Co-operation between plasmin and elastase in elastin degradation by intact murine macrophages

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Intact, thioglycollate-stimulated murine macrophages cultured on an insoluble [³H]-elastin substratum progressively hydrolysed the elastin. Cell lysates had little activity. We compared the effect of various proteinase inhibitors on elastinolysis by either live cells or cell-free, elastase-rich conditioned medium. Only known inhibitors of macrophage elastase blocked the activity of elastase-rich cell-conditioned medium whereas inhibitors of cathepsin B also suppressed intact cell activity. Serum proteinase inhibitors blocked cell-derived soluble elastase activity but not intact cell elastolytic activity. We also observed that plasminogen added to the cell cultures markedly increased elastinolysis by live macrophages or cell-free elastase-rich medium. Purified plasmin alone had no measurable effect on native elastin. Additional experiments indicated that the plasmin enhancement was due to elastin-dependent activation of latent macrophage elastase. These results indicate that live macrophage elastinolysis is a co-operative process involving multiple proteinases, especially a cysteine proteinase(s) and elastase. Plasmin may be a physiological activator of latent macrophage elastase.

Mammalian elastases are recognized by their ability to solubilize mature, insoluble elastin (Werb *et al.*, 1982; Takahashi *et al.*, 1973; Kagan *et al.*, 1972). Stimulated murine macrophages are known to release a true elastase. The enzyme has been purified and characterized (Werb & Gordon, 1975; Banda & Werb, 1981). Most investigations of mouse and human macrophage elastase activity have utilized detergent-treated elastin as a substrate and hence assayed activity of either cell lysates or conditioned medium of cell cultures containing the secreted elastase. However, there is a growing realization that intact cells, and particularly cell surfaces, are important to the expression of proteolytic activity. The activity of several of the coagulation proteinases are facilitated by assembly on platelet or other cell surfaces (Walsh, 1981). Also, cell-bound sperm acrosin is active in the

uterine environment because it is resistant to local proteinase inhibitors whereas free acrosin is not (Brown & Hartree, 1976). Our recent studies of macrophage fibrinolytic activity indicate that the organization of cells, cell-derived enzymes, and fibrin greatly influences fibrin degradation (Chapman *et al.*, 1982). Cell-bound plasminogen activator and fibrin-bound plasmin are relatively resistant to soluble proteinase inhibitors compared with their soluble enzyme forms. Thus, intact macrophages are able to focus fibrin degradation at or near the cell surface when the cells contact fibrin, even in the presence of an excess of serum proteinase inhibitors. The interaction of live macrophages with elastin is less clear. Previous studies examining the degradation of elastin by live macrophages have not used purified substrate (Werb *et al.*, 1980; Jones & Scott-Burden, 1979). We therefore examined whether live mouse macrophages hydrolyse purified insoluble elastin and, if so, how the degradative process compared with that mediated by soluble elastases secreted by these cells. An unexpected finding in the course of these studies was that plasminogen in the culture medium critically influenced the extent of elastin solubilization. We have therefore also examined

Abbreviations used: PBS, phosphate-buffered saline; SDS, sodium dodecyl sulphate; DMEM, Dulbecco's modified Eagle's medium; NPGB, nitrophenyl-*p*-guanidinobenzoate; Z-Phe-Ala-CHN₂, benzyloxycarbonylphenylalanylalanyldiazomethylketone; Msc-Ala-Ala-Pro-Ala-CH₂Cl, methoxysuccinylalanylalanylprolylalanylchloromethylketone; Z-Ala-Arg-Arg-AMC, benzyloxycarbonylalanylarginylarginyl-4-methylcoumarin-7-amide.

the mechanism by which plasmin enhances macrophage elastase activity.

Experimental

Cells and cell culture

C₃H/HeN female mice obtained from the Frederick Cancer Research Center Animal Facility (Frederick, MD, U.S.A.) were injected with 1 ml of thioglycollate medium (Difco) intraperitoneally 3 days before use. Cells were harvested, counted, and made to adhere to either 16mm (Costar) or 35mm (Falcon) culture wells which in some cases had been previously coated with elastin (see below). After 1 h at 37°C in a humidified incubator with air/CO₂ (19:1), cell monolayers were washed three times in PBS and used immediately in experiments. The basic tissue culture medium used for all experiments was DMM. To this culture medium, various sera and other reagents were added as described below. Adherent cell monolayers consistently contained less than 2% polymorphonuclear leucocytes as judged by Giemsa staining.

Reagents

Animal and human sera, plasminogen-free serum and acid-treated serum were prepared and stored as previously described (Chapman *et al.*, 1982). Plasmin, activated by solid phase urokinase, was obtained from Helena Labs (Houston, TX, U.S.A.). The preparation contained only plasmin and the cleavage product of activation as well as a trace of plasminogen as judged by overloaded Coomassie-blue stained SDS gels. Human plasminogen was also obtained from Helena Labs. Human fibrinogen was obtained from Merck (West Point, PA, U.S.A.) and made plasminogen-free by repeated ethanol precipitation in the presence of lysine (Mosesson, 1962). Bovine ligamentum elastin (<400 mesh) and porcine pancreatic elastase, 160 units/mg of protein, were obtained from Elastin Products (St. Louis, MO, U.S.A.). Elastin was ³H-labelled by reductive alkylation with NaB³H₄ (New England Nuclear, Boston, MA, U.S.A.) as described by Gordon *et al.* (1976). The ³H-labelled elastin was washed until the washings contained <0.1% of the total radioactivity, divided into portions and stored at -70°C. All other reagents were of the highest grade commercially available.

Enzyme assays

[³H]Elastin, suspended in water, was sonicated in a Branson 350 sonicator using a microprobe for 45 s at room temperature in the pulse mode at the

lowest energy setting and then dried in 16mm culture wells (Costar) at 47°C. Although sonicated, the elastin appeared particulate by phase- and scanning-electron microscopy. Each well contained approx. (240–400) × 10³ c.p.m. releasable by porcine pancreatic elastase (5 µg/ml). Although there were variations in total elastin/well between plates, the coefficient of variation between wells within a single plate was less than 5%. The specific radioactivity of the [³H]elastin was 1200 c.p.m./µg of protein. Plasmin (25 µg/ml) solubilized less than 0.1% of the total radioactivity. Intact cell elastase activity was assayed by direct culture of macrophages in the elastin-coated chambers. The pre-coated chambers were washed in PBS and 10⁶ thioglycollate-induced macrophages were made to adhere directly to the elastin-coated culture wells. The cells appeared by phase microscopy to adhere both to the elastin and to exposed plastic surfaces. The cells, in duplicate, were incubated in 500 µl of DMM containing various additives for 24–72 h at 37°C. Following the culture period, 300 µl of culture media was removed, spun at 10000g in a Microfuge, and 100 µl aliquots were assayed for degraded elastin by liquid-scintillation counting. Elastase activity is expressed as µg of elastin solubilized in duplicate determinations/24 h per 10⁶ cells. Porcine pancreatic elastase (125 units/mg; 0.1 µg/ml) released 20 µg of elastin in 24 h at 37°C when incubated in the elastin-coated chambers in DMM.

Soluble elastase activity was assayed according to Gordon *et al.* (1976). Macrophages (40 × 10⁶) were made to adhere to a 75 cm² tissue culture flask and incubated for 2 h in 10% (v/v) calf serum. The cell monolayers were washed with PBS and then incubated for 48–72 h in serum-free DMM. The supernatants were then collected, centrifuged to remove cells, and aliquots of the medium were stored at -20°C until use. This medium is known to contain both plasminogen activator and elastase activities (Unkeless *et al.*, 1974; Werb & Gordon, 1975). The cell-free medium (100 µl) was mixed with 100 µl of DMM and 100 µl of [³H]elastin suspended in PBS and incubated in 12mm × 75mm culture tubes for 24 h at 37°C in a shaking water bath. In some experiments, the assay was carried out without agitation in the 16mm culture wells precoated with [³H]elastin. A sample (200 µl) was then centrifuged in a Microfuge at 10000g and 100 µl aliquots were assayed for solubilized elastin by liquid-scintillation counting. The data are expressed as µg of elastin degraded/24 h and represent a mean of duplicate determinations for each experiment. Each culture tube contained approx. 100000 c.p.m. of [³H]elastin releasable by pancreatic elastase (5 µg/ml), corresponding to approx. 85 µg of elastin.

Cathepsin B activity was assayed according to Barrett & Kirschke (1981). After 20–24 h incubation at 37°C in DMM with or without possible inhibitors of lysosomal enzymes, 10^6 macrophages were lysed in 200 μ l of 88 mM-KH₂PO₄/12 mM-Na₂HPO₄ (pH 6.0) containing 1.3 mM-EDTA, 2.7 mM-cysteine and 0.2% Triton X-100. Lysates were then diluted 1:25–1:100 in the same buffer without detergent. Z-Ala-Arg-Arg-AMC (Enzyme Systems Products, Torrence, CA, U.S.A.), 0.1 mM final concentration, was used as substrate in a total reaction volume of 1 ml. After 60 min at 37°C enzyme activity was measured in arbitrary fluorescence units in a Perkin–Elmer spectrofluorimeter. Preliminary data showed that fluorescence was proportional to enzyme concentration over the dilutions tested and that greater than 95% of the fluorescence was blocked by preincubation of cellular lysates with the specific cysteine proteinase inhibitor Z-Phe-Ala-CHN₂ (10 μ M) (Shaw & Dean, 1980).

Protein determination

Protein concentration was determined by the method of Lowry *et al.* (1951) utilizing a bovine albumin standard.

Results

Degradation of [³H]elastin by thioglycollate-induced macrophages

Macrophages cultured in elastin-coated chambers hydrolysed elastin as judged by release of soluble radioactivity. In five separate experiments, 10^6 cells cultured in serum-free DMM hydrolysed an average of 7 μ g of elastin in 24 h. At least some of this elastinolysis is likely to be due to a soluble elastase released by stimulated murine macrophages (Banda & Werb, 1981). Macrophage elastase is known to be a metalloenzyme sensitive to inhibition by the divalent cation chelator EDTA, but not by inhibitors of serine proteinases such as di-isopropyl phosphofluoridate and phenylmethanesulphonyl fluoride. The macrophage soluble elastase is also not sensitive to the active site inhibitor of neutrophil elastase, Msc-Ala-Ala-Pro-Ala-CH₂Cl (Banda & Werb, 1981). We compared the process of elastinolysis mediated by intact cells with that of elastinolysis by elastase secreted by macrophages with respect to inhibition by these and other proteinase inhibitors. Table 1 compares the effects of proteinase inhibitors on the activity of elastase-rich, macrophage-conditioned medium or of intact cells. Elastase activity in the

Table 1. Comparison of the effects of proteinase inhibitors on elastase activity of macrophage-conditioned medium or of intact macrophages

Macrophages were made to adhere to [³H]elastin-coated plastic culture chambers, and incubated in medium containing various proteinase inhibitors at concentrations shown in the Table. Elastase-rich, cell-free conditioned medium was mixed (1:1) with medium containing the various inhibitors and [³H]elastin. After 24 h, aliquots of the media were centrifuged at 10000g and then assayed for degraded elastin by liquid-scintillation counting. Trypan Blue staining of intact cells at the end of the assays showed >90% of cells to be viable at the concentrations of inhibitors shown. Data are expressed as elastolytic or cathepsin B activity of cells, cell lysates, or cell-free media following culture with each inhibitor as a percentage of that observed following culture in medium without inhibitors. Data represent means of at least three experiments for each inhibitor tested.

Proteinase inhibitor	Final concn.	Elastolytic activity (%) in:		Cathepsin B activity (%) in cellular lysate
		Cell-free medium	Intact cells	
None	–	100	100	100
Foetal bovine serum	1%	9	108	105
	10%	7	135	119
Human serum	1%	25	369	106
Soya bean trypsin inhibitor	500 μ g/ml	105	105	67
Trasylol	100 units/ml	100	82	100
EDTA	0.1 mM	20	43	87
	1.0 mM	6	42	83
Phenylmethanesulphonyl fluoride	0.1 mM	97	89	100
	0.5 mM	89	82	100
Msc-Ala-Ala-Pro-Ala-CH ₂ Cl	0.1 mM	98	62	1
	0.5 mM	97	40	0
Z-Phe-Ala-CHN ₂	10 μ M	77	55	3
Chloroquine	25 μ M	95	80	18
	50 μ M	85	69	0

presence of the various proteinase inhibitors is expressed as the percentage of activity of elastase-rich medium or of intact cells incubated without inhibitors. The soluble enzyme activity was nearly completely blocked by 1 mM-EDTA but not by the neutrophil elastase inhibitors Msc-Ala-Ala-Pro-Ala-CH₂Cl or soya bean trypsin inhibitor. This indicates that the elastolytic activity of macrophage-conditioned medium is due to an elastase typical of macrophage-derived, but not neutrophil-derived, elastase. Differences are apparent in the inhibitor profile of intact cells. First, concentrations of serum proteinase inhibitors that inhibited over 90% of the soluble activity had no inhibitory effect on elastinolysis by intact cells. The enhancement over baseline with cells cultured in human serum is examined further below. Second, EDTA had much less inhibitory effect on intact-cell elastase activity than on cell-derived elastase activity. Third, the cysteine proteinase inhibitor Z-Phe-Ala-CHN₂ consistently suppressed intact cell elastinolysis (55% of the activity of cells cultured without Z-Phe-Ala-CHN₂) more than that of elastase-rich medium.

Because of the suppressive effects of Z-Phe-Ala-CHN₂ on intact cell elastinolysis, we directly measured the effect of all the potential inhibitors tested on lysosomal cysteine proteinase activity. We used the cathepsin B substrate Z-Ala-Arg-Arg-AMC. Lysates of thioglycollate-induced macrophages cultured in serum-free DMM had easily measurable cathepsin B activity. The activity was proportional to enzyme concentration over 3-fold dilutions of cellular lysate and inhibitable by 10 μ M-Z-Phe-Ala-CHN₂. As Table 1 shows, both the chloromethylketone (Msc-Ala-Ala-Pro-Ala-CH₂Cl) and the diazomethylketone (Z-Phe-Ala-CHN₂) completely suppressed cathepsin B activity following 18 h culture of macrophages in these reagents. Chloroquine also suppressed intracellular cathepsin B activity. Indeed, all of the reagents which markedly suppressed intracellular cathepsin B also suppressed elastolytic activity. Of the reagents which had no effect on intracellular cathepsin B, only EDTA suppressed elastolytic activity of live cells. These findings indicate that in serum-free conditions, live macrophage elastinolysis is dependent both on a metalloproteinase and a cysteine proteinase(s).

Table 1 also shows much greater elastinolysis by murine macrophages in the presence of human serum than by cells cultured in serum-free medium. This cannot be explained by changes in lysosomal cysteine proteinase activity, at least as judged by the cathepsin B assay. We have thus examined the influence of serum in more detail. Macrophages cultured in elastin-coated chambers hydrolysed the elastin in a time-dependent manner (Fig. 1). As

Fig. 1. Degradation of insoluble [³H]elastin by intact macrophages

Thioglycollate-induced macrophages were cultured in basic tissue culture medium supplemented with 1% dog serum (●), 1% plasminogen-free dog serum (○), 1% plasminogen-free dog serum + human plasminogen (8 μ g/ml) (■), or 1% dog serum + cycloheximide (5 μ g/ml) (▲). An equivalent number of cells lysed in DMM + 0.2% Triton X-100 containing 1% dog serum (Δ) were also incubated for 24–72 h. Data are expressed as μ g of elastin degraded (mean of triplicate determinations).

expected, the extent of lysis was dependent on the culture medium. Cells cultured in 1% plasminogen-free dog serum hydrolysed 5.5 μ g of elastin in 72 h, whereas cells cultured in 1% dog serum hydrolysed over 26 μ g of elastin in 72 h. Plasmin alone had no measurable effect on purified insoluble elastin. The reconstitution of plasminogen-free serum with human plasminogen (8 μ g/ml) restored 70% of the activity observed in 1% dog serum. In addition, progressive elastinolysis depended on the presence of intact cells capable of protein synthesis. Less than 1.5 μ g of elastin was degraded in either lysed cells or intact cells in which protein synthesis was suppressed by cycloheximide (5 μ g/ml).

Because of the striking increase in elastin solubilization by cells cultured in medium contain-

ing plasminogen, we determined whether the proteolytic activity of plasmin mediated the enhancing effect of plasminogen on elastin solubilization. Thioglycollate-induced macrophages are known to express a plasminogen activator and activate plasmin under the culture conditions employed (Chapman *et al.*, 1982; Unkeless *et al.*, 1974). Plasminogen activator is inhibited by NPGB with approx. 50% inhibition occurring at 10^{-8} M-NPGB (Unkeless *et al.*, 1974). NPGB blocks a number of other serine proteinases, including plasmin, but does not inhibit macrophage elastase, a metalloproteinase. Fig. 2 shows that the addition of purified plasminogen to cultures of intact macrophages or of plasmin to elastase-rich cell-conditioned medium in [^{3} H]-elastin-coated chambers increased the elastin degraded at least 2-fold in a 24 h assay. The addition of NPGB, at 10^{-5} M, completely neutralized the enhancing effect of plasminogen or plasmin on macrophage elastolytic activity. Plasmin or plasminogen added to the cell cultures did not change the intracellular cathepsin B activity, measured as described above (results not shown). In additional experiments we observed that

EDTA, an inhibitor of macrophage elastase but not of plasmin, also abrogated the enhancing effect of plasmin on macrophage-conditioned-medium-mediated elastinolysis (results not shown). These results indicate that the proteolytic activity of both elastase(s) and plasmin is required for plasmin enhancement of macrophage elastase activity.

Evidence for activation of latent macrophage elastase

We considered the mechanism by which plasmin enhances macrophage elastase activity. Two major possibilities seemed most likely. First, plasmin could be directly elastolytic in the presence of a true elastase. This mechanism would be similar to reported observations that cathepsin G is elastolytic in the presence of neutrophil elastase (Boudier *et al.*, 1981). However, as stated above, at least 75% of the total lysine residues of mature elastin are oxidized by lysyl oxidase. The P₁ site of plasmin has highest affinity for lysine residues. A second mechanism is the activation of latent macrophage elastase activity by plasmin. Banda & Werb (1981) reported evidence that as much as 80% of elastase activity secreted by thioglycollate-induced murine macrophages is latent and acti-

Fig. 2. Effect of plasminogen on elastinolysis by live macrophages or cell-free conditioned medium

In (a) thioglycollate-induced macrophages were cultured in [^{3} H]elastin-coated culture wells in tissue culture medium containing 1% (v/v) acid-treated, plasminogen-free dog serum. To some cultures purified plasminogen (8 μ g/ml final concentration) or NPGB (10^{-5} M) were added. In (b) elastase-rich cell-free conditioned medium (100 μ l) was incubated with [^{3} H]elastin suspended in PBS (100 μ l) and either DMM (100 μ l) or various concentrations of purified plasmin as indicated in the Figure. After 24 h at 37°C, elastin degradation was measured. Data are expressed as mean ($n = 3$) μ g of elastin degraded/24 h for each concentration of plasminogen or plasmin.

vated in the presence of SDS. However, they did not observe activation of latent activity by plasmin. To test these two possibilities, we neutralized plasmin with NPGB at various times from 5 to 120 min after mixing purified plasmin with elastase-rich cell-conditioned medium at 37°C. NPGB did not neutralize macrophage-derived elastase activity. After neutralizing plasmin, we incubated the medium with [^{3} H]elastin for an additional 20–24 h at 37°C and then measured the extent of elastin degradation. Plasmin preincubated with cell-conditioned medium from 5 to 60 min before neutralization did not cause a subsequent increase in elastase activity (Fig. 3). If anything, there was slightly less elastase activity. However, when we mixed plasmin with cell-conditioned medium in the presence of elastin, a time-dependent increase in elastase activity occurred. By 60 min at 37°C, there was maximal enhancement, and neutralization of plasmin with NPGB at that time did not abrogate the enhancing effect measured at 24 h.

Fig. 3. Evidence of elastin-dependent activation of latent elastase activity by plasmin

Elastase-rich conditioned medium was incubated with plasmin (2.5 μ g/ml) at 37°C either in plastic culture tubes or in [^{3} H]elastin-coated culture wells. After various times as indicated in the Figure NPGB (10^{-5} M) was added. The elastolytic activity of conditioned medium (μ g of elastin/24 h) in the absence of added plasmin is designated 1. The stimulatory effect of preincubation (10–60 min) with plasmin in the presence (●) or absence (○) of elastin is expressed as the ratio of activity with added plasmin to that without plasmin. In some cases EDTA (0.1 mM) and not NPGB was added to the reaction mixture preincubated with elastin (▲).

The increased elastase activity could not be accounted for by elastinolysis simply during the 60 min preincubation before plasmin is neutralized. The increased elastinolysis required continued elastase activity, as judged by the observation that EDTA blocked over 80% of plasmin-enhanced elastinolysis even when the EDTA was added after the 60 min incubation (Fig. 3). Moreover, no measurable degradation of elastin occurred during the first 60 min of the assay (results not shown).

The enhancing effect of plasmin also did not occur if plasmin was incubated with elastin and neutralized by NPGB before the cell-conditioned medium containing elastase was added. We considered the possibility that plasmin could not be neutralized by NPGB once bound to elastin. However, this is unlikely as plasmin added to fibrin-coated chambers was completely neutralized by 10^{-5} M-NPGB. Together these observations suggest that plasmin enhances macrophage elastolytic activity not by directly modifying elastin but by activating a latent elastase. Activation appears to occur only in the presence of elastin. We cannot exclude the possibility that plasmin also has some direct but unmeasured effect on elastin that facilitates elastase activity.

To test further whether plasmin activates a latent elastase or participates directly in the degradation of elastin, we determined whether plasmin would enhance elastin degradation by other elastases. In these experiments, we utilized several sources of soluble elastase activity: cell-free macrophage-conditioned medium, purified porcine pancreatic elastase, *Pseudomonas aeruginosa* conditioned broth and lysates of human neutrophils (Werb *et al.*, 1982). The concentration of each of these reagents was adjusted to an elastolytic activity equivalent to that of macrophage-conditioned medium. In five separate experiments, plasmin (2.3 μ g/ml) enhanced elastolytic activity of cell-free macrophage-conditioned medium as described above (Fig. 2). Plasmin did not enhance the elastolytic activity of neutrophil cell lysates, *Pseudomonas aeruginosa* conditioned broth, or purified pancreatic elastase.

Discussion

In this report we describe an assay of macrophage elastase activity utilizing intact cells and purified, detergent-free insoluble elastin. We use this assay to compare directly the process of elastinolysis by soluble macrophage-derived elastase with that of live cells. Compared with cell-derived elastase-rich medium, the intact cell is less sensitive to natural proteinase inhibitors in the cellular microenvironment (Table 1). This relative

insensitivity to serum proteinase inhibitors can be explained in several ways. Elastase secreted by macrophages in close contact with elastin may bind elastin and thus become resistant to soluble proteinase inhibitors such as α_2 -macroglobulin. There is precedent for this hypothesis. Neutrophil elastase is resistant to α_1 -antiproteinase after binding elastin, and plasmin is resistant to α_2 -antiplasmin after binding fibrin (Reilly & Travis, 1980; Wiman & Collen, 1978). Indeed, we observed that elastin degradation by cells in contact with elastin was 10–30-fold higher than degradation by cells in the same culture wells but physically separate from the elastin (H. A. Chapman, Jr. & O. L. Stone, unpublished work). Werb *et al.* (1980b) also observed that contact promoted macrophage degradation of extracellular matrix proteins. Part of the degradation may also occur inside the cell. We observed a correlation between inhibition of intracellular cathepsin B activity and inhibition of live cell elastinolysis (Table 1). The active site inhibitors of cathepsin B (diazomethyl- and chloromethylketones) inhibited 100% of cathepsin B activity and 60% of elastolytic activity. In contrast, chloroquine consistently suppressed intracellular cathepsin B to a much greater extent than it did elastinolysis (100% versus 30%). This suggests that some of the elastinolysis mediated by cathepsin B (and insensitive to serum proteinase inhibitors) may be expressed as the cell surface. Chloroquine stimulates release of macrophage lysosomal enzymes (Riches *et al.*, 1981). The pH at the surface of adherent macrophages is reportedly acidic, which would allow active enzyme activity at the cell–elastin interface (Etherington, 1980). This interpretation is supported by our inability to block more than 35% of total elastin degradation with an inhibitor of phagocytosis, cytochalasin B (10^{-5} M) (results not shown).

Werb *et al.* (1980a) and Jones & Werb (1980) have previously examined the degradation of metabolically labelled extracellular matrices rich in elastin, glycoprotein, and collagen by intact thioglycollate-induced macrophages cultured on the matrices. They measured elastin degradation by the cells as the difference between [^{3} H]proline released by macrophages and that subsequently released by a purified elastase. They observed that plasminogen accelerated elastin degradation by macrophages but only if the matrices contained glycoprotein. Pretreatment of the matrix with trypsin negated the enhancing effect of plasminogen. They concluded that the only role of plasminogen was to eliminate matrix protein that interfered with the interaction of macrophage elastase with the matrix elastin. Our observations indicate that plasmin is a critical determinant of insoluble elastin degradation by live macrophages even with

a purified elastin substrate (Figs. 1 and 2). Several differences between the previous study and our investigation are apparent. Although the amino acid composition of elastin from various tissues and species are very similar, the three-dimensional structure of elastin purified from ligamentum nuchae and native elastin in an extracellular matrix could be different. The elastin we used had undergone a purification procedure that could conceivably have promoted the observed synergy between plasmin and macrophage elastase. In addition, in the previous investigation, a role for plasmin in solubilization of elastin by macrophages was excluded by neutralization of plasmin with soya bean trypsin inhibitor or α_1 -antiproteinase following the pretreatment of matrices with plasmin. However, plasmin once bound to substrate (in this case glycoprotein) may be resistant to subsequent addition of macromolecular proteinase inhibitors (Wiman & Collen, 1978). Carryover of substrate-bound enzyme into the macrophage culture might then explain the lack of effect of added plasminogen to the matrices. Further studies are needed to determine if our results with ligamentum nuchae elastin apply to nascent extracellular matrix elastin.

The mechanisms of plasmin enhancement of elastase activity are complicated. For elastin deposited within an extracellular matrix, Werb *et al.* (1980a,b) have suggested that plasmin may act to allow access of elastase to elastin. We have also observed a similar phenomenon. Human alveolar macrophages cultured on matrices containing a mixture of fibrin and elastin only degrade the elastin in the presence of plasminogen (Chapman *et al.*, 1984). There may also be a more direct relationship between plasmin and murine macrophage elastase activity. Banda & Werb (1981) reported that macrophages secrete up to 80% of their elastase in a latent form that can be activated by SDS. Data reported here suggest that plasmin also activates latent macrophage elastase activity in the presence of elastin (Fig. 3). This conclusion is supported by our observations that plasmin only enhanced murine macrophage elastase activity and not that of pancreatic, neutrophil or *Pseudomonas*-derived elastase. Plasmin also has much less enhancing effect on human macrophage elastolytic activity, consistent with the reported observations that human macrophages, unlike the murine cells, release very little soluble elastase (Chapman *et al.*, 1984). Previously reported data suggest that plasminogen activator and plasminogen may be involved in the activation of another neutral proteinase, latent collagenase (Werb *et al.*, 1977; Eeckhout & Vaes, 1977). However, proof that plasmin also activates a latent elastase awaits isolation of the latent form of the enzyme.

The data reported in this study indicate that elastin degradation by live macrophages is a co-operative process. Although a number of enzymes are implicated, plasmin and elastase combined are especially effective in the degradation of purified, insoluble elastin. Because elastin is part of a number of structures through which macrophages migrate, e.g., alveolar interstitium and the dermis, the co-operation between plasmin and elastase may facilitate movement of cells within the normal interstitium or within sites of inflammation. In addition, as our data indicate, serum or serum-derived tissue proteinase inhibitors would focus the plasminogen activator and elastase activities near the cell surface. Free elastase in solution is quite sensitive to serum proteinase inhibitors, whereas elastase activity elaborated by cells in contact with elastin was not (Table 1). We have reported similar findings with macrophage plasminogen activator and fibrinolysis (Chapman *et al.*, 1982). Thus, the movement of macrophages in tissues, although dependent on proteolysis, occurs without widespread tissue destruction.

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