A Comparison of Airway and Serum Matrix Metalloproteinase-9 Activity Among Normal Subjects, Asthmatic Patients, and Patients With Asthmatic Mucus Hypersecretion*

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Background: The rate of decline in lung function is increased in asthmatic patients, particularly in those with coexisting asthmatic mucus hypersecretion (AMH), in whom inflammation and the activity of matrix metalloproteinase (MMP)-9 and tissue inhibitor of metalloproteinase (TIMP)-1 in serum and BAL fluid (BALF) may be increased.

Methods: Seven nonasthmatic subjects and 22 asthmatic subjects completed a questionnaire, and underwent lung function testing and bronchoscopy, during which AMH was diagnosed by the presence of mucus plugging. Subjects were classified as follows: mild/moderate asthma; severe asthma; and AMH. In BALF, we measured the differential WBC counts and MMP-9 activity by zymography. We measured total MMP-9 and TIMP-1 activity by enzyme-linked immunosorbent assay in BALF and serum.

Results: The mean (± confidence interval) FEV1 was lower in AMH patients (73 ± 13% predicted) compared with nonasthmatic subjects (95 ± 7%) and patients with mild/moderate asthma (73 ± 9%; p < 0.05), and was similar to that of patients with severe asthma (80 ± 20%). MMP-9 activity was greater in AMH patients and in patients with severe asthma compared with nonasthmatic subjects (p = 0.05 and p = 0.01, respectively), as were TIMP-1 activities (p = 0.001 and p = 0.04, respectively), but MMP-9/TIMP-1 ratios were not. MMP-9 activity increased across the four groups from nonasthmatic subjects to AMH patients (r = 0.58; p = 0.0009), but the differential and total WBC counts were similar. There were no relationships between FEV1 percent predicted and either MMP-9 activity or MMP-9/TIMP-1 ratio. There were no differences in serum MMP-9 activity, which did not correlate with MMP-9 activity in BALF.

Conclusions: AMH and severe asthma were associated with greater proteolytic enzyme activities despite similar airway inflammation, which might play a role in remodeling and accelerated the decline in lung function in these patients. (CHEST 2005; 127:1919–1927)

Key words: asthma; BAL fluid; matrix metalloproteinase; mucus hypersecretion

Abbreviations: AMH = asthmatic mucus hypersecretion; ANOVA = analysis of variance; BALF = BAL fluid; DRR = dose-response ratio; ELISA = enzyme-linked immunosorbent assay; IL = interleukin; MMP = matrix metalloproteinase; PBS = phosphate-buffered saline; TIMP = tissue inhibitor of metalloproteinase

Asthma has many well-known clinical manifestations, one of which is an accelerated decline in spirometric function. This occurs particularly in patients who have severe, longstanding, or adult-onset asthma ultimately leading to chronic, fixed airflow limitation of varying degree.1,2 This is thought to be secondary to structural changes in the airway,3,4 collectively referred to as remodeling, which involves changes in extracellular matrix, collagen, elastin, and airway smooth muscle.4,5 Accelerated decline in spirometric function also occurs in asthmatic subjects who report chronic cough and sputum production due to asthmatic mucus hypersecretion (AMH), but who do not have bronchiectasis, allergic bronchopulmonary aspergillosis, or a history of heavy smoking.6,7

Matrix metalloproteinases (MMPs) play a role in tissue remodeling in diseases such as asthma,8 bronchiectasis,9 rheumatoid arthritis,10 and mesothelioma,11 and in wound healing.12 MMP-9 is a 92-kd collagenase that cleaves type IV collagen, which makes up a substantial portion of the basement
membrane of airways and is regulated by the tissue inhibitor of metalloproteinase (TIMP)-1, which binds to MMPs in a 1:1 ratio. Its activity is increased in the bronchial biopsy specimens, blood, induced sputum, and BAL fluid (BALF) of patients who have severe or uncontrolled asthma.

Because of the association of decline in lung function with AMH, severe asthma, and persistent airway inflammation, we hypothesized that MMP-9 activity would be increased in the blood and airway secretions in asthmatic patients who had severe disease and/or AMH (diagnosed at bronchoscopy), and that it would correlate with airway inflammation. Our aim was to compare MMP-9 activity and TIMP-1 expression in the serum and BALF in healthy subjects, patients with mild/moderate asthma, patients with severe asthma, and patients with AMH. In addition, we compared MMP-9 activity in serum and BALF.

**Materials and Methods**

Asthmatic and nonasthmatic subjects underwent clinical testing in the lung function laboratory and then underwent bronchoscopy within 2 weeks of laboratory testing. Asthmatic subjects were classified as having mucus hypersecretion if they had mucus causing occlusion of the subsegmental or larger airways visualized during bronchoscopy, as described by Thompson et al (bronchoscopists, G.G.K. and K.Y.). The study was approved by the Ethics Review Committee of the Central Sydney Area Health Service (reference X99–0217). Written informed consent was obtained from all subjects.

**Subjects**

Seven nonasthmatic subjects and 22 asthmatic subjects were recruited from among patients and students at the Asthma Centre, Royal Prince Alfred Hospital, and from the University of Sydney. The diagnosis of asthma was based on the World Health Organization/National Heart, Lung, and Blood Institute guidelines. Asthmatic subjects had clinically stable asthma, as defined by stable symptoms and medication usage over the previous 3 months, and had no symptoms of respiratory tract infection in the previous 6 weeks. None of the subjects were current smokers, ex-smokers of > 20 pack-years, or pregnant, or had coexistent chronic cardiac or pulmonary disease.

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**Clinical Assessment of Asthma**

We administered a respiratory questionnaire that included questions on the frequency and nature of asthma symptoms, medication use, and the presence of chronic cough that was productive of phlegm. The dose of inhaled corticosteroids was converted to equivalent doses of beclomethasone dipropionate according to the following equation: 100 μg of beclomethasone dipropionate = 80 μg of budesonide = 50 μg of fluticasone. Skin prick tests were performed with a panel of 14 common aeroallergens applied to the forearm. Skin wheal sizes were considered to be positive if they were ≥ 4 mm in mean diameter, and atopy was defined as the presence of one or more positive reactions.

**Lung Function Tests**

Spirometry was measured in all subjects in accordance with American Thoracic Society criteria (VMAX; SensorMedics; Yorba Linda, CA), and the normal reference values used were those of Morris et al. Nonasthmatic subjects underwent methacholine challenge with doses ranging from 3 to 199 μmol using a nebulizer (model No. 646; DeVilbiss HealthCare; Somerset, PA) and a dosimeter (Rosenthal French; Baltimore, MD). Asthmatic subjects underwent methacholine challenge by the rapid method and received doses from 0.03 to 7.5 μmol administered with a hand-held nebulizer (model No. 45; DeVilbiss). The challenge was stopped in all subjects either after their FEV1 had decreased by ≥ 20% of baseline values or after the final dose had been administered. Results were expressed as the log dose–response ratio (DRR).

**BAL**

Fiberoptic bronchoscopy (Olympus; Tokyo, Japan) was performed under IV sedation after premedication with IV atropine and local anesthesia of the upper airways half an hour before the procedure. BAL was performed in a subsegmental bronchus of the left lower lobe by infusing 50 mL of sterile 0.9% saline solution. BALF was separated into acellular and cellular components by centrifugation (400 g for 10 min at 4°C). The supernatant was further centrifuged (5,000 g for 20 min at 4°C) to remove any debris and was stored at −20°C in 1-mL aliquots for zymography.

The cell pellet was resuspended in phosphate-buffered saline (PBS) solution to its original BALF volume, filtered through nylon gauze (pore size, 60 μm), then diluted 1:1 with 20 μL of Trypan blue for cell counting and viability assessment using a hemocytometer. To standardize cell numbers, after centrifugation (200 g for 10 min at 20°C), PBS solution was added to the pellet to yield approximately 1 × 10⁶ cells/mL. Seventy microliters of the cell suspension was added to a cytospin slide, spun at 5,000 revolutions per minute for 5 min and then stained (Diff Quick solution; Lab Aids; Sydney, NSW, Australia) for differential cell counting. At least 400 inflammatory cells were counted in each slide by two investigators (F.W.S.K. and C.D.) who were blinded to the clinical details of the subjects, and the average was used.

**Gelatin Zymography**

Gelatin zymography was used to assess the activity of MMP-9 in BALF as previously described. The supernatant was diluted two times with nonreducing loading buffer (400 mmol/L Tris-HCl, 5% sodium dodecyl sulfate, 30% glycerol, 0.006% bromo-
phenol blue). Fifteen microliters of equal amounts of the sample (15 μL) were mixed with the loading buffer, and proteins were separated by polyacrylamide gel electrophoresis (0.75 mm; constant current 20 mA) consisting of an 8% sodium dodecyl sulfate solution with 1% gelatin (Bio-Rad). The gels were incubated in a renaturing buffer (2.5% Triton X-100 buffer) for 30 min to remove the sodium dodecyl sulfate. After rinsing, the gels were incubated (37°C for 20 h) in an enzyme activation buffer (50 mmol/L Tris-HCl [pH 7.3], 200 mmol/L NaCl, and 0.02% Tween 20). The gels were then stained with Coomassie brilliant blue R250 stain and destained (5% methanol, 7% acetic acid in PBS solution), and the gelatinolytic activity was detected as clear bands. The molecular weight of the gelatinolytic bands was estimated relative to the prestained molecular-weight markers (see BluePlus2 Prestained Standard; Invitrogen, Carlsbad, CA), and an MMP-9 standard (Quantikine; R&D Systems; Minneapolis, MN) was run in each gel as a positive control.

Zymogram Image Analyses

Zymograms were digitized (Kodak Digital Science Image Station 440; NEN Life Science Products, Inc; Boston, MA), and gelatinolytic activity (Fig 1) was measured using freeware (ImageJ, release beta 1.402; Scion Corporation, Frederick, MD). The gelatinolytic activity was measured as the product of mean optical density multiplied by the area of digestion of the band inside a region of interest that was drawn around the clearly visible digested band (Fig 1). The mean of three measurements was used. Variations in Coomassie brilliant blue staining between different zymograms was standardized by using a single biological standard that had a high level of gelatinolytic activity and that was not from one of the study subjects (Fig 2). A 1:64 dilution of this sample was used in all zymographic assays as the common standard to adjust for small differences by reference to the standard curve in Figure 2 (closed circle).

Enzyme-Linked Immunosorbent Assay

Total MMP-9 and TIMP-1 were measured in BALF and serum by enzyme-linked immunosorbent assay (ELISA) [human MMP-9 and human TIMP-1; Quantikine; R&D Systems] according to the instructions of the manufacturer. The minimum detectable amounts of MMP-9 and TIMP-1 were 0.156 and 0.08 ng/mL, respectively.

Data Analysis

Asthmatic patients were classified by an asthma severity score based on the National Asthma Council Guidelines (Table 1).28 For analyses, subjects were classified into the following four groups: AMH (defined by bronchoscopic appearance, regardless of asthma severity); severe asthma (severity score, 3); mild/moderate asthma (severity score, 1 to 2); or nonasthmatic. Zymographic data and BAL cell counts were reported as the median (interquartile range). The MMP-9 activity data were nonnormally distributed (Shapiro-Wilk coefficient, 0.68; p < 0.0001) due to the zero values; therefore, differences between clinical groupings were examined by Kruskal-Wallis test, and any significant differences were further analyzed by pair-wise comparisons using Mann-Whitney tests. The relationships between MMP activity and inflammatory cell counts, FEV1, and DRR were examined using the Spearman rank correlation test. All other data were presented as the mean (95% confidence intervals) with differences between groups examined using analysis of variance (ANOVA).

Results

The subjects’ demographic and lung function data are shown in Table 2. There were no differences in age among the groups (ANOVA). However, as expected, AMH had poorer mean lung function compared with those who had mild/moderate asthma and healthy subjects (73 ± 13%, 94 ± 9%, and 95 ± 7% confidence intervals of predicted values, respectively; p = 0.01 [ANOVA]). Eight subjects had AMH, of whom two were classified as having mild/moderate asthma. Only five of eight subjects who had AMH underwent bronchial challenge tests, two
other patients had received bronchodilators recently and had normal spirometry findings, while the third patient had an FEV₁ of 50% predicted but did not undergo a bronchodilator test. In the group of patients with severe asthma, three of eight patients underwent bronchial challenge tests, three patients had low baseline FEV₁ values that precluded their undergoing the test (their postbronchodilator FEV₁ values were low), while the remaining two subjects refused to undergo the test but had normal baseline FEV₁ values. Two asthmatic subjects were receiving therapy with continuous oral corticosteroids, and all but two asthmatic subjects were receiving inhaled corticosteroids; one had mild/moderate asthma and the other had severe asthma.

Zymographic Analysis of BALF

Gelatinolytic bands corresponding to molecular weights of 200, 92, and 85 kd were detected in the BALF. Twelve of 22 asthmatic subjects (55%) expressed gelatinolytic proteins with molecular weights of 92 and 85 kd, which corresponded to pro-MMP-9 and active MMP-9 bands, respectively. Only one patient with mild/moderate asthma and no nonasthmatic subjects had measurable MMP-9 activity. The pro-MMP-9 and active MMP-9 bands were always present together. A gelatinolytic band of high molecular weight at 200 kd, which was assumed to be representing dimerized MMP-9 was also found in 12 of 22 of the asthmatic subjects, but none of the control subjects showed such activity.

The results of MMP-9 activity measured by computer-assisted densitometry in the different clinical groups are summarized in Figure 3. There were significant differences in the amounts of pro-MMP-9 and active MMP-9 between groups (p = 0.01 [Kruskal-Wallis test]). MMP-9 activity was greater in patients who had AMH and in patients with severe asthma compared with healthy subjects (p = 0.05 and p = 0.01, respectively). When we compared the MMP-9 activities in patients with AMH and severe asthma to that expressed by patients with mild/moderate asthma, we observed no significant differences (p = 0.08, respectively). However, MMP-9 activity increased across the groups (Fig 3) [r = 0.58; p = 0.0009]. In the asthmatic subjects, the MMP-9 activity was unrelated to the percent predicted FEV₁ and FVC values, the dosage of inhaled steroids, symptoms score, and log DRR to methacholine.

ELISA Analysis of BALF and Serum

The MMP-9 and TIMP-1 levels in BALF measured by ELISA are shown in Figure 4. The findings for MMP-9 are similar to the data obtained by zymography with differences in MMP-9 expression between groups (p = 0.03) and with increasing MMP-9 activity across the groups (rs = 0.54, p = 0.003). Additionally, there were significant dif-

Table 1—Asthma Severity Score*

<table>
<thead>
<tr>
<th>Severity Score</th>
<th>Symptoms</th>
<th>Bronchodilator Use</th>
<th>FEV₁ % predicted</th>
<th>Taking Oral Corticosteroids</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>&lt; Once/mo</td>
<td>&lt; Once/mo</td>
<td>&gt; 80%</td>
<td>No</td>
</tr>
<tr>
<td>2</td>
<td>≥ Once/mo but &lt; daily</td>
<td>&gt; Once/mo but &lt; 3 Times/d</td>
<td>60–80%</td>
<td>No</td>
</tr>
<tr>
<td>3</td>
<td>Daily or nocturnal symptoms &gt; once/wk</td>
<td>≥ 3 Times/d</td>
<td>&lt; 60%</td>
<td>Yes</td>
</tr>
</tbody>
</table>

*Severity score is determined by the highest score in any single category.

Table 2—Demographic and Lung Function Data*

<table>
<thead>
<tr>
<th>Clinical Group</th>
<th>No.</th>
<th>Sex</th>
<th>Age, yr</th>
<th>Atopy, No.</th>
<th>Productive Cough, No.</th>
<th>FEV₁, % predicted</th>
<th>FVC, % predicted</th>
<th>ICS Dose, µg/d</th>
<th>DRR, % fall FEV₁</th>
<th>Ex-Smokers, No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal</td>
<td>7</td>
<td>M</td>
<td>25 ± 11</td>
<td>3</td>
<td>0</td>
<td>94 ± 8</td>
<td>95 ± 5</td>
<td>4.5 (0.24–0.84)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Mild/moderate asthma</td>
<td>6</td>
<td>M</td>
<td>36 ± 7</td>
<td>6</td>
<td>1</td>
<td>94 ± 9</td>
<td>99 ± 9</td>
<td>2,000 (0–2,000)</td>
<td>12.5 (3.0–55.7)</td>
<td>5</td>
</tr>
<tr>
<td>Severe asthma</td>
<td>8</td>
<td>M</td>
<td>39 ± 10</td>
<td>7</td>
<td>6</td>
<td>90 ± 20</td>
<td>99 ± 16</td>
<td>2,000 (0–4,000)</td>
<td>19.4 (3.9–248.8)</td>
<td>1</td>
</tr>
<tr>
<td>AMH</td>
<td>8</td>
<td>M</td>
<td>44 ± 6</td>
<td>6</td>
<td>7</td>
<td>73 ± 13</td>
<td>81 ± 11</td>
<td>2,000 (0–2,000)</td>
<td>6.4 (3.2–47.3)</td>
<td>3</td>
</tr>
</tbody>
</table>

*Values given as mean confidence interval, unless otherwise indicated. M = male; F = female; ICS = inhaled corticosteroid.
†Cough data were missing in one patient in the severe asthma group.
‡Values given as median dose equivalent of beclomethasone dipropionate (range).
§Values given as geometric mean (range).
ferences in TIMP-1 expression (p = 0.01), but there were no differences in MMP-9:TIMP-1 ratios between groups (p = 0.06).

The BALF MMP-9 levels measured by ELISA correlated well with the active MMP-9 and pro-MMP-9 levels measured by zymography (r = 0.93 and r = 0.92, respectively; p = <0.0001). However, there was no correlation between serum and BALF MMP-9 activities measured by ELISA or among TIMP-1 activities. The MMP-9/TIMP-1 ratio was weakly correlated with the asthma severity score (r = 0.45; p < 0.05) but not with the individual MMP-9 or TIMP-1 activities. They were unrelated to DRR.

The median serum MMP-9 concentrations of the healthy subjects, patients with mild/moderate asthma, patients with severe asthma, and patients with AMH were 363 ng/mL (range, 210 to 711 ng/mL), 460 ng/mL (range, 87 to 1,292 ng/mL), 754 ng/mL (range, 136 to 1,089 ng/mL), and 235 ng/mL (range, 93 to 1,242 ng/mL), respectively. The corresponding serum TIMP-1 concentrations were 225 ng/mL (range, 196 to 502 ng/mL), 204 ng/mL (range, 157 to 288 ng/mL) 209 ng/mL (range, 157 to 272 ng/mL), and 211 ng/mL (range, 97 to 253 ng/mL), respectively. Neither serum MMP-9 and TIMP-1 concentrations nor their ratios differed among the groups, nor were they related to asthma severity.

Cell Counts and Total Protein Concentration of BALF

A summary of differential cell counts and total protein in the BALF of patients in the different clinical groups is presented in Table 3. There were no differences in the total number of inflammatory cells or the total protein concentration in BALF among the groups. The percentage of neutrophils was higher in patients who had AMH and severe asthma compared with that in healthy subjects (p = 0.002 and p = 0.002, respectively). The percentage of eosinophils was higher in patients who had AMH and severe asthma compared with that in healthy subjects (p = 0.0006 and p = 0.0003, respectively). The percentage of eosinophils was also greater in patients with severe asthma compared with those with mild/moderate (p = 0.01). Further analysis revealed that the number of neutrophils (but not the number of eosinophils) in asthmatic subjects correlated with MMP-9 activity measured by zymography (r = 0.49; p = 0.008).
In this study, we found elevated MMP-9 activity and TIMP-1 expression in asthmatic patients who had a history of AMH that was confirmed at bronchoscopy or severe asthma, compared to healthy subjects and patients who had mild/moderate asthma. There were no differences in MMP-9/TIMP-1 ratios among groups. The clinical parameters of the AMH group were similar to those of the severe asthma group with the presence of frequent symptoms, comparable lung function, and high inhaled corticosteroid requirements. Thus, this subgroup of asthmatic patients that has evidence of AMH, with copious amounts of mucus present in the large airways seen bronchoscopically, may represent part of the spectrum of severe asthma.

Chronic mucus hypersecretion is a well-recognized pathology in patients who have COPD, in whom it has been found to be associated with exacerbations. In asthma patients, mucus hypersecretion is well-recognized as an important component of the pathogenesis of acute attacks, during which it is thought to cause further narrowing by causing physical obstruction of airways that are already inflamed and narrowed by bronchoconstriction. An increase in the number of goblet cells has also been found in postmortem studies of asthmatic subjects. Lange et al reported that AMH, defined by self-reported production of phlegm during at least 3 months per year for at least two consecutive years, was present in 63% of men and 54% of women who had asthma. More importantly, it has also been associated with a greater decline in lung function in asthmatic patients, regardless of smoking history and initial FEV1 values.

The association between mucus hypersecretion and faster decline in lung function in asthma suggests that there might be more airway inflammation and remodeling. Hence, there may also be greater MMP-9 activity given its association with eosinophilic and neutrophilic inflammation, and its potential role in tissue remodeling by degrading elastin, collagen, and proteoglycan. In two published studies of bronchial biopsies, immunohistochemical staining for MMP-9 was colocalized with neutrophils and eosinophils, although there were no differences between asthmatic subjects, either treated with steroids or untreated, compared with healthy subjects. The activity of MMP-9 in the airway secretions of patients who have AMH has not been reported previously.

We defined mucus hypersecretion as the clear-cut presence of increased mucus in the airways causing airway obstruction, independently of asthma severity and of a history of chronic cough productive of phlegm. This was based on the “bronchitis index,” which was validated as a way of describing the severity of bronchitis seen during bronchoscopy. It is a four-component index that includes the presence of mucus in the airways, with airway occlusion being classified as the highest grade. Although we cannot be completely sure that mucous plugging was chronic, we think it is highly likely since we ensured that all patients were clinically stable (ie, they did not have symptoms of a recent respiratory tract infection), and seven of the eight patients reported having chronic cough and sputum production. If we had defined AMH by both history and bronchoscopic appearance, one patient would be reclassified as having mild/moderate asthma; however, this only increased the differences between the AMH and severe asthma groups, compared with the mild asthma group. We also found a high proportion of subjects who had severe asthma and also had a history of chronic productive cough but no mucous plugging seen at bronchoscopy. This may have been due to selection bias, with patients having chronic cough being more likely to respond to recruiting.

### Table 3—Differential Cells Counts and Total Protein Level in BALF*

<table>
<thead>
<tr>
<th>Cells</th>
<th>Normal (n = 7)</th>
<th>Mild/moderate Asthma (n = 6)</th>
<th>Severe Asthma (n = 8)</th>
<th>AMH (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total cells, $\times 10^6$ cells/mL</td>
<td>0.47 (0.26–1.13)</td>
<td>0.35 (0.14–1.54)</td>
<td>0.33 (0.12–0.58)</td>
<td>0.73 (0.51–0.91)</td>
</tr>
<tr>
<td>Neutrophils, %</td>
<td>2 (1.8–3.6)</td>
<td>3 (1.8–24.3)</td>
<td>23† (5.5–25.5)</td>
<td>20† (4.4–44.6)</td>
</tr>
<tr>
<td>Eosinophils, %</td>
<td>0.3 (0.2–1.5)</td>
<td>1.0 (0.4–5.0)</td>
<td>10‡ (5.1–14.8)</td>
<td>2.5‡ (2.3–3.8)</td>
</tr>
<tr>
<td>Macrophages, %</td>
<td>95 (93–98)</td>
<td>92 (64–96)</td>
<td>62 (57–79)</td>
<td>57 (42–84)</td>
</tr>
<tr>
<td>Lymphocytes, %</td>
<td>0.3 (0.3–0.7)</td>
<td>1.7 (0.1–19.6)</td>
<td>1.5 (0.9–1.6)</td>
<td>1.2 (0.6–16.3)</td>
</tr>
<tr>
<td>Total protein concentration, mg/mL</td>
<td>0.72 (0.23–0.76)</td>
<td>0.50 (0.31–0.97)</td>
<td>0.60 (0.28–1.11)</td>
<td>0.35 (0.07–1.07)</td>
</tr>
</tbody>
</table>

*Values in parentheses are ranges.†p < 0.01 compared with normal group.‡p < 0.001 compared with normal group.§p = 0.01 compared with mild/moderate asthma group.
advertisements and more likely to agree to undergo bronchoscopy. These patients could have had intermittent mucous plugging.

The asthmatic subjects who had mucus hypersecretion had copious amount of sputum in the airways at bronchoscopy. Although it is not possible to determine how much this abnormal mucus secretion contributed to the spirometric abnormality, it was likely that it played a significant role not only by causing physical blockage, but also by increasing surface tension, thereby favoring airway closure during forced expiratory maneuvers. Furthermore, the presence of increased amounts of mucus in the bronchial tree may also impede the deposition of inhaled drugs to the inflamed airways, suggesting that better clearance of secretions might help to improve asthma treatment. AMH might represent a variation of the disease, and, although its causes are unclear, it might include the up-regulation of the T-helper type 2 cytokines interleukin (IL)-4, IL-5, IL-9, and IL-13, which have been implicated in increasing mucus secretion.

Our findings that MMP-9 activity and TIMP-1 expression are increased in the BALF of subjects who had severe asthma compared to healthy subjects and patients with mild/moderate asthma are in accordance with the findings of previous studies. Lemjabbar and colleagues found a 10-fold to 160-fold increase in MMP-9 activity in BALF in patients receiving mechanical ventilation for the treatment of acute severe asthma over that of nonasthmatic subjects. Mattos et al also demonstrated increased MMP-9 activity in the induced sputum of patients who had severe asthma compared with patients who had mild asthma and healthy subjects, although levels of MMP-9 in BALF was not different. Patients who had nocturnal asthma had a fourfold circadian increase in levels of MMP-9 in BALF compared with healthy subjects and asthmatic patients who did not have nocturnal asthma. Interestingly, the investigators observed that these differences in MMP-9 expression disappeared when MMP-9 activity was determined in the afternoon. This observation would indicate that the amount of MMP-9 found in the BALF of asthma patients correlates with the infiltration of inflammatory cells in the lung and therefore most probably is produced by such cell types rather than by resident lung cells.

In regard to the possible source of MMP-9 activity in the BALF of patients who have severe asthma or AMH, we found an increase in the percentage of neutrophils in these subjects, who also had high levels of MMP-9 activity despite receiving high doses of inhaled corticosteroids. This suggests that severe asthma is associated with a persistent airway neutrophilia that is poorly responsive to inhaled steroid treatment. This then provides an ongoing source of MMP-9 and is consistent with previous findings of the poor response of MMP-9 activity to steroid treatment in patients with severe asthma. Neutrophils therefore could play a significant role in severe asthma and also in the inflammation associated with AMH. Thus, neutrophils might be important in asthmatic remodeling given the known presence of MMP-9 in neutrophils and our findings of an association between MMP-9 and the percentage of neutrophils. Interestingly, Corbel et al, in a murine model of asthma, also found that MMP-9 activity measured in BALF increased as the duration of repeated ovalbumin challenges progressed and that this was also correlated with the number of neutrophils in the BALF. This is analogous to chronic allergen exposure, which has also been shown to increase MMP-9 activity in the BALF of asthmatic subjects.

Our severity score was based on our national guidelines, which, like the Global Strategy for Asthma Management and Prevention guidelines, do not include the dose of inhaled steroids being administered. This explains why the doses of inhaled steroids were comparable between the different asthma groups, which perhaps suggests the limitations of such “clinical severity” scores such that they could be considered more appropriately to be measures of asthma control rather than severity.

As metalloproteinase inhibitors are now available for clinical trials in the treatment of diseases like osteoarthritis and rheumatoid arthritis, MMPs may be a target for novel therapy in asthma patients. Mechanical obstruction may play a role in AMH by decreasing mucociliary clearance, by trapping the inflammatory mediators with resulting persistent inflammation, and possibly by preventing the penetration of antiinflammatory medications.

We did not observe any differences among groups in our study in regard to the serum levels of MMP-9 or TIMP-1, suggesting that the increases in MMP-9 activity and TIMP-1 expression are restricted to the inflamed area of the lung. However, other groups have reported increased serum levels of MMP-9 in patients presenting with asthma exacerbations, although there are no published data of any increase in MMP-9 level in the blood of subjects who have severe but clinically stable asthma.

In conclusion, our study shows that MMP-9 levels are high in the BALF of patients who have AMH or severe asthma. However, MMP-9 levels are similarly low in the airways of patients with mild/moderate asthma and in control subjects. No correlation in the levels of MMP-9 or TIMP-1 between serum and BALF was noted. Further studies are needed to assess
whether the removal of the mucus can improve inflammation and remodeling in patients with AMH.

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