Alveolar macrophage-mediated elastolysis: roles of matrix metalloproteinases, cysteine, and serine proteases

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Chronic obstructive pulmonary disease (COPD) is a common lung disease with cigarette smoking as the major etiological factor, but only 15% of smokers develop COPD. Destruction of lung elastin observed in COPD is mediated by many enzymes, including cysteine, serine, and matrix metalloproteinases (MMP). The contribution of these enzymes to the lung elastolytic load, released from alveolar macrophages collected from nonsmokers, healthy smokers, and COPD patients, was examined by radiolabeled elastin as substrate in the presence of specific enzyme inhibitors. The activity of MMP was further examined by zymography and Western blotting. COPD macrophages degraded more elastin than either of the other groups. Elastolysis was greatest in the initial 24 h. Through the 72-h culture period, the contribution to elastolysis of serine elastases decreased, MMP increased, and cysteine elastases remained constant. The increased release of elastolytic enzymes in COPD subjects may explain why some smokers develop COPD. This difference may be due to unknown susceptibility factors. Serine proteases play a significant role; however, other enzymes, particularly the MMP, deserve further investigation.

Chronic obstructive pulmonary disease (COPD) is an increasing burden on the health care resources of the world (1). As yet there is no effective therapy, and its pathophysiology is not well understood (3). The pathological hallmarks of the disease are small airway inflammation and obstruction and destruction of the extracellular matrix (ECM) of the lung parenchyma that eventually lead to emphysema.

The destruction of the ECM is important in the development of gas trapping and airway obstruction. The ECM is a complex mixture of long-chain proteins, each with specific structural properties, and includes collagen, elastin, laminin, and gelatin (16). Although elastin makes up only 2.5% of the dry weight of the lung (45), it is vital for the elastic recoil of the small airways and their ability to resist negative pressure collapse (31, 46). Elastin is relatively conserved throughout life and once damaged or destroyed is difficult to replace (7), since repair to elastin networks often results in malformed and dysfunctional elastin filaments (18).

There are many proteolytic enzymes released in the lung that are capable of degrading elastin. These fall into three groups, serine proteases, cysteine proteases, and matrix metalloproteinases (MMP), based upon their structural and functional chemistry (49). The best known enzyme capable of degrading elastin is neutrophil elastase (NE, NC-IUBMB 3.4.21.37), a serine protease that is released by activated neutrophils. Cathepsins L and S (NC-IUBMB 3.4.22.21 and 27) are elastolytic cysteine proteases, whereas MMP-2 (gelatinase A, NC-IUBMB 3.4.24.24), MMP-9 (gelatinase B, NC-IUBMB 3.4.24.35), and MMP-12 (macrophage metalloelastase, NC-IUBMB 3.4.24.21) are the predominant MMP capable of elastolysis. MMP require the removal of a 10-kDa prodomain for activation to occur and can be found in both the latent and cleaved (mature) states.

The cellular sources of these enzymes include neutrophils, macrophages, and bronchial epithelial cells (23, 39, 41, 44). In COPD there is an increase in the number of neutrophils in sputum and upper airway secretions compared with normal subjects. Macrophages predominate in bronchoalveolar lavage (BAL) fluid, suggesting that they may also play an important role in the underlying inflammation in the distal airway (25, 28). Furthermore, macrophages have also been identified in increased numbers in the parenchyma of subjects with COPD (8). The aim of this study was to examine the elastolytic activity of alveolar macrophages (AM) from BAL fluid obtained from nonsmoking normal subjects, smokers without COPD, and patients with COPD. We also examined the role of specific...
elastolytic enzymes using newly available selective protease inhibitors.

METHODS

Subjects. Ten COPD patients, 10 smokers with normal lung function, and 10 normal nonsmokers were studied. COPD was defined by spirometry according to American Thoracic Society criteria; all were current smokers. Subjects with any allergy or asthma and those taking respiratory medications were excluded. Healthy smokers were defined as smokers with normal lung function who had smoked >20 pack years.

This study was approved by the East Berkshire Health Authority Ethics Committee; subjects gave written informed consent.

Bronchoscopy and BAL. Bronchoscopy and lavage processing were performed as described previously (15).

Preparation of elastin-coated plates. Twenty-five microliters of a solution of [3H]elastin (1 mg/ml) solution (25 μg, 6.25 μCi; Amersham Pharmacia, Amersham, UK) were added to the wells of a 24-well Primaria cell culture plate. The plate was shaken to distribute the elastin across the well and dried overnight. The plate was then washed three times to remove unbound [3H]elastin.

AM culture. After being counted, BAL macrophages were resuspended in RPMI 1640 medium (containing 0.1% BSA, 1% NaHCO3, 1% penicillin, 1% streptomycin) in a concentration of 1 × 106 macrophages/ml and seeded onto the [3H]elastin-coated cell culture plates, and 0.5 ml (500,000 macrophages) was added to each cell culture well. After incubation for 4 h (95% air-5% CO2 vol/vol) at 37°C, nonadherent cells were removed by washing with cell culture medium. Only AM and bronchial epithelial cells will adhere to cell culture plates; neutrophils do not adhere. Preliminary experiments demonstrated that the level of contamination by bronchial epithelial cells was <1%, and no neutrophils were recovered after adherence by scraping. Cell culture supernatants were collected after 24 h of incubation. The resulting supernatant was centrifuged (1,300 g) to remove any cells or debris and stored at −70°C until analyzed. Media and inhibitors were replaced on the cells after every 24-h period.

Measurement of elastin degradation. Cell supernatant (100 μl) was added to a scintillation vial together with 10 ml of Optiphase safe scintillator (Packard, London, UK). We measured elastin degradation by counting supernatant radioactivity using liquid scintillation counting.

Measurement of MMP protein levels and activity. MMP-2 and MMP-9 in the culture supernatant were quantified using commercially available ELISA kits (R&D Systems, Abingdon, UK) according to manufacturer’s instructions.

Semiquantitative measurement of MMP activity was performed by zymography (26, 36). Briefly, samples (15 μl) and standards (1 μg; Amersham Pharmacia Biotech, Little Chalfont, UK) were loaded into 10% polyacrylamide gels (wt/vol) incorporating 0.1% (wt/vol) gelatin substrate. Proteins were subjected to electrophoresis at 125 V for 60 min, and the gels were then washed three times in 20 mM Tris-HCl, pH 7.8, 2.5% (vol/vol) Triton X-100 for 15 min. The gels were then washed twice in 1% Triton X-100 (vol/vol) containing 10 mM CaCl2, 5 μM ZnCl2, pH 7.8, in Tris-HCl and incubated for 18 h at 37°C. After incubation, gels were stained with 1% (wt/vol) Coomassie blue in 45% methanol (vol/vol). Bands of lysis (enzyme activity) were visualized by washing in 25% (vol/vol) methanol, 7.5% acetic acid solution (vol/vol). The zones of lysis in the gels were analyzed using the Gelworks (UVP, Cambridge, UK) software system. Images were taken, and bandwidth and density were measured. Only the bands that corresponded to MMP-2 and -9 were analyzed. Standard curves were generated with known amounts of MMP-2 and MMP-9 protein and were linear at the concentrations measured; results are therefore expressed as %control (1 μg of control MMP run in each gel) activity for each gel. The assay was found to be linear for MMP activity at the concentrations of MMP-9 found in this study (data not shown). Confirmation that the activity seen was due to MMP-2 and MMP-9 was obtained by immunoprecipitation of the samples and standards before these were run in the zymography gels. Antibodies to MMP-2 and MMP-9 (Oncogene Research Products, Cambridge, MA) were incubated with samples and standards and separated using protein A-Sepharose beads. The resulting supernatants were run in zymography gels together with supernatants obtained after separation of the antibodies from the bound MMP-2 and MMP-9.

Supernatant MMP protein levels were also measured by Western blotting (26). After electrophoresis, gels were transferred onto Hybond-enhanced chemiluminescence (ECL) nitrocellulose membrane, blocked overnight, and then incubated for 1 h at 20°C with 1:200 mouse antibodies (PBS, 0.1% BSA) raised against the specific MMP. Membranes were washed and incubated for 1 h with 1:10,000 antibody (1,500,000 goat anti-mouse horseradish peroxidase-linked IgG); the proteins were visualized using ECL solution and developed on X-ray film. Band density was measured using the Gelworks image analysis system (Ultra-Violet Products, Cambridge, UK). Antibodies to MMP-2 and latent and cleaved MMP-9 were purchased from Oncogene Research Products. Antibody to MMP-12 was a kind gift from Dr. S. Shapiro (St. Louis, MO). Media protein levels were measured using the Bradford assay (9).

Statistical methods. Results were compared by one-way analysis of variance with post hoc testing if P < 0.05. Groups were further compared with the Mann-Whitney nonparametric test. All data shown are means ± SE.

RESULTS

Subject classification. The demography and lung function data of the three subjects groups are shown in Table 1. Groups were matched for age, sex, and smoking history. COPD subjects had significantly decreased

Table 1. Subject demography and lung function data

<table>
<thead>
<tr>
<th>Group</th>
<th>Age, yr</th>
<th>Sex Ratio</th>
<th>FEV1,1</th>
<th>FEV1, % predicted</th>
<th>FVC, 1</th>
<th>RV, % predicted</th>
<th>Pack Year</th>
</tr>
</thead>
<tbody>
<tr>
<td>COPD</td>
<td>74.9 ± 1.5</td>
<td>6:4</td>
<td>1.07 ± 0.23</td>
<td>48.2 ± 2.7</td>
<td>2.0 ± 0.3</td>
<td>172.7 ± 14.5</td>
<td>55.3 ± 5.0</td>
</tr>
<tr>
<td>Healthy Smokers</td>
<td>60.2 ± 5.8</td>
<td>7:3</td>
<td>2.55 ± 0.33*</td>
<td>89.4 ± 3.3*</td>
<td>3.2 ± 0.4*</td>
<td>124.7 ± 5.7*</td>
<td>49.0 ± 4.8</td>
</tr>
<tr>
<td>Nonsmokers</td>
<td>61.4 ± 4.8</td>
<td>4:6</td>
<td>2.75 ± 0.44*</td>
<td>92.5 ± 3.3*</td>
<td>3.4 ± 0.6*</td>
<td>104.2 ± 7.2*</td>
<td>0</td>
</tr>
</tbody>
</table>

Data are means ± SE. FEV1, forced expiratory volume in 1 s; FVC, forced vital capacity; RV, residual volume; Pack year, 1 pack year is smoking 1 packet of cigarettes (20) every day for 1 yr; COPD, chronic obstructive pulmonary disease; M, male; F, female. *P < 0.05 vs. COPD subjects.
forced expiratory volume in 1 s (FEV	extsubscript{1}), FEV	extsubscript{1} %predicted, FEV	extsubscript{1}/forced vital capacity (FVC), and FVC measurements. Preliminary studies confirmed that similar numbers of macrophages from each subject group adhered to the culture plates and that no neutrophils were found on the cell culture plates.

BAL cell counts and differential counts are shown in Table 2.

**Macrophage-mediated elastolysis.** At all times points (24, 48, and 72 h) throughout the study period, AM from COPD subjects degraded more elastin than either of the other groups (P < 0.001) (Fig. 1). There was no significant difference in the level of elastin degradation of AM from healthy smokers and nonsmokers. For all groups the amount of \(^{3}\text{H}\)elastin degraded was greatest in the initial 24 h of the study and declined significantly with time (P < 0.01). This decline was not due to AM death, as cell viability measured by trypan blue exclusion was maintained throughout and was similar for all groups (data not shown).

**Inhibitor studies.** To investigate the nature of the elastolysis released from the AM enzyme group, we added specific inhibitors to the culture media: the nonspecific MMP inhibitor batimastat (3 \(\mu\)M), the serine protease inhibitor BAY39-6437 (1 \(\mu\)M), and the cysteine protease inhibitor E-64 (10 \(\mu\)M). The \(K_{i}\) for batimastat against MMP-9 is 1.7 nM and against MMP-2 is 4 nM (11, 17); for E-64 against cathepsin-L it is 129 nM (47) and for BAY39-6437 against neutrophil elastase 101 nM (47). Blank wells were used as radioactive background references. All inhibition is expressed as percentage of inhibition from control well activity.

MMP inhibition was at the lowest in the initial 24 h (30.9 ± 1.6%); this increased at 48 h to 53.8 ± 2.3% and again to 60.2 ± 2.4% at 72 h (Table 3, Fig. 2). The inhibition by the cysteine protease inhibitor (E-64) was consistent throughout the study period at 24, 48, and 72 h with 28.1 ± 1.3, 28.6 ± 1.2, and 29.0 ± 1.0% respectively. Serine protease-mediated elastolysis was greatest for the first 24 h (43.4 ± 1.8%) and declined for the following 48-h period, 14.2 ± 0.6 and 9.3 ± 0.4%, respectively. There was no statistical difference in the inhibition profiles among the three subject groups.

**MMP profile.** All samples were analyzed by ELISA, Western blot, and zymography (Table 4).

**Table 2. BAL differential cell counts and total cell counts from nonsmokers, healthy smokers, and COPD subjects**

<table>
<thead>
<tr>
<th></th>
<th>NS</th>
<th>HS</th>
<th>OS</th>
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<tbody>
<tr>
<td>Total cell count, (\times10^{6})</td>
<td>5.5 ± 0.8*</td>
<td>6.5 ± 1.2</td>
<td>8.9 ± 1.2</td>
</tr>
<tr>
<td>Macrophages, %</td>
<td>86.3 ± 2.4</td>
<td>89.8 ± 2.9</td>
<td>91.4 ± 1.6</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>10.6 ± 2.5</td>
<td>7.9 ± 2.9</td>
<td>6.2 ± 1.8</td>
</tr>
<tr>
<td>Eosinophils, %</td>
<td>0.5 ± 0.13</td>
<td>0.7 ± 0.2</td>
<td>0.8 ± 0.14</td>
</tr>
<tr>
<td>Bronchial epithelial cells, %</td>
<td>2.6 ± 0.6</td>
<td>1.6 ± 0.4</td>
<td>1.6 ± 0.4</td>
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</tbody>
</table>

Data are means ± SE. Total cell count is total number of cells recovered from BAL after a standard 240-ml lavage. BAL, bronchoalveolar lavage; NS, nonsmoker; HS, healthy smoker; OS, COPD subject. *P < 0.05 OS vs. NS.

Fig. 1. A: total alveolar macrophage (AM)-mediated elastolysis for each 24-h time point for chronic obstructive pulmonary disease (COPD) subjects (solid bars), healthy smokers (hatched bars), and nonsmokers (open bars). Means ± SE are shown. AM from subjects with COPD, healthy smokers, and nonsmokers were cultured for 72 h in the presence of tritiated elastin. Medium was collected and replaced every 24 h, and release of radioactivity was measured. B: cumulative elastin degradation over time shown pictorially at each 24-h time point for AM from COPD subjects (▲) compared with AM from healthy smokers (●) and nonsmokers (♦); n = 10 for each group.

MMP-2 could be detected in only four of the samples by ELISA. In contrast, all of the samples had detectable levels of MMP-9. There was an increased MMP-9 protein level after 24 h of incubation for the COPD AM compared with healthy smokers and nonsmokers (8.0 ± 0.4 vs. 5.1 ± 0.4 and 3.8 ± 0.4 ng/ml, respectively, P < 0.001).

Using Western blotting, we were able to quantify the proportions of latent and active MMP-9 protein present in the supernatants. There was a significantly increased level of latent MMP-9 in the COPD AM compared with the other AM supernatants (P < 0.01, see Fig. 3B). Cleaved MMP-9 protein was present in greater levels in all samples, and there was an increased amount of protein present in the COPD samples compared with healthy smoker and nonsmoker supernatant (P < 0.001). There was no difference between the healthy smoker and nonsmoker supernatants.

MMP-2 and MMP-12 were not detectable by Western blotting in any sample examined in this study.

Using zymography, we could detect measurable MMP-2 activity in cell culture supernatants, although enzyme activity was low. There was an increased
MMP-2 activity seen at the 72-kDa position on the zymogram from the COPD supernatants compared with the healthy smoker and nonsmoker supernatants (P < 0.05 and P < 0.001, respectively). There was an increase in MMP-2 activity in the healthy smoker samples when compared with the nonsmokers (P < 0.01). The signal generated by MMP-9 activity on the zymography gels was much greater for all samples than that of MMP-2. Increased MMP-9 activity was demonstrated in the COPD samples compared with healthy smoker and nonsmokers (P < 0.01 and P < 0.001, respectively, for both latent (92 kDa) and cleaved (82 kDa)), and more MMP-9 activity was detectable in the healthy smoker samples than nonsmoker samples (P < 0.001, see Fig. 3A). Confirmation of protein identity was obtained by immunoprecipitation followed by zymography. MMP-2 and MMP-9 antibodies completely abolished activity at 72 and 92 kDa, respectively, on zymography. No activity attributable to MMP-12 could be identified.

**DISCUSSION**

There are several theories of disease causation in COPD. The elastase/antielastase hypothesis proposes that excess destruction of lung tissue (leading to emphysema and airflow obstruction) is due to an imbalance between the destructive enzymes and their inhibitors. This theory was founded after the characterization of α1-antitrypsin deficiency as a rapidly progressive emphysematous-like condition caused by a lack of the major plasma inhibitor of neutrophil elastase (10). The inflammation/repair hypothesis suggests that there is a deficiency in the repair mechanisms in the lungs of affected individuals after enzymatic damage. The repair mechanisms may be overwhelmed by the amount of destruction occurring or may incorrectly repair damaged tissue, affecting lung function (37).

Neither of these hypotheses is exclusive, and, indeed, there is evidence that both are correct to some degree. Excess elastolytic enzyme can cause emphysematous lesions; this has been demonstrated by the administration of pancreatic elastase into hamster lungs, resulting in emphysema-like pathology (6). Mice with deletion of the MMP-12 gene fail to develop emphysema after exposure to cigarette smoke (22), suggesting it is this enzyme that is responsible for the emphysematous lesions seen in this disease. The levels of elastin in emphysematous lungs have been reported as normal or even increased, suggesting that repair/replacement can occur. However, the resulting elastin polymers are malformed and functionally impaired (18).

Our study demonstrates that AM are a significant source of elastolytic enzymes. In these experimental cell culture conditions, AM from subjects with COPD have a markedly increased elastolytic activity compared with smokers with matched smoking history who have normal lung function. Although increased elastolytic activity of macrophages from COPD patients has been previously reported (2), the differences between smokers with and without airflow obstruction are more marked in our study. Subjects were selected according to standard lung function criteria for COPD. It was beyond the scope of this study to select specifically for the presence of emphysema (with computerized tomography scanning), although the finding of a significant increase in residual volume in the COPD subjects suggests that this pathology is present.

In our cell culture model, neutrophils were excluded by the adherence step; therefore, no neutrophil-derived enzyme was contributing to the results. Moreover, macrophages are relatively long-lived cells compared with neutrophils and are capable of synthesizing a wide variety of proteolytic enzymes, and our study examined a relatively long time course. Macrophages also contain preformed granule proteins that can be released into their surroundings or used after opsonization of bacteria.

The important novel finding of our study is the identification of the types of enzymes responsible for the increased elastolytic activity of COPD-derived AM. We
also show that the types of elastolytic enzymes released from AM may change over time. We have used three selective protease inhibitors to identify serine protease (BAY39-6437), cysteine protease (E-64), and MMP (batimastat, BB-94). These inhibitors are selective at the concentrations used in the study for their enzyme class, with no significant cross-class activity. Each of these inhibitors is commercially available, and BB-94 has been evaluated clinically as an antineutrophil therapy. They have all been used extensively in vitro (4, 11, 47).

We hypothesize that the initial, and greatest, release of enzymes occurs as the macrophages release their granules. These contain a balanced mixture of enzymes, including serine, cysteine, and metalloproteinases. This is a potent mixture, capable of degrading a large quantity of elastin (5). With time, the balance of enzyme releases changes, with a decrease in serine proteases and an increase in the MMP. Macrophages do manufacture serine proteases during their development; however, these potent enzymes are not expressed in mature and stimulated macrophages (13, 40). It is thought that the serine proteases released by macrophages may derive from neutrophils. After stimulation, neutrophils release large quantities of serine proteases. These enzymes are neutralized to a certain degree by α1-antitrypsin and cleared by α2-macroglobulin. However, macrophages are capable of engulfing serine proteases, such as neutrophil elastase and cathepsin G, storing these enzymes and then re-releasing them into the interstitium. Indeed, macrophages express a cell surface receptor that specifically binds neutrophil granule glycoproteins such as neutrophil elastase and cathepsin G (12). Unfortunately, further characterization of these enzymes was outside the focus of this study.

Similarly, the finding of significant levels of cysteine protease activity in pure macrophage culture can be partially explained by uptake of neutrophil-derived cathepsin L (36, 40). However, macrophages can manufacture both cathepsin L and cathepsin S after stimulation (41, 42). These are potent elastolytic enzymes that have optimum activity at acidic pH. They are therefore implicated in inflammation-driven elastolysis and ECM destruction (48). Our results are consistent with previous studies; however, this study demonstrates that the amount of the cathepsins released was fairly constant through the time course of our experiments; there is a basal release. It is possible that to upregulate the release of the cathepsins a stimulus other than the presence of elastin is required. This merits further investigation, given the potency of the cysteine proteases.

The amount of elastin degraded also declines with time, possibly because the metalloproteinases are less potent elastolytic enzymes than either the serine or cysteine proteases (49).

Despite the powerful evidence in mice that MMP-12 has a key role in the pathogenesis of emphysema, its role in humans is at present unclear (21). In this series of experiments, we performed Western blots with the same antibody and under the same conditions as investigators who have identified MMP-12. We did not, however, perform casein zymograms, which may be a more sensitive method for the detection of small quantities of MMP-12. Other investigators have also been unable to identify MMP-12 in human tissue (34), al-

Table 4. MMP profile of AM supernatants of three subject groups collected at 24 h

<table>
<thead>
<tr>
<th></th>
<th>COPD</th>
<th>Healthy Smokers</th>
<th>Nonsmokers</th>
</tr>
</thead>
<tbody>
<tr>
<td>MMP-9 (ng/ml, ELISA)</td>
<td>8.0 ± 0.4</td>
<td>5.1 ± 0.37*</td>
<td>3.8 ± 0.35†</td>
</tr>
<tr>
<td>MMP-9 latent (band density, Western blot)</td>
<td>8.448 ± 1.205</td>
<td>3.061 ± 645*</td>
<td>4.583 ± 714*</td>
</tr>
<tr>
<td>MMP-9 cleaved (band density, Western blot)</td>
<td>43.500 ± 5.069</td>
<td>9.409 ± 1.016*</td>
<td>6.424 ± 1.257*</td>
</tr>
<tr>
<td>MMP-2 activity (%control)</td>
<td>3.5 ± 0.3</td>
<td>2.7 ± 0.2*</td>
<td>1.8 ± 0.2†</td>
</tr>
<tr>
<td>MMP-9 activity (%control)</td>
<td>36.5 ± 3.3</td>
<td>22.3 ± 1.4†</td>
<td>10.7 ± 0.8†</td>
</tr>
</tbody>
</table>

All data are means ± SE. MMP-9 protein levels measured by ELISA and Western blot; MMP activity was measured using zymography. *P < 0.05 vs. COPD, †P < 0.05 vs. healthy smokers; n = 10 for all groups.

Fig. 3. A: sample gelatin zymogram demonstrating increased gelatin degradation by MMP-9 (latent and cleaved) derived from AM of subjects with COPD. One microgram of protein was loaded in each lane. Lanes 1, 4: COPD; lanes 2, 5: healthy smokers; lanes 3, 6: nonsmokers; lane 7: latent MMP-2 standard; lane 8: latent MMP-9 standard. B: sample Western blot for latent MMP-9, demonstrating increased amounts of protein in samples from AM from subjects with COPD. Five micrograms of protein were loaded to each lane. Lane 1: MMP-9 + vehicle control; lane 2: nonsmoker; lane 3: healthy smoker; lane 4: COPD; lane 5: nonsmoker; lane 6: healthy smoker; lane 7: COPD.
though MMP-12 mRNA has been found (19). Another possible explanation is that AM need a potent stimulus to produce and release MMP-12, which was not present in our system. Our data suggest that MMP-9 may play a more significant role in elastolysis than MMP-12 from AM in this cell culture model, as it is present in increased amounts and the MMP-9 detected is present in the cleaved (mature) form as demonstrated by Western blotting and zymography. Cleavage is not equivalent to active as cleaved MMP (in the biological system) may still be bound to and inhibited by tissue inhibitor of MMP (TIMP; see below).

Macrophages can be activated in many ways and by many stimuli. Small airway inflammation occurs in COPD, with an overall increase in inflammatory cells and particularly an increase in CD8+ T cells (38). There is evidence for oxidative stress in COPD, and this can both enhance elastolytic enzyme activation and prevent enzyme inhibitor action (27, 35). In our system, elastolysis occurs in the absence of any stimulus other than elastin fragments and macrophage adherence to the cell culture plate. Elastin fragments have previously been shown to be potent chemoattractants for macrophages (24), and it is possible that they are also a stimulus for the release of elastolytic enzymes. Our experiments demonstrate that macrophages from subjects with COPD are more capable of increased elastolysis than those from healthy smokers and nonsmokers in the presence of the same stimulus (elastin fragments and adherence). This finding is consistent with a previous study in subjects with emphysema, although the nature of the elastolytic activity was not determined (32, 33). A study in rats exposed to cigarette smoke also demonstrated increased elastolytic activity of AM (34). The COPD-derived macrophages appear to be intrinsically different, but the reasons for this are not yet certain. The role of macrophage adherence in this is uncertain and merits further investigation.

Macrophages also release the inhibitors of MMP, the TIMP, along with MMP. If the TIMP release is decreased or TIMP function is reduced through the action of oxidants, then excess MMP activity will result (20). To take our study further, it would be necessary to measure TIMP and the levels of TIMP-MMP interaction; this unfortunately was beyond the scope of this present investigation.

Cigarette smoke contains a complex mixture of chemicals, many of which may alter cell function (14, 29, 30). It is possible that the macrophages from individuals susceptible to the effects of cigarette smoke respond in a way different from those of healthy smokers. It may be that smoking stimulates the release of MMP and/or inactivates TIMP, leading to an increase in ECM loss. There is also evidence that despite cessation of smoking, the increased rate of decline of lung function continues, implying that a change has occurred that is irreversible and has long-lasting effects (43). These exsmokers with COPD may be important in elucidating the physiological changes that occur and lead to COPD.

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