

A FAST-ACTING ELASTASE INHIBITOR IN HUMAN MONOCYTES

BY EILEEN REMOLD-O'DONNELL

From The Center for Blood Research, Boston, Massachusetts and the Department of Biological Chemistry, Harvard Medical School, Boston, Massachusetts 02115

The elastase content of neutrophils is very high, i.e. 46 μg per 10^8 cells, or 1.2×10^7 molecules/cell (1). The enzyme, which is localized in granules (2), is a serine proteinase; it functions optimally at the neutral pH of extracellular fluids (3). Neutrophil elastase has broad substrate specificity, capable of cleaving fibrinogen (4), fibronectin (5), immunoglobulin (6), plasminogen (7), growth factors (8), complement (9), and coagulation factors (10), as well as the extracellular structural components elastin, cartilage proteoglycans (11), and type III collagen (12, 13). Elastase of circulating monocytes appears to be identical to neutrophil elastase (14); the enzyme is present at 30-fold lower levels in monocytes (1, 14). Much of the attention focused on neutrophil and monocyte elastase has emphasized its pathological roles, e.g. in the development of pulmonary emphysema (15) and in arthritic lesions (16). A physiological role was demonstrated for elastase of neutrophils as a fibrinolytic agent in circulating blood (1). Moreover, neutrophil elastase was recently found to play a role in the migration of phagocytes from the circulation into inflammatory sites, i.e., it was shown that elastase activity is required for the chemotactic movement of neutrophils through an amnion membrane (17) or through an endothelial cell barrier (18), but is not required for movement through an artificial barrier.

Elastase is clearly a potent proteinase; pathological effects would be anticipated in the absence of regulation. In circulation, released elastase is found in inactive form complexed to the plasma inhibitor α_1 -proteinase inhibitor (α_1 PI, α_1 -antitrypsin)¹ and to a lesser extent to α_2 -macroglobulin (19). At inflammatory sites, phagocytes, i.e. monocyte/macrophages and especially neutrophils, have the capacity to generate high local concentrations of elastase. It is thought (20, 21) that neutrophil elastase at inflammatory sites is inactivated by α_1 PI from circulating blood plasma, and possibly from macrophages (22). A second possibility for regulating elastase activity at inflammatory sites is alteration in the cell content of the proteinase itself. The serine elastase content of monocytes decreases as the cells develop macrophage-like characteristics (23). This study

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¹Abbreviations used in this paper: α_1 PI, α_1 -proteinase inhibitor; FCS, fetal calf serum; HBSS, Hanks' balanced salt solution; MPI, macrophage proteinase inhibitor; NP-40, Nonidet P-40; PAGE, polyacrylamide gel electrophoresis; pAMPSF, *p*-(amidinophenyl)methane sulfonyl fluoride; PBS, phosphate-buffered saline; SDS, sodium dodecyl sulfate.

describes a third capability for regulation of elastase activity at inflammatory sites. We present the first characterization of a fast-acting inhibitor of elastase present in high concentrations in human monocytes.

Materials and Methods

Monocytes. Monocytes were purified from freshly-drawn blood anticoagulated with acid citrate-dextrose obtained from Children's Hospital Medical Center Blood Bank (Boston, MA). The blood was centrifuged in the collection bag at 2,500 rpm for 3 min in a Sorvall HG rotor. The blood bag was placed under pressure in a plasma extractor (Fenwal Labs, Deerfield, IL), and after removal of the platelet-rich plasma, 80 ml was expressed from the top of the packed cells (buffy coat) and diluted with equal volume $\text{Ca}^{++}/\text{Mg}^{++}$ -free Hanks' balanced salt solution (HBSS) with 20–50 $\mu\text{g}/\text{ml}$ leupeptin. All salt solutions and media contained 100 U/ml penicillin and 100 $\mu\text{g}/\text{ml}$ streptomycin. 20 ml portions of buffy coat cells were layered onto 17 ml Ficoll/Hypaque and fractionated by centrifugation. The interface cells (mononuclear cells) were washed by 1–4 cycles of suspending and pelleting at room temperature in $\text{Ca}^{++}/\text{Mg}^{++}$ -free HBSS with 2% fetal calf serum (FCS) to reduce platelet contamination to negligible levels, as judged by opalescence and microscopic examination.

Monocytes were purified on Percoll gradients; $1\text{--}2.5 \times 10^8$ washed mononuclear cells at $2\text{--}5 \times 10^7$ cells/ml in RPMI 1640 were layered onto 37 ml Percoll gradients and centrifuged at 4°C (24). The upper band cells were pelleted twice in RPMI 1640 with 2% FCS at 4°C. This fraction, designated 0-day monocytes, contained the monocytes in 97% average yield as judged by Wright's stain and peroxidase stain (25); the monocyte content in four preparations was 40, 83, 56, and 59%, based on peroxidase staining.

The monocyte preparations were cultured at 2×10^6 cells/ml in RPMI 1640 with 10% FCS as nonadherent cells in Teflon beakers (Nalge Co., Rochester, NY); the medium was partially replenished every 2 d. After 1–10 d the cell suspension was brought to room temperature and pelleted.

Adherence-purified monocytes, used in selected experiments, were prepared by culturing mononuclear cells (2.0×10^7 in 5 ml RPMI with 10% FCS) in $60 \times 15\text{-mm}$ tissue culture dishes (3002; Falcon Labware, Oxnard, CA) for 2 h at 37°C. Nonadherent cells were decanted, and the monolayers were washed twice in warm RPMI with 2% FCS. The adherent monocytes were harvested with or without 7 d culture in RPMI with 10% FCS by collecting the cells into cold HBSS with the aid of a rubber policeman and pelleting.

Lymphocytes. Lymphocytes were purified by culturing washed mononuclear cells for 1 h at 5×10^6 cells/ml in RPMI 1640 with 2% FCS in plastic tissue culture flasks; the nonadherent cells (lymphocytes) were washed by pelleting.

Neutrophils. The Ficoll-Hypaque pellet was suspended in HBSS and combined with an equal volume of 2% dextran in 0.15 M NaCl. After the erythrocytes had sedimented (45 min) the neutrophils were removed and washed by pelleting in HBSS; residual erythrocytes were removed by lysis in water.

Cell Lines. The human histiocytic lymphoma cell line U937 (26, 27), donated by D. Liu (Brigham and Women's Hospital, Boston, MA), was grown in RPMI in 1640 medium with 10% FCS and 0.1% gentamycin. The human lymphoblastoid cell line CEM (28), donated by H. Lazarus (University of Miami Medical School, Miami, FL), was grown in Dulbecco's modified Eagle's medium with 4.5 mg/ml glucose, 10% FCS, 100 U/ml penicillin, and 100 $\mu\text{g}/\text{ml}$ streptomycin. CEM and U937 cells were washed before use by pelleting three times in HBSS.

Lysis of Cells. Cells were washed at room temperature (rather than 4°C) in an effort to maximize removal of serum $\alpha_1\text{PI}$. As a final wash, cells were incubated at $0.5\text{--}2 \times 10^7$ cells/ml for 5–10 min in HBSS at room temperature; they were brought to 4°C and pelleted. Detergent lysates were prepared by extracting 2.5×10^7 cells with 1 ml of 0.5% Nonidet P-40 (NP-40) in phosphate-buffered saline (PBS). Lysates were clarified (20,000 g for 13 min) and stored at -80°C .

Radiolabeling of Elastase with Na^{125}I . Radiolabeling of porcine pancreatic elastase and

human neutrophil elastase by the Iodogen method (29), removal of excess reagents, and evaluation of products were described (30). ^{125}I label associated with protein was found to be >90% covalently bound. Protein concentration was determined by the Folin method (31). The products, ^{125}I -labeled porcine pancreatic elastase with 0.5–1.5 $\mu\text{Ci}/\mu\text{g}$ and ^{125}I -labeled human neutrophil elastase with 1–3 $\mu\text{Ci}/\mu\text{g}$ were stored in portions at -20°C .

Formation of ^{125}I -Elastase-Inhibitor Complex. Cell extracts (15–150 μl) were incubated with 30–200 ng of ^{125}I -elastase in 20 μl of HBSS at 37°C for 20 min; typical reactions contained 60 μl cell extract from 1.5×10^6 cells with 130 ng ^{125}I -labeled porcine pancreatic elastase. The reaction was simultaneously stopped and the mixture prepared for electrophoresis by adding 40 μl sodium dodecyl sulfate (SDS) solubilizing solution (5% SDS, 25% glycerol, 100 $\mu\text{g}/\text{ml}$ bromophenol blue, 8% mercaptoethanol) and heating at 100°C for 1 min. Alternatively, the incubation mixtures were immediately subjected to immunoprecipitation.

Immunoprecipitation. The immunoprecipitating particles, fixed *Staphylococcus aureus* coated with rabbit antiserum to $\alpha_1\text{PI}$ or with normal rabbit serum, were prepared as described (32). The particles were incubated with the preformed ^{125}I -elastase-inhibitor complexes for 60 min at room temperature. The resulting bacteria-antibody-antigen complexes were washed (32) and extracted at 100°C for 1 min with 120 μl of SDS solubilizing solution diluted 1:3 with PBS.

SDS Polyacrylamide Gel Electrophoresis (PAGE). For Laemmli SDS electrophoresis (33), the 2.2-mm resolving slab gels were prepared from 370 mM Tris HCl buffer, pH 8.8, 7.5% acrylamide, 0.2% bisacrylamide, 0.1% SDS. The modified stacking gels were prepared from 60 mM Tris HCl buffer, pH 6.8, 4% acrylamide, 0.1% bisacrylamide, 0.3% SDS. Running buffer was 42 mM Tris/190 mM glycine buffer, pH 8.3, with 0.1% SDS.

For Fairbanks/Laemmli SDS electrophoresis, the procedure (34) was modified to generate 2.2-mm resolving slab gels prepared from 40 mM Tris acetate buffer, pH 7.4, 2 mM EDTA, 6% acrylamide, 0.2% bisacrylamide, 1% SDS. Stacking gels were included as described for Laemmli electrophoresis. Running buffer was 40 mM Tris acetate buffer, pH 7.4, 2 mM EDTA, 1% SDS.

The standard proteins, myosin, β -galactosidase, phosphorylase a, albumin, creatine kinase, carbonic anhydrase, and soybean trypsin inhibitor indicated mol wt of 200,000, 130,000, 94,000, 68,000, 40,000, 29,000, and 22,000, respectively. Gels were dried and subjected to autoradiography.

Materials and Suppliers. The sources of materials are as follows: NP-40 (BDH Chemicals, Ltd., Poole, United Kingdom); Na^{125}I (Amersham Corp., Arlington Heights, IL); Iodogen (Pierce Chemical Co., Rockford, IL); Percoll (colloidal polyvinylpyrrolidone-coated silica, P-1644; Sigma Chemical Co., St. Louis, MO); intensifying screens (Cronex Lightning Plus; E. I. Dupont de Nemours Co., Wilmington, DE); *N,N'*-methylene bisacrylamide and acrylamide (Bio-Rad Laboratories, Richmond, CA); formaldehyde-fixed, heat-killed *Staphylococcus aureus* (The Enzyme Center, Boston, MA); Ficoll-Paque and Sephadex G-25 (Pharmacia Fine Chemicals, Piscataway, NJ); and acid citrate-dextrose, National Institutes of Health formula A (Fenwal Laboratories, Deerfield, IL).

HBSS and $\text{Ca}^{++}/\text{Mg}^{++}$ -free HBSS, RPMI 1640, Dulbecco's modified Eagle's medium, penicillin, streptomycin, and fetal bovine serum were from M. A. Bioproducts, Walkersville, MD. Serum was heat-inactivated (56°C for 1 h). PBS was from Gibco Laboratories, Grand Island, NY, and gentamycin sulfate from U. S. Biochemical Corp., Cleveland, OH.

Homogeneous porcine pancreatic elastase (95–125 U/mg) and human neutrophil elastase purified from purulent human sputum (35) were from Elastin Products Co., Inc., Pacific, MO. *N*-acetyl-Ala-Ala-Pro-Ala- CH_2Cl was kindly supplied by J. Powers (Georgia Institute of Technology). *p*-(amidinophenyl)methanesulfonyl fluoride (*p*AMPSF) was obtained from Chemicon International, Inc., El Segundo, CA. One of the rabbit antisera against $\alpha_1\text{PI}$ was kindly supplied by C. Alper (The Center for Blood Research); another antiserum was from Dako Corp., Santa Barbara, CA.

Results

Detection of Elastase Inhibitor in a Monocyte-like Cell Line. Based on the detection of an elastase inhibitor in macrophages of guinea pigs (30) we hypothesized that human monocytes/macrophages contain a proteinase inhibitor active against elastase. Most circulating blood proteinase inhibitors, as well as the cellular proteinase inhibitor of guinea pig macrophages have the common feature of forming covalent complexes with serine active site proteinases (36). A molecule that forms a complex with ^{125}I -labeled pancreatic elastase was detected in extracts of the human monocyte-like cell line U937, but was not detected in extracts of the human lymphoblastoid cell line CEM (Fig. 1, A–C).

We conclude that the integrity of the active site of elastase is required for complex formation with the U937 component because the reaction is inhibited by diisopropylfluorophosphate, a serine active site inhibitor, and by *N*-acetyl-Ala-Ala-Pro-Ala-chloromethyl ketone, which binds to the elastase active site, but not by *p*-AMPSF, which binds to the active site of arginine-containing serine proteinases (Fig. 1, D–G). Formation of the complex is inhibited by 20-fold excess nonradiolabeled elastase, but not by 20-fold excess ovalbumin (not shown). The ability of the component in U937 cell extracts to form a complex with ^{125}I -elastase is destroyed by boiling in SDS (not shown). The preformed complex, on the other hand, is stable when heated in SDS, suggesting that the linkage is a covalent bond. Bases such as ammonium hydroxide (37) are known to hydrolyze ester bonds of serine proteinase–proteinase inhibitor complexes. The complex of the U937 component with ^{125}I -elastase is cleaved on incubation with 1.5 M ammonium hydroxide at 37°C for 60 min (Fig. 1, H–K), further indicating that the complex is a proteinase–proteinase inhibitor covalent complex. The complex of the U937 proteinase inhibitor with ^{125}I -elastase comigrates on Laemmli SDS-

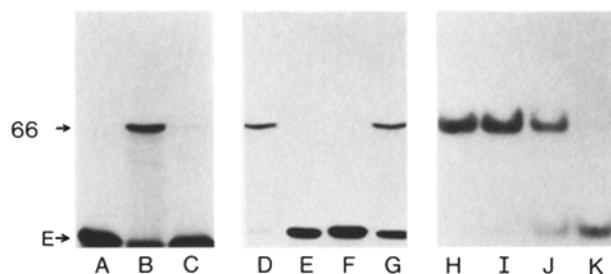


FIGURE 1. A–C, reaction of ^{125}I -labeled porcine pancreatic elastase with a component of human cell lines. Shown is an autoradiograph of a Laemmli SDS-PAGE gel of 33 ng ^{125}I -elastase incubated with: A, no additives; B, the extract of 1.5×10^6 U937 cells; and C, the extract of 1.5×10^6 CEM cells. Arrow indicates the ^{125}I -elastase-inhibitor complex of *M*, 66,000; E designates unreacted ^{125}I -elastase. D–G, effect of synthetic inhibitors on the reaction of ^{125}I -pancreatic elastase with the component in U937 cells. The U937 extract was reacted with ^{125}I -elastase that had been preincubated for 20 min at 30°C with: D, no additives; E, 2 mM diisopropylfluorophosphate; F, 500 μM *N*-acetyl-Ala-Ala-Pro-Ala- CH_2Cl ; and G, 500 μM *p*-AMPSF. The agents introduced as solvents for the synthetic inhibitors (2% ethanol, 0.2% propylene glycol) did not inhibit complex formation (not shown). H–K, cleavage of the complex by ammonium hydroxide. Shown is an autoradiograph of a Fairbanks/Laemmli SDS-PAGE gel of the U937 extract– ^{125}I -pancreatic elastase reaction product, which underwent a second incubation for 60 min with: H, PBS at 4°C; I, PBS at 37°C; J, 1.5 M NH_4OH at 4°C; and K, 1.5 M NH_4OH at 37°C. Diisopropylfluorophosphate at 1 mM was added to all reaction mixtures before the second incubation to prevent proteolysis of the complex.

PAGE with the complex of ^{125}I -elastase with the guinea pig macrophage proteinase inhibitor (MPI) (30) at M_r 66,000 (not shown), suggesting that these are analogous molecules from different species.

Dose Dependence of Complex-formation Assay. The amount of the ^{125}I -elastase-inhibitor complex generated was found to depend on the amount of U937 extract added (Fig. 2A). The complex and unreacted ^{125}I -elastase were quantified by gamma counting after excision from SDS-polyacrylamide gels. Plots of the percent of ^{125}I -elastase converted to complex vs. the amount of extract added gave dose-response curves (Fig. 2B), thus establishing that the ^{125}I -elastase complex formation reaction can be used as a semiquantitative assay for the proteinase inhibitor.

Elastase Inhibitor in Blood Cells. Isolated blood cells were lysed and examined for their content of inhibitor by the ^{125}I -pancreatic elastase complex formation assay. Compared to U937 cell extracts, activity was minimal in lymphocyte extracts, and not detectable in neutrophils (Fig. 3). Surprisingly, elastase inhibitor activity was not detected in extracts of Percoll-purified monocytes (Fig. 3).

Appearance of Elastase Inhibitor in Monocytes Matured in Culture. We questioned whether the elastase inhibitor is expressed by monocytes that have acquired macrophage-like properties by in vitro culture. Percoll-purified monocytes were allowed to mature in culture. The elastase inhibitor activity of

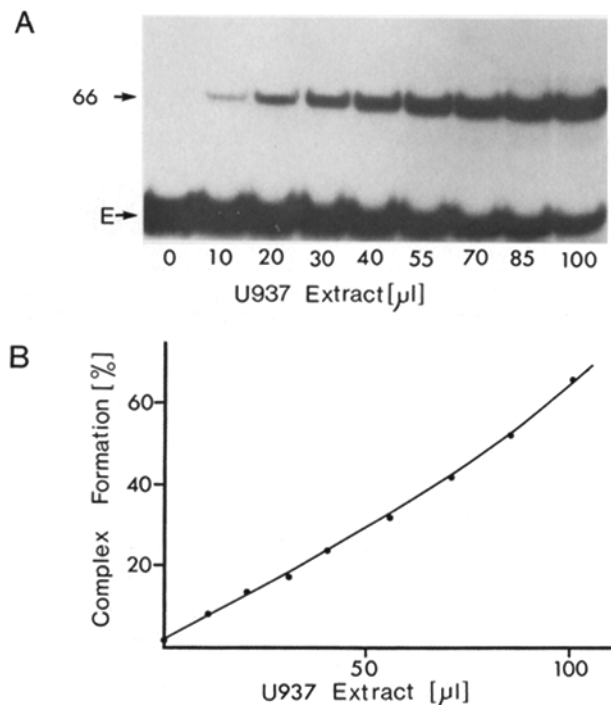


FIGURE 2. Effect of the concentration of U937 extract on the extent of complex formation with ^{125}I -pancreatic elastase. Shown in A is an autoradiograph of a Fairbanks/Laemmli SDS-PAGE gel of 97 ng ^{125}I -elastase reacted with varying amounts of U937 extract, as indicated. Arrow indicates the ^{125}I -elastase-inhibitor complex of M_r 66,000; E designates unreacted elastase. B shows quantitation of the complex measured by gamma counting of the ^{125}I -complex and unreacted ^{125}I -elastase excised from the SDS electrophoresis gel.

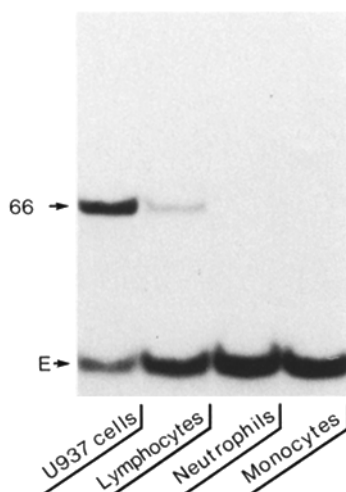


FIGURE 3. Reaction of extracts of human blood cells with ^{125}I -pancreatic elastase. Shown is an autoradiograph of a Fairbanks/Laemmli SDS-PAGE gel of the reaction of 97 ng ^{125}I -elastase with lysates of 1.5×10^6 cells, as indicated. Arrow indicates the ^{125}I -elastase-inhibitor complex of M_r 66,000; *E* designates unreacted ^{125}I -elastase.

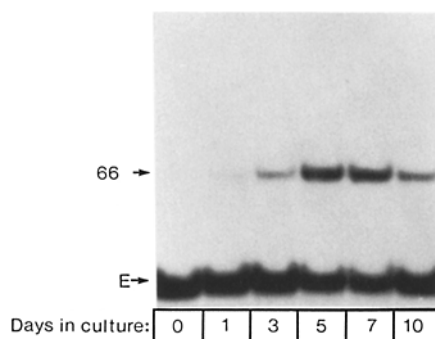


FIGURE 4. Appearance of the elastase inhibitor in monocytes during in vitro culture. Shown is an autoradiograph of a Fairbanks/Laemmli gel containing 130 ng ^{125}I -porcine pancreatic elastase reacted as indicated with extracts of 1.9×10^6 monocytes cultured for 0 d, 1 d, 3 d, 5 d, 7 d, or 10 d.

monocytes increased during culture; it was maximal after 5–7 d (Fig. 4), at which time the inhibitor activity of monocyte extracts was comparable to that of U937 cells (not shown). These findings indicate that the expression of the elastase inhibitor by monocytes requires a maturation process.

Elastase Inhibitor in Adherence-Purified Monocytes. Percoll-purified monocytes are contaminated with varying numbers of lymphocytes (see Materials and Methods). Therefore, it was important to demonstrate that the monocyte is the cell that develops the elastase inhibitor on in vitro culture. Adherence to plastic tissue culture is an alternate method to separate monocytes from lymphocytes; adherence generates monolayers of monocytes in high purity (38, 39). Extracts of freshly-isolated adherence-purified monocytes, like extracts of freshly-isolated Percoll-purified monocytes, did not form a complex with ^{125}I -pancreatic elastase. Extracts of adherence-purified monocytes that were cultured as monolayers for 7 d had elastase-complexing activity that was quantitatively comparable to that

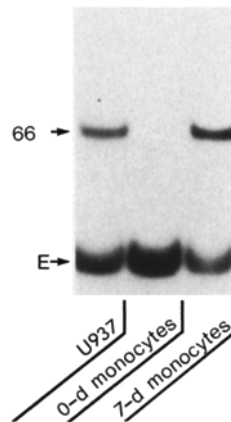


FIGURE 5. Complex formation of ^{125}I -labeled human neutrophil elastase with a component of U937 cells and cultured monocytes. Shown is an autoradiograph of a Fairbanks/Laemmli gel of 44 ng ^{125}I -labeled human leukocyte elastase reacted with extracts of 1.7×10^6 cells, as indicated.

of Percoll-purified monocytes cultured for 7 d (not shown). These findings indicate that the monocyte is the cell that acquires elastase inhibitory activity on *in vitro* culture.

Reaction of the Monocyte/U937 Inhibitor with Elastase of Human Neutrophils. The serine elastase of porcine pancreas, which is readily available, was used as the target proteinase in most experiments. The likely physiological target, however, is the related serine elastase of human neutrophils and monocytes. Therefore, ^{125}I -labeled human neutrophil elastase was used as a target proteinase. Extracts of U937 cells formed a complex with ^{125}I -neutrophil elastase (M_r 66,000) (Fig. 5). This activity was not detectable in freshly-isolated, Percoll-purified monocytes, but extracts of monocytes cultured for 7 d formed a complex with ^{125}I -neutrophil elastase (Fig. 5).

The Monocyte Inhibitor Is Fast-acting. The time course of the reaction with ^{125}I -pancreatic elastase was examined using the extracts of 7-d cultured monocytes as the source of inhibitor. The reaction was found to be complete after 20–40 s at 37°C (Fig. 6). Identical results were obtained with extracts of U937 cells (not shown). These findings characterize the inhibitor in monocytes/U937 cells as a fast-acting inhibitor of elastase.

The Monocyte Elastase Inhibitor Is Not $\alpha_1\text{PI}$. We examined whether the elastase inhibitor detected in this study is the circulating elastase inhibitor $\alpha_1\text{PI}$, since it was previously shown (22) that isolated monocytes synthesize $\alpha_1\text{PI}$. When human serum, as the source of $\alpha_1\text{PI}$, was reacted with ^{125}I -pancreatic elastase, a major complex formed of M_r 80,000. This complex did not comigrate with the ^{125}I -elastase complex formed by extracts of U937 cells or monocytes (Fig. 7, the three left lanes), indicating that the monocyte inhibitor and $\alpha_1\text{PI}$ are nonidentical. Immune precipitation by two different rabbit antisera against human $\alpha_1\text{PI}$ precipitated the complex from serum, but did not precipitate the complex formed by cultured monocytes or U937 cells (Fig. 7, the three right lanes). These findings show that the monocyte elastase inhibitor identified in this study is not $\alpha_1\text{PI}$, and is not immunologically related to $\alpha_1\text{PI}$.

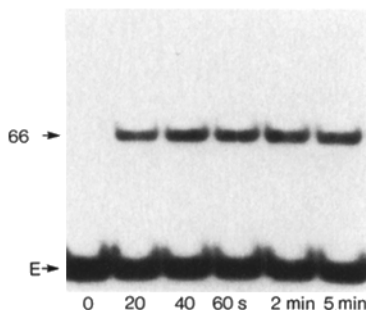


FIGURE 6. Time course of the reaction of ^{125}I -pancreatic elastase with the inhibitor in 6-d cultured monocytes. Shown is an autoradiograph of a Fairbanks/Laemmli SDS electrophoresis gel of 190 ng ^{125}I -labeled pancreatic elastase reacted at 37°C with the extract of 2.5×10^6 6-d cultured monocytes for 0–5 min, as indicated. Note that formation of the ^{125}I -elastase-inhibitor complex is complete after 20–40 s. No further reaction was observed at 5 min of incubation (last lane) or at 20 or 30 min (other experiments, not shown).

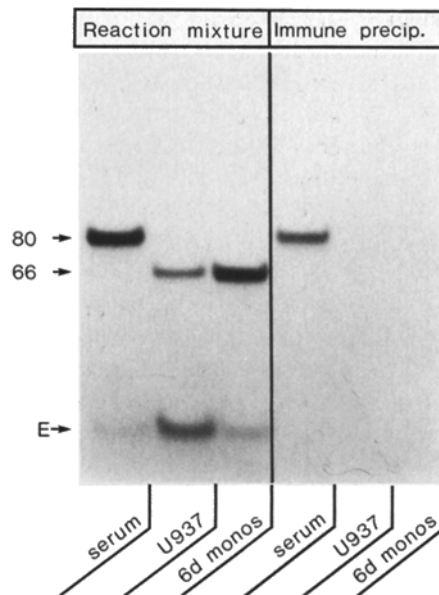


FIGURE 7. Comparison of the ^{125}I -elastase- $\alpha_1\text{PI}$ complex with the ^{125}I -elastase-U937/monocyte inhibitor complex. Shown in the left three lanes of the autoradiograph (Fairbanks/Laemmli gel) are 130 ng ^{125}I -pancreatic elastase reacted as indicated with 0.5 μl normal human serum, or with the extract of 1.5×10^6 U937 cells, or the extract of 10^6 6-d cultured monocytes. Shown in the right three lanes are replicate reaction mixtures immunoprecipitated with rabbit anti-human $\alpha_1\text{PI}$ antiserum. Note that the antiserum precipitates the serum $\alpha_1\text{PI}$ -complex, but does not precipitate the complex formed by the monocyte/U937 inhibitor. Identical results were obtained with another rabbit anti-human $\alpha_1\text{PI}$ antiserum (not shown).

Discussion

This is the first characterization of a proteinase inhibitor molecule of human monocytes that is active against neutrophil elastase. The molecule is detected in cultured monocytes and the monocyte-like cell line U937; it was identified as a proteinase inhibitor based on its ability to form, with the active site of elastase, a complex that is stable in boiling SDS and susceptible to nucleophilic cleavage.

The quantity of complex formed is dependent on: the concentration of monocyte or U937 extract (Fig. 2); the concentration of ^{125}I -elastase (not shown); the temperature (not shown); and the time of incubation (Fig. 6). The mol wt of the elastase inhibitor is estimated at 50,000, which is the M_r of the complex (66,000) minus that of elastase (26,000), with 10,000 arbitrarily added to represent the inhibitor fragment assumed to be released on formation of the complex (36). Based on coelectrophoresis, the monocyte inhibitor appears to be the human analog of the guinea pig component MPI, an inhibitor of elastase and trypsin detected (30) in culture medium and extracts of elicited macrophages.

It has been reported (40) that certain proteinase-proteinase inhibitor complexes cannot be detected on Laemmli electrophoresis gels due to their lability in the primary amine Tris at high pH. To avoid base-catalyzed hydrolysis of the elastase-elastase inhibitor complex, a Fairbanks/Laemmli hybrid electrophoresis system was developed with lower pH and lower concentrations of Tris buffer. The mobilities of pancreatic elastase and seven marker proteins were proportional to the log of their mol wt on these Fairbanks/Laemmli gels. When examined on Fairbanks/Laemmli gels, the conversion of ^{125}I -elastase to the ^{125}I -elastase-inhibitor complex in a reaction driven toward completion by excess U937 extract was >85% complete, demonstrating that the complex is stable in this electrophoresis system. When eight sets of duplicate reaction mixtures were examined in parallel on both electrophoresis systems, the ^{125}I -elastase conversion to complex detected on Laemmli gels was 62% of that detected on Fairbanks/Laemmli gels, indicating that partial hydrolysis occurs in the former system.

The elastase inhibitor is detected in the monocyte-like cell line U937, but is not detected in freshly isolated monocytes. The elastase inhibitor activity becomes detectable in monocytes only after *in vitro* culture; activity is maximal at 5–7 d. Human monocytes cultured *in vitro* are known to acquire macrophage-like characteristics; the cells do not divide, but acquire macrophage-like morphology and increase in size (41). The amounts of certain components increase on *in vitro* maturation of monocytes; these included C2 and factor B (38, 41), and the lysosomal enzymes β -glucosaminidase, β -glucuronidase, and acid phosphatase (42). The amounts of other components, including HLA-DR (43), serine elastase (23), and $\alpha_1\text{PI}$ (22) decrease as the monocyte differentiates in culture.

As mentioned above, the high activity of serine elastase measured in fresh monocyte extracts decreases when monocytes mature in culture, reaching negligible levels at day 6 (27). Both the study of elastase activity (27) and this study of elastase inhibitor were done on unfractionated cell extracts. More refined examination will be required to determine whether absolute levels of elastase decrease on culture, or absolute levels of elastase inhibitor increase, or whether the levels of both are altered.

This study is the first characterization of the major elastase inhibitor molecule of human monocytes. The activity of the elastase inhibitor was detected in several earlier studies, e.g. in the cytosol fraction of human pulmonary macrophages (44). The presence of an elastase inhibitor in U937 cells was inferred from the increase of elastase activity on fractionation of cell extracts (14).

The elastase inhibitor is present in high concentrations in cultured monocytes.

The content was estimated from the number of ^{125}I -elastase molecules added per reaction and the resulting fraction converted to complex. The estimate that this calculation supplies is a minimum estimate because inhibitor molecules may fail to react, or may already be complexed to proteinase, or may react with nonradiolabeled proteinases in the cell extract. The estimated inhibitor content of monocytes cultured for 7 d is $\geq 1.5 \times 10^6$ functional molecules per cell, or 12 μg per 10^8 cells. We can, thus, conclude that the elastase inhibitor molecule is a major component in monocytes, representing $>0.1\%$ of monocyte protein.

It is interesting to compare the monocyte elastase inhibitor with the circulating elastase inhibitor $\alpha_1\text{PI}$. This study shows that the elastase inhibitor of monocyte extracts is not identical to $\alpha_1\text{PI}$, since the two do not comigrate on SDS-electrophoresis (Fig. 7). Moreover, the two are not immunologically related, since the monocyte inhibitor failed to react with heteroantiserum to $\alpha_1\text{PI}$ (Fig. 7). It was previously shown (22) that synthesis of $\alpha_1\text{PI}$ by monocytes is greatest in freshly isolated cells, and declines during 10 d in culture. This pattern is opposite to that observed for the monocyte elastase inhibitor, which increases when monocytes mature in culture (Fig. 4). $\alpha_1\text{PI}$ is plentiful in circulating blood plasma (2 mg/ml) (45), whereas the monocyte inhibitor is either absent or present in concentrations too low to be detected in this study (Fig. 7, first lane). This study detected no $\alpha_1\text{PI}$ in monocyte extracts by an assay that was adequate to detect $\alpha_1\text{PI}$ in diluted serum (Fig. 7). The lack of detection of $\alpha_1\text{PI}$ in monocyte extracts might be due to its low concentration.

As discussed above, elastase inhibitor activity increases as monocytes mature in culture into macrophage-like cells similar to those found at inflammatory sites. It is, thus, likely that the elastase inhibitor is a functional component of the inflammatory macrophage rather than the circulating monocyte. This fits well with the recent finding (17, 18) that elastase activity is required by neutrophils (and presumably by monocytes) for movement in response to chemotactic agents from the circulation to extravascular sites. At extravascular sites, this function is no longer required, and elastase inhibition becomes important. The rapid reaction of the monocyte inhibitor with elastase, and its high concentration in matured monocytes make this molecule an excellent candidate for regulating serine elastase activity at inflammatory sites. Control of elastase activity at inflammatory sites is important because of the high concentrations that can be released, and the large spectrum of proteins susceptible to cleavage (see Introduction). We have not investigated whether the elastase inhibitor is secreted by human monocytes, but MPI, which is the corresponding elastase inhibitor of guinea pig macrophages, was originally detected as a secreted protein (30).

To establish its physiological role, it will be necessary to determine the activity spectrum of the monocyte proteinase inhibitor, i.e. to determine whether the inhibitor has the capacity to act on other proteinases secreted by inflammatory cells, including neutrophil cathepsin G (angiotensin-converting enzyme) (46, 47), and macrophage plasminogen activator (48), cytolytic factor (49), complement components C2 (38) and factor Bb (41). It is not known whether other cells express this elastase inhibitor molecule. We can conclude that the monocyte inhibitor is not identical to protease nexin, an inhibitor in human fibroblasts of trypsin-like proteinases (50), because the latter is inactive against leukocyte

elastase (51). Comparison with proteinase inhibitors found in other cells (52, 53) must await further characterization. We must also determine whether the monocyte elastase inhibitor is the same or related to the inhibitor of plasminogen activator detected in human monocytes (48, 54).

Summary

A proteinase inhibitor active against neutrophil and pancreatic elastase was detected in extracts of cultured human monocytes and the human monocyte-like cell line U937. This component forms a covalent complex with the active site of elastase; the complex is stable in boiling sodium dodecyl sulfate solution, and is susceptible to nucleophilic cleavage.

The activity of the elastase inhibitor is not detected in extracts of freshly isolated monocytes, but becomes detectable when the monocytes are allowed to mature in culture, with maximum levels occurring at 5–7 d. The monocyte inhibitor is fast-acting; its reaction with ^{125}I -labeled elastase is complete in <1 min at 37°C.

Analysis by electrophoresis and studies using a heteroantiserum to α_1 -proteinase inhibitor demonstrated that the elastase inhibitor of monocytes/U937 cells is not identical to α_1 -proteinase inhibitor, the major elastase inhibitor of blood plasma. The extent of conversion of ^{125}I -elastase to the ^{125}I -elastase-inhibitor complex is proportional to the amount of U937 extract or cultured monocyte extract, indicating that this reaction can serve to quantify the elastase inhibitor. The elastase inhibitor is an abundant component in mature monocytes, with $\geq 1.5 \times 10^6$ molecules/cell ($\geq 12 \mu\text{g}$ per 10^8 cells, >0.1% of total cell protein). Its mol wt is estimated at 50,000.

Thus, the monocyte inhibitor should be classified as a putative regulator of neutrophil (and monocyte) elastase activity at inflammatory sites. This designation is based on the properties of the molecule, including its high concentration in maturing monocytes, its affinity for elastase, and its fast reaction with this enzyme.

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