Matrix Metalloproteinase Expression and Production by Alveolar Macrophages in Emphysema

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The aim of this study was to examine the hypothesis that alveolar macrophages represent a significant source of matrix-degrading proteinases in the emphysematous lung. Macrophages from bronchoalveolar lavage fluid of 10 patients with emphysema and 10 normal volunteers were maintained *in vitro* for 24 h and assessed semiquantitatively for mRNA transcript levels of the matrix metalloproteinases (MMPs) gelatinases A and B, macrophage metalloelastase (MME), and interstitial collagenase. Release of these MMPs into the culture medium and secretion of neutrophil elastaselike activity was also assessed. Elevated levels of mRNA transcripts for gelatinase B ($p < 0.0005$) and interstitial collagenase ($p < 0.0005$) were observed in macrophages from emphysematous patients. Increased collagenase ($p < 0.01$) and neutrophil elastaselike activities ($p < 0.001$) were also measured in conditioned medium from patient macrophages. With gelatinase B, complexed forms of the enzyme were secreted by patient but not by control macrophages. No difference in transcript levels of gelatinase A or MME was observed between patient and control samples, and neither enzyme was detected in macrophage-conditioned media from either group. These results directly demonstrate that alveolar macrophages from the emphysematous lung produce elevated quantities of matrix-degrading enzymes with both elastolytic and collagenolytic activities. Finlay GA, O'Driscoll LR, Russell KJ, D'arcy EM, Masterson JB, FitzGerald MX, O'Connor CM. Matrix metalloproteinase expression and production by alveolar macrophages in emphysema. AM J RESPIR CRIT CARE MED 1997;156:240–247.

Pulmonary emphysema, the major contributor to morbidity and mortality in patients with chronic obstructive pulmonary disease, is characterized by progressive destruction of the alveolar matrix. Loss of elastic recoil and histologic evidence of damage to elastin fibers implicates elastic degradation as a key feature in the pathogenesis of this disease. In the 1960s, linkage of early-onset emphysema to genetic deficiency of α_1-protease inhibitor (α_1PI), an inhibitor of neutrophil elastase (NE), led to the development of the elastase-antielastase theory of emphysema (1). This postulates that a shift in the elastase-antielastase balance of the lung leads to the unopposed action of elastase, resulting in parenchymal destruction.

Although a shift in the NE-α_1PI balance in favor of elastolysis may indeed contribute significantly to elastin destruction in emphysema associated with α_1PI deficiency, the situation in smoking-induced emphysema is less clear. There are contradictory reports as to whether or not α_1PI is inactivated in the lungs of cigarette smokers (2–4), and, although levels of NE complexed to α_1PI are elevated in patients with both clinical and subclinical emphysema (5, 6), there is little evidence for the presence of active or "unopposed" NE.

Evidence for the presence of increased numbers of neutrophils in the lungs of patients with emphysema is also controversial (5, 7), and many studies have implicated the alveolar macrophage as the major inflammatory effector cell (8, 9). Di Stefano and coworkers (10) have shown that increased numbers of macrophages in bronchial biopsies correlates with airflow limitation. By comparison, neutrophil numbers were not elevated, and no relationship between neutrophils and disease symptoms was observed. A direct relationship between lung destruction and the number of alveolar macrophages, but not neutrophils, in the alveolar parenchyma has also been reported by Finkelstein and coworkers (11). Thus, significant attention has now focused on the macrophage as a potential source of elastolytic activity in the lung (reviewed in 12 and 13).

A range of proteases produced by the alveolar macrophage are capable of degrading elastin, including the lysosomal cathepsins L and S (13) and the matrix metalloproteinases (MMPs) gelatinase A and gelatinase B, and macrophage metalloelastase (MME) (12). The tight lysosomal compartmentalization of the cathepsins suggests that their major *in vivo* role may be the degradation of endocytosed protein (14), although release of these enzymes after macrophage necrosis could contribute to extracellular matrix degradation in disease situations (13). By contrast to the cathepsins, macrophage MMPs are synthesized and secreted extracellularly. Senior and colleagues (15) have shown that elastin degradation by alveolar macrophages is in-

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hibited by the tissue inhibitor of metalloproteinases, indicating a significant role for MMPs in macrophage-mediated elastolysis. Studies by Janoff and colleagues (16) suggest that as much as 50% of the elastolytic activity in bronchoalveolar lavage (BAL) fluids from smokers may be attributable to MMPs (16).

Recent studies have also implicated another macrophage-derived MMP, interstitial collagenase, in the pathogenesis of emphysema. D'Armiento and coworkers (17) have shown that transgenic mice expressing the human form of intersititial collagenase spontaneously develop emphysema. Although this latter MMP degrades collagen rather than elastin, given the close intermeshing of elastin and collagen in the alveolar matrix, it is probable that the degradation of elastin *in vivo* involves the cooperative action of a range of proteinases. Indeed, we have recently demonstrated that elastin fragmentation in emphysema is accompanied by significant collagen remodeling (18).

Although the studies quoted above indicate that macrophage MMPs have the potential to contribute to tissue destruction in emphysema, to date there is no direct evidence of their production in the emphysematous lung. Thus, the aim of this study was to see if alveolar macrophages from patients with emphysema expressed increased amounts of mRNAs for MMPs or secreted increased amounts of these enzymes.

METHODS

Study Population

Ten patients 43 to 75 yr of age with emphysema confirmed by medical history, chest roentgenography, pulmonary function, and CT scan underwent lung lavage. Four were current smokers and six were ex-smokers who had ceased smoking for a minimum of 10 yr. None of the patients had received inhaled or oral steroids at the time of lavage or in the previous 6 wk, nor did any display significant reversibility of airflow obstruction after inhalation of β_2-agonists. A control group of 10 normal subjects, matched for sex and current smoking status (four smokers, six nonsmokers 43 to 58 yr of age) volunteered for BAL while undergoing a brief general anaesthetic for minor surgery. They were also tested for pulmonary function and underwent chest roentgenography and CT scan to confirm the absence of disease. CT scanning was performed during gentle respiration and scans were acquired at the sternoclavicular joint, the carina, and through the lung bases 2 cm above the dome of the diaphragm. The mean attenuation within each region was calculated and corrected by reference to a water phantom that was placed below each patient to check CT number accuracy on a patient-to-patient basis. No subject in either the patient or the control groups had a history of atopy or episodic wheezing, nocturnal dyspnea, or ankle edema. All subjects denied having significant symptoms of a respiratory tract infection in the preceding 6 wk. Arterial blood gas analysis performed on all subjects while they breathed room air showed no evidence of clinically significant hypoxemia or hypercapnea. All current smokers were lifelong smokers of at least 10 cigarettes per day, and ex-smokers had ceased their habit for at least 10 yr. Nonsmoking control subjects had never smoked. Smokers were asked to refrain from smoking for at least 12 h prior to lavage. Thus, in each case the diagnosis of emphysema was established or excluded and the absence of coexistent disease confirmed. All subjects gave their informed consent, and the project was approved by St. Vincent's Hospital Ethics Committee.

Alveolar Macrophage Preparations

BAL samples were processed under sterile conditions within 1 h of collection. After straining through two layers of surgical gauze to remove debris and mucus, cells were recovered from lavage fluid by centrifugation ($400 \times g$ for 10 min) and suspended at a concentration of 1×10^6 cells/ml in RPMI medium containing 2% fetal calf serum, 2% Hepes, 1% amphotericin B, 0.5% kanamycin, and 1% L-glutamine (GIBCO BRL, Grand Island, NY). Viability was determined after the addition of 50 μl ethidium bromide (0.1%)/acridine orange (0.03%) to a 50-μl aliquot of cell suspension and found to be more than 90% in all cases. Differential counts were determined on Diff-Quik-stained preparations. Alveolar macrophages were isolated by differential attachment to tissue culture flasks (Costar, Cambridge, MA) for 2 h at 37° C. Unadhered cells were then removed and counted, and a slide was prepared for differential analysis to enable calculation of plating efficiency and number of alveolar macrophages adhered. Fresh serum-free medium was added to the adhered cells (> 95% alveolar macrophages in all cases), which were maintained in a humidified atmosphere containing 5% CO_2 and 95% air for a further 24 h, at which time conditioned medium was removed. To avoid postsampling proteolysis in the conditioned medium samples, the serine protease inhibitors diisopropylfluorophosphate (DIPF) (1 mM) and phenylmethylsulfonylfluoride (PMSF) (1 mM) were added to aliquots to be assessed for MMP activity. Aliquots without added inhibitors were also prepared for analysis of serine protease elastase activity. All samples were stored at −70° C until analyzed.

RT-PCR Amplification of RNA from Alveolar Macrophages

To assess MMP expression in alveolar macrophages, total cellular RNA was isolated from adherent macrophages using an Ultraspec-II RNA Isolation System (Biotecx Labs, Inc., Houston, TX). The purity and yield of RNA were determined spectrophotometrically by measuring the absorbance of an aliquot at 260 and 280 nm. When necessary, specimens were concentrated by precipitation in 70% ethanol containing (0.3 M) sodium acetate. To monitor the integrity of the RNA isolated, random samples were examined on agarose gel electrophoresis in the presence of formaldehyde to visualize intact rRNA bands. Reverse transcription (RT) and polymerase chain reactions (PCR) were carried out essentially as described by O'Driscoll and colleagues (19), with minor modifications of the protocols. In brief, RT was performed in a 20-μl volume reaction containing 50 ng oligo dT primers (Promega, Madison, WI), 50 mM TRIS-HCl (pH, 8.3), 75 mM KCl, 3 mM $MgCl_2$, 10 mM dithiothrietrol, 40 U RNasin, 0.5 mM of each deoxynucleotide triphosphate (Promega), and 200 U MMLV reverse transcriptase (GIBCO BRL). Each reaction contained 1 μg of extracted RNA. After completion of RT (37° C for 1 h), the temperature was raised to 95° C for 2 min to inactivate the MMLV reverse transcriptase and denature RNA-cDNA hybrids. PCR was set up by including 5 μl of the formed cDNA in a total volume of 50 μl containing TRIS-HCl (10 mM at pH 9.0), 5 mM KCl, 0.1% Triton X-100, 1.5 mM $MgCl_2$, 0.2 mM of each deoxynucleotide triphosphate, 250 ng each of the target and endogenous control (glyceraldehyde phosphate dehydrogenase, GAPDH) primers (Table 1), and 2.5 U of Taq DNA polymerase (Promega). Where feasible, primer pairs were selected to flank an intron-containing sequence so that DNA contamination, if it occurred, could be diagnosed. The samples were overlaid with mineral oil, and after an initial denaturing step at 94° C for 1.5 min, they were processed for an appropriate number of cycles (20 when coamplifying GADPH with intersititial collagenase, gelatinase A, or MME; 28 when amplifying gelatinase B) at 94° C for 1.5 min, 54° C for 1 min (except for gelatinase B where 57° C was found to be more appropriate), and 72° C for 3 min. At the end of the last cycle the elongation time at 72° C was extended to 10 min. The PCR conditions used were those that in preliminary experiments had been found to be optimal for each set of primer pairs and that ensured that assessment was performed during the exponential phase of the reaction, thus enabling semiquantitative analysis (23). All RT and PCR reactions on samples were performed in duplicate.

For each set of primers employed, amplified products were verified by their predicted sizes. In addition, random samples were digested with an appropriate restriction enzyme (Table 1). All products were visualized after electrophoresis on an ethidium-bromide-stained 3.5% agarose gel. The gels were photographed, and the negatives produced were analyzed by densitometry (Imaging Densitometry Model GS-670; BioRad Laboratories, Richmond, CA). Densitometric absorbances of MMP transcripts were normalized to the corresponding absorbance of the constitutive GADPH product, which, with the exception of gelatinase B, was coamplified with the MMP in the same RT-PCR reaction tube. As the number of PCR cycles required to detect gelatinase B transcripts (28 cycles) was beyond the linear range for GADPH amplification, densitometric absorbances of gelatinase B transcripts were normalized to GADPH products from the same sample and amplified simultaneously (20 cycles) but in a separate reaction tube.

TABLE 1

PRIMERS TO AMPLIFY cDNA FORMED BY REVERSE TRANSCRIPTION OF mRNA TEMPLATES*

mRNA of Interest	Primer Sequences	Amplified cDNA Sequence Length (*bp*); Diagnostic Restriction Enzyme: Products Length (*bp*)
Interstitial collagenase†	(a) 5′ AGATGTGGAGTGCCTGATGT 3′	474
	(b) 5′ TAGCTGAACATCACCACTGA 3′	Sty 1: 295 + 179
Gelatinase A‡	(a) 5′ TGACATCAAGGGCATTTCAGGAGC 3′	180
	(b) 5′ GTCCGCCAAATGAACCGGTCCTTG 3′	Sty 1: 132 + 49
Gelatinase B§	(a) 5′ GGTCCCCCCACTGCTGGCCCTTCTACGGCC 3′	639
	(b) 5′ GTCCTCAGGGCACTGCATGATGTCATAGGT 3′	Hind III: 389 + 250
Macrophage metalloelastase†	(a) 5′ ATATGTCGACATCAACACAT 3′	286
	(b) 5′ ATAAGCAGCTTCAATGCCAG 3′	Dpn I: 170 + 116
GAPDH‖	(a) 5′ TCCATGACAACTTTGGCATCGTGG 3′	380
	(b) 5′ GTTGCTGTTGAAGTCACAGGAGAC 3′	Xba 1: 254 + 126

* All primers were made to order by R&D Systems, Barton Lane, London, United Kingdom.
† Primers selected in this laboratory.
‡ *See* reference 20.
§ *See* reference 21.
‖ *See* reference 22.

Assessment of Protease Activity in Medium Samples Conditioned with Alveolar Macrophages

Aliquots of alveolar-macrophage-conditioned medium were analyzed for MMPs via gelatin zymography, which detects gelatinases A and B, casein zymography, which detects MME and stromelysins, and via assessment of collagenase activity. Serine protease elastase activity was also assessed.

Gelatin zymography was carried out on unconcentrated samples of conditioned medium as described by Overall and coworkers (24). Zones of enzymatic activity were visualized on electrophoretic gels as clear bands against a blue background. Molecular weight markers run concurrently with the samples provided information regarding the molecular weight of the gelatin-degrading bands. Positive standards included a neutrophil lysate and a preparation containing gelatinases A and B from gelatinase-B-transfected HK cells (obtained from Dr. S. McDonnell, Dublin City University, Dublin, Ireland). Densitometry was carried out on negative images of the zymograms using the GDS-8000 Complete Gel Imaging System (Phoretix International, Newcastle-upon-Tyne, UK). To enable densitometric comparison between zymograms all bands were standardized to the gelatinase A/gelatinase B positive standard on each zymogram. Units of gelatinase activity were expressed as arbitrary densitometric units per 10^6 adhered macrophages.

The casein zymogram procedure was as described by Fernandez-Resa and colleagues (25). As with gelatin zymography, zones of enzymatic activity were seen as clear bands against a blue background. Conditioned medium from PMA-stimulated macrophages was used as a positive control, and densitometry was performed as described above.

Prior to analysis for collagenase activity, conditioned media were concentrated tenfold by ultrafiltration on CF25 centriflo membrane cones (Amicon Corp., Danvers, MA). Collagenase activity was determined by measuring the release of radiolabeled fragments from Type-1 collagen as previously described (26). Units of collagenase activity were calculated as micrograms of collagen degraded per minute per milliliter of sample.

Serine protease elastaselike activity was measured in samples concentrated × 10 via centrifugal evaporation (Gyrovap; Howe and Co., Oxon, UK). The NE-sensitive chromogenic peptide, *N*-methoxy-succinyl-Ala-Ala-Pro-val *p*-nitroanilide (Sigma Chemical, St. Louis, MO) was used as substrate, and the assay was performed as previously described (27). To confirm the serine protease nature of the elastolytic activity measured, a selection of samples from both patients and control subjects (n = 5) were also assessed for elastase activity in the presence and absence of 10 mM DIPF/PMSF, which inhibit serine proteases but not MMPs.

Statistical Analysis

The nonparametric Mann-Whitney U test was used for statistical comparison of data between control and patient groups. Multiple and simple regression analysis was used to correlate clinical and enzyme data. In all tests, p values < 0.05 were taken as indicating a significant difference between the groups or a significant correlation (28).

RESULTS

Patient Characteristics

Clinical and lavage characteristics of the patient and control groups are shown in Table 2. As expected, all patients had impaired pulmonary function as indicated by $\%FEV_1$ of predicted normal values $< 75\%$ and areas of low attenuation on

TABLE 2

CLINICAL AND BRONCHOALVEOLAR LAVAGE CHARACTERISTICS OF EMPHYSEMA PATIENTS AND CONTROL SUBJECTS*

	Patients with Emphysema	Control Subjects	p Value
FEV_1, % pred	61 (29–71)	103 (79–11)	$p < 0.001$
FEV_1/FVC, %	69 (44–106)	101 (96–112)	$p < 0.002$
D_{LCO}, % pred	68 (28–103)	95 (81–109)	$p < 0.002$
CT Score, MLDs HU	−836 (−740 to −902)	−698 (−614 to −735)	$p < 0.001$
BAL volume retrieved, ml	80 (16–95)	88 (25–130)	NS
BAL cells recovered, 10^6	12.9 (1.5–36.4)	10.0 (1.0–37.4)	NS
Alveolar macrophages, %	91.75 (81.7–100)	91.4 (81.0–95.2)	NS
Neutrophils, %	1.35 (0–5.7)	1.2 (0–3.7)	NS

* Data are expressed as medians with absolute ranges shown in parentheses.

CT scan. As a group they also displayed decreased $\%FEV_1$/FVC ratios and impaired diffusing capacity, as assessed by D_{LCO}. With respect to BAL characteristics, no significant difference was observed between emphysema and control groups in BAL volume retrieved, total number of cells recovered or type of cell present (Table 2). Plating efficiency and number of macrophages cultured were also similar between the two groups. It was noted, however, that within the patient group current smokers had a significantly higher proportion of alveolar macrophages retrieved on BAL (median, 96.4%; range, 94.2 to 100%) than their ex-smoking counterparts (median, 87.9%; range, 81.7 to 93.3%; $p < 0.01$). Although smokers in the control groups had somewhat higher yields of BAL macrophages (median, 94.2%; range, 90.6 to 95.2%) than did control nonsmokers (median, 88.7%; range, 81.0 to 94.7%), this did not reach the level of statistical significance ($p = 0.09$).

MMP Expression and Production by Alveolar Macrophages

Macrophages from control subjects yielded somewhat higher RNA yields (median, 1.76; range, 0.82 to 1.98 $\mu g/10^6$ macrophages) than did those from patients with emphysema (median, 1.16; range, 0.86 to 1.36 $\mu g/10^6$ macrophages; $p < 0.05$). Agarose gel electrophoresis on random samples demonstrated the presence of intact rRNA bands and the $A_{260/280}$ ratios obtained (Control subjects: median, 1.87; range, 1.64 to 2.00 absorbance units. Patients with emphysema: median, 1.7; range, 1.60 to 1.94 absorbance units) indicated that the purity of the RNA extracted in all cases was well within the acceptable range for RT-PCR. After RT-PCR, the size of the amplified product observed on agarose gel electrophoresis confirmed the absence of DNA contamination in sample reaction mixes. In addition, good reproducibility between duplicate RT and PCR reactions was observed for each sample, indicating little problem with tube-to-tube variation within the reaction systems.

Gelatinase B. Detectable levels of gelatinase B mRNA were expressed by all control subjects and patients with emphysema (Figure 1). Densitometric analysis indicated that transcript levels were significantly higher in macrophages from patients (median, 4.16; range, 3.26 to 8.35 densitometric units/0.25 μgRNA) than from control subjects (median, 1.53; range, 1.00 to 3.2 densitometric units/0.25 μgRNA; $p < 0.0005$). On zymography, latent gelatinase B (92 kD) was shown to be present in macrophage-conditioned media from nine of the 10 patients in the emphysematous group (median, 0.62; range, 0 to 1.91 densitometric units/10^6 macrophages) and in five of the 10 control samples (median, 0.07; range, 0 to 0.88 densitometric units/10^6 macrophages; NS). Although the quantities of gelatinase B released by macrophages did not differ significantly between patient and control groups, it was of interest that bands of gelatinase activity corresponding to 130 and 270 kD were observed in patient samples, whereas only the 92-kD band was seen in samples from control subjects (Figure 2).

Gelatinase A and MME. Gelatinase A and MME gene transcripts were expressed by macrophages from all control subjects and patients with emphysema, but the levels of either transcript did not differ significantly between the groups (Figure 3). No gelatinase A protein (72 kD) was detectable on gelatin zymograms of macrophage-conditioned media from either emphysema or control groups (Figure 2). Similarly, casein zy-

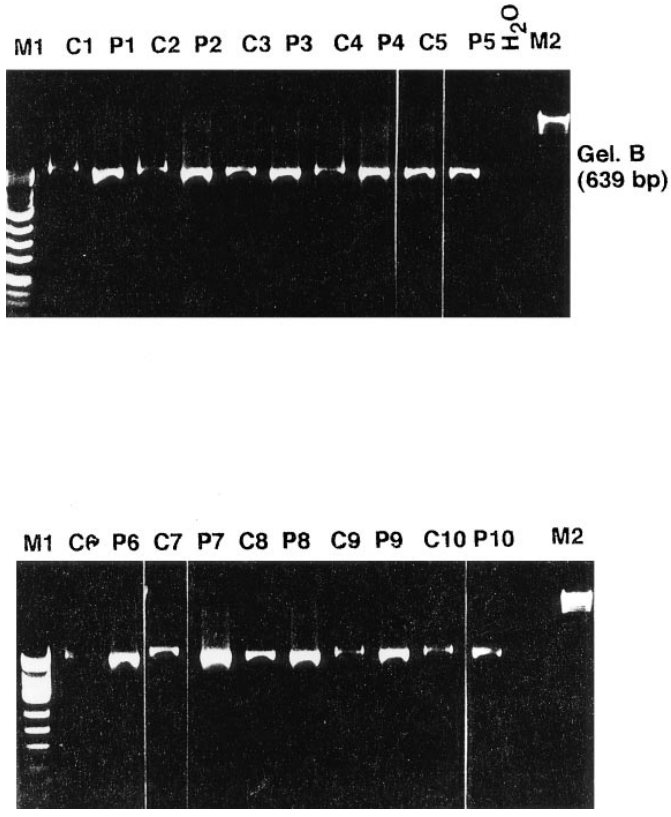

Figure 1. Semiquantitative RT-PCR analysis of gelatinase B (Gel. B) mRNA expressed by alveolar macrophages from patients with emphysema (P1–P10) and control subjects (C1–C10); 28 cycles of PCR amplification of gelatinase B were performed. Reaction tubes were also prepared when RNA was replaced with water as a negative control (H_2O). M1 and M2 indicate molecular weight markers øX174 DNA/Hinf I (Promega) and lambda DNA/EcoR1 + Hind III (Promega) by which the target product sizes were verified. These results are representative of repeat experiments.

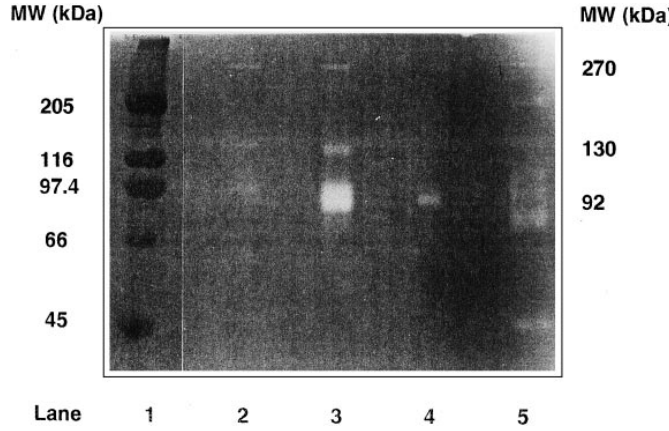

Figure 2. Gelatin zymogram of conditioned media from cultured alveolar macrophages from representative patients with emphysema (lanes 2 and 3) and a control subject (lane 4). Gelatinase bands of 92 kD (latent gelatinase B), 130 kD (neutrophil gelatinase associated lipocalin complex [NGAL]), and 270 kD (higher molecular weight multiple form of gelatinase B) are visible in the patient specimens, but only the 92 kD band was observed in control samples. A positive assay control neutrophil lysate (lane 5) is also shown. No gelatinase A (72 kD) was observed in any sample. MW indicates molecular weight markers (high molecular weight standard marker: 29,000 to 205,000 [Sigma]) by which the band sizes were verified (lane 1).

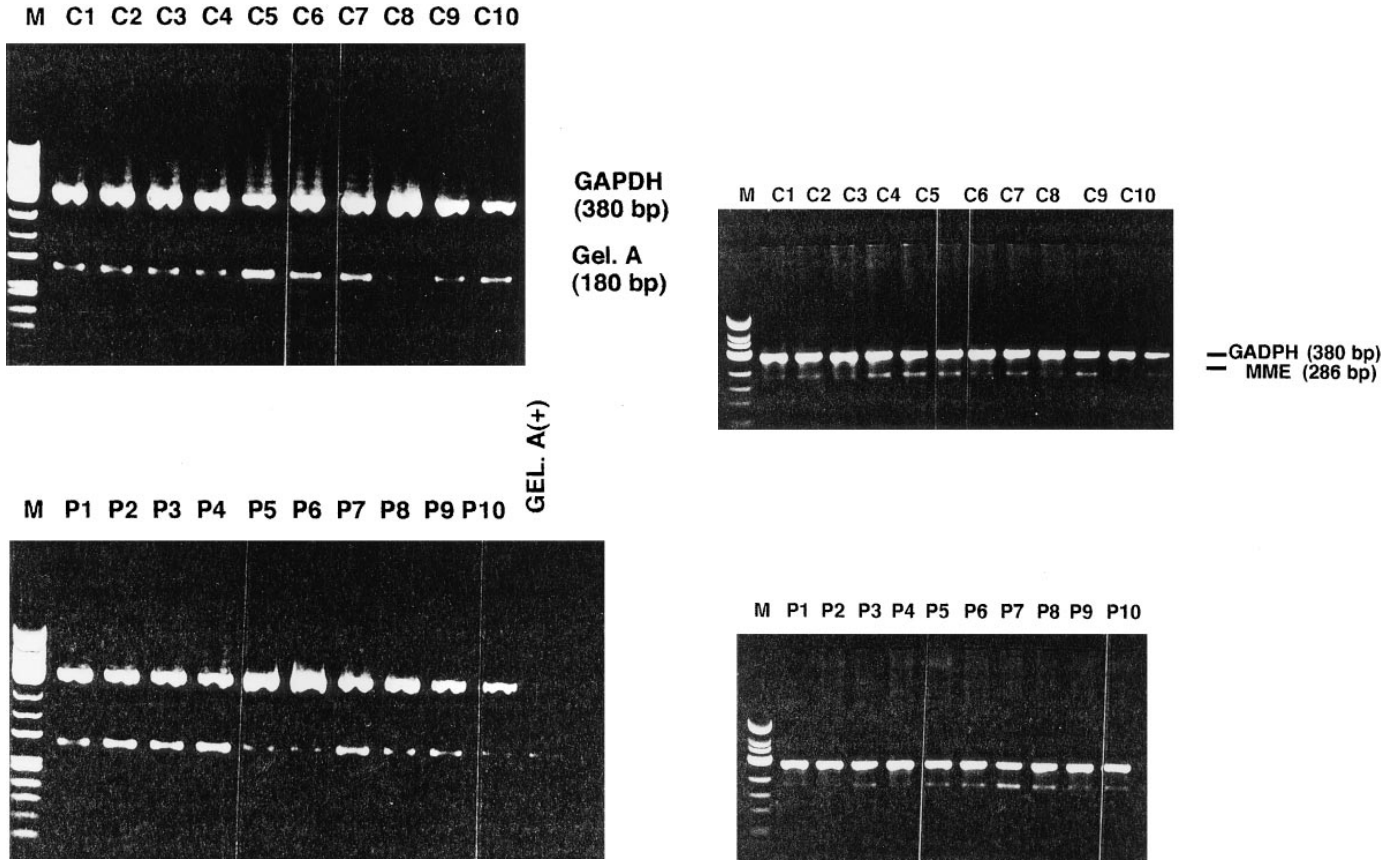

Figure 3. *Left panel*: semiquantitative RT-PCR analysis of gelatinase A (Gel. A) mRNA expressed by alveolar macrophages from patients with emphysema (P1–P10) and control subjects (C1–C10). GAPDH was coamplified with gelatinase A and used to standardize its expression. *p300*, a clone consisting of a 0.305 Kb sequence of gelatinase A in *pBluescript SK* vector (ATCC, Rockville, MD) was amplified simultaneously in a separate reaction tube as a positive control [Gel. A(+)]. These results are representative of repeat experiments. *Right panel*: semiquantitative RT-PCR analysis of MME mRNA expressed by alveolar macrophages from patients with emphysema (P1–P10) and control subjects (C1–C10). GAPDH was coamplified with MME and used to standardize its expression. M indicates molecular weight markers (øX174 DNA/Hinf I [Promega]) by which the target product sizes were verified. These results are representative of repeat experiments.

mograms did not detect the presence of MME in any of the study samples, although clear bands of activity at 55 kD were observed in positive assay control samples (data not shown).

Collagenase. Alveolar macrophages from all 10 patients with emphysema expressed mRNA for interstitial collagenase (median, 3.78; range, 1.97 to 5.02 absorbance/0.25 μgRNA) compared with only two control subjects (median, 0.00; range, 0.00 to 2.02 absorbance/0.25 μgRNA; $p < 0.0005$), both of whom were smokers (Figure 4). Collagenase activity was detected in conditioned media from six patients (median, 0.06; range, 0 to 0.13 units/10^6 macrophages), whereas no activity was measurable in macrophage-conditioned media from any of the control subjects ($p < 0.01$) (Figure 5). Within the patient group, the amount of collagenase released by macrophages was not significantly different between smokers and ex-smokers, and no correlation between levels of the intersititial gene transcript and levels of collagenase activity in the conditioned media was observed.

Neutrophil Elastaselike Activity from Alveolar Macrophages

Although the alveolar macrophage has not been shown to transcribe the NE gene, it can internalize NE by a receptor-mediated process and harbor active enzyme for as long as 5 d (29). Thus, to fully estimate the elastolytic potential of alveolar macrophages from patient and control groups, NE-like activity in macrophage-conditioned media was assessed. Significantly higher levels of NE-like activity were observed in samples from the patient group (median, 0.20; range, 0.09 to 1.1 nU/10^6 macrophages) than in control samples (median, 0.0; range, 0.0 to 0.13 nU/10^6 macrophages; $p < 0.001$) (Figure 6). In a representative population ($n = 3$ patients, $n = 2$ control subjects) the serine protease inhibitors DIPF and PMSF were found to completely inhibit the elastolytic activity observed, confirming that the activity was not attributable to proteases other than serine proteases.

DISCUSSION

The predominance of the alveolar macrophage in the lungs of smokers (7, 8) and their ability to produce and secrete a range of MMPs with elastolytic activity has led to the proposal that these MMPs may play a significant role in elastin degradation in emphysema (12). In this study we demonstrated that alveolar macrophages from a group of patients with emphysema proved by CT scan transcribe significantly more of the elastolytic MMP, gelatinase B, than macrophages from a control group without emphysema. By comparison, no difference in mRNA transcripts for either gelatinase A or MME was observed. These results provide direct evidence that macrophage-derived gelatinase B, but not gelatinase A or MME, is a source of increased elastolytic capacity in the emphysematous lung.

Although gelatinase B transcript levels were elevated in macrophages from patients with emphysema, the amount of

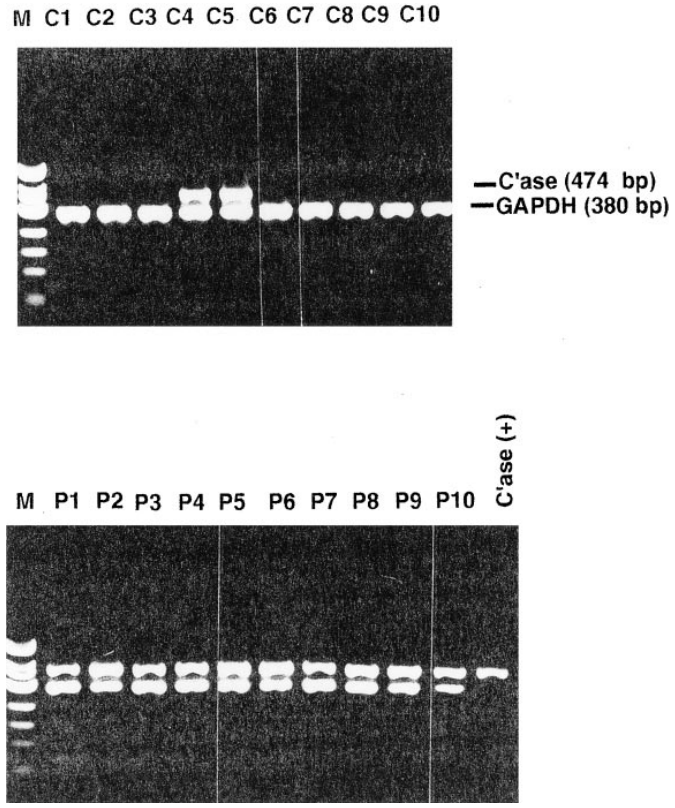

Figure 4. Semiquantitative RT-PCR analysis of interstitial collagenase (C'ase) mRNA expressed by alveolar macrophages from patients with emphysema (P1–P10) and control subjects (C1–C10). GAPDH was coamplified with C'ase and used to standardize its expression. *pCIIase 1,* a clone consisting of the complete coding sequence for fibroblast collagenase in *pSP64* vector (ATCC, Rockville, MD) was amplified simultaneously in a separate reaction tube as a positive control, C'ase (+). M indicates molecular weight markers (øX174 DNA/Hinf I [Promega]) by which the target product sizes were verified. These results are representative of repeat experiments.

92-kD gelatinase B secreted did not differ significantly between patient and control groups. However, additional bands of gelatinolytic activity at molecular weights 130 and 270 kD were observed in patient but not in control samples. This banding pattern is identical to that observed for neutrophil-derived gelatinase B where the band of molecular weight 130 kD has been characterized as gelatinase B in complex with another neutrophil secondary granule protein, lipocalin (30), and the 270-kD bands as a multiple form of gelatinase B (31). Although similar complex formation has not been described for gelatinase B from macrophages, it may be that macrophages can, in a manner analogous to that described for NE (29), internalize lipocalin, which subsequently binds gelatinase B. Alternatively, the complexed forms of gelatinase B may reflect macrophage internalization of preformed gelatinase B complexes released by neutrophils. Although interpretation of the banding pattern observed on zymograms remains speculative, the presence of these extra bands demonstrates that patient macrophages display an additional elastolytic capacity that is not present in control cells. The absence of detectable levels of gelatinase A and MME in macrophage-conditioned media adds to the conclusion from the transcription studies that these MMPs are unlikely contributors to increased elastolysis in emphysema.

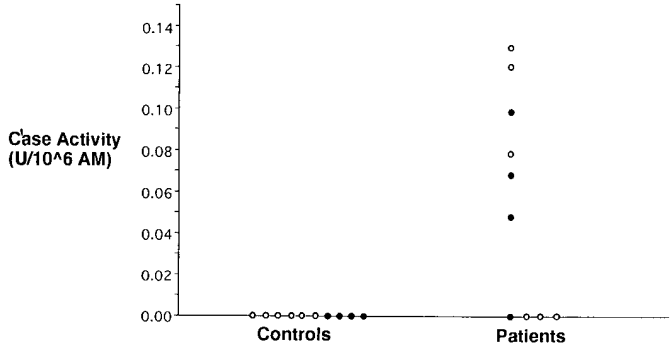

Figure 5. Collagenase (C'ase) activity levels in media conditioned with alveolar macrophages (AM) from patients with emphysema and control subjects, expressed as units per 10^6 AMs. Smokers (*closed circles*) and ex- or nonsmokers (*open circles*) are indicated in each group.

In fully assessing the elastolytic potential of alveolar macrophages from patient and control groups, we found significantly higher levels of NE-like activity in conditioned media samples from patients than in those from control subjects. As indicated earlier, macrophages can internalize NE and retain it for considerable periods of time (29). Thus, the increased levels of NE-like activity in patient samples may reflect increased internalization of NE by patient macrophages *in vivo*. Alternatively, as the monocyte precursors of macrophages contain NE (32) and increased numbers of immature macrophages are present in the lungs of smokers (33), the higher levels of NE-like activity observed may be indicative of the presence of immature macrophages in the emphysematous lung.

The observation by D'Armiento and coworkers (17) that transgenic mice expressing the human form of interstitial collagenase spontaneously develop emphysema prompted the assessment of transcription of this MMP in addition to MMPs with elastolytic activity. Unlike the other MMPs examined where expression was detectable in all study samples, mRNA transcripts for interstitial collagenase were detectable in only 20% of control samples compared with 100% of patient samples. Thus, the presence or absence of mRNA for this MMP more readily distinguished between patient and control groups than any of the elastolytic MMPs. Although the activity assay used to assess collagenase secretion into conditioned media

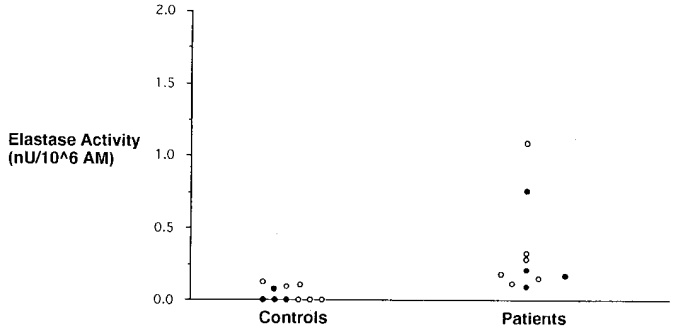

Figure 6. Neutrophil elastase activity levels in media conditioned with alveolar macrophages (AM) from patients with emphysema and control subjects, expressed as nano units per 10^6 AMs. Smokers (*closed circles*) and ex- or nonsmokers (*open circles*) are indicated in each group.

was not specific for interstitial collagenase (it also, indeed preferentially, measures neutrophil collagenase), it was of interest that activity levels also displayed a degree of discrimination between patient and control groups, with activity present in 60% of conditioned media from patient macrophages but in none of the control samples. Given that collagenase does not degrade elastin, it was somewhat surprising that, of all four MMPs examined, the transcription and secretion of this enzyme was most strikingly elevated in patient samples vis-a-vis control samples.

In conjunction with the observations of D'Armiento and coworkers (17) in transgenic mice, the collagenase results from this study are strongly suggestive of a significant role for collagen-digesting MMPs in matrix degradation in emphysema and add weight to the concept that alveolar destruction in this disease involves the cooperation of multiple proteinases (12). Evidence for such a cooperative effect comes from *in vitro* and *in vivo* studies. *In vitro*, matrix degradation has been shown to proceed more rapidly in the presence of both alveolar macrophages and NE than with either NE or macrophages alone (34). In an animal model of spontaneous emphysema, de Santi and colleagues (35) have shown that destruction of lung elastin is associated with the presence of alveolar macrophages containing collagen degradation products. In the human disease, we have recently observed that elastin fragmentation is accompanied by significant collagen remodeling (18). Thus, it would appear that, whereas excessive elastin degradation is an integral component of the emphysematous lesion, it is not the sole—or necessarily even the most dominant—feature underlying matrix degradation in this disease.

In summary, this study has demonstrated for the first time that alveolar macrophages from emphysematous patients transcribe and secrete greater quantities of an elastolytic MMP, gelatinase B, than macrophages from control subjects. It also demonstrates increased transcription of the collagen degrading MMP, interstitial collagenase, and increased release of collagenase activity by macrophages from patients with emphysema compared with that in control subjects. Allied to the observation that emphysematous macrophages release significant quantities of neutrophil elastaselike activity, the study has provided unequivocal evidence that, in smoking-induced emphysema, the major inflammatory cell present in the lung both synthesizes and secretes increased quantities of proteases, which, in combination, can degrade all the components of the alveolar matrix.

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