Effect of pancreatic and leukocyte elastase on hydraulic conductivity in lung interstitial segments


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Houtz, P. K., P. D. Jones, N. E. Aronson, L. M. Richardson, and S. J. Lai-Fook. Effect of pancreatic and leukocyte elastase on hydraulic conductivity in lung interstitial segments. J Appl Physiol 97: 2139–2147, 2004. First published August 6, 2004; doi:10.1152/japplphysiol.00567.2004.—Elastase-induced changes in flow were used to quantify the degradation of lung interstitial elastin. Degassed rabbit lungs were inflated with silicon rubber via airways and vessels. The lungs were cut into 1-cm-thick sections. Two chambers were bonded to each section to enclose the interstitium surrounding an arterial segment. Flow of albumin solution (0–5 g/dl) between the chambers was followed by that of the albumin solution with 0.25 g/dl pancreatic elastase solution. Driving pressure was 5 cmH2O, and mean interstitial pressure was either 0 or 10 cmH2O. Elastase caused an increase in flow in ~70% of the interstitial segments and a reduction in flow in the remaining segments. The elastase-induced response in flow was independent of both albumin concentration and mean interstitial pressure. Leukocyte elastase (5 units/dl) produced flow responses similar to those of 0.25 g/dl pancreatic elastase. The increased flow of leukocyte elastase was reduced by a subsequent flow with 0.25 g/dl pancreatic elastase but enhanced by a subsequent flow with a 10-fold lower concentration. A change in the order of the elastase flows reversed the concentration-dependent responses. This behavior suggests a complex interaction among the interstitial fibers after degradation by pancreatic and leukocyte elastase. Endogenous elastase-induced increases in interstitial permeability might affect blood lymph barrier permeability, whereas elastase-induced cessation of flow might be related to the alveolar septal wall destruction observed in emphysema.

reflection coefficient; rabbit; lung fluid balance; hyaluronidase; emphysema

THE INTERSTITIUM SURROUNDING large pulmonary blood vessels is the major storage site for excess water during the formation of pulmonary edema (26, 29). Thus its transport properties are important to understanding the pathogenesis and resolution of edema. In previous studies with isolated lung interstitial segments, interstitial flow resistance depended on several factors: the degree of hydration, albumin concentration of the solution, electrical charge of the solute, and tissue hyaluronan. Hydraulic conductivity increased with albumin concentration (13), positively charged solute molecules (22), and tissue degradation by hyaluronidase (3, 28, 31) and decreased with negatively charged solute molecules (22). These effects were also observed by interstitial cuff growth and interstitial pressure equilibration in isolated rabbits lungs inflated with liquid (15, 16). More recent experiments showed that the restriction of albumin measured by the transport of tracer 125I-albumin through lung interstitial segments was maximal with Ringer solution and decreased with albumin concentration and increased hydration (31). Similar results were measured in the isolated rat diaphragm (19). The increased hydraulic conductivity and reduced restriction with albumin concentration were attributed to a colloid osmotic pressure-induced interstitial pore expansion.

Elastase has been implicated in the etiology of emphysema and chronic bronchitis and is commonly used in experimental animal models to study the disease (10, 11, 17, 25, 32). The development of emphysema is associated with the destruction of elastic fibers in the interstitium of alveolar septal walls as evidenced by abnormal airspace expansion. In experimental studies of pulmonary emphysema, two types of elastase, polymorphonuclear leukocyte (neutrophil) and pancreatic (serum) elastase are used. In vivo, the destruction of elastic fibers by elastase is thought to result from an imbalance between the normal elastase inhibitors (mainly α1-proteinase inhibitor and α2-macroglobulin) and elastase. In early studies, leukocyte elastase inhibited by α1-antitrypsin but not by pancreatic trypsin inhibitor was implicated in elastolysis during acute arteritis and pulmonary emphysema (10). Neutrophil elastase has been implicated in the pathophysiology of acute lung injury primarily with the involvement of the lung microvascular barrier (11). However, the contribution of the lung interstitium interposed between the lymph and microvascular endothelium has not been extensively studied. One study showed little disruption of the extracellular matrix organization after the formation of edema with pancreatic elastase injected intravenously in rabbits (21). We wondered whether degradation of elastic fibers by elastase would increase lung interstitial hydraulic conductivity and contribute to lung microvascular permeability damage.

Accordingly, we studied the elastase-induced increase in flow through perivascular interstitial segments as a measure of the degradation of interstitial elastic fibers. We measured the change in tracer 125I-albumin concentration with flow through the interstitium to determine the change in interstitial pore size caused by 0.25 g/dl pancreatic elastase solution. Pancreatic elastase increased flow in ~70% of the segments and decreased flow in the remaining segments. In separate experiments, we studied the differences in the flow response between pancreatic elastase and leukocyte elastase. Both 5 units/dl leukocyte elastase and 0.25 g/dl pancreatic elastase solutions were equally effective in producing an increase in flow through lung interstitial segments. However, the effect on flow by leukocyte and pancreatic elastase solutions measured in sequence was highly dependent on the flow order and on the concentration of pancreatic elastase. This behavior suggests a
complex interaction among different interstitial fibers degraded by pancreatic and leukocyte elastase.

**METHODS**

This study was approved by the University of Kentucky Animal Care Committee.

The method of isolating a 1-cm length of perivascular interstitium of rabbit lung has been described in previous studies (3, 13, 22, 28, 31). New Zealand White rabbits (n = 28, 3.0–4.4 kg body wt) were tranquilized with ketamine (50 mg/kg) and xylazine (5 mg/kg) injected intramuscularly. After 5,000 units of heparin were administered intravenously, each animal was euthanized by an overdose of pentobarbital sodium (50 mg/kg) and exsanguination. The chest was opened by a sternotomy, and cannulas were tied into the trachea, right ventricle, and left atrial appendage. The entire thorax was removed by transection at the levels of the neck and diaphragm, and the lungs were inflated to 15–20 cmH₂O with silicon rubber solution (Microfil, Flow Tech, Carver, MA) via reservoirs attached to the cannulas. Different colored solutions were used for the identification of the arteries, vein, and alveolar space on subsequent dissection. The solutions mixed with a catalyst (5%) solidified in 2 h. The two solid caudal lobes were cut into six 1-cm-thick slabs transverse to the main airway. The interstitium surrounding the largest artery of each slab was selected for study. Two chambers were bonded to the sides of each slab to enclose the ends of the arterial segment (Fig. 1). The upstream chamber was positioned adjacent to the larger diameter end of the arterial segment and filled with albumin solution containing radioactive tracer¹²⁵I-albumin (~3,500 counts/s per ml; Perkin-Elmer, Boston, MA). The downstream chamber was connected to a length of PE200 tube and filled with albumin solution without tracer and dyed with blue food coloring (0.2%) to easily see the meniscus in the PE200 tubing. All solutions were made with lactated Ringer solution, adjusted to 7.35–7.40 pH and filtered (0.45-µm pore diameter).

The driving pressure (ΔP), upstream pressure (P₁) minus downstream pressure (P₂), was 5 cmH₂O, and the mean pressure (Pm) was either 0 or 10 cmH₂O. Flow was measured by the distance the meniscus moved along the PE200 tubing at timed intervals. Each 1-cm length of PE200 tubing contained 0.0157 ml.

We studied the effect of pancreatic elastase on flow through the interstitium by the following procedure. Flow of an albumin solution (0, 1, or 5 g/dl) containing tracer (control) was measured first and was followed by the flow of a control solution with 0.25 g/dl pancreatic elastase (porcine pancreatic, Elastin Products, Owensville, MO). These experiments were repeated at both 0 and 10 cmH₂O Pm. For each flow, the volume transferred into the downstream chamber was at least ~0.1 ml to minimize the error due to the interstitial volume in the calculation of downstream tracer concentration (31). At the end of each flow, the downstream chamber solution was collected and the chamber was rinsed to collect any residual tracer. The radioactivity of downstream and upstream solutions was measured with a gamma counter (Wizard 1470, Perkin-Elmer, Billerica, MA). The absorption of the tracer by the plastic chambers was minimized by immersing the chambers in a 5 g/dl albumin solution overnight before the experiment. At the end of each experiment, we dissected the interstitial segment and measured mean vessel diameter using a macroscope with a calibrated scale. The mean diameter was the average of the major and minor diameters of the two ends of the vessel segment.

In 30% of the segments studied, 0.25 g/dl pancreatic elastase solution used in the foregoing experiments produced a reduced flow (see RESULTS). We determined whether this behavior was caused by the type of elastase with the following experiments. Flow of the following solutions was measured through interstitial segments in turn: 5 g/dl albumin solution (control), 5 units/ml human leukocyte elastase (Sigma, St. Louis, MO) in control solution, and pancreatic elastase (0.025 and 0.25 g/dl) in control solution. In separate segments, we reversed the order of the two types of elastase solutions. To determine whether the separate flows of the two types of elastase were crucial, we repeated the experiments with a 5 g/dl albumin solution followed by a mixture of 0.025 g/dl pancreatic elastase and 5 units/ml leukocyte elastase. In these experiments, ΔP was 5 cmH₂O and Pm was 0 cmH₂O.

In separate experiments, we determined the effect of elastase concentration on the flow response. We measured the flow of the following solutions: 5 g/dl albumin solution (control), followed by control solutions with 0.0025, 0.025, and 0.25 g/dl pancreatic elastase in turn. We repeated the experiments with control solution followed by 0.05, 0.5, and 5 units/ml leukocyte elastase solutions in turn. In these experiments, ΔP was 5 cmH₂O and Pm was 0 cmH₂O.

To determine whether the elastase-induced increase in flow was reversible, the flow of the following solutions was measured in turn: 5 g/dl albumin solution (control), control solution with pancreatic elastase (0.25 g/dl), and 5 g/dl albumin solution.

In separate experiments, we studied the effect of hyaluronidase on the pancreatic elastase-induced increase in flow. The flow of the following solutions was measured in turn: Ringer solution (control), control solution with 0.25 g/dl pancreatic elastase, and control solution with 0.02% hyaluronidase (bovine testes hyaluronidase, Sigma). In other experiments, we reversed the order of the elastase and hyaluronidase solutions. In these experiments, ΔP was 5 and Pm was 5 or 10 cmH₂O.

**Statistics.** Data are reported as mean values ± SD in the tables and mean values ± SE in the figures. We used paired and unmatched t-tests where appropriate to test for a significant difference between two groups of measurements. Multiple linear regression analysis was used to determine differences among more than two variables. We accepted P < 0.05 to be significant.

**RESULTS**

Figure 2 shows representative volume-time curves measured in the experiments. The effect of pancreatic elastase on the flow through the interstitial segments was variable and fell into three main groups (Fig. 2). Of the 62 segments studied, 70% of the segments (positive responders) showed either an immediate increase in flow (48%) or a delayed positive response (22%); and 30% of the segments showed a continuous decrease in flow until the flow stopped (negative responders), with elastase after the flow of albumin (control) solution. A linear regression analysis was performed on each volume-time curve, and the slope of the regression equation was used as the flow. Only significant flow values were accepted. The ratio of the flow with elastase to the prior flow of the control solution was used.
as a measure of the increased flow with elastase rather than the absolute flow magnitudes because of the one to three orders of magnitude variation of flow among the segments. The time frame used for the regression of the data was constant for each pair of flows.

Table 1 summarizes the data of flow (Qa) of the control solution (0, 1, and 5 g/dl albumin), flow of control solution with 0.25 g/dl pancreatic elastase (Qe), ratio of Qe to Qa (Qe/Qa), downstream-to-upstream $^{125}$I-albumin concentration ratio (Cout/Cin = $\alpha$, sieving coefficient), and the ratio (Qe/Qa) of Cout/Cin with elastase ($\alpha_{e}$) to Cout/Cin without elastase ($\alpha_{a}$). Qe/Qa values averaged 4.7 and were significantly greater than 1. As measured by Qe/Qa greater than 1, the effect of elastase was to increase flow significantly with 0 and 5 g/dl albumin solution at 0 and 10 cm H2O Pm, but not with 1 g/dl albumin solution at 0 and 10 cm H2O Pm. A multiple linear regression analysis of the entire Qe/Qa data vs. albumin concentration (Calb, g/dl), Pm (cm H2O), and vessel diameter (D, mm) showed a significant increase with D ($P = 0.0007$) but no significant variation with either Calb ($P = 0.78$) or Pm ($P = 0.11$); Qe/Qa = $-3.4 + 0.13$ Calb + 0.30 Pm + 3.2 D, $n = 56$, $R^2 = 0.23$, $P = 0.003$. The significant increase in Qe/Qa with D was due to including the negative responders (Qe/Qa < 1) that were associated mostly with the smaller diameter vessels. When positive responders (Qe/Qa > 1) and negative responders were grouped separately, Qe/Qa in either group showed no significant correlation with vessel diameter.

We studied the flow response to elastase as a function of the initial (control) flow magnitude using a power regression analysis. There was no significant correlation for any group in Table 1. However, the pooled data of Table 1 with 0.25 g/dl pancreatic elastase showed that the flow response as measured by Qe/Qa decreased as the magnitude of the control flow of albumin solution (Qa, ml/h) increased (Fig. 3): Qe/Qa = 0.69 Qa$^{-0.45}$, $n = 63$, $P = 0.0003$. The greater elastase-induced flow response at the lower flows might be related to the kinetics of elastase degradation (see Discussion).

Table 1. Effect of albumin concentration and mean interstitial pressure on 0.25 g/dl pancreatic elastase-induced increase in flow through interstitial segments

<table>
<thead>
<tr>
<th>Calb (g/dl)</th>
<th>Pm = 0 cm H2O</th>
<th>Pm = 10 cm H2O</th>
</tr>
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<tbody>
<tr>
<td>Qa, ml/h</td>
<td>0 (Ringer)</td>
<td>1</td>
</tr>
<tr>
<td>0.21±0.38</td>
<td>1.0±1.3</td>
<td>0.27±0.34</td>
</tr>
<tr>
<td>0.46±0.80</td>
<td>0.61±1.0</td>
<td>0.72±0.66</td>
</tr>
<tr>
<td>$\alpha_{a}$ (Cout/Cin)</td>
<td>0.57±0.25</td>
<td>0.85±0.12</td>
</tr>
<tr>
<td>$\alpha_{e}$ (Cout/Cin)</td>
<td>0.82±0.11</td>
<td>0.88±0.10</td>
</tr>
<tr>
<td>Rp(a), nm</td>
<td>12±9.0</td>
<td>22±16</td>
</tr>
<tr>
<td>Rp(e), nm</td>
<td>24±17</td>
<td>21±9.8</td>
</tr>
<tr>
<td>Qe/Qa</td>
<td>4.2±4.8 (12)*</td>
<td>2.1±4.2 (12)</td>
</tr>
<tr>
<td>$\alpha_{e}/\alpha_{a}$</td>
<td>1.5±0.55 (6)*</td>
<td>1.1±0.19 (9)</td>
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</table>

<table>
<thead>
<tr>
<th>Calb (g/dl)</th>
<th>Pm = 0 cm H2O</th>
<th>Pm = 10 cm H2O</th>
</tr>
</thead>
<tbody>
<tr>
<td>Qa, ml/h</td>
<td>0 (Ringer)</td>
<td>1</td>
</tr>
<tr>
<td>0.17±0.18</td>
<td>3.5±4.3</td>
<td>0.08±0.42</td>
</tr>
<tr>
<td>0.66±0.63</td>
<td>4.0±4.3</td>
<td>0.37±0.20</td>
</tr>
<tr>
<td>0.52±0.058</td>
<td>0.91±0.13</td>
<td>0.81±0.056</td>
</tr>
<tr>
<td>0.94±0.15</td>
<td>0.98±0.067</td>
<td>0.93±0.13</td>
</tr>
<tr>
<td>7.8±0.37</td>
<td>17±5.9</td>
<td>15±2.2</td>
</tr>
<tr>
<td>29±21</td>
<td>28±11</td>
<td>29±14</td>
</tr>
<tr>
<td>8.4±12 (11)*</td>
<td>3.8±6.2 (10)</td>
<td>5.3±3.6 (6)*</td>
</tr>
<tr>
<td>1.9±0.33 (5)*</td>
<td>1.1±0.16 (9)</td>
<td>1.2±0.20 (6)</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of experiments. Driving pressure was 5 cm H2O. Calb, albumin concentration; Pm, mean interstitial pressure; Qa and Qe, albumin and elastase solution flows, respectively; Cout/Cin, downstream-to-upstream $^{125}$I-albumin concentration ratio. Subscripts: a, albumin solution; e, elastase solution. Rp(a), pore radius with albumin solution; Rp(e), pore radius with elastase solution. *Significantly greater than 1 ($P < 0.05$).
The sieving coefficient $C_{elast}/C_{in}$ ($\alpha$) was calculated from the total radioactivity collected from the downstream chamber divided by the volume of solution collected from the upstream chamber. Dilution was equal to the total downstream volume collected divided by the volume of solution that passed into the downstream chamber. Table 1 shows that $\alpha$ values without elastase ($\alpha_a$) were minimal (0.52–0.57) with 0 g/dl $C_{alb}$ at both 0 and 10 cmH2O Pm and increased significantly with 1 and 5 g/dl $C_{alb}$ (0.81–0.91). The effect of elastase was to increase $\alpha_e$ from 0.52–0.57 to 0.82–0.94 with 0 g/dl $C_{alb}$. A similar trend was observed with 1 and 5 g/dl $C_{alb}$ but was in general not significant because of the greater values of $\alpha_a$ measured.

The increase in flow caused by the elastase as measured by $Q_e/Q_a$ was positively correlated with $\alpha_e/\alpha_a$ (Fig. 4). The regression equation was $\alpha_e/\alpha_a = 0.038 Q_e/Q_a + 1.1$, $P = 0.001$. Because $Q_e/Q_a$ was negatively correlated with $Q_a$ (Fig. 3), $\alpha_e/\alpha_a$ was negatively correlated with $Q_a$: $\alpha_e/\alpha_a = 1.1 Q_a^{-0.072}$, $P = 0.0035$. Because $\alpha$ is related to the equivalent pore radius through the reflection coefficient (see Eqs. 1 and 2), the elastase-induced flow was due to a pore size increase caused by the degradation of the elastic fibers.

We modeled the interstitium as a membrane to determine its reflection coefficient ($\sigma$) from measured $\alpha$ values (24, 29):

$$\sigma = 1 - \alpha$$  \hspace{1cm} (1)

This equation is applicable to flow of a solution of a single solute through uniform cylindrical pores with a Peclet number high enough so that $\alpha$ becomes flow independent. Reflection coefficient is related to $a/R_p$, the solute-to-pore radius ratio, as follows (1):

$$\sigma = [1 - (1 - a/R_p)^2]$$  \hspace{1cm} (2)

where $a$ is albumin radius and $R_p$ is pore radius. From the values of $\alpha_a$ and $\alpha_e$ (Table 1), $\sigma$ for albumin without elastase decreased from 0.43–0.48 for the flow of Ringer solution to 0.09–0.19 for 1 and 5 g/dl albumin solution. The effect of elastase was to further decrease $\sigma$ to 0.02–0.18. For an $a$ of 3.5 nm (Eq. 2), the equivalent cylindrical $R_p$ without elastase increased from 8–12 nm with Ringer solution to 15–22 nm with 1 and 5 g/dl albumin solution. The effect of elastase was to increase $R_p$ about twofold (2.2 ± 1.7).

Table 2 summarizes the data of the flow of 5 g/dl albumin solution ($Q_a$, control) followed in turn by the flow of 5 units/dl leukocyte elastase solution and the flow of 0.025 g/dl or 0.25 g/dl pancreatic elastase solutions. Note that of the 12 interstitial segments (Table 2, Fig. 5A), 8 (67%) of the segments responded to leukocyte elastase with an increased flow and produced a further increase in flow with the low-concentration pancreatic elastase. The other four segments that did not increase flow with leukocyte elastase produced an increase in flow with pancreatic elastase. This increased flow with 0.025 g/dl pancreatic elastase after the flow of leukocyte elastase was eliminated with a 10-fold higher concentration pancreatic elastase solution (Table 2, Fig. 5B).

In 12 separate segments (Table 2, Fig. 5C), we reversed the order of the elastase solutions with 0.025 g/dl pancreatic elastase followed by leukocyte elastase. Here, only three (25%) of the segments increased flow with 0.025 g/dl pancreatic elastase, whereas the other nine (75%) had a reduced flow. The subsequent flow with leukocyte elastase produced no additional increase in flow in the 12 segments. Thus the prior flow of the low-concentration pancreatic elastase inhibited the increase in flow of leukocyte elastase observed as the first flow (Table 2, Fig. 5A). By contrast, an increased flow with the higher concentration pancreatic elastase observed in 5 of 12 segments was followed by a further increase in flow with the subsequent leukocyte elastase solution, whereas in the 7 segments with a negative response in flow, the flow was further reduced with the subsequent leukocyte elastase (Table 2, Fig. 5D).

In five segments, there was no increase in flow of a mixture of 0.025 g/dl pancreatic elastase and 5 units/dl leukocyte elastase compared with a prior control flow of a 5 g/dl albumin solution. Thus the inhibition of flow by 0.025 g/dl pancreatic elastase occurred both in the presence of and after the flow of 5 units/dl leukocyte elastase solution.
Similar to the increased response in flow found for 0.25 g/dl pancreatic elastase as flow magnitude decreased (Fig. 3), the pooled data of Table 2 with both pancreatic and leukocyte elastase showed a similar significant correlation: $Q_{p,l}/Q_a = 0.44 \pm 0.02$, $n = 43$, $P = 0.0011$. $Q_{p,l}/Q_a$ values include both $Q_{p}/Q_a$ and $Q_{l}/Q_a$ values. However, an analysis of each group in Table 2 showed that only the data with low-concentration (0.025 g/dl) elastase was significant: $Q_{p}/Q_a = 0.41 \pm 0.05$, $n = 12$, $P = 0.011$.

Table 3 summarizes the data on the irreversibility of the 0.25 g/dl pancreatic elastase-induced increase in flow. Note that elastase increased the flow of a 5 g/dl albumin solution on average 3.6-fold. The subsequent flow of 5 g/dl albumin solution produced no significant change in flow.

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**Table 2. Effect of leukocyte and pancreatic elastase on flow through interstitial segments**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Qa, ml/h</th>
<th>Qp, ml/h</th>
<th>Qp/Qa</th>
<th>Qp/Ql</th>
<th>Qp/Qa</th>
<th>Qp/Ql</th>
</tr>
</thead>
<tbody>
<tr>
<td>Positive responders: 8/12</td>
<td>0.20 ± 0.21</td>
<td>0.27 ± 0.28</td>
<td>1.50 ± 0.35*</td>
<td>0.81 ± 0.96</td>
<td>2.4 ± 1.0*</td>
<td></td>
</tr>
<tr>
<td>Negative responders: 4/12</td>
<td>0.87 ± 0.80</td>
<td>0.52 ± 0.74</td>
<td>0.70 ± 0.42</td>
<td>0.94 ± 1.3</td>
<td>1.8 ± 0.49*</td>
<td></td>
</tr>
<tr>
<td>Leukocyte followed by pancreatic elastase (0.25 g/dl)</td>
<td></td>
<td></td>
<td></td>
<td></td>
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<td></td>
</tr>
<tr>
<td>Positive responders: 7/7</td>
<td>0.46 ± 0.56</td>
<td>0.96 ± 0.84</td>
<td>2.6 ± 1.2*</td>
<td>3.2 ± 7.1</td>
<td>2.4 ± 5.7</td>
<td></td>
</tr>
<tr>
<td>Pancreatic (0.025 g/dl) followed by leukocyte elastase</td>
<td>Qp, ml/h</td>
<td>Qp, ml/h</td>
<td>Qp/Qa</td>
<td>Qp/Ql</td>
<td>Qp/Qa</td>
<td>Qp/Ql</td>
</tr>
<tr>
<td>Positive responders: 3/12</td>
<td>0.09 ± 0.07</td>
<td>0.31 ± 0.29</td>
<td>3.2 ± 2.8</td>
<td>0.15 ± 0.15</td>
<td>0.69 ± 0.46</td>
<td></td>
</tr>
<tr>
<td>Negative responders: 9/12</td>
<td>1.7 ± 3.1</td>
<td>0.44 ± 0.63</td>
<td>0.65 ± 0.35*</td>
<td>0.54 ± 0.68</td>
<td>1.4 ± 1.4</td>
<td></td>
</tr>
<tr>
<td>Pancreatic (0.25 g/dl) followed by leukocyte elastase</td>
<td>Qp, ml/h</td>
<td>Qp, ml/h</td>
<td>Qp/Qa</td>
<td>Qp/Ql</td>
<td>Qp/Qa</td>
<td>Qp/Ql</td>
</tr>
<tr>
<td>Positive responders: 5/12</td>
<td>0.31 ± 0.23</td>
<td>0.78 ± 0.78</td>
<td>2.3 ± 0.85*</td>
<td>30 ± 48</td>
<td>27 ± 28*</td>
<td></td>
</tr>
<tr>
<td>Negative responders: 7/12</td>
<td>1.3 ± 1.3</td>
<td>0.30 ± 0.60</td>
<td>0.15 ± 0.17*</td>
<td>0.15 ± 0.25</td>
<td>0.43 ± 0.15*</td>
<td></td>
</tr>
</tbody>
</table>

Qa, flow of 5 g/dl albumin solution (control); Qp, flow of leukocyte elastase solution; Qp, flow of pancreatic elastase solution. *Significantly different from 1 ($P < 0.05$).

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Fig. 5. A and B: $Q_l/Q_a$, ratio of the flow of leukocyte elastase solution ($Q_l$) to prior flow of albumin solution ($Q_a$) is compared with $Q_p/Q_l$, ratio of the subsequent flow of pancreatic elastase solution ($Q_p$, 0.025 g/dl (A) and 0.25 g/dl (B)) to $Q_l$. Bar represents 1 SE. *Significant increase in flow (ratios compared with 1).

C and D: $Q_p/Q_a$, ratio of the flow of pancreatic elastase solution ($Q_p$, 0.025 g/dl (C) and 0.25 g/dl (D)) is compared with $Q_l/Q_p$, ratio of the subsequent flow of leukocyte elastase solution ($Q_l$) to $Q_p$. Bar represents 1 SE. *Significant change in flow (ratios compared with 1).
was independent of albumin concentration and increased hydration caused by increasing Pm. The increase in hydraulic conductivity was positively correlated with an increase in the sieving coefficient of albumin (Fig. 4) and in the equivalent pore radius. The elastase-induced increase in flow was caused by an irreversible degradation of elastic fibers that produced an increased interstitial pore size. On the basis of the flow response (number of responders), a 5 units/dl leukocyte elastase solution was as effective as a 0.25 g/dl pancreatic elastase solution in degrading lung interstitial elastic fibers. However, the increase in flow by a 5 units/dl leukocyte elastase solution was reduced by a subsequent flow of 0.25 g/dl pancreatic elastase solution but enhanced by a subsequent flow of a lower concentration (0.025 g/dl) pancreatic elastase solution. The effect of pancreatic elastase concentration on the leukocyte elastase response was reversed when the flow order of the two elastase solutions was reversed. These results point to a complexity in the different fiber types that are degraded by pancreatic and leukocyte elastase.

**Methods.** The limitations of the method used to measure hydraulic conductivity through a section of lung perivascular interstitium have been discussed in previous studies (3, 13, 28, 31). First, the flow measured in these segments showed considerable variability that spanned three orders of magnitude. Thus, to study the effect of elastase, the ratio of the flow of solution with elastase to that without elastase was used as the measure of an increased flow. By this measure, each experiment acted as its own control. Second, leaks via spaces between the vessel wall and the silicon rubber that filled the vessel were minimized by directing flow from the large end of the tapered vessel. This procedure produced compression of the silicon rubber that expanded the vessel diameter. This effect plus the normal vessel tension minimized any spaces between the vessel wall and the rubber. Also, the flow direction was opposite to the normal direction of interstitial flow in situ. Any flow through lymphatic vessels would be protected by unidirectional lymphatic valves. Third, we assumed that the flow measured was through the perivascular interstitium and not through the vessel wall that has a much smaller hydraulic conductivity (14) and no permeability response to hyaluronidase (27). Fourth, in the fixation of the interstitium by inflating the lung with silicon rubber, the interstitium was subjected to no interstitial pressure. However, this effect would be negligible because there was no increased interstitial expansion on exposure to liquid at ambient pressure. However, this effect would be negligible because of the small compressibility of water. Fifth, the perivascular interstitium studied was associated with the largest arteries (1–4 mm diameter). The amount of elastin and other compos-
ments that were degraded by elastase might differ around smaller, more peripheral vessels. Sixth, we filled the airways and blood vessels with silicon rubber to form a solid lung, which allowed the separation of the lung into slabs with identifiable perivascular interstitial segments. Morphological studies showed that the rubber was retained within the capillary bed with no structural abnormalities of the endothelial barrier (5). On dissection at the end of each experiment, we observed no rubber within the interstitial tissue.

Comparison with previous results. There are similarities between the response in flow with elastase and that with hyaluronidase. Elastase doubled \( R_p \) as measured by \(^{125}\)I-albumin, similar to that found for hyaluronidase after the flow of hetastarch solution (3). Flow increased fivefold with 0.25 g/dl pancreatic elastase comparable to the sixfold increase measured with hyaluronidase.

The response in flow of elastase solutions observed in the present study differed in several respects compared with that observed with hyaluronidase solutions in previous studies (28, 31). First, the increase in flow with 0.25 g/dl pancreatic elastase solution was independent of the albumin concentration of the solution and the hydration caused by the increase in Pm. By contrast, in previous studies, hyaluronidase increased the interstitial hydraulic conductivity, but the increase was absent with higher albumin concentrations (5 and 10 g/dl) at 0 cmH\(_2\)O Pm. Second, in 22% of the interstitial segments, the flow response with elastase solution was delayed. This effect was not observed with hyaluronidase. Third, in 30% of the interstitial segments, the flow with elastase decreased with time and eventually stopped. This behavior was not observed with hyaluronidase.

Several factors might contribute to the differences in the response between elastase and hyaluronidase. The delayed response might be related to the peculiar cross-linked structure of elastin that produces a nonlinear release of peptides into solution with the rate of hydrolysis of peptides bonds by elastase (25). Another reason might be related to the concentration of the elastase and the hyaluronidase used in the experiments. The higher concentration elastase solution might exert a colloid osmotic pressure similar to that measured for albumin and significantly affect the pore structure at different concentrations. Nonuniformities in the interstitial pore size that depend on both the degree of hydration and albumin concentration might contribute to the differences in the flow responses measured with elastase (31). Whatever the differences in the response in flow due to elastase and hyaluronidase, the flow increase due to pancreatic elastase was not enhanced by a subsequent flow of hyaluronidase. This behavior points to a nonspecificity of the interstitial components that are degraded by elastase (17) and hyaluronidase. Elastase has been reported to have catalytic activity to protein, such as fibrin (17), and to peptide substrates other than elastin (32), whereas hyaluronidase (testes) degrades glycosaminoglycans other than hyaluronan (manufacturer’s specification).

A response of elastic fiber degradation by elastase was to increase the equivalent pore size. These effects were inseparable from those of hyaluronidase. Thus elastic fibers contribute to the structural integrity of the interstitium in a manner similar to that of hyaluronan. One possibility is that elastic fibers and hyaluronan molecules are closely intertwined within the interstitium. This is supported by studies that suggest that hyaluronan either by its binding to elastic fibers or by its physical location adjacent to elastic fibers protects elastin from being degraded by exogenous elastase (4, 30).

An ultrastructural correlate of the equivalent pore radius estimated for flow of albumin solution through pulmonary interstitium has not been defined. The present data suggest an equivalent pore radius of \( \sim 8 \) nm for Ringer solution and \( \sim 16 \) nm for 1–5 g/dl albumin solution, values consistent with previous results (31). A similar increase in tissue pore radius with albumin concentration has been demonstrated in the isolated rat diaphragm (19). In the latter study, a two-pore system with an interconnecting osmotic flow based on the nonoverlapping actin and myosin filament arrangement defined for skeletal muscle was proposed to explain the increase in pore size with diffusion and bulk transport of albumin solutions. A small-pore system (9 nm radius) formed within a network of polysaccharides and glycoprotein has been proposed for umbilical cord (18). However, an ultrastructural basis for a polysaccharide network in the interstitial matrix has not been measured. It is possible that interstitial pores are synonymous with the channels formed between elastic and collagen fibrils. Ultrastructural studies showed that the elastic fibrils have diameters of \( \sim 60 \) nm and are arranged in a network with a center-to-center spacing of \( \sim 80 \) nm (8, 23). A square lattice arrangement of elastic fibrils would produce an interboundary spacing of 20 nm and an equivalent pore radius of 10 nm, near the values estimated in the present study. Two observations suggest that collagen was not primarily responsible for the measured interstitial restriction of albumin. First, in rabbit corneas (7) and synovium (6), the interboundary spacing of collagen fibrils (6, 7) is relatively small (10 nm) and would produce a pore radius of \( \sim 5 \) nm, a value below that estimated in the present study. Second, hyaluronidase reduced the interboundary spacing of collagen fibrils (6), opposite to the expected increase in pore radius associated with an increased hydraulic conductivity (3, 31).

The present studies also showed that with elastic fiber degradation by elastase, the equivalent pore radius doubled. A similar behavior was measured with the degradation by hyaluronidase after the flow of albumin (31) and hetastarch solutions (3). However, there were differences between the elastase and hyaluronidase studies. First, the hetastarch studies showed an equivalent pore radius that was 50% greater for 6% than for 2% hetastarch solution and a doubling of pore radius at both concentrations with hyaluronidase. The 50% increase in pore radius was attributed to a colloid osmotic pressure-induced expansion by the hetastarch solution in the interstitial pores, whereas the doubling of the pore radius with hyaluronidase was attributed in part to a pore expansion caused by a hyaluronan degradation-induced reduction of the colloid osmotic pressure of the tissue outside the pores. By contrast, in the present study, no increase in pore radius was observed between 1 and 5 g/dl albumin solution. In the absence of a colloid osmotic pressure-induced increase in pore radius, the increase in pore radius with elastase could come about by a uniformly spaced degradation of half of the elastic fibrils.

Differences between pancreatic elastase and leukocyte elastase have been reported, particularly in their response to different inhibitors (10). Leukocyte elastase is inhibited by \( \alpha_1 \)-antitrypsin but not by pancreatic trypsin inhibitor. It has been reported that leukocyte elastase produced elastolysis dur-
ing acute pulmonary emphysema (9). However, numerous studies have reported the use of pancreatic elastase in experimental models of pulmonary emphysema (32).

In the present studies, pancreatic elastase of relatively high concentration produced increases in flow that were similar to those of leukocyte elastase. Differences between the responses in flow of leukocyte and pancreatic elastase solutions were found by measuring the sequential effects of leukocyte and pancreatic elastase. The reasons for these responses are speculative. The absence of a dose response in the elastase-induced flow might be related to the differential effects of pancreatic and leukocyte elastase. Several factors might be involved. First, pancreatic and leukocyte elastase might degrade different parts of the elastic fiber. Ultrastructural studies of human lung tissue in vitro showed that leukocyte elastase produced damage to only the periphery of the elastic fiber, whereas pancreatic elastase produced damage to both the periphery and interior of the elastic fiber (20). These effects might also involve the resident time for elastolysis that might be related to the magnitude of the flow (see below). Second, by-products of the degradation process might interact sterically or biochemically to prevent or enhance degradation by elastase. Third, pancreatic and leukocyte elastase might degrade two different fiber types. For example, human leukocyte elastase might be devoid of elastinolytic activity and the flow response might be caused by digestion of collagen fibers (2), although its effects on native collagen are not equivocal (17, 25). This issue might benefit from studies of the differential effects of leukocyte elastase and collagenase on hydraulic conductivity. Finally, the wide variation of the elastase-induced response in flow was found to be related to the hydraulic conductivity of the interstitial segment. The flow with pancreatic elastase increased as the hydraulic conductivity as measured by the control flow magnitude decreased (Fig. 3). This behavior might be related to the kinetics of enzymatic degradation because a low flow would predispose to a greater penetration of the elastase into the elastic fiber, an effect associated with degradation by pancreatic elastase and not by leukocyte elastase (20).

In the present experiments, 30% of the interstitial segments showed either a reduction or cessation of flow with elastase. It is possible that the reduced flow with elastase was another aspect of the degradation process that produced a closure of interstitial pores either by structural collapse or clogging of interstitial pores by the smaller degradation products. Ultrastructural correlates of these effects have not been reported and warrant further study.

Implication of the results. The present studies showed that, in general, elastase produced an increased hydraulic conductivity and reduced reflection coefficient in interstitium surrounding large pulmonary vessels. These effects might be important in the development of pulmonary disease such as acute respiratory distress syndrome that involves the formation of pulmonary edema (11). Increases in endogenous elastase in the etiology of disease might conceivably reduce the interstitial reflection coefficient and produce an overall increase in the blood-lymph barrier permeability. Elastase-induced reduction in flow caused by structural collapse might contribute to destruction of alveolar septal walls and air space expansion observed in emphysematous lungs.

GRANTS

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REFERENCES