Proc. Natl. Acad. Sci. USA
Vol. 92, pp. 3849–3853, April 1995
Medical Sciences

Pericellular mobilization of the tissue-destructive cysteine proteinases, cathepsins B, L, and S, by human monocyte-derived macrophages

VIVEK Y. REDDY, QING-YU ZHANG, AND STEPHEN J. WEISS*

Department of Internal Medicine and Comprehensive Cancer Center, The University of Michigan, Ann Arbor, MI 48109

Communicated by Seymour J. Klebanoff, University of Washington Medical Center, Seattle, WA, December 28, 1994

ABSTRACT **Human macrophages are believed to damage host tissues in chronic inflammatory disease states, but these cells have been reported to express only modest degradative activity *in vitro*. However, while examining the ability of human monocytes to degrade the extracellular matrix component elastin, we identified culture conditions under which the cells matured into a macrophage population that displayed a degradative phenotype hundreds of times more destructive than that previously ascribed to any other cell population. The monocyte-derived macrophages synthesized elastinolytic matrix metalloproteinases (i.e., gelatinase B and matrilysin) as well as cysteine proteinases (i.e., cathepsins B, L, and S), but only the cathepsins were detected in the extracellular milieu as fully processed, mature enzymes by either vital fluorescence or active-site labeling. Consistent with these observations, macrophage-mediated elastinolytic activity was not affected by matrix metalloproteinase inhibitors but could be almost completely abrogated by inhibiting cathepsins L and S. These data demonstrate that human macrophages mobilize cysteine proteinases to arm themselves with a powerful effector mechanism that can participate in the pathophysiologic remodeling of the extracellular matrix.**

In chronic inflammatory disease, monocyte-derived macrophages (MDMs) are postulated to damage host tissues by mobilizing proteolytic enzymes that mediate the pathologic dissolution of the extracellular matrix (1, 2). Interestingly, despite their presumed importance *in vivo* as a destructive cell population, *in vitro* studies indicate that human macrophages display only modest degradative activity via proteolytic processes that remain undefined (2–6). Thus, it has been alternatively proposed that macrophages play a more important, but indirect, role in inflammation by regulating the activity of tissue-destructive accessory cell populations (7, 8). In contrast, we now demonstrate that human monocytes can be induced to undergo a late-onset maturation process in which they express a degradative phenotype hundreds of times more destructive than that previously assigned to other mammalian cell populations. Furthermore, proteolytic activity was linked to the ability of MDMs to secrete fully processed and enzymically active forms of the cysteine proteinases (CPs) cathepsins B, L, and S into the extracellular milieu.

MATERIALS AND METHODS

Elastin Preparation. Elastin (bovine ligamentum nuchae; Elastin Products, St. Louis) was reductively labeled as described (3). Elastin-coated dishes were prepared by drying 2 mg of [^{3}H]elastin onto either one-half or the entire surface of 35-mm bacteriologic plastic Petri dishes (Falcon).

Cell Preparation and Elastinolysis. Adherence-purified human monocytes (9) were cultured atop either [^{3}H]elastin-coated or uncoated dishes in RPMI 1640 medium supplemented with 5–80% autologous human serum or 40% fetal bovine serum (Hyclone) and 100 units of penicillin per ml and 50 units of streptomycin per ml for up to 15 days. Fifteen-day-old MDM cultures (referred to here as MDMs) contained $\approx 5 \times 10^5$ cells per dish with <10% multinucleated cells. Human alveolar macrophages were obtained as described (3). When MDMs were cultured on uncoated dishes, 2–8 mg of [^{3}H]elastin was added as a particulate suspension in the absence or presence of recombinant tissue inhibitor of metalloproteinase 1 or 2 [Synergen (Boulder, CO) and Amgen, respectively], BB-94 (British Biotechnology, Oxford, U.K.), E-64, Z-Phe-Phe-CHN_2 (Z, benzyloxycarbonyl; CHN_2, diazomethane), Z-Phe-Ala-CHN_2, Z-Phe-Ala-CH_2F (CH_2F, fluoromethylketone; all obtained from Enzyme Systems Products, Livermore, CA), Z-Tyr-Ala-CHN_2, Z-[^{125}I]-Tyr-Ala-CHN_2, Z-Phe-Tyr(*o-tert*-butyl)-CHN_2 (gifts of E.N. Shaw, Friedrich Meischer Institute, Basel), or CA-074 (Taisho Biochemicals, Omiya, Japan). All CP inhibitors were added in 0.2% dimethyl sulfoxide, which affected neither CP activity nor elastin degradation. At 24-h intervals, solubilized [^{3}H]elastin was quantitated by β-scintillation counting (3). Results are expressed as means ± SEM.

Northern Blots, Western Blots, and Substrate Gel Electrophoresis. Zymography was performed as described (10) on 24-h serum-free conditioned medium using polyacrylamide gels impregnated with κ-elastin (2 mg/ml) (Elastin Products). For Western blots, samples were separated by SDS/8.5% or 12% PAGE and immunoblotted with rabbit antiserum to either human gelatinase B or matrilysin (gifts of H. G. Welgus, Washington University, St. Louis), rat cathepsin B (gift of J. S. Mort, Shriner's Hospital, Montreal), human cathepsin L (gift of R. W. Mason, Virginia Polytechnic Institute), human cathepsin S (gift of H. A. Chapman, Harvard, or H. Kirschke, Martin Luther University, Halle, Germany), or nonimmune rabbit serum. Human cathepsin B and L standards were obtained from Athens Research & Technology (Athens, GA), while recombinant cathepsin S was provided by H. A. Chapman. For Northern blots, total RNA (10 μg) from mature MDMs was electrophoresed on a 1.2% agarose gel, blotted, and hybridized with ^{32}P-labeled cDNA probes for cathepsin B, L, or S as described (11).

Active-Site Labeling. MDMs were incubated with 0.1 μM Z-[^{125}I]Tyr-Ala-CHN_2 or Z-[I]Tyr-Ala-CHN_2 (12) for 3–24 h, and either membrane-associated proteins (eluted by acid glycine; ref. 13) or aliquots of serum-free conditioned medium were separated by SDS/12% PAGE and visualized by autoradiography. Cell lysis during acid-glycine treatment was <0.1% as defined by the release of [^{35}S]methionine-labeled intracellular enzymes. Z-[^{125}I]Tyr-Ala-CHN_2-labeled proteins

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. §1734 solely to indicate this fact.

Abbreviations: CP, cysteine proteinase; MDM, monocyte-derived macrophage.

*To whom reprint requests should be addressed.

were applied to a MA7S HPLC column (Bio-Rad) and eluted with a linear 0–500 mM NaCl gradient.

Vital Fluorescence Staining for Cathepsin B Activity. MDMs were incubated with 2 mM Z-Ala-Arg-Arg-MNA (MNA, 4-methoxy-2-naphthylamide; Enzyme Systems Products) and 1 mM 5-nitrosalicylaldehyde at pH 7.4 (14). After a 20-min incubation at 25°C, the reaction was stopped with *N*-ethylmaleimide, and selected samples were incubated with either crystal violet or trypan blue (0.5 mg/ml) for 1 min at 25°C (15). Fluorescent conjugates were visualized on a Bio-Rad MRC 500 confocal microscope.

RESULTS

Elastinolytic Potential of Human MDMs. To monitor the proteolytic potential of maturing MDMs, monocytes were cultured on insoluble elastin-coated dishes for 1–15 days. In 10% autologous serum, MDMs displayed little elastinolytic potential until days 10–15 when they degraded ≈150 μg of elastin per 10^6 cells per 24 h (Fig. 1*A*). Once elastinolytic activity was elicited, proteolysis was not further affected by varying the serum concentration from 0% to 80% (unpublished observation).

While the large relative size of the elastin fragments (i.e., 37–140 μm) relative to the MDMs precluded phagocytosis, the cells were tightly adherent to the insoluble substrate (Fig. 1*B*). Indeed, MDM–elastin contact was a prerequisite for elastinolytic activity since proteolysis was completely inhibited (in the absence or presence of serum) when cells and substrate

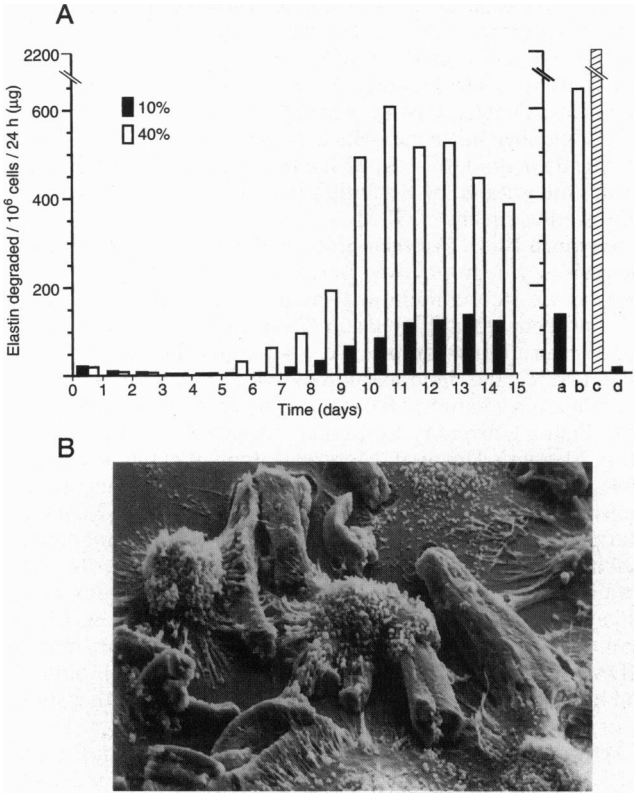

FIG. 1. MDM-mediated elastin degradation. (*A*) MDMs were allowed to mature, either alone (*Right*) or in the presence of 2 mg of [^{3}H]elastin (*Left*) in the indicated concentrations of human serum. Elastinolysis was monitored daily (*Left*) as well as after 2 or 8 mg of [^{3}H]elastin was added to 15-day-old MDM cultures or alveolar macrophages (*Right*). Results are from a single representative study. Bars: a, 10% human serum; b, 40% human serum; c, 40% human serum plus excess elastin; d, alveolar macrophages. (*B*) Fifteen-day-old MDMs were incubated with elastin and visualized by scanning electron microscopy. (×570.)

were plated on opposing sides of the culture dishes ($n = 3$). The expression of the proteolytic phenotype was not, however, dependent on the presence of elastin during the 15-day culture period since comparable activity was detected when (*i*) MDM monolayers were cultured alone and a suspension of elastin was added on day 15 (Fig. 1*A*) or (*ii*) 15-day-old MDMs were cultured alone and reseeded atop elastin-coated dishes (unpublished observation).

Because monocyte differentiation can be affected by serum components (16), the effect of varying the serum concentration or serum source used during the maturation process on degradative activity was determined. At an optimal concentration of 40% serum, the rate of onset and magnitude of elastinolytic activity increased significantly (Fig. 1*A*). [In comparison (and in agreement with previous findings; refs. 2–6), alveolar macrophages cultured in 40% autologous serum for 1–10 days degraded no more than 3.5 μg of elastin per 10^6 cells per 24 h ($n = 2$).] When the elastin load presented to the MDMs was increased from 2 to 8 mg (to determine whether the substrate concentration was limiting), elastinolysis reached a peak level of >2 mg degraded per 10^6 cells per 24 h (Fig. 1*A*). Increasing the size of the elastin fragments to a mean of >140 μm (provided by Elastin Products) did not affect elastinolytic activity (unpublished observation). In paired experiments, MDMs cultured in 40%, 10%, or 5% serum and challenged with 8 mg of elastin degraded 2572 ± 440 μg, 932 ± 46 μg, and 156 ± 48 μg of elastin per 10^6 cells per 24 h, respectively ($n = 4$). In contrast, monocytes cultured for 15 days in 40% fetal bovine serum maximally degraded 198 ± 60 μg per 10^6 cells per 24 h ($n = 3$).

Identification of Elastinolytic Proteinases in MDMs. MDMs have been previously reported to secrete elastinolytic matrix metalloproteinases (2, 17–19). Zymography and Western blot analyses of MDM-conditioned medium demonstrated that the cells secreted gelatinase B and matrilysin (Fig. 2*A*). Significantly, however, both enzymes were routinely recovered in their latent precursor forms as assessed by Western blotting or zymography and the smaller sized enzymically active species (17–19) could not be detected (Fig. 2*A*). Furthermore, the elastinolytic potential of MDMs proved completely insensitive to matrix metalloproteinase inhibitors (17, 20) including TIMP-1 (10 μg/ml), TIMP-2 (10 μg/ml), or 1 μM BB-94 (102% ± 3%, 98% ± 4%, and 96% ± 4% of control, respectively; $n \geq 4$). Similarly, although oxidants and/or serine proteinases have been implicated in other models of leukocyte-mediated proteolysis (21), neither superoxide dismutase (100 μg/ml), catalase (100 μg/ml), N^G-monomethyl-L-arginine (1 mM), α_1-proteinase inhibitor (250 μg/ml), α_1-antichymotrypsin (250 μg/ml), secretory leukoproteinase inhibitor (100 μg/ml), nor aprotinin (100 μg/ml) affected elastinolysis ($n \geq 4$).

In addition to secreting neutral pH optima proteinases, MDMs have previously been reported to secrete lysosomal hydrolases and glycosidases (22). Although these enzymes do not express proteolytic activity, these findings suggested that lysosomal proteinases might be cosecreted. Three members of the lysosomal CP family—cathepsins B, L, and S—express elastinolytic activity under cell-free conditions (23), but the ability of MDMs to synthesize and secrete active forms of these proteinases has not been previously demonstrated. As shown in Fig. 2*B*, Northern blot analysis demonstrated that MDMs expressed ≈2.2- and ≈4.0-kb cathepsin B transcripts (24), an ≈1.7-kb cathepsin L transcript (25), and ≈4.0- and ≈1.7-kb transcripts for cathepsin S (11, 26, 27). Western blots of MDM lysates (Fig. 2*C*) demonstrated that cathepsins B, L, and S could be detected as proform precursors (≈45 kDa, ≈43 kDa, and ≈37 kDa, respectively), which underwent further processing to generate either the mature single-chain enzymes (≈31 kDa, ≈34 kDa, and ≈25 kDa for cathepsins B, L, and S, respectively) or the two-chain, ≈25-kDa forms of cathepsins B and L (for review, see refs. 23–25). Significantly, whereas all

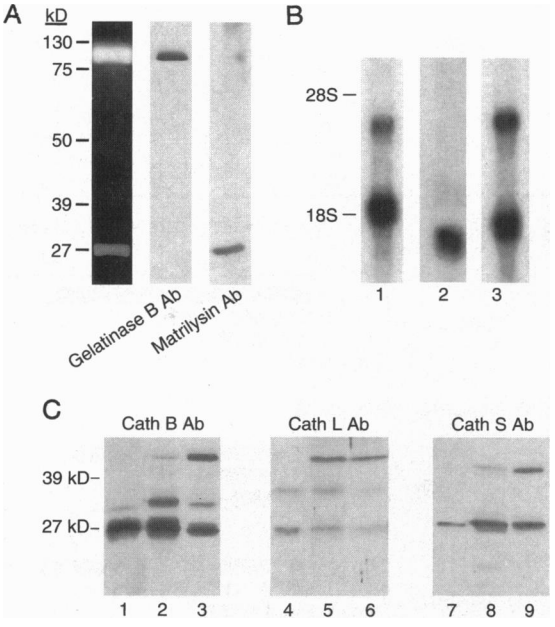

FIG. 2. Secretion of elastinolytic proteinases by MDMs. (*A*) Identification of metalloelastases in MDM-conditioned medium. Two elastinolytic proteinases were detected in MDM supernatants by κ-elastin zymography (lane 1) and identified by Western blotting as gelatinase B and matrilysin (lanes 2 and 3, respectively). (*B*) Northern blots of total RNA from MDMs. The blot was hybridized with cathepsin B (lane 1), L (lane 2), or S (lane 3) cDNA probes. The ≈4.0-kb cathepsin S mRNA has not been previously described, but multiple mRNAs with different 3′ untranslated extensions for other CPs have been reported (24). Monocytes contained barely detectable levels of B, L, or S transcripts (unpublished observation). (*C*) Secretion of mature forms of cathepsins B, L, and S. Western blots of MDM lysates (lanes 2, 5, and 8) and MDM-conditioned medium (lanes 3, 6, and 9) identified proforms as well as mature forms of cathepsins B, L, and S. The mature single- and two-chain forms of purified cathepsins B and L, as well as the single-chain form of cathepsin S, are shown in lanes 1, 4, and 7, respectively.

earlier studies have detected only proforms of cathepsin B or L secreted by macrophages (28–31), MDM cultures secreted both the proforms and active forms of all three CPs into the extracellular milieu (Fig. 2*C*). Pulse–chase analyses demonstrated that the mature forms of the CPs were secreted within 2 h after a 30-min pulse (unpublished observation).

Secretion of Enzymically Active CPs by MDMs. Given that active CPs may be unstable at neutral pH or undergo rapid inhibition by extracellular antiproteinases (23–25), the immunologic detection of processed CPs does not establish enzymic activity. Hence, MDMs were examined by vital fluorescence staining with the cathepsin B substrate Z-Ala-Ala-Arg-MNA (14). Intracellular cathepsin B activity could be directly visualized *in situ* and was distributed in a punctate pattern characteristic of the lysosomal compartment (Fig. 3*A*). In contrast, in MDM–elastin cocultures, large quantities of the fluorescent product also accumulated immediately adjacent to the elastin particles via a process inhibitable by the CP inhibitor Z-Phe-Phe-CHN_2 (Fig. 3 *B* and *D*). The extracellular location of the cathepsin B reaction product was confirmed by the ability of the membrane-impermeant fluorescent quenchers (15), crystal violet (Fig. 3*C*) or trypan blue (unpublished observation), to eliminate elastin-associated fluorescence without affecting the intracellular MDM-associated product.

Because specific fluorescent substrates are available only for cathepsin B, the extracellular distribution of cathepsins L and S was alternatively examined with the active-site probe Z-[^{125}I]Tyr-Ala-CHN_2—an affinity label that specifically reacts with enzymically active CPs (23, 32, 33). When actively degrading MDM cultures were incubated with the probe, at least four radiolabeled proteins could be either eluted from the plasma membrane or recovered in the medium (Fig. 4 *Inset*). After cation-exchange chromatography and Western blotting, five active forms of CPs were identified: the single-chain as well as the two-chain active forms of cathepsins B and L and the single-chain active form of cathepsin S (Fig. 4). Significantly, when cell populations that did not express elastinolytic activity were active-site labeled (e.g., alveolar macrophages, 5-day-old MDMs, endothelial cells, or fibroblasts), CPs were detected only in intracellular compartments (unpublished observation).

Effect of CP Inhibitors on the Elastinolytic Activity of MDMs. To directly establish the role of cathepsins B, L, and S in elastin degradation, MDMs were incubated with structurally dissimilar CP inhibitors (20, 23, 26, 33). The cognate CPs affected in the treated cells were then identified by the consequent loss in Z-[^{125}I]Tyr-Ala-CHN_2 labeling, and the impact on the cell's proteolytic potential was determined. While not altering (*i*) the synthesis, secretion, or activity of secreted gelatinase B or matrilysin; or (*ii*) CP synthesis or secretion (unpublished observation), pan-specific CP inhibitors (i.e., E-64, Z-Phe-Ala-CHN_2, Z-Phe-Phe-CHN_2, Z-[^{125}I]Tyr-Ala-CHN_2, or Z-Phe-Ala-CH_2F) completely abrogated active-site labeling (Fig. 5*A*) and blocked elastin degradation (Fig. 5*B*). When CP inhibitors were removed from MDM cultures, elastinolytic activity returned to control levels within 24 h (unpublished observation). Finally, to determine the relative roles of the CPs in elastinolysis, cells were incubated with CA-074 (20), which specifically inhibited cathepsin B (Fig. 5*A*), or Z-Phe-Tyr[*o-tert*-butyl]-CHN_2 (36, 37), which affected only cathepsin L and S activities (Fig. 5*A*). Significantly, while cathepsin B inhibition did not affect elastinolysis, the com-

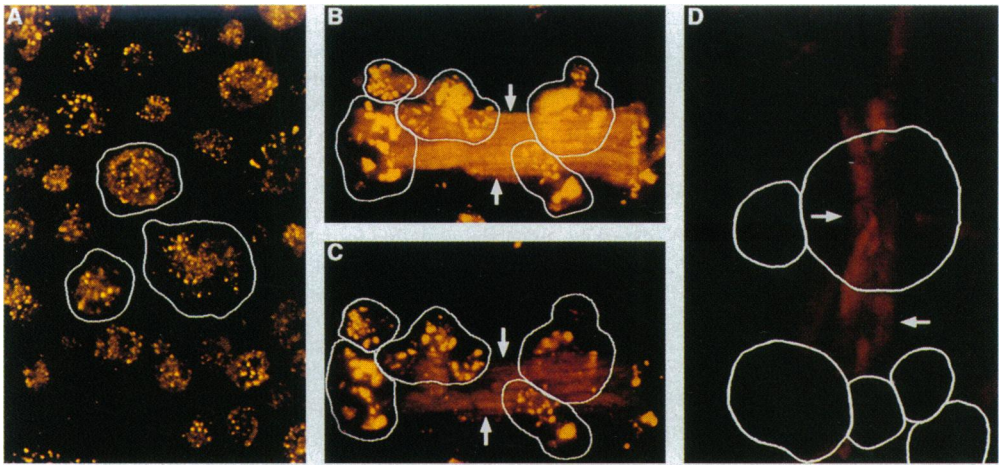

FIG. 3. Vital fluorescence staining for extracellular cathepsin B activity. MDMs (cells outlined by solid white lines) were incubated with either Z-Ala-Arg-Arg-MNA alone (*A*) or after addition of elastin particles (*B–D*; margins of the elastin fragment are indicated by arrows). In *C*, crystal violet was added; in *D*, the cells were preincubated for 30 min with 100 μM Z-Phe-Phe-CHN_2 (23). Quenching agents did not affect the intracellular fluorescence in control MDM cultures.

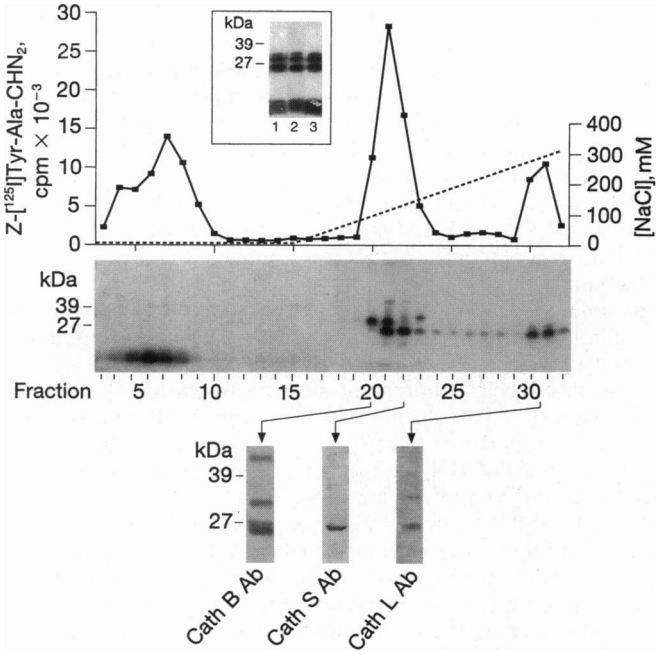

FIG. 4. Active-site labeling of extracellular CPs with Z-[^{125}I]Tyr-Ala-CHN_2. MDMs were probed with Z-[^{125}I]Tyr-Ala-CHN_2, and four radiolabeled bands (≈6, ≈25, ≈31, and ≈34 kDa) were detected by SDS/PAGE (*i*) on the plasma membrane (*Inset*, lane 1), and (*ii*) secreted into the conditioned medium after a 3-h (*Inset*, lane 2), or a 16-h (*Inset*, lane 3) incubation. After HPLC separation of the secreted CPs, the radiolabeled fractions resolved into four fractions characteristic of cathepsins B, L, and S (32). The ≈6-kDa band (fractions 3–8) represents the light chain of the two-chain form of cathepsin B (23, 24), while the ≈31-kDa single-chain form of cathepsin B (23, 24) was detected in fractions 20–21 by Western blotting (Cath B Ab). Because the active site of cathepsin B resides in the ≈6-kDa light chain (23, 24), the ≈25-kDa immunoreactive heavy chain (which can appear as a doublet; ref. 34) does not react with Z-[^{125}I]Tyr-Ala-CHN_2. The 45-kDa band (Cath B Ab) is the proform of cathepsin B (23, 24), a small portion of which may exist in a catalytically active form (ref. 35; seen labeled by the iodinated probe in fraction 21). The ≈25-kDa band was resolved into two fractions (i.e., fractions 21–23 and 30–32) that were identified as the single-chain form of cathepsin S and the heavy chain of the two-chain form of cathepsin L by Western blotting (Cath S Ab and Cath L Ab, respectively). Unlike cathepsin B, cathepsin S exists only as a single-chain species (26, 27), while the active site of cathepsin L is found in the 25-kDa heavy chain of the enzyme (23, 32). The small quantity of single-chain cathepsin L (≈34 kDa) detected in the immunoblot was not labeled with Z-[^{125}I]Tyr-Ala-CHN_2 (fractions 30–32) and may represent catalytically inactive enzyme. Similar results were obtained with the plasma membrane-bound pool of ^{125}I-labeled CPs (data not shown).

bined inhibition of cathepsins L and S almost completely ablated proteolysis (Fig. 5*B*).

DISCUSSION

We have demonstrated that human MDMs can actively deploy CPs to express an elastin-degrading phenotype hundreds of times more destructive than that previously ascribed to other cell populations (3–6, 38, 39). Although alveolar macrophages are commonly used for mechanistic analyses of macrophage-mediated tissue damage (3–6), MDMs are known to display functional heterogeneity *in vitro* and *in vivo* (40). *In vitro*, serum constituents, adhesive interactions with the substratum, and autocrine factors are presumed to have acted in concert to initiate the complex gene program that yielded the degradative phenotype described here. Indeed, using a subtractive hybridization protocol, we have recently identified hundreds of gene products that are selectively expressed in matrix-destructive MDMs (unpublished observation).† While the *in vivo* situations in which this phenotype would likely be encountered remain to be determined, our results clearly outline the MDM's degradative potential.

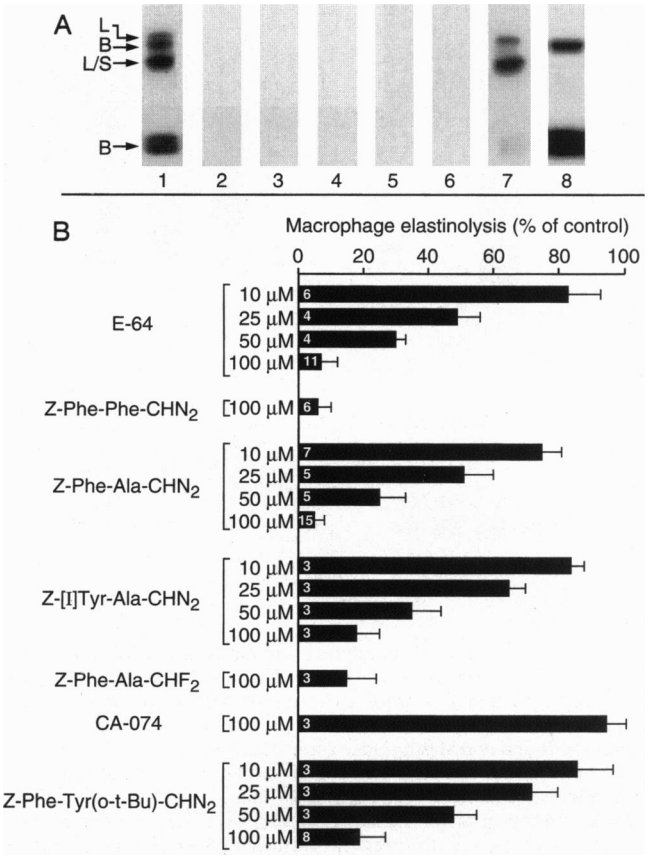

FIG. 5. Role of cathepsins B, L, and S in MDM-mediated elastin degradation. (*A*) Inhibitory profile of a panel of CP inhibitors. MDM CPs were labeled with Z-[^{125}I]Tyr-Ala-CHN_2 in the absence (lane 1) or presence of the following inhibitors (3-h preincubation) (100 μM): E-64 (lane 2), Z-Phe-Phe-CHN_2 (lane 3), Z-Phe-Ala-CHN_2 (lane 4), Z-[^{125}I]Tyr-Ala-CHN_2 (lane 5), Z-Phe-Ala-CH_2F (lane 6), CA-074 (lane 7), and Z-Phe-Tyr(*o-tert*-butyl)-CHN_2 (lane 8). Plasma membrane-bound and intracellular CPs were similarly affected (data not shown). (*B*) Inhibition of MDM-mediated elastinolysis by CP inhibitors. MDMs were incubated with elastin in the presence of CP inhibitors at the indicated concentrations. Results are expressed as percentage control elastinolysis (means ± SEM) of the indicated number of experiments. Control elastinolysis was 1624 ± 94 μg of elastin degraded per 10^6 cells per 24 h (n = 22).

Circumstantial evidence has recently been forwarded to implicate CPs in tissue-destructive states including chronic lung damage, arthritis, bone resorption, and metastasis (e.g., see refs. 20, 23–26, 36, 41, and 42). Of note, however, while alveolar macrophages, synoviocytes, osteoclasts, and tumor cells have all been found to secrete inactive precursors of cathepsin B or L under defined conditions, in virtually all reported cases active forms of the CPs were not detected either in the extracellular medium or on the cell surface (28–31, 43–49). In the few studies in which mature forms of the CPs were identified extracellularly (e.g., see refs. 24 and 50–52), the samples had undergone extensive experimental manipulation prior to assay, including exposure to acid pH—a condition known to promote the autoactivation of CP proforms (53, 54). Furthermore, the ability of the intact cell to display matrix-destructive activity was either not examined or could not be confirmed (24, 50–52). Nonetheless, CP inhibitors have been shown to suppress a range of degradative

†MDMs have been found to express a newly discovered member of the CP family (58), but its role in elastinolysis has not yet been defined.

events *in vitro* as well as *in vivo* (e.g., see refs. 3–5, 20, 36, 41, and 42). However, in light of the fact that active forms of CPs were not detected extracellularly, others have concluded that intracellular CPs may regulate the activities of downstream proteolytic effectors (20, 42, 55, 56). The demonstration that MDMs secrete and use active forms of multiple CPs during elastinolysis is direct evidence that CPs directly participate in matrix-destructive events.

The mechanisms by which MDMs regulate extracellular CP activities remain speculative, but two possibilities deserve comment. First, all known CPs display acid pH optima (23). Indeed, having noted that the pH of the medium in MDM cultures rapidly falls after challenge with elastin, we have found that the cell–elastin interface is rapidly acidified by a vacuolar-type H^+-ATPase. Although osteoclasts may similarly acidify pericellular zones, MDMs did not acidify their subjacent compartment, did not form ruffled cell borders or clear zones, were insensitive to herbimycin A, and failed to respond to calcitonin (unpublished observation). Second, macrophages also secrete the potent CP inhibitor cystatin C (57), and cathepsin B, L, and S–cystatin C complexes were detected in the MDM-conditioned medium (unpublished observation). Thus, we posit that MDMs form sequestered, acidic microenvironments into which CPs are deposited. As the proteinases diffuse from these sites, their activities would be quenched rapidly by the combination of deleterious pH changes and exposure to antiproteinases. However, within acidic zones, the CPs could collaborate to degrade almost all extracellular matrix components (23–25). The ability of MDMs to mobilize these endoproteases not only highlights their potential role in inflammation but also suggests that therapeutic interventions directed against CPs or the processes that regulate their secretion could exert significant tissue-salvaging effects.

We thank H. A. Chapman, H. Kirschke, R. W. Mason, E. N. Shaw, and M.-N. Hamilton for helpful discussions. V.Y.R. was supported by a Medical Student Fellowship from the Howard Hughes Medical Institute. This study was supported by a grant from the National Institutes of Health (R37-AI 21301).

1. Johnston, R. B. (1988) *N. Engl. J. Med.* **318,** 747–752.
2. Shapiro, S. D., Campbell, E. J., Welgus, H. G. & Senior, R. M. (1990) *Ann. N.Y. Acad. Sci.* **624,** 9–80.
3. Chapman, H. A., Jr., Stone, O. L. & Vavrin, Z. (1984) *J. Clin. Invest.* **73,** 806–815.
4. Chapman, H. A. & Stone, L. E. (1984) *J. Clin. Invest.* **74,** 1693–1700.
5. Chapman, H. A., Jr., Reilly, J. J., Jr., & Kobzik, L. (1988) *Am. Rev. Respir. Dis.* **137,** 412–419.
6. Senior, R. M., Connolly, N. L., Cury, J. D., Welgus, H. G. & Campbell, E. J. (1989) *Am. Rev. Respir. Dis.* **139,** 1251–1256.
7. Vaes, G. (1980) *Agents Actions* **10,** 474–490.
8. Circollo, A., Welgus, H. G., Pierce, F. N., Kramer, J. & Strunk, R. C. (1991) *J. Biol. Chem.* **266,** 12283–12288.
9. Pawlowski, N. A., Abraham, E. L., Pointer, S., Scott, W. A. & Cohn, Z. A. (1985) *Proc. Natl. Acad. Sci. USA* **82,** 8208–8212.
10. Desrochers, P. D., Mookhtiar, K., Van Wart, H. E., Hasty, K. A. & Weiss, S. J. (1992) *J. Biol. Chem.* **267,** 5005–5012.
11. Shi, G.-P., Webb, A. C., Foster, K. E., Knoll, J. H. M., Lemere, C. A., Munger, J. S. & Chapman, H. A., Jr. (1994) *J. Biol. Chem.* **269,** 11530–11536.
12. Mason, R. W., Wilcox, D., Wikstrom, P. & Shaw, E. N. (1989) *Biochem. J.* **257,** 125–129.
13. Stephens, R. W., Pollanen, J., Tapiovaara, H., Leung, U.-C., Sim, P.-S., Salonen, E.-M., Ronne, E., Behrendt, N., Dano, K. & Vaheri, A. (1989) *J. Cell Biol.* **108,** 1987–1995.
14. Van Noorden, C. J. F., Vogels, I. M. C., Everts, V. & Beertsen, W. (1987) *Histochem. J.* **19,** 483–487.
15. Kindzelskii, A. L., Xue, W., Todd, R. F., III, Boxer, L. A. & Petty, H. R. (1994) *Blood* **83,** 1650–1655.
16. Kreutz, M. & Andreesen, R. (1990) *Blood* **12,** 2457–2465.
17. Birkedal-Hansen, H., Moore, W. G. I., Bodden, M. K., Windsor, L. J., Birkedal-Hansen, B., DeCarlo, A. & Engler, J. A. (1993) *Crit. Rev. Oral Biol. Med.* **4,** 197–250.
18. Senior, R. M., Griffin, G. L., Fliszar, C. J., Shapiro, S. D., Goldberg, G. I. & Welgus, H. G. (1991) *J. Biol. Chem.* **266,** 7870–7875.
19. Busiek, D. F., Ross, P. F., McDonnel, S., Murphy, G., Matrisian, L. M. & Welgus, H. G. (1992) *J. Biol. Chem.* **267,** 9087–9092.
20. Buttle, D. J., Handley, C. J., Ilic, M. Z., Saklatvala, J., Murata, M. & Barrett, A. J. (1993) *Arthritis Rheum.* **36,** 1709–1717.
21. Weiss, S. J. (1989) *N. Engl. J. Med.* **320,** 365–376.
22. McCarthy, K., Musson, R. A. & Henson, P. M. (1982) *J. Reticuloendothel. Soc.* **31,** 131–144.
23. Mason, R. W. & Wilcox, D. (1993) in *Endosomes and Lysosomes: A Dynamic Relationship—Advances in Cell and Molecular Biology of Membranes*, eds. Storrie, B. & Murphy, R. F. (JAI, Greenwich, CT), pp. 81–116.
24. Sloane, B. F., Moin, K., Krepela, E. & Rozhin, J. (1990) *Cancer Metastasis Rev.* **9,** 333–352.
25. Kane, S. E. & Gottesman, M. M. (1990) *Semin. Cancer Biol.* **1,** 127–136.
26. Shi, G.-P., Munger, J. S., Meara, J. P., Rich, D. H. & Chapman, H. A. (1992) *J. Biol. Chem.* **267,** 7258–7262.
27. Wiederanders, B., Bromme, D., Kirschke, H., Figura, K. V., Schmidt, B. & Peters, C. (1992) *J. Biol. Chem.* **267,** 13708–13713.
28. Portnoy, D. A., Erickson, A. H., Kochan, J., Ravetch, J. V. & Unkeless, J. C. (1986) *J. Biol. Chem.* **261,** 14697–14703.
29. Kominami, E., Tsukahara, T., Hara, K. & Katunuma, N. (1988) *FEBS Lett.* **231,** 225–228.
30. Reilly, J. J., Mason, R. W., Chen, P., Joseph, L. J., Sukhatme, V. P., Yee, R. & Chapman, H. A., Jr. (1989) *Biochem. J.* **257,** 493–498.
31. Reilly, J. J., Jr., Chen, P., Sailor, L. Z., Wilcox, D., Mason, R. W. & Chapman, H. A., Jr. (1991) *Am. J. Physiol.* **261,** L41–L48.
32. Mason, R. W., Bartholomew, L. T. & Hardwick, B. S. (1989) *Biochem. J.* **263,** 945–949.
33. Xin, X.-Q., Gunesekera, B. & Mason, R. W. (1992) *Arch. Biochem. Biophys.* **299,** 334–339.
34. Mach, L., Stuwe, K., Hagen, A., Ballaun, C. & Glossl, J. (1992) *Biochem. J.* **282,** 577–582.
35. Buttle, D. J., Bonner, B. C., Burnett, D. & Barrett, A. J. (1988) *Biochem. J.* **254,** 693–699.
36. Baricos, W. H., Cortez, S. L., Le, Q. C., Wu, L.-T., Shaw, E., Hanada, K. & Shah, S. V. (1991) *Arch. Biochem. Biophys.* **288,** 468–472.
37. Shaw, E., Mohanty, S., Colic, A., Stoka, V. & Turk, V. (1993) *FEBS Lett.* **334,** 340–342.
38. Yusa, T., Blood, C. H. & Zetter, B. R. (1989) *Am. Rev. Respir. Dis.* **140,** 1458–1462.
39. Kobayashi, J., Wigle, D., Childs, T., Zhu, L., Keeley, F. W. & Rabinovitch, M. (1994) *J. Cell. Physiol.* **160,** 121–131.
40. Laszlo, D. J., Henson, P. M., Remigio, L. K., Weinstein, L., Sable, C., Noble, P. W. & Riches, D. W. H. (1993) *Am. J. Pathol.* **143,** 587–597.
41. Esser, R. E., Angelo, R. A., Murphey, M. D., Watts, L. M., Thornburg, L. P., Palmer, J. T., Talhouk, J. W. & Smith, R. E. (1994) *Arthritis Rheum.* **37,** 236–247.
42. Everts, V., Delaisse, J.-M., Korper, W., Niehof, A., Vaes, G. & Beerstsen, W. (1992) *J. Cell. Physiol.* **150,** 221–231.
43. Dong, J., Prence, E. M. & Sahagian, G. G. (1989) *J. Biol. Chem.* **264,** 7377–7383.
44. Achkar, C., Gong, Q., Frankfater, A. & Bajkowski, A. S. (1990) *J. Biol. Chem.* **265,** 13650–13654.
45. Delaisse, J.-M., Ledent, P. & Vaes, G. (1991) *Biochem. J.* **279,** 167–174.
46. Cullen, B. M., Halliday, I. M., Kay, G., Nelson, J. & Walker, B. (1992) *Biochem. J.* **283,** 461–465.
47. Huet, G., Flipo, R.-M., Colin, C., Janin, A., Hemon, B., Collyn-D'Hooghe, M., Lafyatis, R., Duquesnoy, B. & Degand, P. (1993) *Arthritis Rheum.* **36,** 772–780.
48. Tagami, K., Kakegawa, H., Kamioka, H., Sumitani, K., Kawata, T., Lenarcic, B., Turk, V. & Katunuma, N. (1994) *FEBS Lett.* **342,** 308–312.
49. Keppler, D., Waridel, P., Abrahamson, M., Bachmann, D., Berdoz, J. & Sordat, B. (1994) *Biochim. Biophys. Acta* **1226,** 117–125.
50. Maciewicz, R. A., Wardale, R. J., Etherington, D. J. & Paraskeva, C. (1989) *Int. J. Cancer* **43,** 478–486.
51. Sloane, B. F., Rozhin, J., Krepela, E., Ziegler, G. & Sameni, M. (1991) *Biomed. Biochim. Acta* **50,** 549–554.
52. Corticchiato, O., Cajot, J.-F., Abrahamson, M., Chan, S. J. & Keppler, D. (1992) *Int. J. Cancer* **52,** 645–652.
53. Rowan, A. D., Mason, P., Mach, L. & Mort, J. S. (1992) *J. Biol. Chem.* **267,** 15993–15999.
54. Mason, R. W. & Massey, S. D. (1992) *Biochem. Biophys. Res. Commun.* **189,** 1659–1666.
55. Goretzki, L., Schmitt, M., Mann, K., Calvete, J., Chucholowski, N., Kramer, M., Gunzler, W. A., Janicke, F. & Graeff, H. (1992) *FEBS Lett.* **297,** 112–118.
56. Kobayashi, H., Moniwa, N., Sugimura, M., Shinohara, H., Ohi, H. & Terao, T. (1993) *Biochim. Biophys. Acta* **1178,** 55–62.
57. Chapman, H. A., Jr., Reilly, J. J., Jr., Yee, R. & Grubb, A. (1990) *Am. Rev. Respir. Dis.* **141,** 698–705.
58. Shi, G. P., Chapman, H. A., Bhairi, S. M., DeLeeuw, C., Reddy, V. Y. & Weiss, S. J. (1995) *FEBS Lett.* **357,** 129–134.