Cathepsin D-Like Activity in Neutrophils and Monocytes

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Received 29 September 1988/Accepted 15 February 1989

Monocytes-macrophages and polymorphonuclear leukocytes contain an acid proteolytic enzyme that cleaves tritiated hemoglobin. The monocyte-macrophage-derived enzymatic activity was completely inhibited by pepstatin A, a property of cathepsin D. Monocyte-derived macrophages developed detectable cathepsin D-like activity after 5 days in culture, and this activity coincided with the appearance of other known indicators of macrophage maturation. The cathepsin D activity further increased significantly with time after day 5 of culture. The proteinase activity extracted from neutrophils was only partially inhibitable by pepstatin A, which indicates that this activity is contributed by more than one proteolytic enzyme, including cathepsin D. Cathepsin D activity demonstrated in neutrophils and macrophages may be an important marker of phagocyte function.

Cathepsin D is a ubiquitous enzyme which occurs in all vertebrate species and is the major lysosomal aspartic proteinase in most human cells (4). The enzyme has chondro-lytic (14) and collagenolytic (9) functions in tissue, while at the intracellular level it is known to degrade proteins within lysosomes (6). Cathepsin D plays a role in cytotoxic processes during myocardial ischemia (7), chronic liver diseases (13), and rheumatoid joint involvement (15). It is localized within lysosomes of polymorphonuclear leukocytes, monocytes, and macrophages (16).

The main substrates for laboratory studies of cathepsin D are hemoglobin (4) and serum albumin (5). The catalytic property of the enzyme is maximal in an acidic environment, which indicates that its physiologic site of action is more likely to be within lysosomes. By using tritiated hemoglobin, sensitive assays for cathepsin D activity have been developed and we have recently demonstrated that acid-denatured serum enhances this activity (11). To further determine the potential role of cathepsin D in inflammatory responses, we measured neutrophil, monocyte, and monocyte-derived macrophage cathepsin D activities.

Pepstatin A, formic acid (free acid and sodium salt), and Triton X-100 were obtained from Sigma Chemical Co., St. Louis, Mo. Certified reagent-grade ethanol, methanol, formaldehyde, trichloroacetic acid, and sodium acetate were obtained from Fisher Scientific Co., Pittsburgh, Pa. Pepstatin A and radiolabeled hemoglobin substrate were prepared as recently described (11).

Ten microliters of 1.0 M formate buffer (pH 3.5) or 10⁻⁵ mol of pepstatin A per liter in 1.0 M formate buffer (pH 3.5) was delivered to triplicate wells of a V-well microtiter plate. Samples (20 μl) containing cathepsin D (standard or cell lysate) or buffer (for buffer control) were added to wells containing formate buffer or pepstatin in formate. Twenty microliters of hemoglobin substrate at 2.5 to 5.0 mg/ml was added to all of the wells (the enzyme-substrate ratio was 1:200 to 1:400 [wt/wt]), and the plate was incubated at 37°C for the desired time (30 min of incubation was optimal under the conditions described). After incubation, 50 μl of ice-cold 10% trichloroacetic acid was added to each well to stop the reaction and precipitate residual proteins. The plates were centrifuged to pellet the precipitate, and 50 μl of the clear trichloroacetic acid-soluble digest was measured for evidence of proteolysis by detection of tritium-labeled peptide fragments by using a beta counter and liquid scintillation.

Peripheral blood neutrophils, monocytes, and monocyte-derived macrophages from healthy donors were isolated, and cell suspensions were prepared by using previously reported techniques from our laboratory (1, 2). Monocyte-derived macrophage cultures were maintained at 37°C in 5% CO₂ for 2 to 3 weeks. At various times, media were harvested and the cells were washed twice with phosphate-buffered saline and then lysed or the media were replaced. The lysing buffer was 0.1 M acetal buffer (pH 4.5)-0.1% Triton X-100 or 1.0 M formate buffer (pH 3.5)-0.1% Triton X-100.

Lysates of monocyte-macrophage cultures were sequentially tested for cathepsin D activity at days 2, 5, 8, and 12 of culture and showed significant specific proteolysis that started on day 5 of culture. The activity continued to increase during subsequent days in vitro (Fig. 1). By day 12, the activity had increased sevenfold compared with the activity at day 5.

While monocyte-derived acid proteolysis of hemoglobin was completely inhibited by pepstatin A, which is a known specific inhibitor of cathepsin D, proteolysis by neutrophil lysates was only partially inhibited. This partial inhibition was independent of pH or enzyme concentration (Fig. 2).

We have previously found that Triton X-100, as well as other hydrophobic agents, enhances cathepsin D activity in vitro (11). To rule out the possibility that Triton X-100 is the factor which enhanced the enzymatic activity of the lysates, we compared the enzymatic activity of cultured monocytes-macrophages that were lysed with Triton X-100 with that of those that were lysed with hypotonic shock or sonication. Figure 3 shows a slight increase in the proteolytic activity of lysates prepared with the detergent compared with the other methods. The detergent enhancement was significantly pH.

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FIG. 1. Human monocyte-derived macrophage cathepsin D activity during in vitro culture. Normal human monocytes (2 × 10⁶ cells plated per well) in Dulbecco modified Eagle medium–10% fetal bovine serum–10% horse serum were used. The culture medium was changed on the days when cathepsin D was determined (days 2, 5, and 8). Hb, Hemoglobin; mcg, micrograms.

FIG. 2. Comparison of cathepsin D (CD) activities at pHs 3.12, 3.66, and 4.14 with or without 5 μg of pepstatin A per ml A, 1.7 × 10⁶ neutrophils per ml; B, 3.4 × 10⁶ neutrophils per ml; M, 2.0 × 10⁶ monocyte-derived macrophages (day 7) per ml. PMN, Polymorphonuclear leukocytes; mcg, micrograms; Hb, hemoglobin.

FIG. 3. Effects of various extraction procedures on human monocyte-derived macrophage cathepsin D activity. Normal human monocytes (10⁶ cells plated per well in Dulbecco modified Eagle medium–10% fetal bovine serum–10% horse serum) were maintained in culture for 7 days. Hb, Hemoglobin; mcg, micrograms. Probably responsible for the observed enhancement. Watabe and Yago (18) have shown that phospholipids activate cathepsin D proteolysis in vitro. Several secretory products are present in macrophages or monocyte-derived macrophages which are not easily detected in monocytes. These include acid hydrolases, such as acid phosphatase, N-acetyl β-glucosaminidase, β-glucoronidase, and mannosidase (12, 17); complement components C1q, C2, and C4 (19); and large glycoprotein molecules, such as fibronectin (3, 8) and α-2 macroglobulin (10). The increase in cathepsin D activity starting at day 5 of monocyte culture coincides with appearance of the known indices of maturation and relates to the observation that the enzyme is present in macrophages but not in freshly isolated monocytes. Cathepsin D activity is thus an inducible marker of macrophage differentiation.

We observed only partial pepstatin inhibition of cathepsin D activity in neutrophil lysates compared with monocyte-macrophage lysates. This finding suggests that neutrophils contain, in addition to cathepsin D, an acid proteolytic enzyme that cleaves hemoglobin at pH 3.5 and is different from cathepsin D. Since the macrophage-derived activity is completely inhabitable by pepstatin A, its enzyme activity is conferred by cathepsin D or other acid aspartic proteinases. Cathepsin D is present in lysates of neutrophils and monocyte-macrophage macrophages, and its activity is evident with radiolabeled hemoglobin as a substrate. It is very likely that the enzyme has an important role in inflammatory processes mediated by these cells. Inflammatory cells migrate and concentrate at the inflammatory foci and then degranulate and undergo a cell lysis process. Under these conditions, the acid proteolytic enzyme is able to exert its maximal effect on inflamed tissues.

This study was supported by Public Health Service grants NS-17752 and HL-27068 from the National Institutes of Health and grant RG-1919-A-1 from the National Multiple Sclerosis Society.

We acknowledge the expert technical help of Lee A. Burnham and the dedicated secretarial assistance of Shree Lighty.

LITERATURE CITED


