Mechanics, nonlinearity, and failure strength of lung tissue in a mouse model of emphysema: possible role of collagen remodeling

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Ito, Satoru, Edward P. Ingenito, Kelly K. Brewer, Lauren D. Black, Harikrishnan Parameswaran, Kenneth R. Lutchen, and Béla Suki. Mechanics, nonlinearity, and failure strength of lung tissue in a mouse model of emphysema: possible role of collagen remodeling. J Appl Physiol 98: 503–511, 2005. First published October 1, 2004; doi:10.1152/japplphysiol.00590.2004.—Enlargement of the respiratory air spaces is associated with the breakdown and reorganization of the connective tissue fiber network during the development of pulmonary emphysema. In this study, a mouse (C57BL/6) model of emphysema was developed by direct instillation of 1.2 IU of porcine pancreatic elastase (PPE) and compared with control mice treated with saline. The PPE treatment caused 95% alveolar enlargement (P<0.001) associated with a 29% lower elastance along the quasi-static pressure-volume curves (P<0.001). Respiratory mechanics were measured at several positive end-expiratory pressures in the closed-chest condition. The dynamic tissue elastance was 19% lower (P<0.001), hysteresivity was 9% higher (P<0.05), and harmonic distortion, a measure of collagen-related dynamic nonlinearity, was 33% higher in the PPE-treated group (P<0.001). Whole lung hydroxyproline content, which represents the total collagen content, was 48% higher (P<0.01), and α-elastin content was 13% lower (P=0.16) in the PPE-treated group. There was no significant difference in airway resistance (P=0.7). The failure stress at which isolated parenchymal tissues break during stretching was 40% lower in the PPE-treated mice (P=0.002). These findings suggest that, after elastolytic injury, abnormal collagen remodeling may play a significant role in all aspects of lung functional changes and mechanical forces, leading to progressive emphysema.

elastase; extracellular matrix; failure stress; resistance

PULMONARY EMPHYSEMA IS CHARACTERIZED by permanent destruction of the respiratory bronchioles, alveolar ducts, and alveolar walls, leading to hyperexpansion and loss of elastic recoil (1). The most widely accepted hypothesis of how tissue destruction occurs in emphysema is that an imbalance of protease and antiprotease activity exists within the lung that ultimately leads to enzymatic degradation of elastin (38, 45). However, changes consistent with emphysema can also result from abnormality of the collagen matrix (4, 8, 12, 21, 23, 25, 35, 49). Recent studies have provided evidence that significant remodeling of the extracellular matrix (ECM), including collagen, elastin, and proteoglycans, occurs during the development of emphysema both in humans (4, 12, 48, 49) and in experimental rodent models (12, 21, 25, 52). Therefore, besides elastin degradation, the biological breakdown and subsequent remodeling of collagen during the abnormal repair of the lung tissue would play a role in both the progressive nature of this disease (24, 52) and the physiologic functioning of the lung (21, 44).

Among the constituents of the ECM components, elastin and collagen appear to account for most of the viscoelastic mechanical properties of the lung tissue strips (33, 46, 53). Elastic fibers behave more linearly than collagen fibers (28, 36). Therefore, one would predict that the nonlinear behavior of the lung could change when alterations occur in the relative amounts of elastin and collagen in the connective tissue.

We hypothesized that, if alterations occur in the collagen fiber network during the development and progress of emphysema, then both the nonlinear viscoelastic properties of the lung tissue and the strength of alveolar walls should change. To test this hypothesis, we measured the nonlinear and viscoelastic properties of the whole lung in a mouse model of emphysema induced by direct instillation of porcine pancreatic elastase (PPE) (26). To characterize lung elasticity and airway function, we measured the quasi-static pressure-volume (P-V) curves and the forced oscillatory impedance of the respiratory system. We calculated a dynamic nonlinearity index, called harmonic distortion (42), as well as measured the failure stress of parenchymal tissue strips. Finally, to assess changes in composition, we also determined the total amounts of elastin and collagen in the lung.

METHODS

Animal preparation. Two groups of male C57BL/6 mice (Charles River, Boston, MA), weighing 23–25 g, were studied. Animals were treated with direct instillation of either 1.2 IU of PPE (Sigma, St. Louis, MO) dissolved in 90 μl of sterile saline (n=18) or the same amount of saline (control) (n=18) (26). Three weeks after the treatment, experiments were performed. All animal procedures were approved by the Animal Care and Use Committees of Boston University and Harvard Medical School.

Measurement of respiratory mechanics. The detailed methods are described elsewhere (20). The control (n=8) and PPE-treated (n=9) mice were deeply anesthetized by intraperitoneal injection of pentobarbital sodium (70 mg/kg) and then tracheostomized and cannulated in the supine position. The cannula was connected to a computer-controlled ventilator (Flexivent, SCIREQ, Montreal, Canada). Mice were mechanically ventilated with room air; a tidal volume of 8 ml/kg at a frequency of 240 breaths/min was used. After stabilization, the quasi-static P-V curve of the respiratory system was measured as follows. After inflation of the lungs to total lung capacity, defined as a tracheal pressure of 25 cmH2O, mice were mechanically ventilated under the same condition for 5 s. After a 3-s delay, slow volume inflation (0.1 ml/s, total of 1.2 ml) starting from end-expira-
Impedance (Zin) of the network is obtained as (20)

$$Z_{ti}$$ connected in series. Hantos et al. (16) introduced the constant-phase parallel, where each compartment is composed of an airway resistance represented the airway tree by a set of airway pathways arranged in tissue elastance model of the lung (20). Briefly, in this model, we be expected in emphysema. Specifically, we applied a heterogeneous and removed from the respiratory impedance of the mice (20).

The frequency response of the system was obtained, and the measured impedance spectra were off-line corrected for any phase ances. The frequency response of the system was obtained, and the measured impedance spectra were off-line corrected for any phase difference between pressure and flow. Additionally, the flow-dependent impedance of the tracheal cannula was characterized separately delivered to obtain meaningful units for the parameters G and H (3). The volume delivered are similar to normal spontaneous tidal volume and hence provides smooth estimates of the input impedance (43). The difference criterion, which eliminates harmonic distortion and minimizes frequencies in the OVW are selected according to a nonsum-nondif- taining energy from 0.5 to 15 Hz as described previously (27). The ventilation waveform (OVW), which is a broadband waveform con- consecutive inflations of the lungs to total lung capacity.

Impedance measurements. Impedance data collection was made by interrupting mechanical ventilation for 6 s by use of the optimal ventilation waveform (OVW), which is a broadband waveform containing energy from 0.5 to 15 Hz as described previously (27). The frequencies in the OVW are selected according to a nonsum-nondifference criterion, which eliminates harmonic distortion and minimizes cross talk among the frequencies present in the input flow waveform and hence provides smooth estimates of the input impedance (43). The volumes delivered are similar to normal spontaneous tidal volume values; hence, the method provides information on the mechanical properties during conditions mimicking breathing. In our experiment, we matched the peak-to-peak OVW amplitude to the tidal volume delivered by the mechanical ventilator. The ventilator displacement and cylinder pressure signals were low-pass filtered at 30 Hz and sampled at 256 Hz. With the use of Fourier analysis, impedance spectra were calculated on overlapping blocks of pressure, and flow data were calculated as the ratio of the cross-power spectrum of pressure and flow and the autopower spectrum of flow. The forced-oscillatory system was calibrated by measuring the input impedance of known analogs, including tubes and bottles with known impedances. The frequency response of the system was obtained, and the measured impedance spectra were off-line corrected for any phase difference between pressure and flow. Additionally, the flow-dependent impedance of the tracheal cannula was characterized separately and removed from the respiratory impedance of the mice (20).

Mathematical modeling. Data were analyzed with a model that allows for specific alterations in the lung tissue similar to what might be expected in emphysema. Specifically, we applied a heterogeneous tissue elastance model of the lung (20). Briefly, in this model, we represented the airway tree by a set of airway pathways arranged in parallel, where each compartment is composed of an airway resistance (Raw), an airway inertance (Iaw), and a linear tissue impedance (Zti) connected in series. Hantos et al. (16) introduced the constant-phase model and described Zti as

$$Z_{ti}(\omega) = (G - jH)/\omega^n, \text{ with } \alpha = 2\pi \arctan (H/G)$$

$$Z_{ti}(\omega) = (G - jH)/\omega^n, \quad (1)$$

where $$\omega_0$$ and $$\omega$$ are the normalized and absolute circular frequency, G and H are the coefficients of tissue damping and elastance, respectively, $$j$$ is the imaginary unit, and the exponent $$\alpha$$ describes the frequency dependence of tissue resistance [equal to G/(\omega/\omega_0)^\alpha] and tissue elastance [equal to H/(\omega/\omega_0)^\alpha].$$ The normalization factor $$\omega_0 = 1 \text{ rad/s is introduced to obtain meaningful units for the parameters G and H (3). The impedance of the airways (Zaw) in each pathway is given by}$$

$$Zaw(\omega) = Raw + jOmega$$

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The Raw, Iaw, and hysteresis (14), defined as the ratio of $$\eta = G/H$$, of the tissue elements were the same in each pathway, whereas the elastance (H) of the tissue elements followed a hyperbolic distri- bution between a minimum (H_{min}) and a maximum (H_{max}). The input impedance (Z_{in}) of the network is obtained as (20)

$$Z_{in} = \frac{FZaw}{F + ln(Z_{min} + Zaw)}$$

where F, Z_{min} and Z_{max} are given by F = ln(H_{max}/H_{min}), Z_{min} (\omega) = (\eta - j)H_{min}/\omega^n, and Z_{max} (\omega) = (\eta - j)H_{max}/\omega^n.

By minimizing the root mean square difference between the model and the data (6), the five parameters (Raw, Iaw, $$\eta$$, H_{min}, and H_{max}) were determined. The mean $$\eta$$ value ($$\eta_{\text{mean}}$$) was estimated as the expected value of the distribution function and was calculated from the estimates of H_{min} and H_{max} as

$$\eta_{\text{mean}} = \frac{H_{\text{max}} - H_{\text{min}}}{F}$$

(4)

The tissue damping, G, was calculated as $$G = \eta H_{\text{mean}}$$.

Dynamic nonlinearity of respiratory mechanics. When applying a broadband input, one way to probe system nonlinearity is to measure how the elastic and viscous moduli depend on the amplitude of the input. Another way of quantifying nonlinearity is via the so-called harmonic distortion index, which estimates the amount of both harmonic distortion and cross talk in the output signal resulting from system nonlinearities (42). For a broadband input, the coefficient of harmonic distortion ($$k_d$$) is defined as (54)

$$k_d = \sqrt{P_{\text{tot}}/P_{\text{tot}}/100 (\%)$$

where $$P_{\text{tot}}$$ is the total power in the output and $$P_{\text{out}}$$ is the output power due to system nonlinearities only, i.e., the power at noninput frequencies. Because only nonlinearities and noise can produce output energy at noninput frequencies, the values of $$k_d$$ were also corrected for nonzero energy at noninput frequencies (54). The advantage of using $$k_d$$ is that it can be calculated from a single impedance measurement, whereas the traditional method of characterizing nonlinear- ity requires the measurement of the moduli at several distinct amplitudes. The $$k_d$$ in a linear system is zero. In a nonlinear system driven by sinusoids, the $$k_d$$ measures the distortion and cross talk due to system nonlinearities.

Measurements of mechanical failure of lung tissues. To assess the strength of the alveolar wall in the parenchyma, failure tests of lung tissue strips were carried out. Similar to the measurements of respiratory function, an additional dose of pentobarbital sodium (70 mg/kg) was injected intraperitoneally to each animal (n = 6 in each group); the thorax was then opened, and the animals were exsanguinated by severing the inferior vena cava. The heart, lungs, and trachea were carefully dissected en bloc and rinsed in PBS (Sigma). The experimen- tal setup was described previously (53). Parenchymal tissue strips having dimensions of 2.0–3.0 mm × 0.7–1.0 mm × 0.7–1.0 mm in length, width, and thickness, respectively, were carefully prepared from each lung, and the pleura were removed with the use of a razor. Each end of the tissue strip was fixed by cyanoacrylate glue to small metal plates attached to straight steel wires. The assembly was placed in a horizontal tissue bath filled with PBS at room temperature, with one wire attached to a computer-controlled lever arm containing a force transducer (model 300B, Aurora, ON, Canada). We followed the method reported by Tanaka and Ludwig (47). Briefly, each strip was stretched at a rate of 0.2 mm/s until the sample separated into two pieces. Due to the limitation of the maximum displacement of the lever arm, the strips were first stretched to approximately three times their unstretched length before the displacement was recorded and force signals were started. The strain was defined as the total dis- placement in length normalized by the unstretched length of the samples. A transient decrease in the force (see arrows in Fig. 8) was defined as "the failure stress," indicating that fibers in the alveolar walls started to break during stretching. Two or three strips were matched from each lung, and the pleura were removed from each animal, and the average value of the failure stress was used for statistical analysis.

Bronchoalveolar lavage. Whole lung lavages were performed with 1 ml of PBS twice via the tracheal cannula after impedance and P-V curve measurements were completed (n = 5 in each group). The return volume was measured, and the bronchoalveolar lavage fluid (BALF) was centrifuged. The cell pellet was resuspended in red blood cell lysis buffer (0.01% NH4Cl) and brought up to the initial lavaged volume for total cell count by hemocytometry. Slides contain-
ing 500–1,000 cells were prepared using a cytocentrifuge and stained with rapid Wright’s stain. Differential cell analysis was performed by manual counting under a light microscope.

**Lung histology and morphometry.** After mediastinal dissection, the lungs were perfused with 10% buffered formalin via the tracheal cannula at an airway pressure of 25 cmH2O for at least 20 min (n = 5 in each group). The fixed lungs were embedded in paraffin, sectioned, and stained with hematoxylin and eosin for histological analyses. The average distance between alveolar walls, the mean linear intercept (MLI), was calculated according to established methods (10) using a light microscope. For each pair of lungs, 10 histological fields were evaluated.

**BALF elastase-like activity.** Elastase-like activity in BALF was measured by following the protocol of Dhami et al. (9). Briefly, BALF samples were lyophilized and reconstituted in water to make a fivefold concentrated solution. An assay buffer of 0.2 M Tris-HCl, pH 8.0, was prepared; 100 μl of assay buffer, 50 μl of substrate (0.5 mg/ml of N-succinyl-Ala-Ala-Ala-p-nitroanilide), and 50 μl of BALF were then added. All samples were assayed in duplicate. EDTA (10 mM) was added to the samples to inhibit metalloelastase (13). Negative controls of 150 μl of assay buffer and 50 μl of substrate were used. We assessed background absorbance of each BALF sample by incubating 150 μl of assay buffer with 50 μl of each sample. This value was then subtracted from the absorbance of the test wells. We measured the absorbance of the wells at 405-nm wavelength using a spectrophotometer.

**Whole lung collagen content.** Collagen content was assessed by measuring the hydroxyproline content of the tissue as previously described by Woessner (51). Lungs from the control (n = 7) and the PPE-treated (n = 7) mice were lyophilized for 12 h to dry weight, measured, and minced. The lung sample was then hydrolyzed with 4 ml of 6 N HCl at 100°C for 6 h. One milliliter of the hydrolysate was then taken and evaporated. The powder was reconstituted with 1 ml of distilled H2O and reevaporated. The powder was then reconstituted with 5 ml of distilled H2O. Hydroxyproline (Sigma) standard solutions of 0–10 μg/ml were prepared. Sample solution (2 ml) was taken and oxidized with 1 ml of chloramine-T (Sigma) for 20 min. The reaction was then stopped with 1 ml of 3.15 M perchloric acid. After 5 min, 1 ml of p-dimethylaminobenzaldehyde solution was added. The sample was vortexed, incubated in a 60°C bath, and then cooled under tap water for 5 min. The absorbency of the solutions was determined at 557 nm using a spectrophotometer. The hydroxyproline concentration was determined from the standard curve.

**Whole lung elastin levels.** The lyophilized samples were placed into 1 ml of 0.25 M oxalic acid. The suspension was then heated at 100°C for 1 h. The specimen was centrifuged, and the supernatant was collected. The above procedure was repeated for a total of five times until all the insoluble elastin had been converted into a soluble product (α-elastin) (7). One milliliter of the collected supernatants for each mouse was then dialyzed against water using 15,000 molecular-weight cutoff dialysis membrane (Spectrum, Houston, TX). The levels of α-elastin were then determined with the use of Fastin-elastin assay (Biocolor, Belfast, Northern Ireland) following the specific protocol outlined in the kit.

**Statistical analysis.** All data were expressed as means ± SD. Student’s t-test and repeated-measures two-way ANOVA were used to evaluate the significance of differences between means and variances, with P < 0.05 as the level of significance.

**RESULTS**

**Histopathology.** Figure 1 shows representative alveolar structures of a control and a PPE-treated lung stained with hematoxylin and eosin 3 wk after initial treatment. Significant enlargement of the alveolar air spaces was observed in lung samples from PPE-treated mice compared with control mice. Both the mean and the SD of the MLI in the PPE-treated group (83 ± 21 μm) were significantly larger than in the control group (43 ± 3 μm) (P = 0.001). There were no significant differences in body weight between PPE-treated (24.9 ± 0.9 g) and control groups (25.2 ± 1.1 g).

**BALF analysis.** There were no significant differences in BALF total cell numbers between PPE-treated (3.4 ± 1.0 × 10⁴ /ml) and control groups (3.8 ± 0.9 × 10⁴ /ml), and over 95% of the cells were macrophages in both groups. No eosinophils and only 0.5% neutrophils were observed in both groups. Additionally, no elastase-like activity was detected in BALF of the control and PPE-treated mice, suggesting that direct elastolytic activity of PPE had already diminished at 3 wk after treatment as reported previously (40).

**P-V curves and dynamic respiratory mechanics.** Figure 2 compares the quasi-static P-V curves in the two groups obtained during volume-controlled inflation from end-expiratory volume. The pressure was significantly lower in the PPE-treated mice than in the control mice (P < 0.001). The average...
quasi-static elastance value, defined as a slope between 0 and 1.15 ml of inflated volume, was significantly lower in the PPE-treated mice (18.8 \pm 2.8 \text{ cmH}_2\text{O/ml}) than in the control mice (26.5 \pm 1.7 \text{ cmH}_2\text{O/ml}) (P < 0.001).

Figure 3 shows representative cases of the dynamic respiratory system resistance and elastance (calculated from the reactance where elastance = \(-2\pi f \times \text{reactance}\)) as a function of frequency and the fits of the mathematical model (20) to the data in representative control and PPE-treated mice at PEEP = 3 cmH\text{O}. The measured data were fit well by the model at all PEEP levels in both groups. As a function of PEEP, all the values of tissue H parameters, H\text{min}, H\text{max} and H\text{mean}, were significantly PEEP dependent (P < 0.001) and decreased with increasing PEEP in both groups (Fig. 4). The values of H\text{min}, H\text{max}, and H\text{mean} were significantly lower in the PPE-treated group than in the control group (P < 0.001) (Fig. 4). The largest difference in H\text{max} between the control and the PPE-treated mice was at the highest (9 cmH\text{O}) PEEP. Hysteresivity (\(\eta\)) was significantly PEEP dependent in the PPE-treated mice (P < 0.001) but not in the control mice and was significantly higher in the PPE-treated mice at PEEP \(\geq\) 6 cmH\text{O} (Fig. 5A). Raw was significantly PEEP dependent (P < 0.001), but there was no significant difference between the groups (P = 0.78) (Fig. 5B). G was significantly PEEP dependent (P < 0.001) and significantly lower in the PPE-treated mice (P < 0.001) (Fig. 5C).

Dynamic nonlinearity. The \(k_d\), a measure of dynamic nonlinearity of the respiratory system, was significantly PEEP dependent (P < 0.001) and decreased, implying more linear behavior when PEEP was increased in both groups (Fig. 6). The \(k_d\) was significantly larger in the PPE-treated group than in...
the control group ($P < 0.001$). Figure 7 shows the linear relationships between $H_{\text{min}}$ or $H_{\text{max}}$ and $k_d$, including data from all PEEP levels. There were significant correlations between $H_{\text{min}}$ and $k_d$ in both the PPE-treated ($k_d = 0.51 H_{\text{min}} - 1.56$ ($P < 0.001$, $r = 0.83$) and control groups ($k_d = 0.36 H_{\text{min}} - 2.21$) ($P < 0.001$, $r = 0.90$) (Fig. 7A). The slopes of the two lines were significantly different ($P < 0.05$). There were also significant correlations between $H_{\text{max}}$ and $k_d$ in both the PPE-treated ($k_d = 0.14 H_{\text{max}} - 0.82$) ($P < 0.001$, $r = 0.86$) (Fig. 7B). The two lines were nearly parallel, and there was no significant difference in slopes between the two lines ($P = 0.48$); however, the intercept of the PPE-treated group was relatively higher, nearly reaching a statistical level ($P = 0.07$). There were also significant correlations between $H_{\text{mean}}$ and $k_d$.
to PPE leads to a remodeling of the fiber network that significantly alters the mechanical properties and the nonlinear mechanical behavior of the whole organ.

**Collagen remodeling in emphysema.** In emphysema, the loss of elastin from the alveolar walls appears to be a major event in clinical pathology (1). The breakdown and the degradation of collagen fibers have also been reported in both human patients (39) and rodent models of emphysema (9, 35, 52). In addition, several studies have demonstrated that, after the destruction of alveolar walls, remodeling of collagen fibers as a result of an abnormal repair process contributes to the pathogenesis of emphysema (12, 17, 21, 22, 25, 49). Increases in total amount of collagen of the lungs have been reported in human patients (23, 35). In the present mouse model of emphysema, we also observed a 45% statistically significant increase in total collagen content and a 13% decrease in total elastin content of the whole lung (Table 2), suggesting that collagen remodeling within the lung was indirectly triggered after the elastolytic injury. Previous studies have shown that, after the onset and initial progression of emphysema due to the proteolytic injury caused by PPE, synthesis of collagen by lung fibroblasts is considered to be upregulated as part of the repair process of the damaged lung (15, 22). However, the repair process does not restore normal structure and function to the lung leading to pathology and altered physiology (24). Because the extracellular assembly of collagen molecules to fibrils and fibers is sensitive to the composition of the surrounding matrix (22, 24), it is conceivable that the structure and mechanical properties of the newly synthesized collagen differ from those that occur during normal growth. Although the details of this abnormal repair process at the molecular level are beyond the scope of the present study, it is important to discuss the physiological consequences.

**Alveolar wall and fiber failure in emphysema.** It has long been proposed that mechanical failure of the alveolar walls plays a pivotal role in the progression of emphysema (50). Recently, Kononov et al. (21) observed the failure of a single alveolar wall in a rat model of PPE-induced emphysema. However, to our knowledge, this is the first study to quantify the failure stress of parenchymal tissue strips from emphysematous lungs. We found evidence that the emphysematous

**DISCUSSION**

The primary findings of this study are that after PPE treatment of mice 1) lung elastance decreased and hysteresivity increased, 2) parenchymal tissue fibers failed at a lower stress than shown in the control group, 3) dynamic nonlinearity characterized by \( k_d \) increased, and 4) total collagen content increased, whereas there was a tendency of loss of elastin of whole lungs in the emphysema group. These physiological observations support the hypothesis that exposure of the lung to PPE leads to a remodeling of the fiber network that significantly alters the mechanical properties and the nonlinear mechanical behavior of the whole organ.

**Table 1. Tissue fiber rupture test**

<table>
<thead>
<tr>
<th>Failure Stress, kPa</th>
<th>Strain</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>11.9±1.4</td>
</tr>
<tr>
<td>PPE-treated</td>
<td>7.2±2.4</td>
</tr>
</tbody>
</table>

Values are means ± SD (n = 6 mice/group). PPE, porcine pancreatic elastase.

**Table 2. Lung collagen and elastin contents**

<table>
<thead>
<tr>
<th></th>
<th>Dried Lung Weight, mg</th>
<th>Hydroxyproline, ( \mu g/\mu g )</th>
<th>( \alpha )-Elastin, ( \mu g/\mu g )</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>13.5±3.8</td>
<td>4.38±0.83</td>
<td>47.2±6.0</td>
</tr>
<tr>
<td>PPE-treated</td>
<td>13.1±2.4</td>
<td>6.38±1.33</td>
<td>41.2±8.8</td>
</tr>
</tbody>
</table>

Values are means ± SD. Hydroxyproline and \( \alpha \)-elastin contents are expressed as \( \mu g/\mu g \) of dried lung weight (n = 7 mice/group).
lungs tissue breaks at the same strain but at a stress 40% smaller than the normal tissue (Table 1). In an attempt to interpret these results, we first note that normal collagen fibers are stiffer and stronger than other connective tissue constituents (36). As a consequence, the amount and organization of collagen in the alveolar walls should play a crucial role in determining the stiffness and the failure properties of the lung tissue. For example, when the collagen content of normal lung tissue strips was decreased via in vitro digestion using collagenase, the stiffness of the tissue dropped to 40% of its value before digestion (53). Alternatively, normal developmental changes of the lung increase both the stiffness and the failure strength of the alveolar walls as well as collagen contents during maturation. Indeed, Tanaka and Ludwig (47) reported that the failure stress of normal lung tissue from baby rats was 18 kPa, which increased to 28 kPa in adult rats. At the same time, in a follow-up study, Tanaka et al. (46) also found that the collagen content of the lung increased from 18% to ~38% during this normal maturation process. Thus, because in this model of emphysema we found a 45% increase in lung collagen (Table 2), one would expect the stiffness and the failure stress of the tissue to increase. Surprisingly, however, our data showed just the opposite behavior: despite the increase in collagen content, both lung elastance and the failure stress decreased by ~30% (Figs. 2 and 4) and 40% (Table 1), respectively, indicating that the total amount of collagen in the lung tissue is not the primary determinant of the mechanical properties in the diseased state.

In an attempt to resolve the apparent contradiction between increased collagen content and decreased elastance and failure stress in the emphysematous tissue, we first discuss the relation of lung stiffness and alveolar structure. Because the MLI nearly doubled in the emphysematous group, the number of alveolar walls per unit volume that can resist the deformation of the tissue strip could have decreased. This mechanism alone could account for the lower elastance and lower failure stress even if the mechanical properties of the alveolar walls were similar to those of the normal tissue. However, it is likely that not all of the increase in MLI is due to alveolar wall rupture. The PPE-treated lung is softer, and, at the fixation pressure of 25 cmH₂O, the alveoli would be more extended than those in the normal lung. Thus the number of alveolar walls per unit volume in the tissue strip is not necessarily smaller in the PPE-treated lung than in the normal lung. Using microscopic imaging, Brewer et al. (3) recently reported that individual alveolar walls from PPE-treated rats, which also involved collagen remodeling (21), appeared softer and more extensible than those from normal rats. These observations suggest that, despite the increased collagen content, the alveolar walls and the collagen fibers are likely to be weaker in the emphysematous lung as a consequence of the process of degrading and remodeling. Indeed, the ultrastructure of collagen from human emphysematous lungs reveals thickened and disorganized fibrils after remodeling (12). Our data then suggest that the stiffness and the failure properties of the remodeled fibers must decrease compared with normal collagen fibers.

The reduction in the failure stress of collagen has an important effect on how the structure of the lung evolves during the progression of emphysema. Suki et al. (44) recently developed a fiber network model and argued that, because mechanical forces influence the process of tissue breakdown, the alveolar structure must be very heterogeneous and the alveolar walls around the perimeter of severe emphysema lesions or the walls that separate such lesions may be overstretched. In agreement with these predictions, the heterogeneity of the alveolar dimensions was found to be much larger in the emphysematous than in the control lungs, both in the present study and in previous studies (10, 20, 34). Therefore, the alveolar walls in the emphysematous lung may have to oppose larger stresses locally, and, as a result, the increased local stresses can promote rupture of the remodeled walls (44), which in turn results in a decreased failure stress, as observed in Table 1. We thus conclude that mechanical forces are expected to play an important role in the progression of emphysema once the collagen matrix has undergone a critical amount of remodeling.

**Lung mechanical properties.** The consequences of alterations in the ECM of alveolar walls can be traced to organ-level changes in the mechanical properties of the lung. Because the chest wall is very soft in the mouse, at least 90% of H is due to the lung parenchyma (37). Additionally, any change in H must be related to a change in lung mechanics; hence, in the discussion that follows, we assume that changes in H largely reflect changes in lung mechanics. We have recently developed a new mathematical model that assumes a continuous distribution of H between a minimum and a maximum value (H<sub>min</sub> and H<sub>max</sub>, respectively) (20). We found that all H-related parameters (Fig. 4) as well as the static elastance (Fig. 2) decreased in the emphysematous mice compared with controls. The H<sub>max</sub> represents the stiffest regional elastance in the lung, and the collagen should be the most important determinant of its value. Thus the lower H<sub>max</sub> values in the PPE-treated mice suggest that the ultrastructural changes of remodeled collagen weaken the fibers and the alveolar walls, in agreement with analyses of the failure tests. On the other hand, we speculate that H<sub>min</sub> represents the softest regional elastance, which may be related to the loss of alveolar walls in that region. Thus it is likely that the lower value of H<sub>min</sub> is a functional consequence of the increased MLI in the emphysematous mice.

The hysteresivity is a material property of the lung tissue (14), and it also depends on the microscopic constituents of the alveolar walls. Indeed, changes in ECM composition can cause a change in the hysteresivity in the parenchymal tissue level (33, 53). In the present study, hysteresivity of the PPE-treated mice was higher than that of the control mice, as observed in TGF-α transgenic emphysematous mice (32), in mild emphysematous mice induced by nebulized PPE-treatment (20), and in rats (3). In parenchymal tissues of normal guinea pigs, hysteresivity after in vitro digestion with collagenase was significantly higher than that after digestion with elastase (53). This suggests that, in the normal lung tissue, the larger the elastin-to-collagen ratio the larger the value of hysteresivity. However, compared with controls, hysteresivity of the emphysematous mice increased (Fig. 5A), whereas collagen content also increased (Table 2). Together, the hysteresivity and elastance results suggest that remodeling in emphysema produces weak and viscous alveolar walls that also fail at lower stresses than those of the normal lung.

The values of Raw decreased with increasing PEEP most likely due to the increasing diameters of the airways with lung inflation in both groups (Fig. 5B). Although increased PEEP values, which suggest an underlying airway obstruction, were measured in sheep with experimental emphysema after papa
Hmax) in both the PPE-treated and the control mice (Fig. 7), as in emphysema. Recoil in emphysema has also not been well characterized. The extent to which lung surfactant contributes to the disease. The extent to which lung surfactant contributes to the development of emphysema actually contribute to the development of the disease. The extent to which lung surfactant contributes to recoil in emphysema has also not been well characterized. Further studies would be needed to clarify the role of surfactant in emphysema.

Dynamic nonlinearities. Another important physiological finding of this study is that the tissues responsible for governing elastic recoil in the emphysematous lungs also displayed significantly greater nonlinear behavior than control lungs. The mechanical behavior of the normal lung tissue has been characterized as nonlinear (28, 29, 42, 53), and the origin of dynamic nonlinearity has been investigated in various organs (11). In the respiratory system, dynamic nonlinearity is likely related to the ECM components, including the nonlinear viscoelastic collagen and its interactions with the linear viscoelastic elastin, and the viscous ground substance, including mainly proteoglycans (29). Because elastic fibers behave more linearly than collagen fibers (28, 36), tissue nonlinearity could be more related to collagen and, in particular, the extent to which collagen fibers are stretched in the alveolar walls. Thus the dynamic nonlinear behavior of the lung tissue can be considered as a global in vivo assay of collagen function in the intact lung. Although the nonlinearity is certainly related to collagen, it is also possible that the physical interaction between collagen and elastin also influences nonlinear behavior.

In the present study, we demonstrated for the first time that dynamic nonlinearity of the whole lung, as characterized by $k_d$ (42), is linearly related to lung elastance parameters ($H_{\text{min}}$ and $H_{\text{max}}$) in both the PPE-treated and the control mice (Fig. 7), as found for normal tissue strips (53). More importantly, despite a decrease in $H$, $k_d$ increased with emphysema compared with normal lungs. Specifically, the relationship between $k_d$ and $H_{\text{min}}$ as well as $H_{\text{max}}$ shifted to the left in emphysema. This can be accounted for by changes in ECM components. As discussed above, a decrease in elastance and an increase in collagen content suggest that the new collagen in the remodelled alveolar walls must be less stiff than the normal collagen. An increase in $k_d$ on the other hand suggests that the collagen fibers are either more stretched or inherently different from normal with respect to their nonlinear mechanical behavior in the emphysematous alveolar wall.

One may argue that, in contrast to tissue strips, in the whole lung, airflow closure also contributes to harmonic distortion. If a significant portion of the lung is blocked by airway closure and tidal volume remains the same, then a smaller lung will receive the same tidal volume and the lung becomes over-stretched. In fact, the $k_d$ was largest at PEEP = 0 in both groups (Fig. 6), which is the condition where recruitment and derecruitment could occur most during oscillations. Furthermore, the decrease in $k_d$ with PEEP implies gradual recruitment. However, neither lung elastance nor $k_d$ decreased when PEEP was increased from 6 to 9 cmH2O. Thus these data suggest that, above 6 cmH2O PEEP, recruitment did not occur and hence it could not influence our data. Therefore, we believe that the relation between $k_d$ and $H$, and consequently the above interpretation of the results, is insensitive to airflow closure at least at the higher PEEPs included in this study.

In summary, we have characterized the respiratory and lung mechanical properties of a mouse model of emphysema induced by PPE. We observed a decrease in lung elastance and failure strength of the alveolar walls as well as an increase in hysteresivity, dynamic nonlinearity, and total lung collagen content. These results suggest that significant collagen remodeling takes place within the alveolar wall, which produces weak but more nonlinear fibers and alveoli that are locally over-stretched and hence prone to mechanical failure. These alterations in the micromechanics of the alveolar walls significantly affect organ-level lung function in the mouse.

GRANTS
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