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Immune Consequences of Trauma, Shock, and Sepsis

Mechanisms and Therapeutic Approaches

With 185 Figures and 92 Tables

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Pathobiochemical Mechanisms in Inflammation

M. Jochum and H. Fritz

Introduction

The primary response of the organism to an inflammatory stimulus (tissue destruction after multiple trauma, invasive microbae, endotoxins of exogenous and endogenous origin, immune complexes etc.) is directed physiologically towards inactivating and eliminating the agent and to initiating the process of healing and repair. The activation of the complex interacting humoral and cellular defence mechanisms necessary for this purpose carries with it, however, the risk of damaging healthy tissue and thus perpetuating the inflammatory process. The relation of stimuli, mediators, effectors, and inhibitors to each other finally determines the effectiveness or failure of the inflammatory response [1].

Out of the multitude of factors investigated hitherto, proteolytic enzymes, both of the plasma cascade systems (clotting, fibrinolysis, complement, kallikrein/kinin system) and also of lysosomal origin [from polymorphonuclear (PMN) granulocytes, monocytes, macrophages, mast cells], have proved to be potent mediators of inflammation [2].

Mediators of the Humoral and Cellular Systems

The contact between proenzymes of the cascade systems and damaged vascular endothelium together with the activators liberated from those cells results in the formation of system-specific proteases, whose activity is largely responsible for hemostasis and closure of the wound. Some of these proteolytic enzymes (plasma kallikrein, thrombin, plasmin, complement esterases), however, also produce additional mediators of inflammation such as the vasoactive kinins, the hemostatic and edema-forming fibrin monomers and fibrin peptides, or the anaphylactic complement factors (C3a, C4a and C5a) (Fig. 1). System-specific proteases themselves, together with a series of polypeptides formed as a result of their proteolytic activity, act as potent chemotaxins and bring about the sequestration and activation of inflammatory cells (particularly PMN granulocytes) in the area of the wound [2, 3].

The primary task of these cells is to remove the inflammatory stimulus by phagocytosis and thus to limit the inflammatory process to a significant extent [2–4]. Even during the binding and ingestion of "foreign" material in the infective focus, however, the phagocytic cells also liberate numerous aggressive substances (oxygen radicals, hydrolytic and proteolytic enzymes, etc.) into the surrounding

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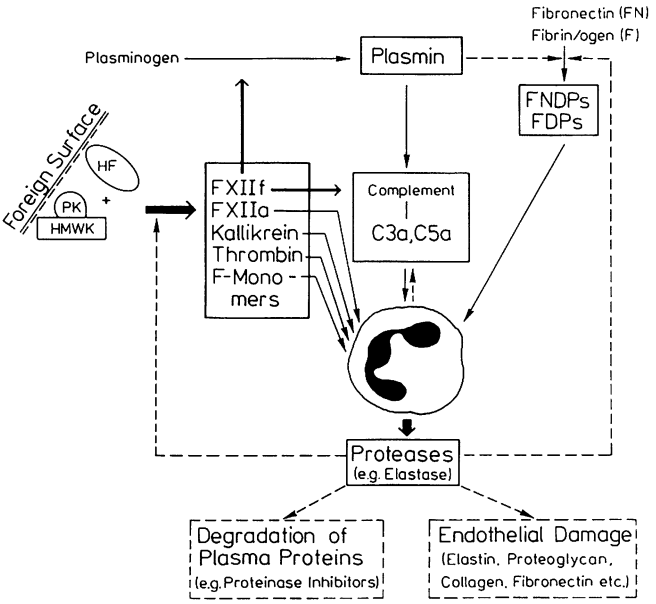

Fig. 1. The interaction between humoral system (clotting, fibrinolysis, complement, kallikrein/kinin system) and the polymorphonuclear (PMN) granulocytes; FNDP = Fibronectin degradation product; FDP = Fibrin/ogen degradation product; FXIIf = Factor XIIf; FXIIa = Factor XIIa; HF = Hageman factor; HMWK = high molecular weight kininogen; PK = Prokallikrein

environment. Once there, these substances are able to cause lasting damage to structural elements (basement membranes, elastin, collagen, fibronectin etc.) and to humoral factors (particularly proteins of the cascade systems). In this way they augment the inflammatory process to a significant degree [5, 6].

When there is restricted activity of an inflammatory stimulus, activation of the proteolytic mediators of inflammation remains confined mainly to the local event by potent protease inhibitors [7]. If the primary defense mechanisms of the organism are not, however, in a position to counteract a massive stimulus (e.g., invasive micro-organisms in severe infection) in time, the increased formation of system-specific proteases and the liberation of proteolytic enzymes and oxidizing substances from inflammatory cells eventually leads to the exhaustion of the functional capacity of the inhibitory regulators. As a consequence, the destructive processes caused by proteases and the systemic manifestations of a local inflammatory process can no longer be prevented adequately or at all [5].

With regard to the pathological mechanisms involved in severe inflammation, the neutral proteases, elastase and cathepsin G, from the azurophilic granules of the PMN granulocytes, stand out as being significant among the lysosomal enzymes currently recognized. They are not only numerically predominant, but also have practically no substrate specificity [8]. After extracellular liberation, these proteases inactivate a series of plasma proteins, such as the protease inhibitors antithrombin III, α_2-plasmin inhibitor, and C1 inactivator [9, 10] only by a very few proteolytic cleavages if they are not inhibited immediately by their natural antagonists, α_1-protease inhibitor, α_1-antichymotrypsin and α_2-macroglobulin,

Myeloperoxidase, another enzyme localized in the azurophilic granules, catalyzes the reaction of hydrogen peroxide (H_2O_2) with chloride ions (Cl^-) in the phagolysosomes. Thus it produces highly bactericidal oxidizing substances which also have extracellular activity and destroy humoral and structurally-bound proteins [11].

The antibacterial activity of lactoferrin, a protein formed mainly in the specific granules of granulocytes, but which is also produced by cells such as those of the glandular epithelium, is also well documented. It depends, in part, upon the catalytic function of the protein in the formation of highly reactive hydroxyl radicals [12]. Patients with lactoferrin deficiency suffer recurrent infections. Lactoferrin has been described as having a large number of other activities in addition, but many of these effects have not been confirmed by other investigators. The functional role of lactoferrin in inflammation can, therefore, be regarded as still being largely unclarified [12].

Studies in Patients and Experimental Animals

Methodology

Fundamental information relating to the extent to which proteases participate in an inflammatory process is obtained from the following studies:

1. Quantitative demonstration of the liberation of lysosomal proteins and activation of system-specific proteolytic enzymes.
2. Simultaneous demonstration of the consumption of protease inhibitors and plasma factors susceptible to proteolytic degradation.
3. Use of specific exogenous protease inhibitors and antioxidants in therapeutic studies in experimental animals.

Measurement of the lysosomal PMN granulocyte proteins elastase [13], myeloperoxidase [14], and lactoferrin [15] was carried out by means of highly specific sandwich enzyme immuno assays (ELISA). The normal concentrations of these proteins in the cells and plasma are shown in Fig. 2. With regard to elastase, it should be noted that the protease released extracellularly into the circulation can no longer be demonstrated as the active enzyme, but only as an inactive complex with α_1-protease inhibitor (α_1 PI). The levels mentioned in the following discussion relate, however, only to the elastase moiety and not to the whole complex.

Protease inhibitors and plasma factors susceptible to proteolytic degradation were quantified in accordance with the methods outlined in [5].

The methods used in the therapeutic studies are extensively described in [16] and [17].

Clinical Studies

Abdominal Surgery and Sepsis

In order to measure exactly the rapid liberation and elimination of elastase during the acute stages of inflammation, blood samples were taken at frequent intervals (6–12 hourly) from patients undergoing abdominal surgery in a prospective clini-

	Elastase	Myeloperoxidase	Lactoferrin
Localisation	azurophilic	PMN Granula azurophilic	specific Body Secretions Glandular Epithelial Cells
M_r	30 000	145 000	70 000
Concentration			
$\mu g/10^6$ PMNs	4- 7	2- 3	4- 7
ng/ml Plasma	60-120	20-60	100-300
Method	Enzyme Immunoassay (ELISA)		
			
	E-α_1PI	MPO	LF

Fig. 2. Localization, method of determination, and normal concentrations of the lysosomal proteins elastase (complexed with α_1-protease inhibitor; *E-α_1 PI)*, myeloperoxidase (*MPO*), and lactoferrin (*LF*)

cal study (Director: Professor Duswald, City Surgical Clinic, University of Munich) and the amount of complexed elastase in the plasma was measured [18]. Patients with no postoperative infection (group A) had only a moderate increase (up to three times) after operation in comparison with the preoperative levels (60–120 ng/ml), while in patients with sepsis, markedly increased concentrations were repeatedly determined in the course of the sepsis. At the time of diagnosis of sepsis, concentrations of complexed elastase were measured and these showed a mean six fold increase in group B (postoperative sepsis which was eventually overcome) or tenfold increase in group C (postoperative sepsis with a fatal outcome). Individual peak levels up to 2500 ng/ml occurred in both groups. In patients with persisting sepsis (group C) the E-α_1 PI level remained significantly elevated, as a mean, until death, while the recovery phase in group B was accompanied by an obvious return to normal levels. At the same time as complexed elastase increased in the plasma, a significant reduction in the inhibitory activity of antithrombin III and α_2-macroglobulin together with the fibrin-stabilizing activity of factor XIII was detected in the patients with sepsis. The reduced activity of the factors named above at the onset of sepsis was restored to normal again in patients who overcame infection (group B), while a further decrease occured in those who perished (group C).

Multiple Trauma

In patients with multiple trauma, the concentration of the complexed elastase in plasma correlated with the degree of severity of the injury [19]. The group of patients under investigation (Director of the study: Dr. Dittmer, Surgical Clinic Munich-Großhadern, University of Munich) with widely differing injuries showed very high increases in the mean levels of elastase (up to ten times normal) up to about 12 h after the accident, followed by a well-defined return to normal. Independent of each other, the whole group could be divided into three individual identical groups on the basis of clinical criteria, aided by a scale of severity as well as in accordance with the peak amount of elastase liberated. With these patients,

it was not possible to establish a correlation between the concentration of the elastase and the consumption of circulating plasma factors because of the large volumes of blood transfused, particularly in the early days following multiple injury.

Sequential studies of the bronchoalveolar lavage fluid of eight patients with multiple trauma (the samples were collected for us by Dr. Joka, Surgical Clinic, University of Essen) revealed a possible pathogenetic relationship between a permeability injury in the lung and the release of lysosomal proteins together with the oxygen products formed during the "respiratory burst" [20]. Even before the increase in extracellular fluid in the lung (as a measure of pulmonary edema), extremely high concentrations of complexed elastase, together with myeloperoxidase and lactoferrin, could be measured in the lavage fluid in some patients. Since the latter two proteins mentioned are involved in secondary oxidative processes which, among other things, inactivate α_1-protease inhibitor [7], the demonstration of enzymatically active elastase, in addition to E-α_1 PI-complex, in a number of these samples is entirely comprehensible (Fig. 3).

General Surgical Patients and Bacterial Infections

In an extensive clinical study (Director of the study: Dr. Inthorn, Surgical Clinic Munich-Großhadern, University of Munich), the effect of operative trauma and bacterial infection of varying degrees of severity upon the release of granulocyte contents and upon humoral mediators of the inflammatory process was investigated [21]. Elastase and lactoferrin were measured in almost identical amounts in plasma in individual cases. The time course of the release of both proteins showed a pattern of behavior that was often to a large degree identical; myeloperoxidase was generally detected in substantially smaller amounts than the other two lysosomal factors. On general review, of the measurements, however, the amount of elastase showed a continual rise corresponding to the extent of the inflammation, while mild and moderate inflammation could not be differentiated on the basis of lactoferrin and myeloperoxidase; the extracellular levels of these two proteins only reflected the degree of severity of the infection when sepsis was present (Fig. 4). The activation of humoral cascade systems in accordance with the severity of the inflammation was clearly documented by the increases in the anaphylatoxin C3a in plasma and by the consumption of plasma prokallikrein, prothrombin, antithrombin III, and fibronectin.

Therapeutic Studies in Experimental Animals

The results shown in the clinical studies described above may be seen as a clear demonstration of the release of lysosomal enzymes from granulocytes and the ensuing extracellular proteolysis or oxidation of vital structural and humoral proteins in the course of a severe inflammatory process. As a means of avoiding a deleterious endogenous imbalance of proteases and protease inhibitors, it appeared to us that the early administration of effective exogenous inhibitors and antioxidants in animal experiments was promising.

In a preliminary study of sepsis in young pigs (Director of the study: Dr. Welter, City Surgical Clinic, University of Munich), we were able to show that

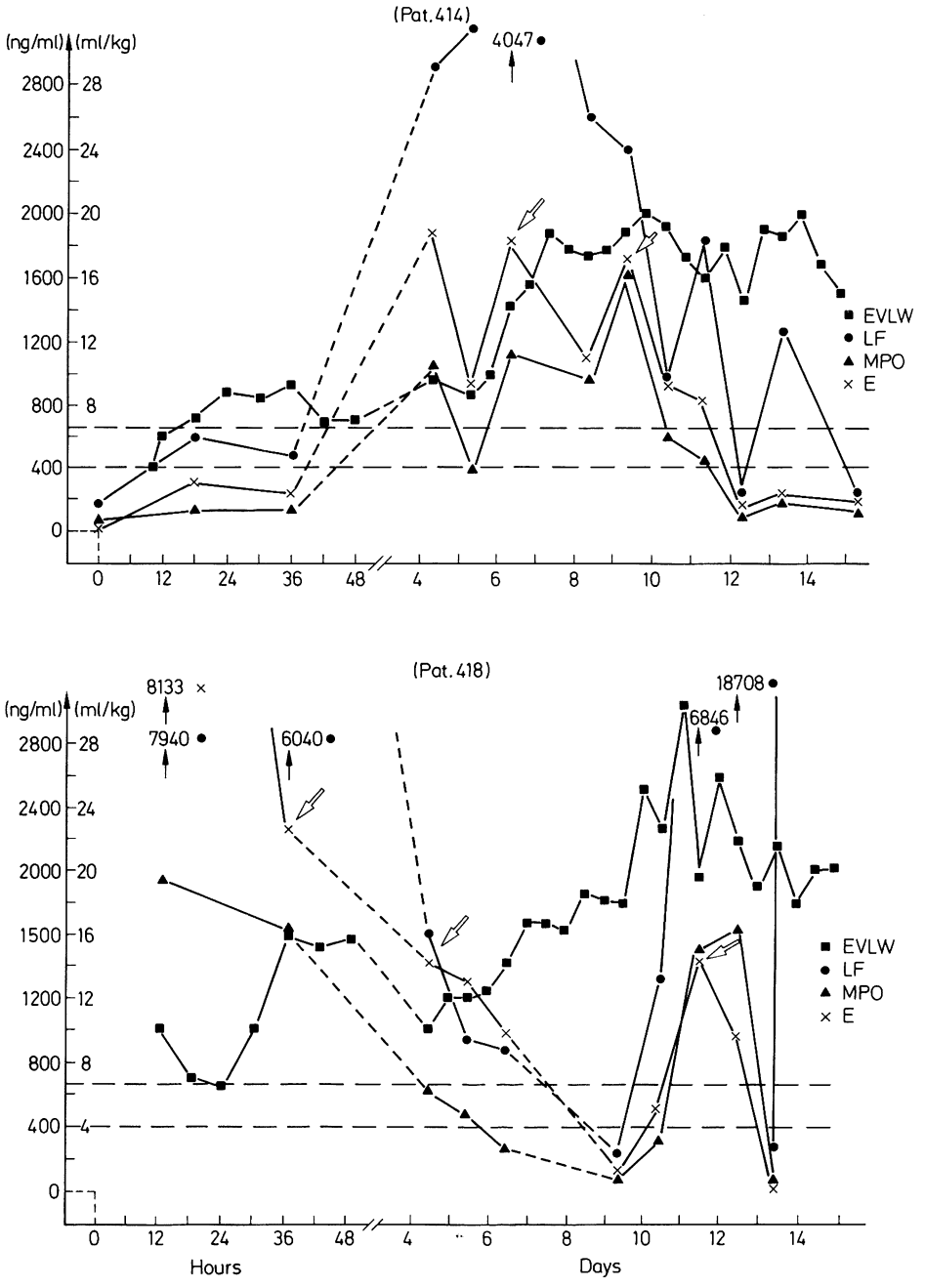

Fig. 3. Granulocyte elastase (*crosses*) in complex with α_1-protease inhibitor and in proteolytically active form (*arrows*) together with myeloperoxidase (*triangles*) and lactoferrin (*circles*) in sequential bronchoalveolar lavage samples from two patients with multiple trauma. The change in extravascular lung water is added (*squares*) as a measure of pulmonary edema

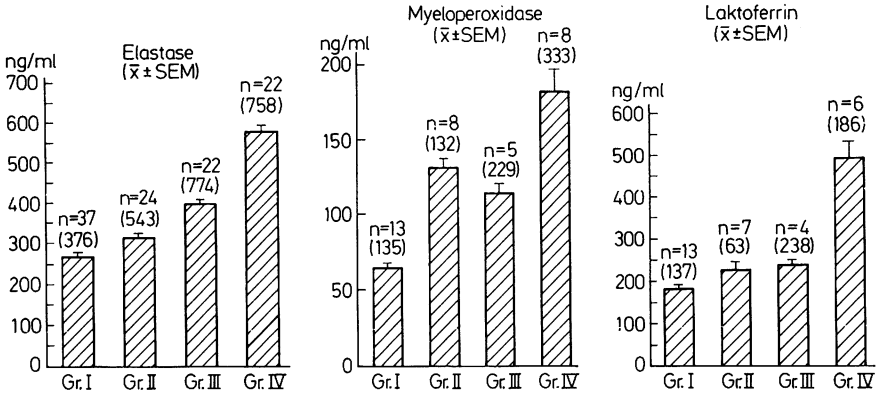

Fig. 4. Increasing concentrations of granulocytic proteins in the plasma of patients following operative trauma with varying degrees of severity of postoperative inflammation. The number of patients (*n*) and the actual number of samples (in brackets) tested in each severity group are shown. *Gr. I*, uncomplicated course; *Gr. II*, a wound infection, locally confined peritonitis, basal pneumonia; *Gr. III*, extensive soft tissue inflammation, peritonitis >1 quadrant, severe bronchial pneumonia; *Gr. IV*, clinically diagnosed sepsis

the relatively specific recombinant elastase cathepsin G inhibitor Eglin (isolated initially from the leech) significantly reduced consumption of protease inhibitors (antithrombin III, α_2-macroglobulin) and other plasma proteins (e.g., factor XIII) and also the formation of interstitial pulmonary edema [16]. Meanwhile, we found in a more sophisticated model of sepsis (Director of the study: Dr. Siebeck, City Surgical Clinic, University of Munich), that Eglin in suitable doses clearly prevented edema in general in the organism and thus is active in counteracting sepsis-related hypotension and multiple organ failure [22].

We achieved similar, though less obviously therapeutic effects by the administration of C1 inactivator, of an inhibitor of system-specific proteases (complement esterases, plasma kallikrein, plasmin), and of the antioxidant superoxide dismutase [17]. A further improvement in inflammatory symptoms may therefore be achievable in future by the combined administration of protease inhibitors and antioxidants.

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