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Emergency Surgery

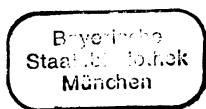
Trends, Techniques, Results

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Pathomechanisms in Septic Shock

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Introduction

Applications of new biochemical techniques in clinical research undoubtedly demonstrate that a variety of pathobiochemical mechanisms is involved in the development of multiple organ failure in septic shock. Elucidation of these pathomechanisms may provide more successful prophylaxis and therapy of septic shock in future.

This report deals with the release of lysosomal proteins connected with substantial imbalance of proteinases and proteinase inhibitors during severe inflammation.

Lysosomal Proteins

Lysosomal factors are liberated from various body cells under pathological conditions. In this respect, especially polymorphonuclear (PMN) granulocytes, which are attracted during severe inflammatory processes in high amounts to the primary shock organs (lungs, liver, and kidneys) are of great interest. These cells are equipped with a powerful proteolytic, hydrolytic and oxidizing potential in their lysosomes enabling the intracellular protein catabolism as well as the degradation of phagocytized extracellular material in the phagolysosomes (1). Of the lysosomal proteinases known so far, elastase, the neutral proteinase from PMN granulocytes, deserves special interest because of its very low cleavage specificity (2).

Myeloperoxidase, which is also localized in the azurophilic granules, catalyzes the reaction of hydrogen peroxide (H_2O_2) with chloride ions (Cl^-) in the phagolysosomes, thus forming various oxidants which are strongly bactericidal (3).

The antibacterial effect of lactoferrin, which is primarily present in the specific granules of granulocytes but also in body cells such as glandular epithelial cells, is well established. Patients with recurrent infections as a consequence of lack of specific granules and lactoferrin have been described only recently. Moreover, various promoting and inhibiting effects of lactoferrin have been proposed. However, most of the studies could not be confirmed by others and even contradictory results have been reported. Hence, at present, the functional role of lactoferrin in the molecular aspects of inflammation is still an open question (4).

Activation and Consumption of Blood Proteins

If the aforementioned proteins – especially the proteinases – are released extracellularly, the inflammatory response may be enhanced via two major routes (5):

Selective proteolysis leads to proenzyme and/or cofactor activation of the blood systems (clotting, fibrinolysis, complement, kallikrein/kinin) and to the formation of biologically highly potent peptides such as kinins,

anaphylatoxins and fibrin/ogen degradation products. Unspecific proteolysis especially due to the action of elastase and cathepsin G destroys not only blood system factors, immunoglobulins and other proteins, but also proteinase inhibitors simply by proteolytic digestion.

Normally, selective activation of blood systems and unspecific proteolysis are kept under control by potent and specific plasma proteinase inhibitors which will not be mentioned in detail here (6). Remarkably, the proteinase inhibitors represent approximately 60% of the residual plasma proteins after removal of albumin and the immunoglobulins. This is an indirect indication upon the significance of proteinase inhibitors as regulatory proteins of the organism.

During severe inflammatory processes, however, the consumption of proteinase inhibitors due to complexation with their target enzymes and/or proteolytic degradation may be overwhelming thus leading to a fatal imbalance of proteinases and their inhibitors (5). For example, α_1 -proteinase inhibitor, the major antagonist of neutrophil elastase, is proteolytically inactivated by a lysosomal metallo-enzyme from macrophages, by the lysosomal thiol proteinase cathepsin B, and by a bacterial elastase as well (6).

Moreover, oxidation of the methionine residue in the enzyme-reactive site of α_1 -proteinase inhibitor (α_1 PI) leads to a significant reduction of the affinity of this inhibitor to neutrophil elastase (7). Such oxidizing agents, for example superoxide anion, hydroxyl radicals, and hydrogen peroxide, are produced in high amounts in the phagolysosomes to facilitate together with myeloperoxidase intracellular protein breakdown. If they are released simultaneously with the lysosomal enzymes under pathological conditions, they may impair locally the inhibition of extracellularly liberated elastase, because the complex formed with oxidized α_1 PI is readily dissociated by substrates exhibiting high affinity for elastase (e.g. elastin). This might lead to

tissue injury following rapid accumulation of polymorphonuclear granulocytes in the lungs during the inflammatory response.

Clinical Studies

a) Major surgery and septicemia

In a first approach we used the PMN granulocytic elastase (E) as a marker of such pathological release reactions.

The liberated enzyme is present in the circulation primarily in form of the elastase- α_1 -proteinase inhibitor complex (E- α_1 PI). A small amount of neutrophil elastase may be bound also to α_2 -macroglobulin (α_2 M), however, compared to the E- α_1 PI complex, the E- α_2 M complex is much more rapidly eliminated from the circulation.

Evaluation of the amount of complexed elastase in the clinical studies performed so far was done with an enzyme-linked immunoassay (8), now commercially available.

In our first prospective clinical study, plasma levels of the E- α_1 PI complex were measured at suitable intervals in patients subjected to major abdominal surgery, followed either by uncomplicated recovery (group A) or by septicemia (groups B and C). Patients of group B survived the infection, whereas patients of group C died due to severe septicemia or septic shock (9). One characteristic curve of each group is presented in figure 1.

The patient without postoperative infection showed only a moderate (up to threefold) increase of the preoperative E- α_1 PI value following surgery, whereas the septic patients exhibited multiple elevated levels in the septic phase. Therefore, blood specimens should be taken at least every 6 to 12 hours during the acute phase of an inflammatory process.

At the time of diagnosis of septicemia highly significantly elevated E- α_1 PI levels were measured corresponding to an up to sixfold or

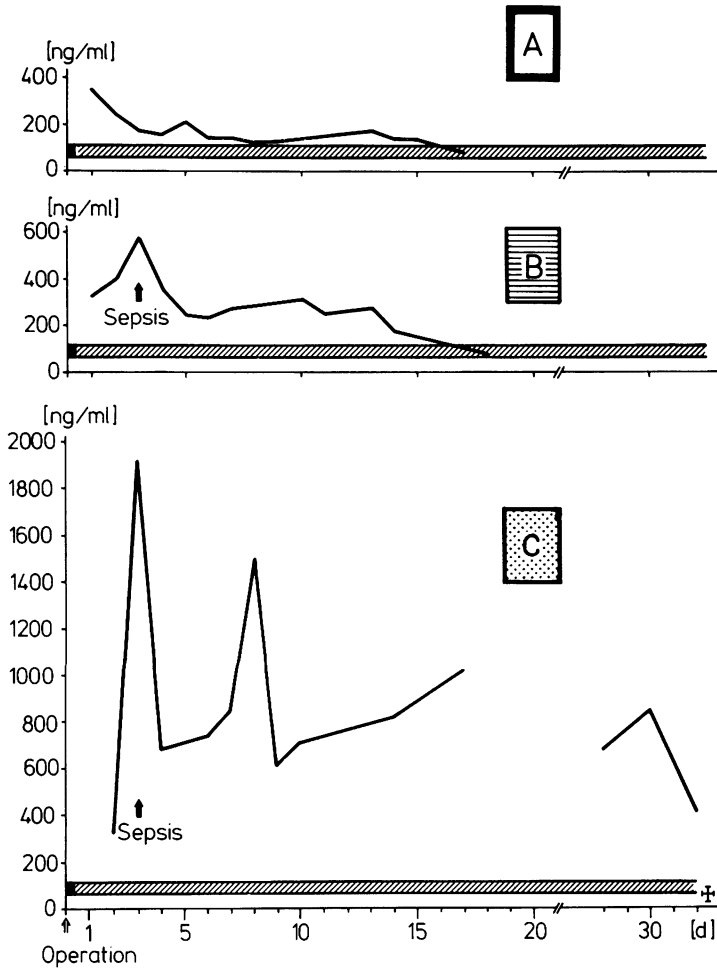


Figure 1. Plasma levels of elastase in complex with α_1 -proteinase inhibitor (E- α_1 PI) in patients subjected to major surgery. A: patient without postoperative infection, B: patient surviving postoperative septicemia, C: patient dying due to septicemia. (hatched area = normal range). Only the amount of elastase (ng/ml) is indicated.

even tenfold mean increase in groups B and C. Individual peak levels were found to be as high as 2500 ng/ml in both groups.

In patients with persisting septicemia the E- α_1 PI levels remained high until lethal outcome (group C), whereas recovery from septicemia was reflected by a concomitant decrease of the E- α_1 PI levels to the normal range (group B).

A clear relationship could be demonstrated between the amount of elastase liberated into the circulation and the decrease of activity of antithrombin III, factor XIIIa, and α_2 -macro-

globulin. Interestingly, these proteins are known to be very sensitive substrates of elastase in vitro (10). All factors normalized in patients who overcame the infection but remained pathologically low in those who died.

b) Multiple trauma

Data obtained very recently (figure 2), show a concomitant release of elastase, myeloperoxidase, and lactoferrin in a patient suffering from severe multiple trauma, which itself is

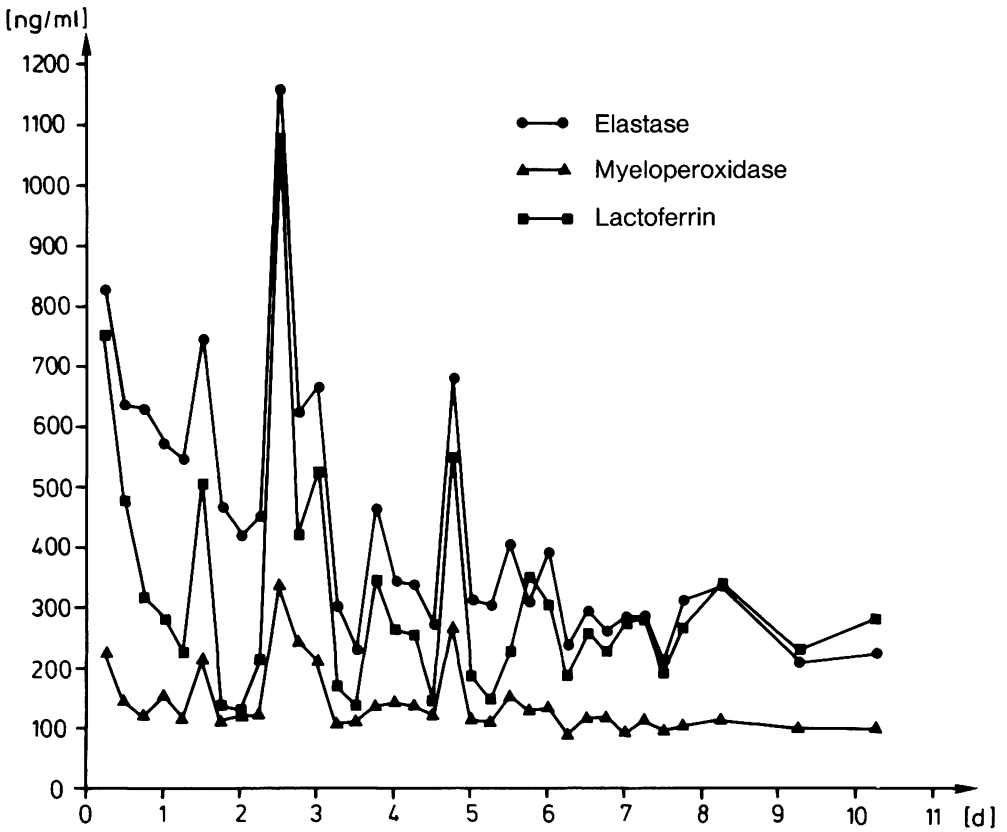


Figure 2. Plasma levels of complexed elastase (with α_1 PI), myeloperoxidase and lactoferrin in a patient suffering from severe multiple trauma. (Normal range: complexed elastase = 60–120 ng/ml, myeloperoxidase = 20–60 ng/ml, lactoferrin = 100–300 ng/ml).

already indicated by the highly elevated levels of all three granulocytic proteins at the time of the first measurement (6 hours posttrauma). The varying amounts of these proteins reflected quite perfectly the severity as well as the amelioration of the posttraumatic course. Much to our surprise, a good correlation existed in the patient's plasma not only between the azurophilic elastase and myeloperoxidase levels but also between these enzymes and lactoferrin from the specific granules. This suggests that both types of granules have been equally involved in phagocytosis and

extracellular liberation of the lysosomal proteins in the underlying kind of inflammation. Whether the same holds true for other inflammatory processes is currently under evaluation.

Conclusion

In general, elevated plasma levels of granulocytic lysosomal proteins indicate participation of polymorphonuclear granulocytes in an inflammatory event taking place elsewhere in

the organism. The amount of the E- α_1 PI complex as well as of myeloperoxidase and lactoferrin seems to reflect the intensity of both the inflammatory stimulus and the response of the neutrophils.

In our opinion, lysosomal and other cell-derived proteinases are preferential candidates to offer means for differential diagnosis but also for therapeutic approaches with suitable exogenous proteinase inhibitors as it is presented by *Welter et al.* (Pathobiochemical mechanism in experimental sepsis: Influence of the cloned elastase inhibitor eglin) and *Siebeck et al.* (The role of Cl-esterase inhibitor during early septicemia) in this issue.

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