Upregulation of Gelatinases A and B, Collagenases 1 and 2, and Increased Parenchymal Cell Death in COPD*

Lourdes Segura-Valdez, PhD; Annie Pardo, PhD; Miguel Gaxiola, MD; Bruce D. Uhal, PhD; Carina Becerril, MSc; and Moisés Selman, MD, FCCP

Background: A central feature in the pathogenesis of COPD is the inflammation coexisting with an abnormal protease/antiprotease balance. However, the possible role of different serine and metalloproteinases remains controversial.

Patients and measurements: We examined the expression of gelatinases A and B (matrix metalloproteinase [MMP]-2 and MMP-9); collagenases 1, 2, and 3 (MMP-1, MMP-8, and MMP-13); as well as the presence of apoptosis in lung tissues of 10 COPD patients and 5 control subjects. In addition, gelatinase-A and gelatinase-B activities were assessed in BAL obtained from eight COPD patients, and from six healthy nonsmokers and six healthy smoker control subjects.

Setting: Tertiary referral center and university laboratories of biochemistry, and lung cell kinetics.

Results: Immunohistochemical analysis of COPD lungs showed a markedly increased expression of collagenases 1 and 2, and gelatinases A and B, while collagenase 3 was not found. Neutrophils exhibited a positive signal for collagenase 2 and gelatinase B, whereas collagenase 1 and gelatinase A were revealed mainly in macrophages and epithelial cells. BAL gelatin zymography showed a moderate increase of progelatinase-A activity and intense bands corresponding to progelatinase B. In situ end labeling of fragmented DNA displayed foci of positive endothelial cells, although some alveolar epithelial, interstitial, and inflammatory cells also revealed intranuclear staining.

Conclusion: These findings suggest that there is an upregulation of collagenase 1 and 2 and gelatinases A and B, and an increase in endothelial and epithelial cell death, which may contribute to the pathogenesis of COPD through the remodeling of airways and alveolar structures.

(CHEST 2000; 117:684–694)

Key words: apoptosis; collagenases; COPD; emphysema; gelatinases; metalloproteinases

Abbreviations: EDTA = ethylenediaminetetraacetic acid; ISEL = in situ end labeling; MMP = matrix metalloproteinase; PBS = phosphate-buffered saline; SDS = sodium dodecyl sulfate

COPD is a major clinical disorder usually associated with cigarette smoking. The disease is characterized by a chronic, slowly progressive airway obstructive disorder resulting from a combination of pulmonary emphysema and irreversible reduction in the caliber of small airways of the lung.1 Usual findings include chronic bronchitis, small airway disease, and severe widespread centriacinar emphysema, either alone or associated with panacinar emphysema. Emphysema is an anatomically defined condition characterized by abnormal and permanent airspace enlargement beyond the terminal bronchioles accompanied by destruction of the alveolar walls.2

The most accepted theory for the pathogenesis of emphysema involves a proteinase/antiproteinase imbalance. In this context, there are a number of studies supporting that excessive elastolytic activity,3,4 and more recently collagenolytic activity,5–7 participating in the connective tissue destruction of the lung parenchyma. The main cellular sources of enzymatic activity in the lower respiratory tract are neutrophils and alveolar macrophages, both of which are increased in the smoker’s lung.8,9 However, in situ evidence of inflammatory cells producing pro-
teolytic enzymes is scanty. In addition, studies have been primarily focused on emphysematous lesions, but the possible participation of enzymatic breakdown in airways remodeling has not been explored.

Elastolytic activity has been attributed in the human disease to neutrophil serine elastase, and in a mouse experimental model to macrophage metalloelastase. Nevertheless, gelatinases A and B (matrix metalloproteinase [MMP]-2 and MMP-9), a subgroup of the matrix metalloproteinases family, include elastin in their substrate specificity, and therefore might also play a role in the rupture of the elastic fibers. 

In this context, a recent work performed on emphysematous samples obtained by partial lung resection showed increased expression of gelatinase A.

Concerning collagenase, although macrophages are the main cells suspected to be responsible for this exaggerated enzymatic activity, data have been obtained in the human disease only with cells from BAL.

On the other hand, changes in lung extracellular matrix and exposure to oxidant injury as occurs in COPD may provoke cell death by apoptosis, and this process may eventually contribute to the chronic lung damage. To our knowledge, cell death has not been evaluated in pulmonary tissues of human COPD.

With these precedents, the aim of this study was to explore the occurrence of apoptosis and the expression and localization of gelatinases A and B, and neutrophil and macrophage collagenases in lungs from patients with COPD.

Materials and Methods

Study Population

We studied the expression and localization of immunoreactive MMP-1, MMP-2, MMP-8, MMP-9, and MMP-13 in paraffin-embedded lung tissues collected from 10 male individuals with COPD (group 1). Seven of them were obtained from autopsies of patients with prolonged and terminal lung disease (mean age ± SD, 66.8 ± 5.5 years), while the other three derived from lung volume reduction surgery (ages, 59, 56, and 55 years). Male individuals who died from causes other than lung diseases were used as control subjects (mean age, 56 ± 5.1 years).

In addition, gelatinase-A and gelatinase-B activities were assessed in the BAL obtained from eight male COPD patients (group 2; mean age, 60.9 ± 4.2 years), six healthy nonsmoker volunteers (mean age, 37 ± 5.0 years), and six healthy smokers volunteers (mean age, 39.5 ± 7.8 years). The study was approved by the Ethical Committee of the Institute.

The diagnosis of COPD was established in both groups by medical history and pulmonary function tests. We used the American Thoracic Society criteria to define COPD, a history of productive cough for 3 months in each of 2 successive years, and FEV₁/FVC ratio < 0.70, the total lung capacity being > 80% of the predicted value. The presence of emphysematous lesions was confirmed in group 1 by autopsy findings, and in group 2 by CT scans. All patients were heavy smokers (group 1, 45.8 ± 7.5 pack-years; group 2, 49.5 ± 5.1 pack-years), and all of them had severe airflow limitation with FEV₁ < 50% predicted.

BAL

BAL samples were obtained with slight modifications, as described elsewhere. One of the subsegmental bronchi of the middle lobe was lavaged with six 50-mL aliquots of sterile saline solution. The recovered fluid was measured, strained through surgical gauze to remove mucus and debris, and centrifuged at 400g for 10 min at 4°C. Cell pellets were resuspended in 5 mL of phosphate-buffered saline (PBS) solution, and the CBC count was measured in a hemocytometer. Aliquots were fixed in carbowax, and three slides per sample were stained with hematoxylin-eosin, Giemsa, and toluidine blue, and used for differential cell count. The supernatants were stored at −70°C until use.

BAL Gelatin Zymography

Sodium dodecyl sulfate (SDS) polyacrylamide gels containing gelatin (1 mg/mL) were used to identify proteins with gelatinolytic activity from BAL supernatants. Each lane was loaded with 20 μL of sample. After electrophoresis, the gels were incubated in a solution of 2.5% Triton X-100 (Sigma; St. Louis, MO) for 30 min, washed extensively with water, and incubated overnight at 37°C in glycin, 100 mM pH 7.6, containing 10 mM CaCl₂ and 50 nm ZnCl₂. The gels were stained with Coomassie Brilliant Blue R250 (Sigma) and destained in a solution of 7.5% acetic acid and 5% methanol. Zones of enzymatic activity appeared as clear bands against a blue background. Serum-free conditioned medium from human lung fibroblasts was used as gelatinase A marker, and serum-free conditioned medium from phorbol 12-myristate 13-acetate-stimulated U2-OS cells as marker of gelatinase B. Identical gels were incubated in the presence of 20 mM ethylenediaminetetraacetic acid (EDTA). Gelatinolytic activities were quantified using software (Kodak Digital Science 1D Image Analysis Software; Eastman Kodak; Rochester, NY) that quantifies the surface and intensity of lysis bands. Results were expressed in progelatinase A and B arbitrary units: 18 h/20 μL BAL/10,000.

Immunohistochemistry

Collagenase 1 (MMP-1), gelatinases A and B (MMP-2, MMP-9); collagenase 2 (MMP-8); and collagenase 3 (MMP-13) were analyzed by immunohistochemistry, using specific monoclonal primary antibodies.

Tissue sections (3 to 5 μm) were deparaffinized and then rehydrated and blocked with 3% H₂O₂ in methanol for 30 min followed by universal blocking (normal serum, 1.5%; Vector Laboratories; Burlingame, CA). Prior to the immune reaction, antigen retrieval with 0.1 mol/L citrate buffer, pH 6.0, was performed. Primary antibodies—MMP-8, MMP-13 (Fuji Chemical Industries; Toyama, Japan), and MMP-2 (Calbiochem; San Diego, CA) at 5 μg/mL concentration, and MMP-1 and MMP-9 (Fuji Chemical Industries) at 10 μg/mL concentration—were applied and incubated at 4°C overnight. Detection was made by using goat antimouse biotinylated secondary antibody (Dako; Carpinteria, CA). The positive intracytoplasm staining was revealed with chromogenic enzymes coupled to streptavidin, either peroxidase (Vector Laboratories) for MMP-1, MMP-2, and MMP-8, or alkaline phosphatase (Dako) for MMP-9. Negative controls were carried out incubating with nonimmune sera (1.5%). All samples were counterstained with hematoxylin.

CHEST / 117/3 / MARCH, 2000 685
In Situ End Labeling

The in situ end labeling (ISEL) of fragmented DNA was performed as described elsewhere. Tissue sections were deparaffinized by being passed through xylene, 1:1 xylene-alcohol, 100% alcohol, and 70% alcohol for 10 min each. Ethanol was removed by rinsing in distilled water for at least 10 min. The coverslips were then placed in 0.23% periodic acid (Sigma) for 30 min at 20°C. Samples were rinsed once in water and three times in 0.15 mol/L PBS solution for 4 min each, and were then incubated in saline-sodium citrate solution (0.3 mol/L NaCl and 30 mM sodium citrate in water, pH 7.0) at 80°C for 20 min. After four rinses in PBS solution and four rinses in buffer A (50 mM Tris HCl, 5 mM MgCl₂, 10 mM β-mercaptoethanol, and 0.005% bovine serum albumin in water, pH 7.5), the coverslips were incubated at 18°C for 2 h with ISEL solution (0.001 mM biotinylated dUTP, 20 U/mL of DNA polymerase I, and 0.01 mM each dATP, dCTP, and dGTP in buffer A). Afterward, the sections were rinsed thoroughly five times with buffer A and three additional times in 0.5 mol/L PBS solution. Detection of incorporated dUTP was achieved with a fast blue chromogen system. The tissues were rinsed in distilled water three times, counterstained with eosin, and mounted under Fluoromount solution (Southern Biotechnology Associates; Birmingham, AL).

Data Analysis

All data are expressed as mean ± SD. Because some cell subpopulations recovered in BAL fluid did not follow a normal distribution, comparisons were made through nonparametric tests (Kruskal-Wallis plus Mann-Whitney U test). Correction for multiple comparison was done by Bonferroni’s method. Values of p < 0.05 were considered as statistically significant.

RESULTS

Mean (±SD) values of age, smoking history, and lung functional characteristics are shown in Table 1. All patients were heavy smokers and displayed severe airflow limitation, but no statistical differences were found in the functional respiratory tests of both groups. On microscopic examination, lung tissues of group 1 exhibited varied, but usually severe, airway inflammation, goblet cell hyperplasia, and focal squamous metaplasia. In peripheral airways, variable degrees of wall inflammation, fibrosis, and smooth muscle hypertrophy were noticed. Alveolar walls were broken down with enlargement of the alveolar spaces. Some patients exhibited features of lung infection with important neutrophil infiltration in the bronchial epithelium and bronchial glands, and large numbers of intraluminal neutrophils.

Immunohistochemistry

All COPD lungs showed a positive signal for collagenases 1 and 2, while collagenase 3 was not detected. Figure 1 illustrates the results obtained with MMP-1: the immunoreactive collagenase 1 was localized primarily in alveolar and interstitial macrophages (top left, A and top right, B); frequently in bronchial epithelial cells (upper left, C and upper right, D); in some foci of subepithelial fibroblast-like cells (lower left, E and lower right, F); and in endothelial cells (bottom left, G).

On the other hand, numerous neutrophils exhibited a strong intracytoplasmic signal for collagenase 2 (Fig 2). In general, clusters of neutrophils located in the alveolar septa and alveolar spaces, as well as in bronchial epithelium, were usually positive.

Gelatinases A and B were also increased in COPD lungs, both in airways and lung parenchyma. A strong signal for immunoreactive gelatinase B was observed in neutrophils, usually located in similar areas as described for collagenase 2; many of these positively stained neutrophils were infiltrating the alveolar walls (Fig 3, top left, A; top right, B; and middle left, C). Additionally, intravascular neutrophils stained with anti-MMP-9 were also seen (Fig 3, middle right, D). Immunoreactive gelatinase A was revealed in mononuclear inflammatory cells and epithelial cells (Fig 4).

Some control lungs displayed scattered positive inflammatory cells for the MMPs, whereas immunoreactivity was absent in others. In general, neutrophils in normal lungs, when present, showed MMP-8 and MMP-9 staining (Fig 2, bottom right, E; Fig 3, bottom left, E). Alveolar macrophages in normal lungs were occasionally positive for MMP-1 and usually negative for MMP-2 (Fig 1, bottom center, H and bottom right, I). Control samples incubated with nonimmune sera were negative (Fig 3, bottom right, F; Fig 4, bottom right, D).

ISEL

ISEL of fragmented DNA in sections of COPD lungs showed several foci of labeled cells (Fig 5, top left, A; top right, B; lower left, E; and bottom left, G). Most cells giving a positive signal were endothelial cells from capillaries and arterioles (Fig 5, upper left, C and upper right, D). Less frequently, prominent intranuclear staining was also revealed in alveolar epithelial cells, interstitial cells, and inflammatory cells such as neutrophils and lymphocytes (Fig 5, top right, B; bottom left, G).

Table 1—Characteristics of COPD Groups*

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Group 1 (n = 10)</th>
<th>Group 2 (n = 8)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age, yr</td>
<td>63.8 ± 6.7</td>
<td>60.9 ± 4.2</td>
<td>NS</td>
</tr>
<tr>
<td>Smoking pack-yr</td>
<td>45.8 ± 7.5</td>
<td>49.5 ± 5.1</td>
<td>NS</td>
</tr>
<tr>
<td>FEV₁, % predicted</td>
<td>35.6 ± 12.2</td>
<td>43.2 ± 4.7</td>
<td>NS</td>
</tr>
<tr>
<td>FEV₁/FVC, %</td>
<td>42.3 ± 6.6</td>
<td>48.5 ± 7.0</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SD; NS = not significant.
lower right, F). Control lungs were negative or displayed occasional scattered positive cells (Fig 5, bottom right, H).

**BAL Fluid**

A significant lower volume of BAL fluid was recovered from COPD patients as compared to smokers and nonsmoker control subjects (COPD patients, $141 \pm 34.6$ mL vs smokers, $205 \pm 61$ mL and nonsmokers, $234 \pm 34.1$ mL; $p < 0.05$ and $p < 0.01$, respectively). No significant differences in protein concentration were found between the three groups (COPD, $60.3 \pm 22.4$ μg/mL; smokers, $48.9 \pm 21.5$ μg/mL; and nonsmokers, $40.1 \pm 12.4$ μg/mL).
The number and types of cells obtained in the lung lavage are shown in Table 2. COPD patients showed an increase in total cells compared with nonsmoker control subjects. Because some cell subpopulations recovered in BAL fluid did not follow a normal distribution, comparisons were made through non-parametric tests. COPD patients exhibited a significant increment in the percentage of neutrophils when compared with nonsmoker control subjects, while no differences in the percentage of macrophages, lymphocytes, and eosinophils were detected. No significant differences were found in cell profile between COPD and healthy smokers.

**Gelatin Zymography of BAL Fluid**

To identify BAL gelatinolytic activities, aliquots adjusted to 20 μL of lavage fluid were analyzed by gelatin substrate gel zymography. A representative gelatin zymogram comparing COPD with nonsmoker and smoker control subjects is shown in Figure 6. BAL control samples showed faint bands of 72-kd and 92-kd activities corresponding to progelatinase A and progelatinase B, respectively (Fig 6, top, lanes C1 and C2). BAL fluid obtained from smokers showed a twofold increase of 92-kd gelatinolytic activity (Fig 6, top, lanes S1 to S3). COPD patients revealed a marked increase of BAL progelatinase-B activity (Fig 6, top, lanes P1 to P7), and in some samples, the activated form of 86 kd was also evident. In addition, gelatinolytic bands of higher molecular weight, likely representing lipocalin-associated progelatinase-B specific of neutrophils, were also noticed. Densitometric analysis showed a seven-fold and 13-fold increase of progelatinase-B activity in comparison with healthy smokers and nonsmoker control subjects, respectively (Fig 6, bottom). By contrast, progelatinase-A activity displayed only a moderate increase as compared to smoker and nonsmoker control samples (twofold and threefold, respectively). All gelatinolytic bands were fully inhibited by EDTA (not shown).

**Figure 6.** BAL control samples showed faint bands of 72-kd and 92-kd activities corresponding to progelatinase A and progelatinase B, respectively (Fig 6, top, lanes C1 and C2). BAL fluid obtained from smokers showed a twofold increase of 92-kd gelatinolytic activity (Fig 6, top, lanes S1 to S3). COPD patients revealed a marked increase of BAL progelatinase-B activity (Fig 6, top, lanes P1 to P7), and in some samples, the activated form of 86 kd was also evident. In addition, gelatinolytic bands of higher molecular weight, likely representing lipocalin-associated progelatinase-B specific of neutrophils, were also noticed. Densitometric analysis showed a seven-fold and 13-fold increase of progelatinase-B activity in comparison with healthy smokers and nonsmoker control subjects, respectively (Fig 6, bottom). By contrast, progelatinase-A activity displayed only a moderate increase as compared to smoker and nonsmoker control samples (twofold and threefold, respectively). All gelatinolytic bands were fully inhibited by EDTA (not shown).
COPD is a chronic respiratory disorder characterized by slowly progressive airflow obstruction. It usually occurs in heavy smokers, and it is associated with variable degrees of bronchial inflammation and mucus gland hyperplasia, small airway inflammation and fibrosis, and emphysema. Inflammation in COPD is a central and complex pathologic process involving different immune and inflammatory cells, although the precise role of each of them in the pathogenesis is still controversial. According to some evidence, an increased total number of activated T lymphocytes and macrophages characterizes airways and alveolar inflammation in the absence of an exacerbation by infection. Actually, macrophages are usually the most abundant inflammatory cells found in BAL fluid of cigarette smokers, as well as in respiratory bronchioles where emphysematous changes are first manifested. However, lung biopsy and lavage samples obtained from COPD patients also contain increased numbers of neutrophils and a weak but significant relationship between neutrophil sequestration and microscopic emphysema has been also found. Moreover, there is evidence suggesting that cigarette smoking provokes an increased lung neutrophil traffic in human and experimental animals, probably through an up-regulation of interleukin-8 and neutrophil adhesion molecules. Additionally, the clinical course of COPD is recurrently interrupted by acute exacerbations, often provoked by bacterial infections, which occur particularly during the winter months. In these cases, the inflammatory response involves mainly neutrophils whose products can further promote the lung damage.

The continuous presence of activated T lympho-

Figure 3. Immunostaining of MMP-9 in COPD lung tissues revealed by fast red chromogen and counterstained with hematoxylin. Top left, A: immunoreactive gelatinase B was seen in numerous neutrophils infiltrating the alveolar walls (×20). Top right, B and center left, C: high-power magnification of the same area shown in top left, A (×100). Center right, D: immunoreactive MMP-9 in intravascular neutrophils (×40). Bottom left, E: normal lung showing a positive intravascular neutrophil (arrow; ×40). Bottom right, F: negative control section without the primary antibody (×20).
cytes, macrophages, and neutrophils in the upper and lower respiratory tract of COPD patients may have a variety of potential deleterious consequences. However, the mechanisms by which these immune and inflammatory cells participate in the impair and remodeling of the airways and the parenchymal architecture are not precisely known. Macrophages and neutrophils enhance the oxidative stress, and release a variety of proteolytic enzymes. Oxidants react with many cellular components, oxidizing proteins, lipids, and DNA bases; and enzymes may degrade the extracellular matrix molecules that constitute the structural framework of the lung architecture. However, the enzymes participating in the proteolytic lung injury are still under debate, and most evidence suggests a high level of complexity, with the possible participation of both serine and metalloproteinases.3–7,10

Our findings support the notion that several metalloproteinases may play a role in the pathogenesis of COPD. Using immunohistochemistry, we found an increased number of neutrophils in airways and alveolar spaces and alveolar walls, which is accompanied by a marked upregulation of collagenase 2 and gelatinase B. In addition, alveolar macrophages, interstitial cells, and epithelial cells were expressing collagenase 1 and gelatinase A. Moreover, increased gelatinase-A, but mainly gelatinase-B activities were noticed in BAL fluid from COPD patients. Our results differ with a recent report suggesting that immunoreactivity against MMP-1, MMP-8, and MMP-9 is absent in emphysematous tissues; although in the same study, increased collagenolytic and gelatinolytic activities were clearly seen.12 Likewise, it has been demonstrated that alveolar macrophages obtained from BAL of patients with emphysema produce elevated quantities of MMP-1 and MMP-9.7

Our findings are consistent with the concept that chronic exposure to tobacco smoke provokes an increased traffic of neutrophils and macrophages in the smoker’s lungs, which in turn are activated and release a number of molecules including MMPs. The excessive release of MMPs into the airway and parenchymal microenvironments can account for matrix remodeling and basement membrane disruption in both the upper and lower respiratory tract. Actually, a prominent signal was observed in the cells located in the alveolar spaces and alveolar walls, as well as in the epithelial and subepithelial regions of the airways. Interstitial collagenases are involved in fibrillar collagen degradation cleaving the triple helical region of collagen types I and III (localized in the extracellular matrix of the lung parenchyma) and collagen type II (located in the cartilage of the airways) generating three-fourth and one-fourth collagen fragments.36 Gelatinases have the capacity to degrade type-IV collagen, the major structural com-

Figure 4. Immunohistochemical localization of MMP-2. A positive label was revealed by using 3,3′-diaminobenzidine, and samples were counterstained with hematoxylin. Top left, A: positive immunoreactive macrophages located in the interstitial spaces (× 40). Top right, B (× 100) and bottom left, C (× 63): high-power magnifications showing immunolabeled macrophages (arrowhead; top right, B) and putative epithelial cells (arrow; top right, B). Bottom right, D: negative control omitting the primary antibody (× 40).
ponent of basement membranes, and are also able to degrade insoluble elastin. Therefore, excessive collagenases and gelatinases activities may have a profound effect on the major extracellular matrix components of the lungs, provoking interstitial fibrillar collagen degradation and contributing to the breakdown of elastic fibers. This effect may explain why (during the development of the emphysematous lesions that are an integral part of COPD) the alveolar walls are completely destroyed and (in more advanced stages) the abnormal spaces may coalesce into larger bullae.

In addition, the expression of some of these MMPs, ie, gelatinase B, may also participate in inflammatory cell migration across basement membrane.

Importantly, however, emphysematous lesions in COPD involve not only extracellular matrix destruction but also the loss of cellular components, including epithelial and endothelial cells, through mecha-

![Figure 5](image-url)
nisms that are not yet determined. In this study, we present for the first time evidence of increased apoptosis in lungs of COPD patients. In general, endothelial cells from capillaries and arterioles, and occasionally alveolar epithelial cells, interstitial cells, and inflammatory cells, exhibited prominent intranuclear staining by ISEL of fragmented DNA. This finding supports the notion that apoptosis may account, at least partially, for the loss of pulmonary capillaries and alveoli during the development of emphysema.

Excessive production of oxidants and disruption of the normal epithelial cell-matrix interactions provoked by an upregulation of serine and MMPs activities might initiate apoptotic and/or necrotic pathways in COPD. In other processes, it has been suggested that metalloproteinases, in addition to playing a role in extracellular matrix degradation, can alter cellular functions, including induction of apoptosis. For example, the degradation of basement membrane may undergo unscheduled apoptosis in the mammary alveolar epithelium during pregnancy. Likewise, homozygous mice with a null mutation of gelatinase-B gene show a normal development of hypertrophic chondrocytes, but with delayed apoptosis.

Concerning COPD, there is some evidence suggesting that leukocyte elastase can induce apoptotic

<table>
<thead>
<tr>
<th>BAL Fluid Findings</th>
<th>COPD, group 2 (n = 8)</th>
<th>Healthy Smokers (n = 6)</th>
<th>Healthy Nonsmokers (n = 6)</th>
<th>p Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cells, ( \times 10^6 )</td>
<td>19.8 ± 9.0</td>
<td>13.9 ± 6.1</td>
<td>10.8 ± 3.7</td>
<td>&lt; 0.05†</td>
</tr>
<tr>
<td>Macrophages, %</td>
<td>68.1 ± 19.2</td>
<td>86.0 ± 5.6</td>
<td>82.3 ± 11.5</td>
<td>NS</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>19.1 ± 20.3</td>
<td>10.8 ± 4.2</td>
<td>16.3 ± 11.4</td>
<td>NS</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>12.5 ± 9.32</td>
<td>3.2 ± 1.5</td>
<td>1.1 ± 1.1</td>
<td>&lt; 0.001*</td>
</tr>
<tr>
<td>Eosinophils, %</td>
<td>0.23 ± 0.46</td>
<td>0.7 ± 0.5</td>
<td>0</td>
<td>NS</td>
</tr>
</tbody>
</table>

*Data are expressed as mean ± SD; see Table 1 for abbreviation.†COPD vs nonsmoker controls.

![Figure 6](http://journal.publications.chestnet.org/pdfaccess.ashx?url=/data/journals/chest/21941/)
cell death. Additionally, activated T lymphocytes, which are increased in COPD and seem to correlate with the degree of emphysema, might also provoke apoptosis. However, further studies are needed to determine the mechanisms underlying the process of cell death in COPD.

In conclusion, our findings demonstrate that COPD lungs exhibit markedly increased production of matrix-degrading enzymes, such as gelatinases A and B, as well as collagenses 1 and 2. In addition, areas of endothelial and epithelial cell death are often present in the lung parenchyma. Both pathologic processes may play a role in the pathogenesis of COPD through the remodeling of airways and alveolar-capillary structures.

References


