inactivation ($t_{1/2} = 0.6$ msec) of its major target enzyme, the PMN elastase.

In contrast, α_2 -macroglobulin (α_2 M) – a potent inhibitor of all classes of proteinases (serine, cysteine, metallo, and carboxyl proteinases) – is a slow-reacting elastase inhibitor with a much lower molar concentration in plasma compared to α_1 PI. Because of its high M_r (725,000 D) α_2 M is normally restricted to the circulation. It is found in other body fluids (e.g. bronchial and peritoneal secretions) in relevant amounts only when the permeability barrier is

already disturbed by the action of inflammatory mediators^{13, 14}. Moreover, α_2 M-elastase complexes retain proteolytic activity (at least against small substrates) and are eliminated slowly from local body fluids. Therefore, they may still contribute to proteolytic degradation in such inflammatory environments. Taking into account all these facts, α_2 M is not usually considered to be an efficient PMN elastase inhibitor in vivo.

Inter- α -trypsin inhibitor (ITI), a complex of three different protein species¹⁵, shares physiological properties with α_2 M in so far as the low molecular mass inhibitory portion of the complex shows a small molar concentration in plasma and a slow elastase inactivating capability¹⁶. Unlike α_2 M, however, this low molecular mass inhibitory part (M, 30,000) increases significantly in the acute phase state and may reach local inflammatory sites in amounts sufficient to act as a relevant elastase inactivator.

Whereas the three elastase antagonists are primarily produced and secreted into the circulation by the liver, the mucus proteinase inhibitor (MPI) is a main inhibitory component of local mucous secretions from various tissues¹⁷. MPI causes rapid inhibition of the neutral PMN proteinases, elastase and cathepsin G, if released from activated cells in the nose, upper airways, cervix, and seminal plasma. It represents together with α_1 PI the main antiprotease shield of the upper airways (MPI > α_1 PI) and the lung (α_1 PI > MPI).

Although α_1 PI and MPI differ significantly concerning their molecular mass they have

	α_1 -PI	α_2 M	ITI	MPI
M, (D)	52,000	725,000	30,000 ¹	11,700
Plasma-Concentration (g/l)	1.35	2.60	0.01	0.05 ²
μ mol/l	26	3.6	0.3	4
Localisation	systemic local	systemic	systemic local	local
Inactivation of Elastase: $t_{1/2}$	0.6 msec	7.5 msec	?	8 sec
Primary Enzyme Specificity	PMN- Elastase	non-specific	(PMN- Elastase)	PMN-Cathepsin G- Elastase

¹inhibitory active component

²in mucous secretions

Table 1:
Elastase Inhibitors of
Human Origin

several interesting properties in common (Table 1). As potent elastase inhibitors their P₁-P₁'-reactive site contains methionine (Met-Ser in α_1 PI and Leu-Met in MPI), an amino acid residue, which can easily be oxidized by phagocyte oxygen species. Such an oxidation slows down dramatically the inhibition rate of elastase as well as the stability of the elastase/inhibitor complexes^{17, 18}. Hence, replacement of the oxidized inhibitor may occur by a substrate with higher affinity to elastase, for example lung elastin, which can thus be degraded by the enzyme without restraint. Slow inhibition by the oxidized inhibitor enables the destruction of numerous proteins until the elastase activity is eventually abolished.

In addition, both elastase antagonists can be inactivated by cysteine and metallo proteinases escaping from activated macrophages as well as by metallo enzymes from different bacteria¹¹. Thus, during severe infectious diseases α_1 PI and MPI may rapidly lose their inhibitory function at least in local inflamed areas. On the one

hand, this would enable an uncontrolled destructive elastase action leading to the development of multiple organ failure. On the other hand, granulocyte migration through elastin matrices might be favoured by inactivation of the elastase inhibitors in close vicinity to the stimulated cells¹¹.

Therefore, the following points should be considered in view of the development of therapeutically useful elastase inhibitors as potential antiinflammatory drugs in acute diseases like ARDS or septicemia¹¹:

- The inhibitor should have a medium molecular mass (10,000–55,000 D) to ensure easy diffusion into the tissues but also a half-life time which is long enough to establish a sufficiently high inhibitory potential within the vascular system and locally, as well.
- The inhibitor should be negatively charged to minimize its concentration around the outer neutrophil cell membrane. Thereby its uptake by neutrophils and its interference with the intracellular protein break-

Neutrophil Elastase: Further Clinical Aspects

We only can characterize septicemia by its clinical appearance, without finding a satisfying pathogenetic definition of the process. It is widely accepted that sepsis is associated with dysfunction or failure of the immune system and with microcirculatory disturbances, and that sepsis is a mediator disease. But our knowledge about the exact trigger events and interactions, responsible for the breakdown of regulating and compensating mechanisms ending up in the disastrous multi organ failure is still incomplete. We still lack the all-important parameter for the clinical assessment of septic patients. However, the determination of elastase can provide some useful clinical information (Table 1).

The elastase level can help to differentiate between shock due to a cardiac cause or pulmonary embolism and a septic complication. This differential diagnosis is often difficult particularly in older-age intensive care patients, and has substantial impact on the therapeutic consequences. Moreover, the elastase level has been shown to be of value in the estimation of the prognosis of septic patients in internal medicine, abdominal surgery and traumatology. Since in the single patient not so much a high initial elastase value, but a delayed and incomplete decrease is a sign of bad prognosis, serial determinations during the course are advisable.

For improving therapy in septic shock a major consideration is the use of inhibitors, and at the present stage, the best choice are the physiologic inhibitors. An important inhibitor in the physiologic control of coagulation is antithrombin III. The

substitution of antithrombin has been shown to be effective in the treatment of disseminated intravascular coagulation in controlled studies. Since in septic shock a disturbance of several other mediator systems and an enhanced proteolysis due to enzymes such as elastase is present, it should be considered to combine the substitution of antithrombin and fresh frozen plasma. In an uncontrolled study on the treatment of coagulation disturbances in septic shock we found in those patients receiving antithrombin III and fresh frozen plasma not only a normalization of hemostasis and a decrease of thrombin-antithrombin-complex (TAT), but also a decrease of elastase levels and a better survival rate than in septic shock patients without coagulation disturbance who did not receive substitution*.

Thus the assessment of elastase levels appears to be not only helpful in the clinical treatment of patients at risk for septic complications, but also for monitoring studies aimed at the improvement of the still frustrating therapy of septic shock. R.S.

Table 1:
Plasma levels (mean values \pm SEM) of neutrophil elastase and thrombin-antithrombin III (TAT) complexes in various diseases

	Elastase ($\mu\text{g/l}$)	TAT ($\mu\text{g/l}$)
normal persons	123 (± 37) (n = 15)	2.4 (± 0.1) (n = 44)
pulmonary embolism (n = 24)	188 (± 13)	9.3 (± 2.6)
acute myocardial infarction (AMI) (n = 33)	118 (± 13)	11.6 (± 2.7)
AMI with shock (n = 15)	401 (± 93)	18.2 (± 4.0)
myelogenous leukemia (n = 13)	407 (± 74)*	22.7 (± 5.7)
septic shock	1892 (± 236)	16.2 (± 3.9)

Elastase levels were determined by PNM-Elastase ELISA; *the standardization has been changed in the meantime in a way that currently about one third of the former given values is measured with the test. - TAT levels were determined by Enzygnost® TAT-ELISA
Seitz, R., R. Egbring, Dept. of Hematology and Oncology, Internal Medical Center, University Marburg

* Seitz, R. et al., Eur. J. Haematol. 43: 22 (1989).

down might be diminished. Susceptibility to inactivation by oxidants and/or proteolysis by lysosomal cysteine proteases could additionally prevent such undesired effects on the intracellular metabolism. Outside the cell, this feature might also assure the granulocyte movement towards the infectious focus, especially during chronic inflammation.

● The inhibitor should be highly specific for neutrophil elastase allowing an effective competition with natural protein substrates and minimizing unspecific toxicity. Concerning the latter, the immunotolerance to the host should be also as high as possible.

Of the currently discussed and also experimentally studied elastase inhibitors just a few seem to fulfil such requirements – at least in part.

Obviously, an adequate systemic or local supplementation of the body's exhausted inhibitory potential with α_1 PI and MPI of human origin offers the best choice. At present, unfortunately, only α_1 PI can be obtained by plasma fractionation, albeit in amounts, which are even not high enough for treatment of emphysema patients with inborn α_1 PI-deficiency. For short-term application in acute deficiency states like ARDS or septicemia sufficient production of α_1 PI and MPI by genetic engineering^{17, 19} is, therefore, of urgent priority. In this respect, it also has to be considered whether the replacement of methionine at the reactive site P₁-P_{1'}-position²⁰ by non-oxidizable amino acids (e.g. valine or leucine) is at least worthwhile for short-term therapy to save

material compared to the use of the original variant, which can be consumed also by oxidation.

Concerning the variety of non-human origin elastase inhibitors (eglin, variants of aprotinin, etc.) being currently created by recombinant DNA technology¹¹, it is obvious that these products may primarily bear problems of potential antigenicity at least in long-term therapy. Moreover, due to their relatively small molecular size (M_r between 6,000–8,000 D) they are rapidly eliminated from the circulation. To establish an adequate inhibitor potential, a continuous i.v.-infusion seems to be unavoidable. Notwithstanding these drawbacks, recombinant eglin has already turned out to be an efficient therapeutic drug in ameliorating several signs of *E. coli*-induced septicemia in pigs²¹. No side-effects could be detected in these short-term experiments.

Last not least, many synthetic inhibitors of granulocyte elastase (including peptidyl chloromethyl ketones, azapeptides, amino acid-derived latent isocyanates, and aryl-sulfonyl fluorides) have been developed and studied in experimental animal models^{22, 23}. Although they have shown a more or less effective protection against elastase-induced tissue destruction, severe problems of toxicity due to low target enzyme specificity and interference with intracellular protein catabolism, short bioavailability because of rapid elimination of such small molecules (M_r below 500 D), antigenicity, etc. have to be overcome before these compounds can be accepted for clinical therapeutic use.

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Authors' address:

M. Jochum, M. D., H. Fritz, M. D.,
Abt. Klin. Chemie und Klin. Biochemie
Chirurgische Universitäts-Klinik
Innenstadt, Nußbaumstr. 20,
D-8000 München 2, FRG