Synthesis and processing of cathepsin L, an elastase, by human alveolar macrophages

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Cathepsin L was partially purified from lysates of freshly isolated macrophages lavaged from lungs of apparently healthy adults and found to be chromatographically and catalytically identical with liver cathepsin L. Western-blotting analysis showed that lung macrophages contain significant levels of a precursor of cathepsin L (43 kDa) in addition to mature enzyme (25 kDa). After culturing for a further 24 h, the precursor disappeared and a new band, corresponding to 34 kDa, appeared, suggesting that the precursor had been processed to an intermediate form of cathepsin L. Biosynthetic labelling of macrophages *in vitro* with [³⁵S]methionine followed by immunoprecipitation with the cathepsin L antibody confirmed that the cells synthesize cathepsin L as a 43 kDa precursor that is then processed to the mature form (25 kDa) via a 34 kDa intermediate. The precursor, but not the processed forms, was released into the culture medium. During culture *in vitro* the 34 kDa intermediate accumulated, and little enzyme was processed to the 24 kDa form, consistent with the immunoblot data. Human lung macrophages contain a 1.5 kb transcript of cathepsin L mRNA, whereas none is detectable in human monocytes. These results establish that differentiation of human macrophages within the lung is accompanied by synthesis and expression of an elastolytic enzyme, cathepsin L. The altered processing of cathepsin L observed during cultivation *in vitro* suggests caution in the assessment of the elastolytic potential of human macrophages based on assay *in vitro*.

INTRODUCTION

Recent studies have established that human macrophages can degrade elastin and have demonstrated several characteristics of the process that differ markedly from the elastinolysis mediated by human neutrophils or rodent macrophages [1,2]. Rather than producing a soluble neutral elastase, human macrophages degrade elastin by a process that requires cell–substrate contact and involves one or more acidic cysteine proteinases, in co-operation with the neutral proteinases plasminogen activator and plasmin [2–5]. The direct solubilization of elastin is dependent upon acidic cysteine proteinases, implying that a local acidic environment is required. Recent studies of macrophages and osteoclasts suggest that these cells have the capability of generating such an environment [6,7].

Human macrophages are known to synthesize and express at least one enzyme of the cysteine proteinase class, namely cathepsin B [8]. We recently examined the elastolytic activity of cathepsin B as well as that of cathepsin L purified from human liver and found that, whereas cathepsin B was not elastolytic, cathepsin L was nearly as elastolytic as purified pancreatic elastase, albeit at a different pH optimum [9]. To what extent macrophages express cathepsin L is unclear. Some investigators have found immunoreactive protein consistent with cathepsin L in rat macrophages, and other

investigators have not [10,11]. Recently reported data that a cDNA clone for a cysteine proteinase originally found in transformed mouse fibroblasts but now also in mouse peritoneal macrophages shows similarity to the N-terminal sequence for human liver cathepsin L suggests that mouse macrophages synthesize cathepsin L [12–14]. The mouse fibroblast protein has also been shown to be catalytically identical with cathepsin L [15]. However, no data have been published with respect to the possible existence of cathepsin L in human macrophages. In view of the known important differences in expression of elastolytic enzymes between murine and human macrophages, we examined the synthesis and processing of cathepsin L by human macrophages.

EXPERIMENTAL

Cell preparation

Healthy community volunteers underwent, with informed consent, transbronchoscopic lavage as previously described [1]. Volunteers were apparently healthy young individuals, including both smokers (one or two packs/day) and non-smokers, and the mean total and differential cell counts were similar to those previously reported. The basic tissue-culture medium used to wash and culture cells was Dulbecco's modified Eagle's medium supplemented with 20 mM-Hepes, streptomycin (100 µg/ml)

Abbreviations used: Z-Phe-Arg-MHMec, benzyloxycarbonylphenylalanylarginine 7-(4-methyl)coumarylamide; Z-Phe-Phe-CHN₂, benzyloxycarbonylphenylalanylphenylalanyldiazomethane.

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and penicillin (100 units/ml). The pooled cellular pellets were counted with a haemocytometer, washed, and either lysed for chromatographic purification of cathepsin L or used intact for pulse-chase experiments.

Enzyme assays

Cathepsin L activity was determined by measuring cleavage of the synthetic fluorogenic substrate benzyl-oxycarbonylphenylalaninylarginine 7-(4-methyl)coumarylamide (Z-Phe-Arg-NHMec; Bachem, Torrance, CA, U.S.A.). These assays were performed, and fluorescence measured in, a 110 S Perkin-Elmer fluorescence spectrophotometer as previously described [5]. Assays were routinely performed in the presence and absence of benzylloxycarbonylphenylalanylphenylalanyldiazomethane (Z-Phe-Phe-CHN₂) (10 μ M final concn.). Data are expressed as fluorescence units inhibited by the diazomethane.

Partial purification of human macrophage cathepsin L

Cellular lysates were prepared for molecule-sieve and ion-exchange chromatography as described by Mason *et al.* [16], with some modifications. Briefly, macrophages were lysed in 1% Triton X-100/20 mM-acetate/1 mM-EDTA, pH 4.2, for 4 h at 37 °C, followed by overnight incubation at 4 °C. Lysates of 25–30 million macrophages were clarified by centrifugation and were then fractionated on a 2.5 cm $\times$ 70 cm column of Sephadex G-75 equilibrated in 20 mM-acetate, pH 5.5, containing 1 mM-EDTA and 0.05% Triton X-100. Fractions containing cathepsin L activity were further chromatographed on a Mono S cation-exchange column (Pharmacia, Piscataway, NJ, U.S.A.). The column was equilibrated in 20 mM-acetate, containing 1 mM-EDTA, and eluted with a gradient of NaCl (0–500 mM) generated by Waters 510 h.p.l.c. pumps and controller. Portions of 2 ml fractions from this column were assayed for cathepsin L activity and immunoreactivity.

Immunoprecipitation, Western blotting and gel electrophoresis

Labelling, immunoprecipitation and autoradiography were carried out as described previously [17], with some modifications. Briefly, 10⁷ alveolar macrophages/25 cm² flask were labelled in 5 ml of methionine-free tissue-culture medium containing bovine serum albumin (1 mg/ml) and [³⁵S]methionine (100 μ Ci/ml) for 2–6 h at 37 °C. Overnight labelling experiments were done as outlined above, with the addition of 10% (v/v) of media containing methionine. The cell monolayers were then washed and lysed in 1% Triton X-100 in phosphate-buffered saline (6.4 mM-sodium phosphate/0.136 M-NaCl, pH 7.4) containing 10 mM-EDTA, 2 mM-phenylmethanesulphonyl fluoride and Trasylol (100 units/ml). The lysates were subjected to three cycles of freeze-thawing and then clarified by centrifugation. In some experiments the pellet fraction containing membranous fractions of the cell lysate was resuspended in 0.2% SDS in phosphate-buffered saline, pH 7.4, and immunoprecipitated. All immunoprecipitations were done after clarification with Protein A-Sepharose (Pharmacia) and 15 μ l of normal rabbit serum to minimize non-specific binding. The immunoprecipitations consisted of a volume of supernatant, lysate or membrane pellets from labelled cells containing approx. 10⁷ c.p.m. in 0.2% SDS and 1% Triton X-100 suspended in the lysis buffer. After 2 h

incubation at room temperature with 15 μ l of antibody, 6.25 mg of Protein A-Sepharose was added to each sample, and the beads were subsequently washed and boiled to elute bound complexes and electrophoresed in SDS/10%-(w/v)-polyacrylamide gels. Fractions of cellular lysates after molecular-sieve chromatography were prepared for immunoblotting by freeze-drying. Lysates of whole cells were immunoblotted after lysis in 1% Triton X-100 in phosphate-buffered saline, pH 7.4, and extraction of cellular protein as described in [18]. The concentrated material was resuspended in sample buffer and loaded on to SDS/10%-(w/v)-polyacrylamide gels. The electrophoresed proteins were then transferred to nitrocellulose in a Bio-Rad Transblot apparatus using buffers as described by Burnette [19]. Non-fat dry milk (5%, w/v) was used subsequently to block non-specific protein adherence during the antibody staining. Nitrocellulose containing the transferred proteins was stained with either a previously characterized rabbit cathepsin L antibody [14,16] or with a sheep anti-(cathepsin B) antibody (kindly given by Dr. David Burnett, Department of Medicine, University of Birmingham, Birmingham, U.K.). In the case of the rabbit antibody, the staining was revealed by autoradiography after application of a ¹²⁵I-labelled goat antibody against rabbit IgG and extensive washing. In the case of the sheep antibody, visualization was by peroxidase-coupled goat antibody against sheep IgG.

Northern blotting

Total cellular RNA was extracted from purified blood monocytes or alveolar macrophages by the guanidinium isothiocyanate/CsCl method [20]. RNA was electrophoresed (15 μ g/lane) in formaldehyde/agarose gels and transferred to nitrocellulose [21]. The cDNA probe used was an 800-bp cDNA of cathepsin L isolated from a human kidney cDNA library and subcloned in plasmid PUC13 [22]. The filters were baked, prehybridized, and hybridized with the ³²P-labelled nick-translated cDNA as described by Fertsch *et al.* [23]. The filters were washed and exposed to Kodak XR-5 film at –70 °C for 24–48 h.

RESULTS

Partial purification of human macrophage cathepsin L

A total of 25–30 million macrophages were lysed and subjected to molecular sieving through Sephadex G-75. The eluted fractions were assayed for cathepsin L activity. A broad peak of activity against Z-Phe-Arg-NHMec was found in the fractions corresponding to the 15–30 kDa range. This activity was blocked by a 10 μ M final concentration of Z-Phe-Phe-CHN₂. We also determined the immunochemical distribution of cathepsins B and L in the various fractions from this column. The technique of immunoblotting was used to assess the distribution of antigen in the column fractions. Cathepsin L antigen was detected in the fractions comprising the first part (25–30 kDa) of the peak of activity. The specificity of this immunoblotting technique was confirmed by performing blocking experiments. A representative immunoblot of a Sephadex G-75 fraction containing cathepsin L antigen is shown in Fig. 1(b). The major immunoreactive band was 25 kDa under reducing conditions in the immunoblot. This is identical with the reported heavy-chain size of cathepsin L purified from human liver [16]. Cathepsin

Fig. 1. Isolation of macrophage cathepsin L

Fractions of the sizing chromatography found to be rich in cathepsin L antigen were applied to a cation-exchange column (Mono S). (a) Elution (linear salt gradient) pattern as judged by activity against Z-Phe-Arg-NHMec at pH 5.5. Eluted material was diluted into enzyme assay buffer directly without further manipulation. (b) Immunoblots of a representative Sephadex G-75 fraction and of the two peaks of enzyme activity separated by h.p.l.c., as shown in (a). There was no cathepsin B immunoreactivity in h.p.l.c. fraction 14.

B antigen was observed overlapping with the elution of cathepsin L in the apparent 20–30 kDa region of the fractionation. Similar results were found in each of four separate determinations. To separate cathepsins B and L, portions of the Sephadex G-75 fractions containing both proteins (196–224 ml from the Sephadex G-75 eluate) were further subjected to cation-exchange chromatography. Enzyme activity after ion-exchange chromatography was measured at pH 5.5 with the fluorogenic substrate Z-Phe-Arg-NHMec. Three peaks of enzyme activity were eluted from the cation-exchange column. The fraction passing unimpeded through the column probably represents unbound cathepsin B. Two peaks were eluted during the salt gradient: one at approx. 125 mM-NaCl and a second at approx. 380 mM-NaCl. These are the typical positions of cathepsins B and L respectively. The specific cysteine proteinase inhibitor Z-Phe-Phe-CHN₂ blocked all enzyme activity. Immunoblotting of fractions 5 and 14 for both cathepsin L and cathepsin B demonstrated that fraction 5 contained only cathepsin B and fraction 14 contained only cathepsin L (Fig. 1b). That this isolated enzyme is actually cathepsin L was confirmed by performing blocking experiments with the immunoblotting (results not shown). Limited availability of cells prevented further purification of this enzyme.

Western blotting of human alveolar macrophages

The experiments described above show that human macrophages contain an active enzyme of the cysteine-proteinase class with physical, catalytic and immunochemical properties identical with those reported for cathepsin L purified from human liver. The method used to isolate cathepsin L required an autolytic step to release active enzyme. To determine whether this procedure caused any significant proteolysis of cathepsin L, we analysed non-autolysed extracts of freshly isolated macrophages by Western blotting. Immunoreactivity

with antibody raised to purified human liver cathepsin L showed that most of the cathepsin L in human macrophages had the same molecular mass as the purified enzyme, indicating that the purification procedure had not significantly degraded the enzyme (Fig. 2, lane 1). An additional band of 43 kDa was also seen, which probably represents a precursor of cathepsin L. In contrast, after 24 h culture, there is a marked loss of the 43 kDa form

Fig. 2. Molecular forms of steady-state cathepsin L antigen as a function of time in culture

Lysates of alveolar macrophages (10^7 cells/lane) were prepared after initial adherence (lane 1) or after 24 h culture (lane 2) and then immunoblotted and stained for cathepsin L as described in the text.

Fig. 3. Northern blotting of human monocyte and alveolar-macrophage RNA

Total cellular RNA from freshly isolated blood monocytes (lane 1) or alveolar macrophages (lane 2) was electrophoresed, transferred to nitrocellulose and hybridized with a cDNA to human cathepsin L (right-hand panel). The size of the hybridizing mRNA was estimated by comparison with a RNA ladder run concurrently (not shown). The panel on the left illustrates a photograph under u.v. illumination of the total RNA after electrophoresis and indicates that similar amounts of total RNA from both monocytes and macrophages were analysed.

(Fig. 2, lane 2). The 25 kDa form seems to persist at 24 h, in spite of the fact that there is no detectable precursor and clear accumulation of a 34 kDa intermediate. Accumulation of the 34 kDa intermediate during 24 h culture *in vitro* was observed in three separate experiments. In one of these, the amount of the 25 kDa form was markedly less (results not shown).

Analysis of cathepsin L mRNA in blood monocytes and alveolar macrophages

Because macrophage cathepsin L could possibly be accounted for by uptake of extracellular enzyme rather than biosynthesis, we examined gene expression in freshly isolated alveolar macrophages. Recently reported data show that cellular cathepsin L mRNA is 1.5 kb in length in stimulated blood lymphocytes and breast adenocarcinoma cells [22]. In addition, kidney tissue RNA has a ~4-kb mRNA hybridizing with cDNA of cathepsin L. Northern blots of equivalent amounts of total cellular RNA from freshly isolated blood monocytes and alveolar macrophages were hybridized with a cathepsin L cDNA. As indicated in Fig. 3, alveolar macrophages contain only the 1.5-kb mRNA, whereas monocytes have no detectable cathepsin L mRNA. The left-hand panel in Fig. 3 depicts the electrophoresed total cellular RNA showing equivalent 18 S and 28 S RNA in the two lanes.

Synthesis of cathepsin L by human alveolar macrophages *in vitro*

Freshly lavaged macrophages were allowed to adhere and then immediately labelled for 2 h with [³⁵S]-methionine. Culture medium and lysates were then immunoprecipitated and the recovered antigens revealed

Fig. 4. Immunoprecipitation of human alveolar macrophages after a 2 h label with [³⁵S]methionine

Freshly isolated macrophages were allowed to adhere and then immediately labelled for 2 h in methionine-free medium. Membrane pellets (lanes 1 and 2) or culture media (lanes 3 and 4) were then immunoprecipitated with either cathepsin L antibody (lanes 1 and 3) or α_1 -proteinase-inhibitor antibody (lanes 2 and 4) as control. The arrow shows the major labelled band at 43 kDa to be found in the whole lysate, the membrane fraction and the culture medium. Reaction of this protein with the anti-(cathepsin L) antibody is totally blocked by exogenous purified liver cathepsin L. The asterisk (*) shows a 21 kDa doublet in the cell-associated fractions reactive with the cathepsin L antibody. This protein is not blocked by purified liver cathepsin L and is assumed to be a contaminant.

by autoradiography. The autoradiographs in Fig. 4 show the immunoprecipitated proteins from both the cell membrane fraction (lane 1) and the culture medium (lane 3) after a 2 h label. In both cases a polyclonal antibody to α_1 -proteinase inhibitor served as a control (lanes 2 and 4). Longer exposure times were required to demonstrate the presence of α_1 -proteinase inhibitor. In this single experiment two major bands reactive with cathepsin L antibody are shown to be 43 kDa (arrow) and 21 kDa (*) under reducing conditions. The only form released into the culture medium was the 43 kDa protein (lane 3). Blocking experiments with purified cathepsin L (30–50 μ g) only removed the 43 kDa band (result not shown), indicating that the 21 kDa band represents non-specific reaction with the L antibody. Lysosomal enzymes such as cathepsins B and D are known to be synthesized as larger precursor proteins, which are then processed to a smaller, active, enzyme [24–26]. We thus performed pulse-chase experiments to monitor the processing of the 43 kDa band that most likely represents a precursor to the active form of cathepsin L. Data in Fig. 5 show that processing of the cathepsin L precursor (labelled during

Fig. 5. Immunoprecipitation of cathepsin L in human macrophages after pulse-chase labels

Freshly isolated macrophages were labelled for 2 or 24 h as indicated in the Figure. The live cells were then cultured in tissue-culture medium supplemented with 10% fetal-bovine serum for various times as shown ('Chase'). Lysates of the cells were prepared and the cathepsin L antigen identified by immunoprecipitation as described in the text. Notably, no immunoreactive material in the 25 kDa region is identifiable, except after a 24 h chase of overnight-labelled cells. The 21 kDa proteins identified in Fig. 3 were also evident on these immunoprecipitations, but have been 'cropped' from the Figure in order to focus on the specific bands reactive with the cathepsin L antibody. Molecular masses in kDa are indicated on the extreme right and left of the Figure.

a 2 h pulse) occurs very slowly, with no 25 kDa immunoreactive protein visible after 24 h. A 34 kDa protein is visible, however, and progressively increases in time with loss of the 43 kDa form. We have not observed the appearance of the 25 kDa form during labelling periods varying from 2 to 24 h. The autoradiograph in Fig. 5 also shows the relative amounts of the 43 kDa precursor and the 34 kDa intermediate after a 24 h label. Clearly most of the label had accumulated in the 34 kDa form. After an additional 24 h chase, some 25 kDa protein was apparent, although clearly most of the label was still in the 34 kDa form. Additional blocking experiments with purified hepatic cathepsin L showed that both the 34 and the 25 kDa form seen in these experiments completely block (results not shown). Identical biosynthetic experiments with the cathepsin L antibody also showed no synthesis of cathepsin L by freshly isolated blood monocytes.

The disparity between cathepsin L antigen distribution in freshly isolated macrophages, in which the antigen is in either a 43 kDa or 25 kDa form, and the '*in vitro*' biosynthetic data, which demonstrate slow processing and the accumulation of a 34 kDa intermediate, suggests that cathepsin L processing *in vitro* differs from that *in vivo*. The accumulation of increased amounts of the 34 kDa intermediate in the 24 h immunoblots (Fig. 5) also supports this conclusion. Additional studies showed that the altered processing of cathepsin L *in vitro* in tissue-culture medium supplemented with either 10% (w/v) fetal-bovine serum or 10–20% human serum was identical (results not shown).

DISCUSSION

We have shown that human macrophages contain cathepsin L that is essentially identical with the enzyme purified from human liver. It has a molecular mass of 30 kDa and consists of a 25 kDa heavy chain containing the enzyme active site and a 5 kDa light chain linked by a disulphide bridge [16]. Our pulse-chase experiments, done under reducing conditions, show that human macrophages synthesize cathepsin L as a 43 kDa precursor and process the precursor to its fully active 25 kDa form via a 34 kDa intermediate. The 5 kDa light chain was not identified. The 43 kDa precursor form is secreted and some is also membrane-bound. The membrane-bound form could not be removed by either acid treatment or high-salt extraction, suggesting that it is tightly bound. The mechanism by which this form of cathepsin L is membrane-bound is not known. These data are analogous to those obtained in recently reported work examining the biosynthesis of cathepsin B. Cathepsin B is known to be synthesized as a larger precursor with subsequent processing to multiple molecular-mass forms [24]. Moreover, the precursor form of cathepsin B as well as higher molecular-mass forms of a number of lysosomal enzymes are known to be secreted by cells in culture [25,26]. A protein originally described by Gal & Gottesman [12] as a major excreted protein of transformed mouse fibroblasts has been shown to be a cysteine proteinase that is the mouse form of cathepsin L [12,13]. In studies of the biosynthesis and distribution of this enzyme, Portnoy *et al.* [13] described its biosynthesis in mouse peritoneal macrophages. These investigators reported a 36 kDa precursor converted by the cell *in vitro* into a 21 kDa protein via a 28 kDa intermediate. Similar to our observations with human cathepsin L, the 36 kDa precursor is secreted. Thus the pattern of synthesis and processing of cathepsin L is similar in mice and humans, although the actual sizes of the proteins differ somewhat, suggesting species variation.

The observed time course of generation of mature cathepsin L by human macrophages in culture is anomalous with respect to previously reported biosynthetic data for either mouse cathepsin L in macrophages or for lysosomal enzymes in general. Portnoy and colleagues [13] found that thioglycollate-induced mouse macrophages generated a mature 21 kDa cysteine proteinase, most likely mouse cathepsin L, within 2 h of synthesis of the precursor form. Although the rates of processing of precursor to mature forms vary among different lysosomal enzymes, in all reported cases of which we are aware, cells in culture process newly synthesized precursor to a mature enzymically active form within 24 h of synthesis [25,26]. We have not observed any 25 kDa enzyme during 2–6 h labelling periods, and only traces of enzyme during an overnight labelling period. More importantly, both the pulse-chase and extended labelling (24 h) data show that newly synthesized cathepsin L accumulates as the 34 kDa intermediate. That the accumulation of the 34 kDa intermediate *in vitro* represents an acquired processing defect is indicated by immunoblots of extracts of freshly lavaged macrophages, which show the major antigenic forms of cathepsin L to be the 43 kDa precursor and the 25 kDa mature enzyme, with only minor amounts of the 34 kDa intermediate. Immunoblots of extracts of human macrophages after

24 h of culture show near-total loss of the 43 kDa form of cathepsin L compared with fresh cells (Fig. 2). Moreover, increased steady-state levels of the 34 kDa intermediate on the blots are apparent. Altered processing of cathepsin L by alveolar macrophages upon cultivation *in vitro* may be part of a pattern of altered phenotype previously reported to occur with cultivation of these cells *in vitro* [27]. These findings raise cautions with respect to interpretation of functional studies of human alveolar-macrophage proteolytic activities based on assays requiring culture *in vitro*.

Circumstantial evidence suggests that the expression of cathepsin L by human alveolar macrophages is a regulated process. First, the biosynthesis of cathepsin L is not simply a concomitant of differentiation toward mononuclear phagocytes. Monocytes do not express cathepsin L mRNA or protein. Differentiation of the monocyte-like cell line U937 to macrophages by stimulation with phorbol myristate acetate is not accompanied by expression of cathepsin L mRNA, even though other cysteine proteinases, such as cathepsin B, are expressed (J. Reilly & H. Chapman, unpublished work). This suggests that cathepsin L induction may require specific signals that at present remain unknown. Alterations in the steady-state levels of the various forms of cathepsin L upon cultivation of alveolar macrophages *in vitro* also suggest that cathepsin L expression is a regulated, rather than a constitutive, function of macrophages.

The demonstration that human alveolar macrophages express the elastinolytic enzyme cathepsin L suggests that this enzyme contributes, at least in part, and perhaps wholly, to the elastinolytic activity of live macrophages. The fact that the pH optimum of cathepsin L is 5.5 precluded the possibility of attempting to block macrophage-associated elastinolysis with anti-(cathepsin L) antibody, as the antibody will not bind antigen at such an acidic pH. Raising the pH to facilitate such binding results in irreversible inactivation of cathepsin L [9]. Therefore the fraction of macrophage elastinolytic activity accounted for by cathepsin L remains to be defined.

Previously reported observations imply that degradation of insoluble extracellular elastin by live human macrophages can occur extracellularly [2,28]. Results reported here show that, in fact, cathepsin L does traffic to the cell surface, as indicated by the demonstrated release of the 43 kDa precursor *in vitro* (Fig. 4). Previous observations have shown that macrophages and osteoclasts, macrophage-like cells, can acidify the interface between the cell surface and a surface to which the cells are adherent, probably by means of an ATP-dependent proton pump [6,7]. There is evidence that cysteine proteinases are involved in extracellular bone-matrix metabolism by osteoclasts [29]. These observations, viewed together with our data that cathepsin L is made and released by macrophages, provide a plausible pathway by which macrophages can degrade structural proteins such as elastin or collagen [28,30,31] before, or instead of, internalization into a true lysosomal compartment. This may be particularly important in view of the lack of appreciable elastinolytic or collagenolytic activity at neutral pH expressed by human macrophages. This pathway is dependent on multiple regulated steps, including those demonstrated here, such as synthesis and processing of cathepsin L, as well as other potentially regulated steps such as local acidification.

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