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Host Defense Dysfunction in Trauma, Shock and Sepsis

Mechanisms and Therapeutic Approaches

With 416 Figures and 153 Tables

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The Role of Lysosomal Cysteine Proteinases as Markers of Macrophage Activation and as Nonspecific Mediators of Inflammation

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Introduction

A major portion of the lysosomal proteinases responsible for intracellular degradation of phagocytized proteins are cysteine proteinases, the "acid" cathepsins B, H, L, and S (see [1] for review). As we have shown previously [2, 3], increased cysteine proteinase activity is found in blood plasma of polytraumatized and septic patients as well as in local inflammatory secretions such as bronchoalveolar lavage fluid and peritonitis exudate. Most of this activity is due to cathepsin B, which is relatively stable at neutral pH (half-life about 30 min at pH 7.4) and is protected in the form of reversible complexes with its endogenenous protein inhibitors (stefins, cystatins, and kininogens). Here we report some evidence for the role of cathepsin B as a marker of macrophage activation and of lysosomal cysteine proteinases as potential nonspecific mediators of inflammation.

Materials and Methods

Cysteine proteinase activity and complexed polymorphonuclear (PMN) elastase were determined by fluorometric assay and enzyme-linked immunosorbent assay as described [2, 3]. The organization of clinical studies and sampling protocols were reported in detail elsewhere [4, 5]. Proteolysis of fluorescein isothiocyanate IgG (Sigma) by human cathepsin B (Medor, Herrsching, FRG) and peritonitis exudate was performed as described [6].

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Results and Discussion

Cathepsin B as Marker of Macrophage Activation. PMN granulocytes contain only minute amounts of cathepsin B (1.5 ng/10^6 cells), whereas high levels of cathepsin B, L, and H have been found in monocytes, macrophages, and other cells of the reticuloendothelial system [3, 7, 8]. Therefore cathepsin B may be considered as a marker for the activity of the monocyte/macrophage system. This hypothesis was supported by different patterns of release of cathepsin B and PMN elastase during inflammation in patients.

Cathepsin B of blood plasma after major trauma reaches its maximum at least 6 h earlier than complexed PMN elastase (Fig. 1). This early cathepsin B activity of blood plasma proved to be a sensitive and specific parameter for the prediction of subsequent multiple organ failure (Nast-Kolb et al., this volume). In all groups of patients, cathepsin B activity returned to only slightly elevated levels within 3 days after the trauma. Early cathepsin B activity does not discriminate between lethal and reversible organ failure. A nearly constant elastase/cathepsin B ratio around 300 was observed during the first 3 days in all patients, followed by a slow decrease in the groups with reversible organ failure and without organ complications. In patients developing lethal organ failure the elastase/cathepsin B ratio increased to 500–600 and remained high after the 3rd day (Fig. 2).

As determined with cell lysates, an elastase/cathepsin B ratio of 3000–6000 would be expected if both enzymes were released from PMN granulocytes [3].

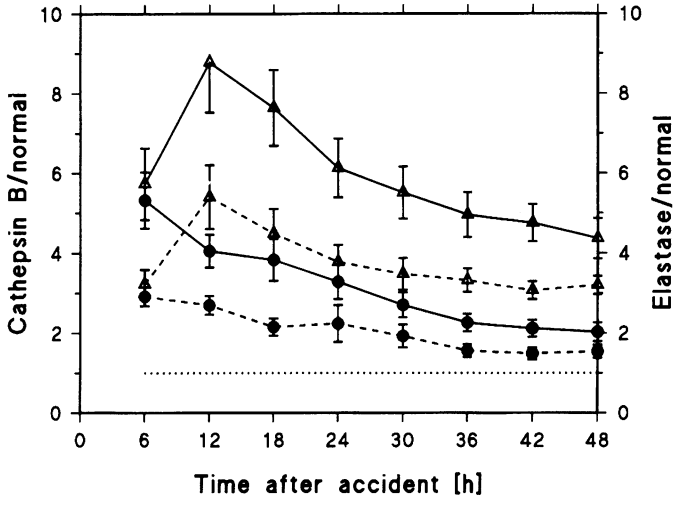

Fig. 1. Cathepsin B activity (●) and complexed PMN elastase (△) of blood plasma in the early phase after major trauma. Both enzymes were expressed as multiples of their normal values (50 mU/l for cathepsin B, 90 ng/ml for PMN elastase). Mean values ± SEM of 29 patients without organ complications (----) and 29 patients with reversible multiple organ failure (———)

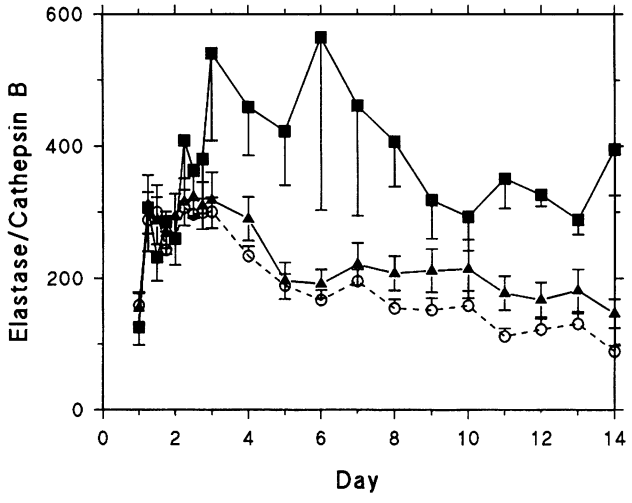

Fig. 2. Elastase/cathepsin B ratio in blood plasma of patients after severe multiple injury. Mean values ± SEM for 29 patients without organ complications (○), 29 patients with reversible multiple organ failure (▲), and 11 patients with lethal multiple organ failure (■)

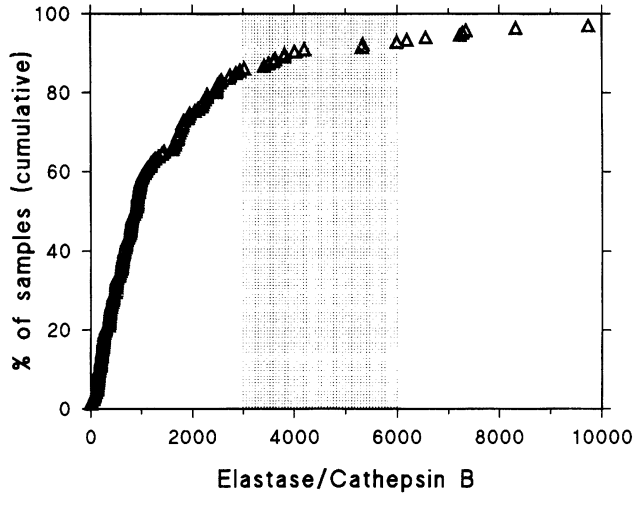

Fig. 3. Elastase/cathepsin B ratio in peritonitis exudates (n = 166). Cumulative percentage of samples as a function of increasing elastase/cathepsin B ratio. *Shaded area*, range of elastase/cathepsin B ratios found within lysates of PMN granulocytes from healthy persons

This ratio was indeed found in a few peritonitis exudates containing high numbers of neutrophils (Fig. 3). In the majority of samples, however, much lower elastase/cathepsin B ratios were observed, supporting the origin of cathepsin B from cells other than neutrophils. Elastase/cathepsin B ratios close

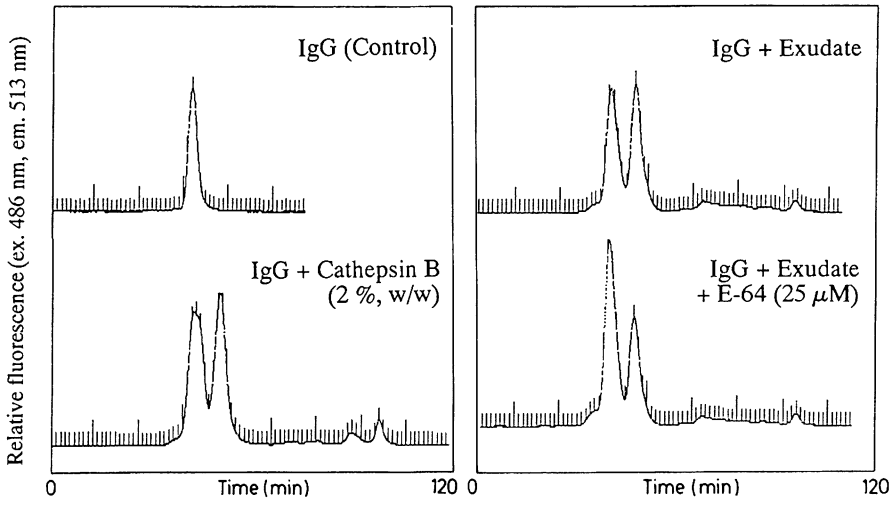

Fig. 4. Degradation of immunoglobulin G (IgG) by isolated human cathepsin B and by E-64 sensitive proteinase activity in peritonitis exudate. Fast gel chromatography of fluorescein isothiocyanate labeled IgG and its fragments on a Superose 12 column

to 1 were found in bronchoalveolar lavage fluid containing a large number of alveolar macrophages.

Cysteine Proteinases as Nonspecific Mediators of Inflammation. Direct evidence for a role of cysteine proteinases in proteolytic pathomechanisms of inflammation comes from studies with peritonitis exudates. Peritonitis exudates reveal strong proteolytic activity which is paralleled by deficient opsonic capacity and high levels of the lysosomal proteinases PMN elastase and cathepsin B [6]. Using fluorescence-labeled protein, degradation of IgG by cell-free supernatant of peritonitis exudate was demonstrated in vitro (Fig. 4). Part of this degradation can be prevented by addition of E-64, a specific inactivator of cysteine proteinases, indicating that lysosomal cysteine proteinases are involved. Catalytic amounts of isolated human cathepsin B (see Fig. 4) and L are able to proteolyse IgG in vitro effectively.

Conclusions

Cathepsin B seems to be a useful marker for the activity of phagocytes of the monocyte/macrophage system that can be followed easily in blood plasma and inflammatory secretions of patients. The observed very early release of cathepsin B after blunt major injury raises new questions about the role of macrophages in the reaction sequence of the inflammatory process.

Cysteine proteinases of monocytes/macrophages may play a role as non-specific mediators of inflammation comparable to that of the serine proteinases elastase and cathepsin G from PMN granulocytes.

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References

1. Barett AJ, Buttle DJ, Mason RW (1988) Lysosomal cysteine proteinases. In: ISI atlas of science: biochemistry. Institute for Scientific Information, Philadelphia, pp 256–260
2. Assfalg-Machleidt I, Jochum M, Klaubert W, Inthorn D, Machleidt W (1988) Enzymatically active cathepsin B dissociating from its inhibitor complexes is elevated in blood plasma of patients with septic shock and some malignant tumors. Biol Chem Hoppe Seyler 369 [Suppl]: 263–269
3. Assfalg-Machleidt I, Jochum M, Nast-Kolb D, Siebeck M, Billling A, Joka T. 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
4. Nast-Kolb D, Waydhas C, Jochum M, Spannagl M, Duswald KH, Schweiberer L (1990) Günstigster Operationszeitpunkt für die Versorgung von Femurschaftfrakturen bei Polytrauma. Chirurg 61:259–265
5. Billing A, Fröhlich D, Kortmann H, Jochum M (1989) Die Insuffizienz der intraabdominellen Infektabwehr bei eitrigen Peritonitis—Folge einer gestörten Fremdkörperopsonierung. Klin Wochenschr 67:349–356
6. Billing A, Fröhlich D, Assfalg-Machleidt I, Machleidt W, Jochum M (1991) Proteolysis of defensive proteins in peritonitis exudate: pathobiochemical aspects and therapeutical approach. Biomed Biochim Acta 50:399–402
7. Kominami E, Tsukahara T, Bando Y, Katunuma N (1985) Distribution of cathepsins B and H in rat tissues and peripheral blood cells. J Biochem 98:87–93
8. Howie AJ, Burnett D, Crocker J (1985) The distribution of cathepsin B in human tissues. J Pathol 145:307–314