Ex Vivo Sputum Analysis Reveals Impairment of Protease-dependent Mucus Degradation by Plasma Proteins in Acute Asthma

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***Rationale*: Airway mucus plugs, composed of mucin glycoproteins mixed with plasma proteins, are an important cause of airway obstruction in acute severe asthma, and they are poorly treated with current therapies.**

***Objectives*: To investigate mechanisms of airway mucus clearance in health and in acute severe asthma.**

***Methods*: We collected airway mucus from patients with asthma and nonasthmatic control subjects, using sputum induction or tracheal aspiration. We used rheological methods complemented by centrifugation-based mucin size profiling and immunoblotting to characterize the physical properties of the mucus gel, the size profiles of mucins, and the degradation products of albumin in airway mucus.**

***Measurements and Main Results*: Repeated *ex vivo* measures of size and entanglement of mucin polymers in airway mucus from nonasthmatic control subjects showed that the mucus gel is normally degraded by proteases and that albumin inhibits this degradation. In airway mucus collected from patients with asthma at various time points during acute asthma exacerbation, protease-driven mucus degradation was inhibited at the height of exacerbation but was restored during recovery. In immunoblots of human serum albumin digested by neutrophil elastase and in immunoblots of airway mucus, we found that albumin was a substrate of neutrophil elastase and that products of albumin degradation were abundant in airway mucus during acute asthma exacerbation.**

***Conclusions*: Rheological methods complemented by centrifugation-based mucin size profiling of airway mucins in health and acute asthma reveal that mucin degradation is inhibited in acute asthma, and that an excess of plasma proteins present in acute asthma inhibits the degradation of mucins in a protease-dependent manner. These findings identify a novel mechanism whereby plasma exudation may impair airway mucus clearance.**

Keywords: airway mucus; rheology; neutrophil elastase; plasma; asthma exacerbation

AT A GLANCE COMMENTARY

Scientific Knowledge on the Subject

Airway mucus plugs are an important cause of airway obstruction in acute asthma exacerbations, and are poorly treated with current therapies.

What This Study Adds to the Field

We show that inhibition of protease-dependent mucin digestion occurs in acute asthma and that plasma proteins may mediate this inhibition by competing with mucin substrates for proteolysis. These data provide mechanistic insights into the pathophysiology of mucus impaction of the airways in acute asthma and highlight potential areas for the development of mucolytic therapies.

Autopsy studies of fatal asthma from as early as the 1920s clearly show that intralumenal accumulation of mucus is an important cause of airway obstruction (1, 2). Dunnill described "numerous grey, glistening, mucus plugs scattered throughout the airway passages," and he noted that "pathologically the outstanding feature of the asthmatic lung lies in the failure of clearance of the bronchial secretions" (2). Despite these long-standing insights into the cause of fatal asphyxiation in acute asthma, there has been little progress in understanding the mechanisms of mucus plug formation in acute asthma as well as a lack of specific treatments targeting this pathologic feature.

In acute severe asthma, there is hypersecretion of mucin glycoproteins from airway mucus cells (3, 4), leakage of plasma from highly permeable bronchial blood vessels (5, 6), and accumulation of inflammatory cells and inflammatory cell debris (7, 8). As a consequence, airway mucus in acute asthma is characterized by high concentrations of mucins, plasma proteins, and inflammatory cells. The resultant pathologic mucus is difficult to clear effectively, as demonstrated by studies showing impaired mucociliary clearance during severe asthma exacerbations followed by improvement of clearance during asthma recovery (9). The cephalad movement of airway mucus propelled by the coordinated, rhythmic beating of epithelial cilia relies not only on ciliary motility but also on the optimal rheological properties of the mucus. Mucus gel elasticity is necessary for cilia to transmit kinetic energy to the mucus layer for forward propulsion, but high elastic recoil would impede mucociliary clearance by the resistance to extrusion from goblet cells as well as the resistance to propulsion by epithelial cilia (10). A rheological balance between elasticity and viscosity is therefore necessary, and this balance is likely to be perturbed during acute asthma exacerbation, when mucus is produced that is abnormal both in volume and in rheological properties.

The major macromolecular components conferring mucus with its gel properties are mucin glycoproteins, which are large,

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heavily glycosylated protein polymers (11, 12). Thus, any mechanism of mucus clearance and turnover in the healthy or asthmatic airway involves degradation of these mucin polymers. Previous studies showed that neutrophil elastase degrades porcine gastric mucin (13), and we hypothesized that proteolytic degradation of airway mucins might promote mucus clearance in the healthy airway. Inhibition of this mechanism in acute asthma would then decrease mucus clearance and promote mucus plug formation. Testing this hypothesis directly *in vivo* poses multiple challenges, so we addressed it in *ex vivo* studies of airway mucus from patients in acute asthma exacerbation and from nonasthmatic control subjects. In these *ex vivo* studies we used rheological methods complemented by centrifugation-based mucin size profiling to determine physical and biochemical characteristics of the mucus gel in health and disease and under different experimental conditions.

Rheological measurements of viscous and elastic moduli elucidate the microstructure of fluids, including the degree of cross-linking between protein polymers. The viscous and elastic moduli of a fluid are determined by the molecular weight of its components and the architecture formed by intra- and intermolecular interactions. These physical characteristics of airway mucus depend heavily on polymeric, highly glycosylated mucin glycoproteins (mucins). Rheometers probe fluid microstructure by measuring its response to strain (fluid displacement) over a range of oscillatory frequencies. The response of the fluid is measured as the elastic (G′) and viscous (G″) moduli. The elastic modulus can be related to the density of molecular cross-links, whereas the viscous modulus can be related to molecular weight. In our study, we measured the elastic and viscous moduli of airway mucus to determine changes in mucin cross-linking and size. This approach allowed us to determine whether airway mucins are susceptible to proteolytic degradation and to explore whether mucin degradation is altered in acute asthma.

Some of the results of this study have been previously reported in abstract form at American Thoracic Society international conferences (14, 15).

METHODS

Additional detail for all methods and materials is provided in the online supplement.

Subjects

Induced sputum, spontaneously expectorated sputum, or tracheal aspirates were collected according to protocols and informed consent procedures approved by the Committee on Human Research at the University of California, San Francisco (UCSF).

Control subjects for sputum induction. All nonasthmatic control subjects were nonsmokers without a history of lung disease or upper respiratory tract infection within the 6 weeks before enrollment. Sputum was induced from eight subjects (one male, seven females; ages 23–51 yr) on multiple study visits separated by at least 2 days. Analysis of induced sputum was performed at the first visit to ensure acceptable quality (squamous cells $< 50\%$ and a rheological signature distinct from saliva).

Control subjects for tracheal aspirates. We enrolled four nonsmokers (three males, one female; ages 42–55 yr) without a history of lung disease or recent upper respiratory tract infection and who were scheduled to undergo elective nonpulmonary surgery.

Subjects with asthma in acute exacerbation. Patients with asthma diagnosed with acute asthma exacerbation by emergency room or intensive care physicians at the UCSF Moffitt-Long Hospital were enrolled. None had radiographic evidence of pneumonia. Six patients with acute asthma (two males, four females; ages 25–75 yr) provided sputum or tracheal aspirates for rheological studies. In four patients, samples were also collected during the recovery phase of exacerbation, usually either just before discharge, 24–72 hours after presentation to the emergency department ($n = 3$), or in one case 3 weeks later when the subject had fully recovered from a prolonged exacerbation and presented for follow-up to our asthma clinical research center to provide a spontaneously expectorated sputum sample.

In addition, six patients with acute asthma provided sputum or tracheal aspirates for analysis of albumin degradation products.

Procedures

Sputum induction. Sputum induction in nonasthmatic subjects was performed with nebulized 3% saline for 20 minutes, using the methods previously described by our laboratory (16). The total and differential cell counts were measured as previously described (17).

Tracheal aspiration. Tracheal aspirates were collected with a 14-French closed tracheal suction catheter and a sputum trap, after induction of anesthesia and intubation.

Measurement of elastic modulus (G′), viscous modulus (G″), entanglement molecular weight (M_e), and entanglement density (V_e). Rheological measurements were made with a cone-and-plate rheometer (AR2000; TA Instruments; New Castle, DE) on airway mucus samples equilibrated to 4°C and maintained by the peltier plate. Elastic (G′) and viscous (G″) moduli were calculated from the measured response of the samples to the oscillating angular displacement. All data were from frequency sweeps performed at a strain of 1% to 5%, within the linear viscoelastic range, at an angular frequency of 0.8 Hz (5 rad/s).

The entanglement molecular weight (M_e) is the molecular weight of polymer segments between cross-links. M_e is calculated from G′ within the plateau region of a frequency sweep (G_p) (Figure 1). For all data presented in Figures 2–4, G_p was determined at 0.8 Hz (5 rad/s).

The entanglement density (V_e) of mucin polymers within a mucus gel is the number of cross-link junctions among polymers in a unit volume and is calculated from the M_e. Gels with highly dense entanglements are difficult to deform, whereas those with low density are easily stretched and ruptured. Healthy airway mucus has a low

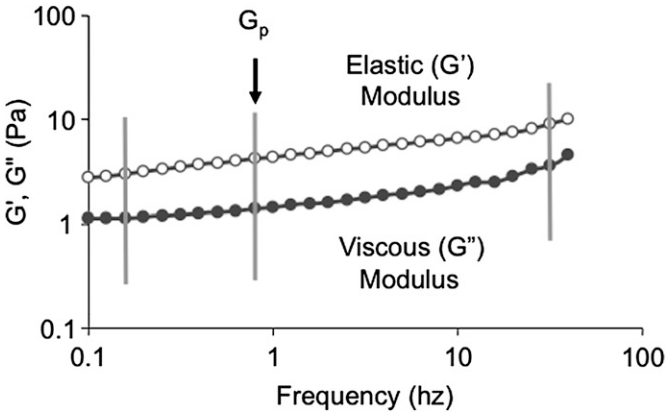

Figure 1. Healthy airway mucus is a cross-linked gel. The elastic modulus (G′) predominates over the viscous modulus (G″) across a broad range of frequencies in this typical frequency sweep from healthy induced sputum. The G′ predominance and plateau as well as the identical dependence of G′ and G″ on frequency (G′ and G″ are parallel lines) are hallmarks of a cross-linked gel. We used G′ and G″ at 0.8 Hz (*arrow, vertical line*) to facilitate comparison of these moduli between samples for all experiments. For calculations of the cough clearance index (CCI), we used G′ and G″ at high frequency (16.0 Hz); for calculations of the mucociliary clearance index (MCI), we used G′ and G″ at low frequency (0.2 Hz) (*vertical lines*). These high and low frequencies approximate the frequencies in the airways due to cough and ciliary beat, respectively. The frequency sweep plateau in this rheological signature of healthy sputum also enables direct determination of entanglement density and entanglement molecular weight from G_p, the plateau modulus.

density of entanglement, and thus its cross-links are easily disrupted, facilitating degradation and promoting mucus clearance, depending on the degree and permanence of disruption.

G′ and G″ can also predict mucus clearance using two indices, the mucociliary clearance index (MCI) and the cough clearance index (CCI). MCI and CCI are calculated using G*, the complex modulus, and tan δ at 1 and 100 rad/second (CCI) to approximate the low frequencies of ciliary beat and higher frequencies generated in the airway during cough. MCI and CCI are derived from *in vitro* (18) and mechanical (18–20) models and are comparable to mucus clearance measured *in vivo* with radiolabeled tracers (20, 21), although they are not direct measures of clearance.

Serial measures of elastic modulus (G′) and viscous modulus (G″) of airway mucus. Aliquots of induced sputum or tracheal aspirates were maintained at 4°C and 37°C for 24 hours. G′ and G″ were measured at different time points, including at baseline and at 4 or 24 hours of incubation. All data are graphed as a percentage of baseline, which equals [(24 h/baseline) · 100] for G′ and G″. To determine whether proteases degrade airway mucins, we used a protease inhibitor cocktail (Complete mini protease inhibitor with EDTA; Roche, Nutley, NJ) with 0.9% NaCl for control, both added to healthy induced sputum at 10% (vol/vol). Samples were incubated at 37°C for 24 hours, and G′ and G″ were measured at baseline and after 24 hours of incubation. To determine whether neutrophil elastase degrades airway mucins, we added purified human neutrophil elastase (Sigma-Aldrich, St. Louis, MO) or 0.9% NaCl to healthy induced sputum at 10% (vol/vol) (elastase concentration, 30 μg/ml) and incubated samples at 37°C. G′ and G″ were measured at baseline and after incubation for 4 hours, a time point at which G′ or G″ does not normally change.

To determine whether albumin increases sputum viscoelasticity and inhibits temperature-dependent degradation, we added human serum albumin (HSA) (AlbuRx 25%; ZLB Behring, King of Prussia, PA) at 80 mg/ml or 0.9% NaCl to induced sputum at 50% (vol/vol) and incubated for 24 hours at 37°C. G′ and G″ were measured at baseline and after 24 hours of incubation. To ensure that the measured inhibitory effect of HSA was not due to contamination of HSA by trace protein impurities, we used high-performance anion-exchange chromatography (MonoQ HR 5/5; GE Health Science Bioscience Corp., Piscataway, NJ) to purify HSA, which was added to healthy induced sputum at 50 mg/ml, with 180 mM NaCl and 3.4 mM Tris-HCl (pH 7.9) as the buffer control. Reagents were added at 50% (vol/vol), and sputum was incubated for 24 hours at 37°C with measurements made at baseline and 24 hours.

Rate zonal centrifugation. Guanidine hydrochloride (GuHCl, 8 M) was added to sputum samples at equal volume. Samples were then layered onto preformed GuHCl gradients (6–8 M) and centrifuged in a Beckman SW40 swing-out rotor. Tubes were emptied from the top, fractions transferred to nitrocellulose, and glycoproteins were detected with anti-MUC5AC polyclonal antiserum (MAN5ACI) (22).

Gel electrophoresis. (*1*) Digestion of albumin by human neutrophil elastase: To determine whether HSA is a substrate of human neutrophil elastase (HNE), chromatographically purified HSA (described previously) was incubated with HNE (enzyme-to-substrate molar ratio of 1:1) at 37°C in phosphate-buffered saline for 30, 120, and 360 minutes. Undiluted HSA incubated at 120 and 360 minutes was used as the control. Albumin was detected with a mouse monoclonal antibody to HSA (GeneTex, Inc., San Antonio, TX) after reducing and nonreducing SDS–PAGE. (*2*) Albumin degradation products in acute asthma: To detect albumin degradation products in airway mucus from patients with asthma in acute exacerbation, samples were processed in cell lysis buffer (RIPA buffer; Pierce, Rockford, IL) immediately after collection and subjected to reducing SDS–PAGE. Albumin was detected by immunoblotting with an HSA antibody as described previously.

Statistics

For comparison of baseline rheological properties of asthmatic and healthy mucus (Figure 2), the rank-sum test was used for all between-group comparisons, with $P < 0.05$ considered significant.

Log-transformed data were used for all other statistical analyses because of the nonnormality of the percentages and to maintain consistency with the rheological convention of evaluating elastic (G′) and viscous (G″) moduli logarithmically. The *t* test was used for between-group comparisons, with $P < 0.05$ considered significant. Logarithmic transformation of G′, G″, and V_e was performed for rheological data presented in Figures 3 and 4 and results are expressed as mean ± SEM or as median and interquartile range, as appropriate.

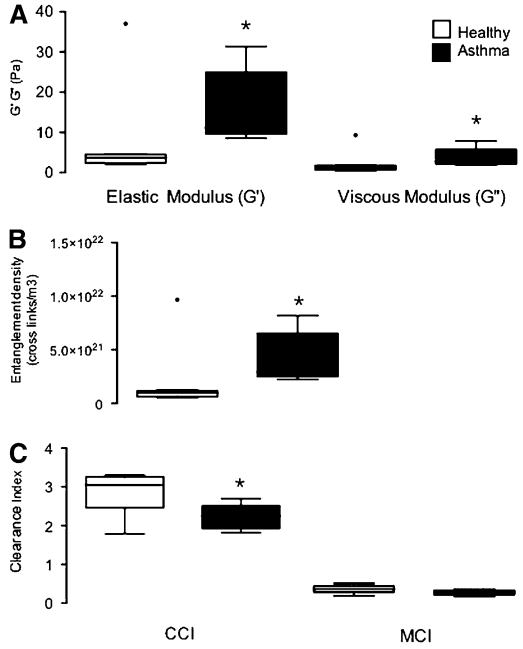

Figure 2. Airway mucus from patients with asthma in acute exacerbation has abnormal rheological properties. (*A*) Elastic and viscous moduli of acute asthmatic mucus (*solid boxes*) are greater than those of healthy mucus (*open boxes*). The greater increase in elastic modulus compared with the viscous modulus indicates that the predominant abnormality in asthmatic mucus is increased cross-linking and entanglement of mucins, not increased concentration of mucins. (*B*) Entanglement density is significantly greater in asthmatic than in healthy mucus. (*C*) The cough clearance index (CCI) is decreased in acute asthma, but the mucociliary clearance index (MCI) is not. Data are from eight healthy subjects and five subjects with asthma and are summarized as box plots with medians, range, and outliers. *$P < 0.05$ versus healthy controls by the rank-sum test.

RESULTS

Rheological Signature of Airway Mucus in Acute Asthma Indicates Increased Cross-linking of Mucin Polymers and Reduced Cough Clearance

In induced sputum and tracheal aspirates from healthy subjects, we found that the elastic modulus (G′, a measure of polymer cross-linking and entanglement) predominated over the viscous modulus (G″, a measure of polymer size or length) (Figure 1). This rheological signature is characteristic of cross-linked mucin polymers in a mucus gel. The number of cross-linked junctions (entanglement density) in these healthy samples ranged from 5×10^{20} to 10×10^{22} m^{-3}, indicating a lightly entangled network.

In sputum and tracheal aspirates collected from patients with asthma during severe exacerbations of asthma, we found that the elastic and viscous moduli were significantly higher than normal (Figure 2A). Furthermore, the predominant abnormality in acute asthma was increased cross-linking of mucin polymers (reflected by the markedly increased elastic response), rather than high concentrations of mucins (reflected by the less markedly increased viscous response). The increased elastic

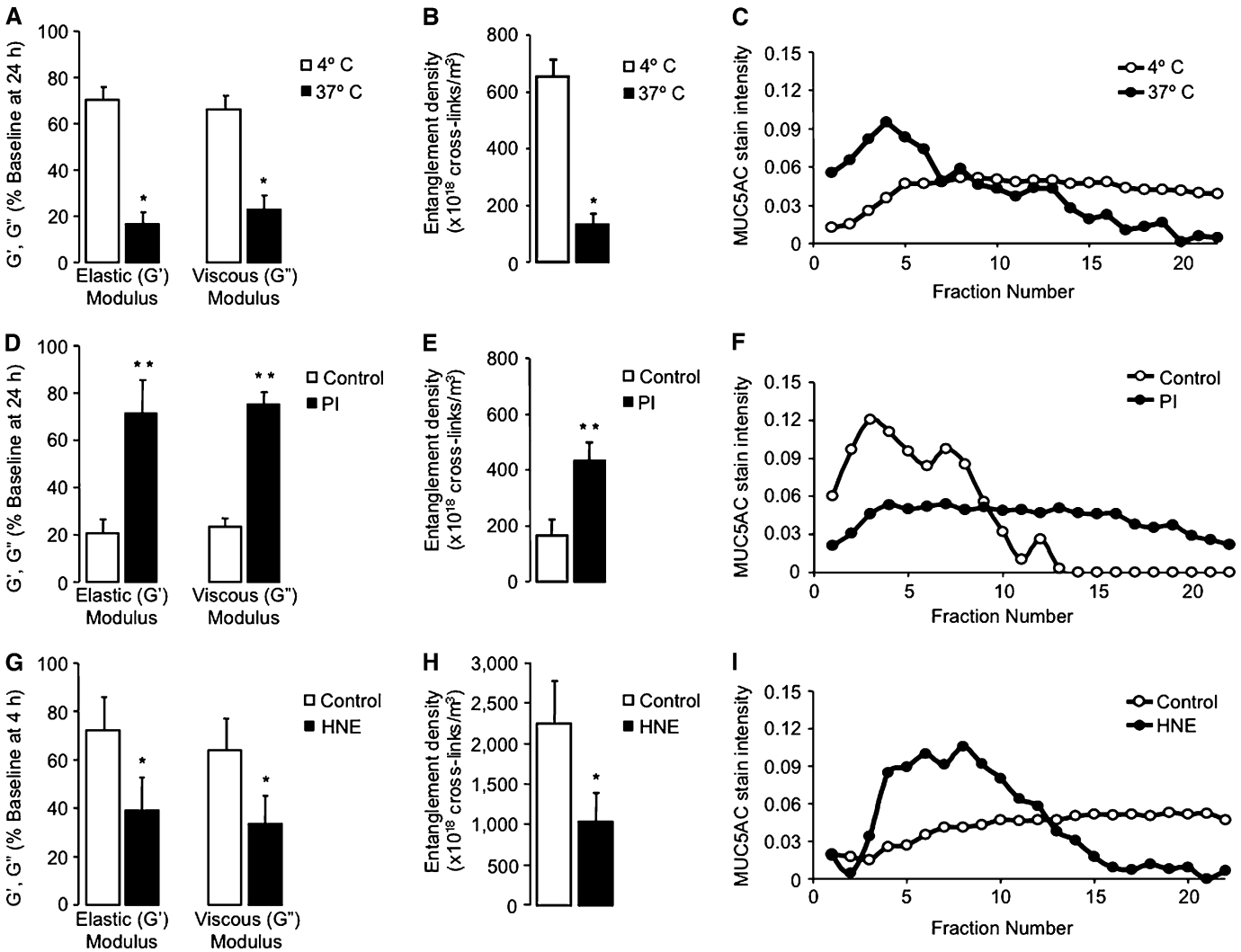

Figure 3. Healthy airway mucus is degraded by proteases. (*A* and *B*) Time- and temperature-dependent changes in the rheological properties of sputum from healthy subjects. (*A*) Sputum incubated at 37°C for 24 hours has markedly lower elastic (G′) and viscous (G″) moduli and (*B*) entanglement density than sputum incubated at 4°C. Data are from five healthy subjects. *$P < 0.05$ versus 4°C. (*C*) Time- and temperature-dependent changes in a representative size profile of mucin polymers in healthy sputum. After incubation at 4°C and 37°C, sputum samples were subjected to rate zonal centrifugation and fractions were transferred to nitrocellulose, followed by staining with MAN5ACI (MUC5AC polyclonal antiserum) (37). The size profiles of the 4°C and 37°C samples are markedly different, with the 37°C sample having predominantly smaller mucins. (*D–F*) Effect of protease inhibition on the rheological properties and mucin size profiles of healthy sputum. (*D*) Unlike sputum incubated with 0.9% NaCl (control), sputum incubated with protease inhibitors (PI) does not significantly decline in G′ or G″ over 24 hours and (*E*) does not change in entanglement density or (*F*) mucin size. Data for (*D*) and (*E*) are from three healthy subjects. **$P < 0.01$ versus saline control. (*G–I*) Effect of neutrophil elastase on the rheological properties and mucin size profiles of healthy sputum. (*G*) Unlike sputum incubated with 0.9% NaCl, sputum incubated with human neutrophil elastase (HNE) for 4 hours at 37°C significantly declines in G′ and G″ and (*H*) entanglement density and (*I*) shows a predominance of smaller mucins after rate zonal centrifugation. Data for (*G*) and (*H*) are from four healthy subjects and are presented as means ± SEM. *$P < 0.05$ versus saline control. The size profiles shown in (*F*) and (*I*) are from the induced sputum of two separate healthy subjects. The *t* test using log-transformed data was performed for all between-group comparisons.

response in airway mucus in acute asthma reflects increased cross-linking of mucin polymers. Consistent with this interpretation, the entanglement density of mucin polymers in the asthmatic mucus gel was markedly increased (Figure 2B), approximating values for cross-linking and entanglement in fibrin clots (23). In addition, we found that the cough clearance index (derived using values for elastic and viscous moduli measured at high oscillatory frequencies) was significantly decreased in the asthmatic samples (Figure 2C), an abnormality that will promote mucus accumulation because cough is the predominant form of clearance in the setting of mucus hypersecretion (24).

Airway Mucus Is Normally Degraded by Proteases, But This Mechanism Is Inhibited in Acute Asthma and Is Restored in Asthma Recovery

The rheological properties of healthy sputum changed significantly in samples incubated *ex vivo* for 24 hours at 37°C. Specifically, we found that mucin polymer cross-linking, length, and entanglement decreased significantly as evidenced by repeated measures of the elastic and viscous moduli (Figures 3A and 3B) and confirmed by rate zonal centrifugation, which showed a slower average sedimentation rate at 24 hours of incubation, indicating decreased mucin size (Figure 3C). These changes did not occur in healthy sputum incubated at 4°C

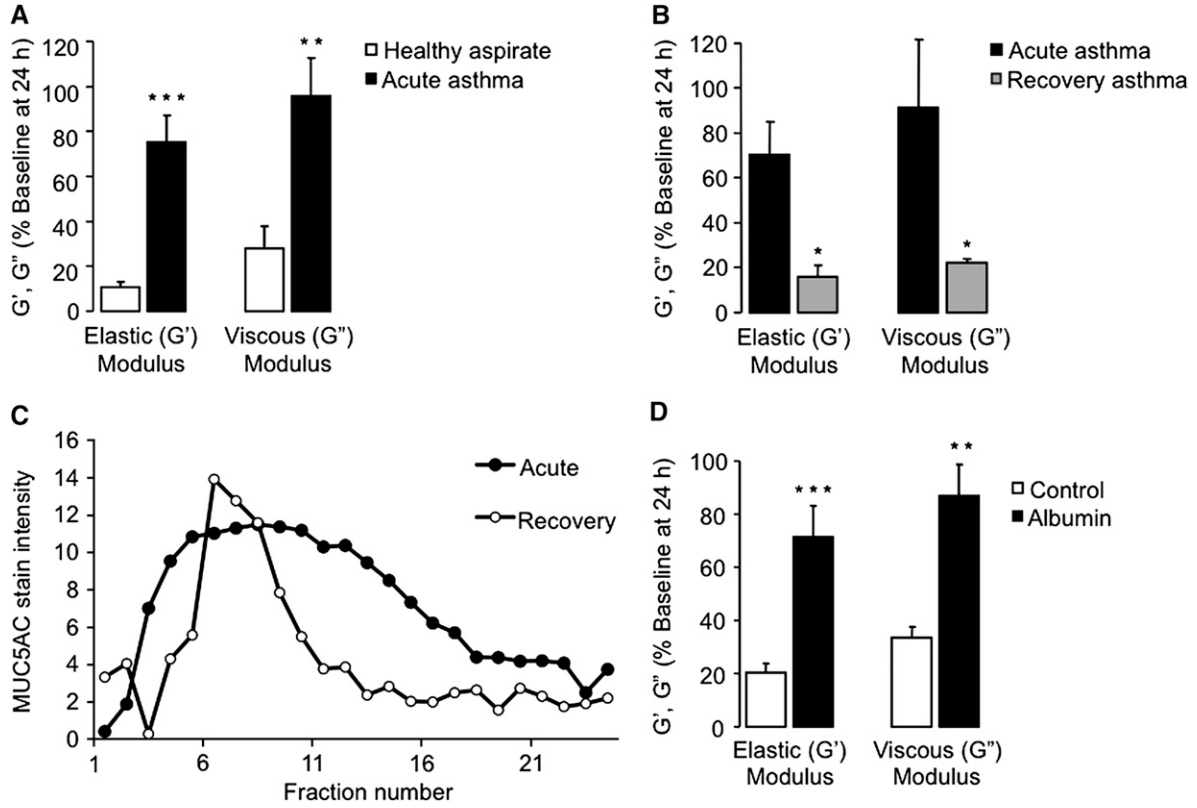

Figure 4. Asthmatic airway mucus resists proteolytic degradation. (*A* and *B*) Time- and temperature-dependent changes in the rheological properties of airway mucus from patients with asthma in the acute and recovery phases of exacerbation. (*A*) Airway mucus collected from patients with asthma early in the course of an exacerbation is markedly resistant to degradation when incubated at 37°C for 24 hours compared with tracheal aspirates from healthy subjects. Data are from four healthy subjects and five patients with asthma. ***$P < 0.001$ and **$P < 0.01$ versus healthy control subjects. (*B*) Comparison of airway mucus collected early in the hospital course of asthma exacerbation (acute asthma) and later in the hospital course (recovery asthma) shows that in the asthma recovery samples, elastic (G′) and viscous (G″) moduli decline to levels similar to those measured in healthy subjects. Data are from paired samples from three patients with asthma. *$P < 0.05$ versus acute asthma. (*C*) Size profile of mucin polymers in sputum samples from a subject with asthma during the acute and recovery phases of asthma exacerbation. Both sputum samples were subjected to rate zonal centrifugation and fractions were transferred to nitrocellulose, followed by staining with MUC5AC polyclonal antiserum. The size profiles of the acute and recovery samples are markedly different, with the recovery sample having predominantly smaller mucins. (*D*) Effects of albumin on the rheological properties of healthy sputum. Unlike sputum incubated with 0.9% NaCl, sputum incubated with human serum albumin does not significantly decline in G′ or G″ over 24 hours. Data are from five healthy subjects and are presented as means ± SEM. ***$P < 0.001$ and **$P < 0.01$ versus saline control. The *t* test using log-transformed data was performed for all between-group comparisons.

(Figures 3A–3C), nor did they occur in healthy sputum incubated with protease inhibitors (Figures 3D–3F). These data show that airway mucus is normally degraded by proteases. A candidate mucin-degrading protease is neutrophil elastase, based on its effects on hog gastric mucin (13), and we therefore investigated whether human neutrophil elastase can digest airway mucins. In healthy mucus mixed with elastase for 4 hours, we found significant reductions from baseline in mucin polymer cross-linking, length, and entanglement (Figures 3G and 3H), along with a decrease in mucin size (Figure 3I).

Having demonstrated protease-dependent degradation of healthy airway mucus, we next considered whether this mechanism is impaired in acute asthma. For these experiments, we studied spontaneously expectorated sputum or tracheal aspirates from patients with asthma, collected during asthma exacerbation, and we used tracheal aspirates from patients undergoing nonpulmonary surgery as control subjects. In the control samples, we found reductions in mucin polymer cross-linking and length after 24 hours at 37°C (Figure 4A), confirming the rheological changes measured in healthy induced sputum (Figure 3A) and making it unlikely that the changes in induced sputum are due to salivary proteases or oral microbes. In contrast to the rheological behavior of the control samples, measures of mucin cross-linking and length in sputum or tracheal aspirates collected from patients with asthma during an acute exacerbation did not change significantly over 24 hours at 37°C (Figure 4A). These data show that mucus degradation is inhibited in the airway at the height of an asthma exacerbation. We considered the possibility that mucus degradation is restored during asthma recovery. To investigate this possibility, we studied spontaneously expectorated sputum collected from three hospitalized patients with asthma during their recovery phase, shortly before their discharge from the hospital and 24–72 hours after their initial presentation to the emergency department. In these recovery samples, we found reductions in mucin polymer cross-linking and length after 24 hours at 37°C that were similar to those measured in healthy airway mucus samples (Figure 4B). Taken together, these data show that the inhibition of mucus degradation that occurs during the acute phase of asthma exacerbation is overcome during the recovery phase. We confirmed this interpretation by rate zonal centrifugation in a subject with asthma from whom we collected spontaneously expectorated sputum while he was being treated for an acute asthma exacerbation in the emergency room

("acute sputum sample") and another spontaneously expectorated sputum sample 3 weeks later, when he was fully recovered ("recovery sputum sample"). We found a much slower average sedimentation rate in the recovery sputum sample than in the acute sputum sample, indicating a much smaller mucin size profile in the recovery sputum sample (Figure 4C).

Albumin Inhibits Protease-driven Mucus Degradation

During acute asthma exacerbations, plasma proteins exude into the airway at increased concentrations (5, 6), and we hypothesized that these proteins could inhibit normal mucus degradation. To investigate this possibility, we added human serum albumin (the most abundant protein in plasma [25]) to healthy sputum and measured elastic and viscous moduli over 24 hours. Albumin increased the elastic and viscous moduli immediately after addition to sputum (data not shown), a finding noted previously by others (26, 27). However, albumin also prevented the usual declines in mucin cross-linking and length measured over 24 hours at 37°C (Figure 4D). To confirm that the inhibition of mucus degradation by human serum albumin was not due to contamination of the albumin preparation by trace protein impurities, we added chromatographically purified albumin to healthy sputum and found that this highly purified albumin also inhibited mucus degradation (data not shown).

Albumin Is a Substrate of Neutrophil Elastase, and Albumin Degradation Products Are Abundant in Sputum Samples in Acute Asthma

The finding that albumin inhibits mucus degradation in acute asthma led us to examine whether albumin is a substrate of human neutrophil elastase. Using immunoblots, we found that albumin incubated with purified human neutrophil elastase yielded albumin degradation products (Figure 5A). We then looked for albumin degradation products in sputum and tracheal aspirates from patients in acute asthma exacerbation. In immunoblots generated using equal volume loading and equal weight loading, we found that these products were abundant in airway mucus collected during acute exacerbation and that some of these fragments corresponded in size to the products of elastase-digested albumin (Figure 5B; and *see* Figure E1 in the online supplement).

DISCUSSION

Acute severe asthma is characterized by hypersecretion of mucins from airway mucus cells and exudation of plasma proteins from leaky blood vessels (5, 28–30). Decreased mucus turnover in acute asthma may therefore be a consequence of unfavorable interactions between abnormally high concentrations of mucins and plasma proteins. To explore how the turnover of pathologic mucus in acute severe asthma differs from normal, we used a range of rheological and biochemical methods to characterize airway secretions from nonasthmatic control subjects and from patients with asthma experiencing severe exacerbations. By making repeated *ex vivo* measures of mucin polymer cross-linking, length, and entanglement in airway mucus from control subjects, we found that the airway mucus gel is normally degraded by proteases. In contrast, these same *ex vivo* measures made in samples of airway mucus collected from patients at the height of acute severe asthma exacerbations showed no significant decrease in mucin polymer cross-linking, length, or entanglement. However, mucus collected from patients with asthma during recovery from an exacerbation showed *ex vivo* changes in mucin measures that were similar to control. These data reveal a mechanism of protease-dependent mucus clearance in the healthy airway that is impaired in acute asthma exacerbation but restored during asthma recovery.

We show that airway mucus is digested by proteases such as neutrophil elastase, resulting in marked decreases in mucin polymer cross-linking, length, and entanglement. Protease digestion may represent a mechanism to optimize the viscoelastic properties of mucus for its transport by the mucociliary apparatus. We hypothesized that protease-driven digestion of mucus is inhibited by plasma proteins, which are at increased concentrations in airway mucus during acute asthma exacerbation (6, 31). Albumin comprises at least 60% of plasma proteins (26), and albumin concentrations are higher than normal in airway secretions in acute asthma (25). We found that albumin mixed with mucus inhibits its normal degradation and that albumin is a substrate of neutrophil elastase. Furthermore, airway mucus from patients with asthma in exacerbation has abundant albumin degradation products that are absent in mucus from control subjects. Thus, exudation of albumin and other plasma proteins into the airway during acute asthma may alter the optimal ratio between proteases and their mucin substrates, thereby inhibiting degradation of mucus and promoting mucus plugging.

We previously showed that neutrophils and neutrophil elastase levels are increased in airway secretions from patients with asthma intubated during acute exacerbation when compared with nonasthmatic control subjects, and that both neutrophils and neutrophil elastase levels are even further increased during recovery (32). In light of the findings reported here, we now interpret the increase in neutrophils and neutrophil elastase during asthma recovery as a necessary response to facilitate mucus clearance. Specifically, the combination of the data we report here, and our previous data on neutrophilic airway inflammation in acute asthma (32), lead us to postulate that increased levels of neutrophil elastase in the recovering asthmatic airway overcome the resistance to mucin degradation imposed by the high concentrations of albumin. In this scheme (represented in Figure 6), neutrophil proteases help to restore airway patency by digesting mucus plugs.

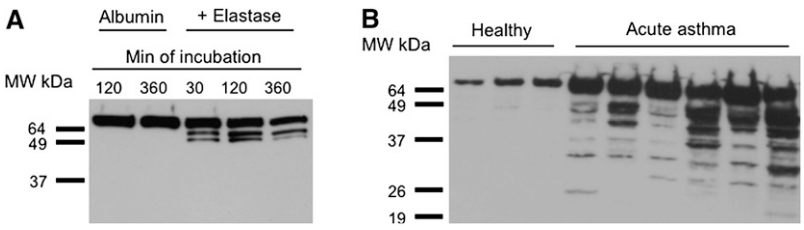

Figure 5. Albumin is a substrate of neutrophil elastase, and albumin degradation products are abundant in sputum samples in acute asthma. (*A*) Degradation of albumin by elastase. Chromatographically purified human serum albumin was incubated for 120 and 360 minutes (undiluted control) and with purified human neutrophil elastase (1:1 molar ratio) for 30, 120, and 360 minutes at 37°C in phosphate-buffered saline. Degradation products were subjected to nonreducing (data not shown) and reducing SDS–PAGE. (*B*) Abundant albumin degradation products in airway mucus from patients with asthma in acute exacerbation but not in healthy sputum. The immunoblot shows equal volume loading (4 μl each) of sputum samples that had been diluted fourfold during processing.

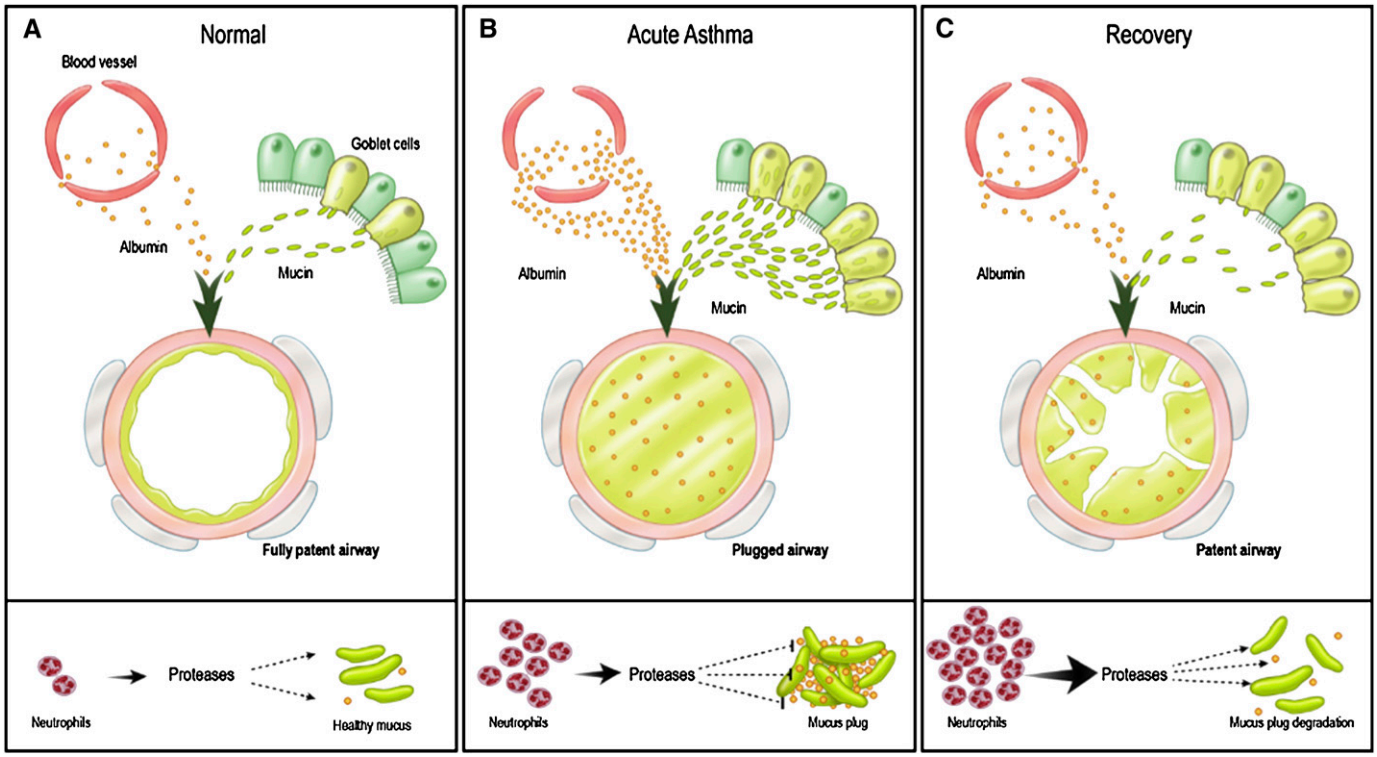

Figure 6. Schematic diagram illustrating a proposed mechanism of mucus degradation in health and in acute asthma. (*A*) Healthy airway mucus lines the patent airway and has optimal clearance via the mucociliary escalator. The mucus gel is formed mainly by mucins, and the sufficient neutrophil protease activity optimizes its rheological properties to enable effective transport. (*B*) Mucus clearance is reduced in acute asthma, and mucus plugs occlude the airway. Protease-dependent degradation of mucins is inhibited by high concentrations of albumin and other plasma proteins, which function as alternative substrates for neutrophil proteases. (*C*) Mucus clearance improves during asthma recovery, a necessary mechanism to restore airway patency. Neutrophils, increased in number, secrete proteases that overcome the inhibition of mucin degradation imposed by high concentrations of plasma proteins.

Our data raise the possibility that a previously unconsidered mechanism for the beneficial effect of corticosteroids in acute asthma is corticosteroid-mediated increases in neutrophil numbers in the airway, because it has been shown that glucocorticoids cause a dose-dependent inhibition of apoptosis leading to increased survival of neutrophils (33). In addition, because β-adrenergic agonists are thought to inhibit bronchovascular permeability (34), it is possible that β-agonist treatment during acute severe asthma not only relaxes airway smooth muscle but also reduces plasma leakage into the airways.

Current treatments for mucus plugging of the airway in acute asthma are limited, and there are no effective mucolytic treatments for acute asthma (29). Our data provide a mechanism to explain how the combination of increased mucin secretion and increased bronchovascular permeability in acute asthma creates pathologic mucus that resists degradation and is prone to occlusive plugs. These findings identify novel mechanisms for mucus clearance in health and disease and suggest new approaches for mucolytic therapy in asthma. Specifically, our data provide a rationale for considering protease-based mucolytic therapy in acute asthma, a treatment approach that would parallel the effective protease-based therapy for fibrin clots in occlusive coronary vascular disease (35, 36).

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