Differences in acute lung response to elastase instillation in two rodent species may determine differences in severity of emphysema development

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Submitted 15 March 2011; accepted in final form 13 April 2011

Vecchiola A, de la Llera JF, Ramírez R, Olmos P, Herrera CI, Borzone G. Differences in acute lung response to elastase instillation in two rodent species may determine differences in severity of emphysema development. *Am J Physiol Regul Integr Comp Physiol* 301: R148–R158, 2011. First published April 13, 2011; doi:10.1152/ajpregu.00133.2011.—Elastase intratracheal instillation induces early emphysema in rodents. However, Syrian Golden hamsters develop more severe emphysema than Sprague-Dawley rats. We have reported species differences in oxidant/antioxidant balance modulating antiprotease function early after instillation. We now hypothesize that other components of the initial lung response to elastase might also be species-dependent. Sprague-Dawley rats and Syrian Golden hamsters received a single dose of pancreatic elastase (0.55 U/100 g body wt) to study acute lung injury biomarkers. Using serum, lung, and bronchoalveolar lavage fluid (BALF) samples, we evaluated changes in alveolar-capillary permeability, alpha 1-antitrypsin (α1-AT) concentration and activity, glutathione content, and proinflammatory cytokines. Rats showed a large increase in alveolar-capillary permeability and few hemorrhagic changes, whereas hamsters exhibited large hemorrhagic changes (P < 0.01) and mild transendothelial passage of proteins. Western blots showed a 30-fold increase in BALF α1-AT concentration in rats and only a 7-fold increase in hamsters (P < 0.001), with [α1-AT-elastase] complexes only in rats, suggesting differences in antiprotease function. This was confirmed by the α1-AT bioassay showing 20-fold higher α1-AT activity in rats and only twofold increase in hamsters (P < 0.001). In rats, results were preceded by a 3-, 60-, and 20-fold increase in IL-6, IL-1β, and TNF-α respectively (P < 0.001). In hamsters, only IL-1β and TNF-α showed mild increases. All parameters studied were back to baseline by 4 days. In conclusion, several components of the initial lung response showed species differences. Cytokine release pattern and functional inhibition of α1-AT were the most significant components differing among species and could account for differences in susceptibility to elastase.

α1-antitrypsin; elastase; animal models; acute lung injury; hemorrhage; experimental

However, very little is known about the molecular, cellular, or biochemical variations in the early stages of acute lung injury that could predict one kind of evolution or the other. A previous study conducted in our laboratory showed that 4 mo after the intratracheal instillation (IT) of a similar single dose of elastase per kilogram of body weight, Syrian Golden hamsters develop severe pulmonary emphysema with profound alterations in respiratory mechanics, whereas Sprague-Dawley rats develop mild emphysema with lesser functional impact (4, 6). Although the molecular-cellular basis of susceptibility of these species to elastase is not known, we have reported evidence for species differences in serum and lung antiprotease protection (6) and in oxidant-antioxidant imbalance modulating antiprotease function at early stages after elastase instillation (7). We have suggested that these differences in the initial lung response to the instilled elastase could play a major role determining the type and magnitude of the resulting emphysema. In this regard, Lucey et al. (26) long ago showed that destruction of alveolar walls after IT instillation of pancreatic elastase into the lungs of hamsters becomes evident once the initial edema, hemorrhage, and inflammation have disappeared. We speculate that since alveolar destruction differs in these species, lung response preceding this destruction is also likely to be different. Thus, we now hypothesize that rat and hamster species differences in the severity of emphysema development after elastase treatment are determined very early after instillation and that not only oxidant-antioxidant imbalance modulating antiprotease function, but also proinflammatory mediator release and other biochemical markers might show species differences.

To date, no attempts have been made to comprehensively examine the effect of elastase on proinflammatory mediator release, alveolar-capillary barrier derangement, and on oxidant and antiprotease responses. Moreover, studies specifically designed to evaluate variations between rodent species in the type or magnitude of the elastase-induced acute lung injury are unavailable. Indeed, results obtained in a particular species are often generalized to others (27).

Taking advantage of the existence of species differences in emphysema development after exposure to elastase, we undertook a comprehensive study of the temporal relationship of changes in 1) proinflammatory mediator release, 2) alveolar-capillary permeability and hemorrhage, 3) α1-AT concentration and activity, and 4) lung tissue and bronchoalveolar lavage fluid (BALF) total GSH content, to assess the hypothesis that species differences might exist in several components of the initial lung response to the instilled elastase.
The study of species variations in the pattern of the initial pulmonary response to elastase instillation might improve our understanding of susceptibility differences to other injurious agents that damage the lungs by mechanisms involving protease release in humans and may provide useful insights about new approaches for the treatment of acute lung injury that could prevent the development of chronic lung damage.

MATERIALS AND METHODS

Experimental Model of Intratracheal Instillation of Elastase

The study was performed according to the protocol submitted to and approved by the Animal Research Ethics Committee of the Pontificia Universidad Católica de Chile. Adult male Sprague-Dawley rats of 260 ± 10 g body wt (± 53 days of age) and male Syrian Golden hamsters of 100 ± 10 g body wt (48–55 days of age) received a single intratracheal dose of porcine pancreatic elastase (0.55 U/100 g body wt diluted in 0.7 ml or 0.3 ml of 0.15 M NaCl for rats or hamsters, respectively; Sigma, St. Louis, MO). By utilizing existing power formulas that predict lung weight from body wt (lung weight = 11.3 × body wt (kg)−0.99) (14), we calculated that hamster lung weight was one-third that of rat lung weight. Thus, on the basis of either total body weight or estimated lung weight, hamsters received one-third of the rat dose. The elastase solution was prepared under sterile conditions immediately prior to administration. Previously, rats and hamsters were anesthetized with an intraperitoneal injection of a ketamine-xylazine solution (0.8 ml for rats and 0.3 ml for hamsters) containing 8 mg/ml of ketamine and 1 mg/ml of xylazine.

A small incision was performed in the suprasternal notch to separate cervical muscles and expose the trachea. The elastase solution was injected directly into the trachea with a 25G hypodermic needle. After recovering from anesthesia, animals were kept in a nursery with a 12:12-h dark-light regime and with water and food consumption of standard rodent chow ad libitum. In this study, six groups of animals for each species were studied (4 h, 12 h, 24 h, 48 h, 4 days, and 7 days) after intratracheal instillation of elastase (n = 7 or 8 per group, plus 20% lethality). Lethality rate was similar in both species, occurred in most cases prior to 24 h, and was within the range of what has been published in the very few papers that report it (41, 42) with values around 25%.

The control group (n = 10 for each species) consisted of normal rodents that did not undergo surgery. They were kept under similar conditions as the experimental groups and anesthetized at the time of death.

Sample Collection

Immediately after anesthesia with the ketamine-xylazine solution, a midline laparotomy was performed for the removal of 1–2 ml of blood from the inferior vena cava to obtain serum and to exanguinate the animal. Subsequently, the lungs were excised in block, and the trachea was cannulated for the performance of bronchoalveolar lavage of both lungs. Three aliquots of 0.15 M saline, either 1 or 3 ml for hamsters and rats, respectively, were instilled, immediately aspirated, mixed, and then stored at −80°C.

In another group of animals (n = 10 for each time point for each species), the left lung was excised, immediately weighed (wet weight), and dried in an oven at 80°C until stable weight (dry weight) was obtained. This allowed for the calculation of the dry-to-wet weight ratio. The right lung was used for histological evaluation of hemorrhage.

Total Protein Content in Bronchoalveolar Lavage Fluid

It was measured using the bicinchoninic acid method (Sigma) (44). Protein standards were obtained by dilution of a stock solution of BSA (Invitrogen, Carlsbad, CA). Results were expressed as times over control values for each species.

SDS-PAGE of BALF and Serum Samples

This method was used to separate proteins and study changes in protein composition of these fluids using the Coomassie blue stain and for Western blot analysis of alpha 1-antitrypsin (α1-AT) in BALF.

Western Blot Analysis of α1-AT in BALF

Thirty micrograms of BALF samples were separated by 10% SDS-PAGE and immobilized onto NO2-cellulose membranes (0.45-μm pore; Thermo Scientific, Rockford, IL). Membranes were probed with an anti-human α1-AT rabbit antibody (Sigma, Mississauga, ON, Canada) and then with a goat anti-rabbit peroxidase-conjugated antibody (Thermo Scientific, Rockford, IL). α1-AT was visualized by enhanced chemiluminescence (SuperSignal Pico Chemiluminescent Substrate kit; Thermo Scientific) and Kodak X-ray film. Densitometric analysis was performed using the Image J 5 Program. Equal loading was controlled by Ponceau staining.

α1-AT Bioactivity Measured by the Elastase Inhibitory Capacity Colorimetric Assay

The inhibition that BALF samples exerted on the in vitro elastase-induced N-succinyl-Ala-Ala-Ala-p-nitroanilide (Sigma) hydrolysis test was studied according to Bieth and Wermuth (3) and Ying and Simon (56). The rate of p-nitroanilide released at 25°C was followed for 3.5 min at 405-nm wavelength using a microplate spectrophotometer reader (BIO-TEK Instruments, Winooski, VT). The assay was carried out in triplicate. Results were expressed as times over control values for each species.

Lung hemorrhage. Lung hemorrhage was evaluated by hemoglobin content in BALF and histological assessment. Absorbance at 414 nm of a BALF sample dilution was measured. Results were expressed as times of increase above control values of optical density. For histological assessment, lung fixation was obtained by filling the lungs through the tracheal cannula to 25 cmH2O with 10% neutral pH-buffered formaldehyde solution. The trachea was then occluded, and the fixation was allowed to continue for 2–4 wk prior to the morphological study. For each animal, three whole lung longitudinal sections were embedded in paraffin, sectioned at 5 μm, and stained with hematoxylin-and-eosin for analysis by light microscopy at ×40 magnification.

Total Glutathione Content

Lung tissue samples homogenized in 5% sulfosalicylic acid and BALF samples were assayed for total GSH content using the DTNB-GSSG reductase recycling assay, modified by Griffith (20). Results were expressed as a percentage of the control values.

BALF Proinflammatory Cytokines

IL-6, IL-1β, and TNF-α concentrations were measured in duplicate with commercially available ELISA kits (Quantikine, R&D Systems, Minneapolis, MN), according to the manufacturer’s instructions. Microplates were read using a microplate reader (BIO-TEK Instruments, Winooski, VT).

Statistical Analysis

Data are presented as means ± SE. One-way ANOVA and a Tukey post hoc test were used (47). Nonlinear regression analysis and curve fitting were performed using Graph Pad Prism 5. A value of P < 0.05 was considered statistically significant.
RESULTS

Markers of Increased Alveolar-Capillary Permeability at Different Times After IT Instillation of Elastase

**BALF total protein content.** Quantitative changes in BALF total protein concentration at different times after IT elastase instillation are shown in Fig. 1A. Results obtained were expressed as times over control value for each species. In rats, total protein content was increased four-fold at 4 h ($P<0.01$) and 12 h ($P<0.001$) after elastase treatment and was back to control value at 4 days.

In hamsters, a larger variability in total protein content was observed after elastase treatment. In this species, total protein content reached a peak later than in the rat, with 8 times the control value at 24 h ($P<0.001$) and 48 h ($P<0.01$) and returned to control value at day 7 after instillation.

**BALF SDS-PAGE.** Qualitative changes in BALF total protein content are shown in Fig. 1B. This figure shows a representative Coomassie blue-stained gel, illustrating changes in the electrophoretic profile of rat and hamster serum and BALF proteins after elastase treatment. For each of the species, BALF protein profile (B) was compared with the profile of serum proteins (S) at each time after elastase instillation.

In rats, BALF and serum samples from control animals differed in the content and type of proteins present; whereas very few proteins were visible in BALF, a wide variety of proteins were present in the serum. At 4 and 12 h after elastase treatment, there was a significant change in the electrophoretic profile of BALF with the addition of proteins of different molecular sizes, acquiring an electrophoretic pattern similar to that of the serum. This supports a significant increase in transendothelial passage of serum proteins due to increased alveolar-capillary permeability. This phenomenon persisted up to 24 h. Large-molecular-weight proteins disappeared from the BALF at 48 h, and its electrophoretic profile became similar to that of control animals at 4 and 7 days.

Similar to control rats, control hamsters exhibited very low protein content in BALF, with significant differences compared with the serum. Four hours after elastase treatment, new protein bands appeared in BALF. At 12 and 24 h, the change in the electrophoretic pattern of BALF was more significant than at 4 h, but unlike the rat, the protein profile did not resemble the one seen in the serum. Smaller-molecular-weight proteins predominated in this species. At 4 and 7 days, the BALF protein profile was still different than the one in the control animal.

**Lung dry-to-wet-weight ratio.** Table 1 shows that the lung dry-to-wet-weight ratio after elastase treatment did not significantly differ from control values in both species.
Pulmonary Hemorrhage

Hemorrhage was evaluated by measuring the content of hemoglobin in BALF and visualizing red blood cells in histological sections. Fig. 2A illustrates changes in hemoglobin concentration in BALF after elastase instillation in both species.

In the rat, a statistically significant transient increase in hemoglobin concentration was found at 12 h ($P < 0.001$). At this time, optical density was three times that in controls.

In the hamster, the increase in hemoglobin concentration occurred earlier and was of a larger magnitude than in rats. The highest hemoglobin concentration in this species was observed at 4 h after instillation, reaching 8 times the value of controls ($P < 0.001$). At 24 h, it was still 6 times above control values ($P < 0.001$) and returned to baseline values at 4 days.

Although with differences in magnitude, in both species, the time course of changes in Hb concentration resemble the time course of changes in total protein content (Fig. 1A). Because the hamster BALF contains significantly higher Hb levels than the rat BALF, hemoglobin contributes significantly more in hamster than in rats to total protein content.

Fig. 2, B and C, shows representative lung histological sections stained with hematoxylin and eosin at different times after elastase instillation for rats and hamsters, respectively. In addition to inflammatory cell infiltration (PMNs) and hyaline membrane formation, a large number of red blood cells were found between 4 and 24 h in both species. However, red blood cells were more prominent in the hamster, the species that also showed more destruction of alveolar septa.

Lung Antiprotease Protection Prior to and After IT Elastase Instillation

BALF $\alpha_1$-AT concentration by Western blot analysis. A representative Western blot of BALF samples from rats and hamsters is shown in Fig. 3A. Samples of BALF were obtained from control and elastase-treated animals at 4, 12, 24, and 48 h and at 4 and 7 days after elastase instillation. BALF from the control rat showed two immunoreactive bands of $\alpha_1$-AT in the 55–60 kDa molecular size range, corresponding to the native $\alpha_1$-AT described in rodents (25). After elastase treatment, the immunoreactivity of these bands increased, and new forms of immunoreactive $\alpha_1$-AT appeared, starting at 4 h and up to 24 h: (1) an ~88-kDa band corresponding to the [$\alpha_1$-AT-elastase] complex (48) and (2) a 45–50-kDa band, corresponding to the proteolytic fragment of $\alpha_1$-AT, derived from the interaction between elastase and $\alpha_1$-AT, similar to what has been described in humans (32) and rodents (48). The immunoreactive pattern of BALF $\alpha_1$-AT resembled the one in control animals at 4 days.

BALF from the control hamster showed a faint immunoreactive band of $\alpha_1$-AT in the 55–60-kDa range. After elastase treatment, the immunoreactivity of this band increased at 4 h and gradually decreased from 12 h to undetectable levels at 4 days. In this species, the ~88-kDa band corresponding to the
[\alpha_1\text{-AT-elastase}] complex was not found under conditions similar to the rat; however, in some of the animals, it was possible to find the proteolytic fragment.

Figure 3B shows equivalent protein loading using Ponceau staining. Figure 3C shows the densitometric analysis of Western blots (n = 5 for each time point and for both species). In rats, a significant increase in BALF total [\alpha_1\text{-AT}] immunoreactivity starting at 4 h was seen. At this time point, [\alpha_1\text{-AT}] immunoreactivity reached a maximum of 30 times the value found in controls (P < 0.001). Although not statistically significant, at 48 h and 7 days, immunoreactivity was still above control values. In the hamster, total [\alpha_1\text{-AT}] immunoreactivity increased seven times the value of the controls at 4 h (P < 0.05) and slowly decreased to reach control values at 48 h to 4 days. Hatched bars in Fig. 3C show the contribution of the proteolytic fragment derived from the [\alpha_1\text{-AT-elastase}] interaction to BALF total immunoreactivity. In rats, this band appeared at 4 h and remained present until day 7, whereas in hamsters, it was present only in some animals at 4 h.

**BALF \alpha_1\text{-AT bioactivity (elastase inhibitory capacity).** Figure 4A shows changes in BALF [\alpha_1\text{-AT}] bioactivity after elastase treatment in both species. Under basal conditions, rat BALF [\alpha_1\text{-AT}] activity was 2 times higher than in hamsters (inset, P < 0.001). In rats, there was an abrupt increase in [\alpha_1\text{-AT}] activity between 4 and 12 h after instillation (P < 0.001), reaching 10–20 times the control values, respectively, and returning close to basal levels at 48 h. In the hamster, on the other hand, we found a very mild increase in BALF [\alpha_1\text{-AT}] activity at 24 h (P < 0.01) and at 4 and 7 days (P < 0.01).

**Correlation between BALF [\alpha_1\text{-AT}] immunoreactivity and activity.** Figure 4B shows the relationship between BALF [\alpha_1\text{-AT}] immunoreactivity and its activity. For the rat, results fit an exponential function of the form $Y = Y_0 0.3727x$. The curve shows an exponential growth of [\alpha_1\text{-AT}] activity for immunoreactivity above 15 (arbitrary units) and mild activity for immunoreactivity below 15 in this species. The highest values of [\alpha_1\text{-AT}] immunoreactivity correlating with the highest values of activity correspond to the 4-, 12-, and 24-h time points, when the largest increase in alveolar-capillary permeability occurred.

On the other hand, in the hamster, no correlation was found between the low BALF [\alpha_1\text{-AT}] immunoreactivity and its low activity. Although there was an increase in alveolar-capillary permeability in this species, transendothelial passage of [\alpha_1\text{-AT}] was lower than in rats (Fig. 3).

**Lung Total Glutathione**

Table 2 shows changes in total glutathione content in lung tissue and in BALF after elastase instillation in rats and hamsters. In rats, no significant changes in total GSH were found over time in either lung tissue or BALF. In hamsters, whereas lung tissue GSH showed a prolonged 25% drop in total GSH content (P < 0.01), confirming previous results (7), BALF total GSH increased significantly (P < 0.05).

**Proinflammatory Cytokines in BALF**

Figure 5 shows changes in IL-6, IL-1β, and TNF-α concentration in BALF after elastase treatment in both species.

**IL-6.** In rats, there was an increase in IL-6 levels from 4 h (P < 0.01) and up to 24 h (P < 0.05) after elastase instillation. In this species, IL-6 levels returned close to control values at 48 h. In hamsters, on the other hand, no increase in IL-6 levels was seen at any time after elastase treatment.

**IL-1β.** In rats IL-1β levels were elevated almost 60 times over the control values at 4 and 12 h (P < 0.001) and returned to control values at 48 h. In hamsters, there was only a four-fold increase in IL-1β levels at 12 h (P < 0.05).

**TNF-α.** In rats at 4 h, TNF-α levels were 24 times higher than in controls (P < 0.001), persisted elevated at 12 h, and progressively decreased to reach control values at 48 h. In hamsters, levels of TNF-α followed a similar pattern than in rats, although changes were of a lower magnitude. The highest
value of this cytokine was seen at 4 h \( (P < 0.05) \), with a progressive reduction to reach control values at 4 days.

The peak in the three cytokines measured is a very early event that precedes 1) peaks in total protein content and \( \alpha_1 \)-AT concentration and activity, and 2) the restoration to control values of most of the markers of acute lung injury induced by elastase.

Figure 6 shows species differences in proinflammatory cytokines and in elastase handling by \( \alpha_1 \)-AT at the alveolar level for both species.

Figure 7 summarizes the time course of the changes in all components of the initial lung response to elastase that were evaluated in this study. The short duration and the relative magnitude of the changes in the parameters studied are shown for each species.

**DISCUSSION**

Despite the large number of publications dealing with the model of IT instillation of elastase, we have very little insights on molecular, cellular, or biochemical variations in early stages of acute lung injury that could be related to increased susceptibility to emphysema development. Our aim was to study several biomarkers of acute lung injury after IT elastase instillation.

We found that Sprague-Dawley rats and Syrian Golden hamsters, two species that differ in the magnitude of emphysema development after a single IT instillation of elastase, also show species variations in several markers of elastase-induced acute lung injury. They differ in the magnitude of the alveolar-capillary permeability derangement, lung hemorrhage, cytokine release, GSH status, and antiprotease function. The most significant species variations were seen in \( \alpha_1 \)-AT concentration and function and in the pattern of cytokine release. Whereas in the rat, the large increase in alveolar-capillary permeability was associated with a large transendothelial passage of active \( \alpha_1 \)-AT to the alveolar spaces, in the hamster, to the contrary, \( \alpha_1 \)-AT was found in low concentration and mostly in an inactive form. With regard to proinflammatory mediators, rats exhibited a large increase in IL-6, IL-1\( \beta \), and TNF-\( \alpha \) alveolar concentration, whereas hamsters showed only a mild increase in IL-1\( \beta \) and TNF-\( \alpha \) with no change in IL-6.

Animal models of IT instillation of elastase have been extensively used to study mechanisms involved in the pathogenesis of emphysema downstream protease release through inducing in a short period of time a protease/antiprotease imbalance. However, most of the studies have focused on

Table 2. **Species differences in lung and BALF GSH content after IT elastase instillation**

<table>
<thead>
<tr>
<th></th>
<th>Rats Total GSH</th>
<th>BALF Total GSH</th>
<th>Hamsters Total GSH</th>
<th>BALF Total GSH</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Mean ( \pm ) SE</td>
<td>Mean ( \pm ) SE</td>
<td>Mean ( \pm ) SE</td>
<td>Mean ( \pm ) SE</td>
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<tr>
<td>Control</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>4 h</td>
<td>91 ( \pm ) 10.9</td>
<td>138 ( \pm ) 11.5</td>
<td>93 ( \pm ) 5.2</td>
<td>422( * ) ( \pm ) 11.8</td>
</tr>
<tr>
<td>12 h</td>
<td>103 ( \pm ) 3.3</td>
<td>53 ( \pm ) 7.6</td>
<td>74( * ) ( \pm ) 4.5</td>
<td></td>
</tr>
<tr>
<td>24 h</td>
<td>105 ( \pm ) 5.1</td>
<td>106 ( \pm ) 6.8</td>
<td>81( * ) ( \pm ) 5.7</td>
<td>214( * ) ( \pm ) 12.3</td>
</tr>
<tr>
<td>4 day</td>
<td>94 ( \pm ) 7.5</td>
<td>89 ( \pm ) 5.1</td>
<td>87( * ) ( \pm ) 4.1</td>
<td>93 ( \pm ) 5.7</td>
</tr>
<tr>
<td>7 day</td>
<td></td>
<td></td>
<td>75( * ) ( \pm ) 9.1</td>
<td>179 ( \pm ) 11.6</td>
</tr>
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</table>

BALF, bronchoalveolar lavage fluid. \(* P < 0.05; \dagger P < 0.01; n = 6–10.\)
elucidating ultrastructural changes induced by elastase (15, 31, 37) and biochemical changes affecting the extracellular matrix, namely turnover of elastin, proteoglycans, and collagen (51). Furthermore, most of the studies have been done in hamsters, and their results have been extrapolated to rodents in general (45, 46). Although the recent work by Inoue et al. (22) evaluated for the first time BALF proinflammatory changes in this model, the work was done in rats only. Studies evaluating species or strain variations in the initial lung response after IT elastase instillation are not available.

Elastin has generally been regarded as the target molecule in this model. However, elastase can degrade most of the connective tissue components of the lungs (51) and can also make other components susceptible to degradation by other enzymes (51, 53). It has been shown that elastase destroys the proteoglycan content of the alveoli, mainly of the basement membranes (51) with loss of binding properties of these proteoglycans to other extracellular matrix components (35). These proteolytic effects on the extracellular matrix add to other direct destructive effects of elastase, such as injury to the epithelial cells (38) and endothelial cell detachment (37, 43) to damage the alveolar-capillary permeability barrier, producing transendothelial fluid extravasation and hemorrhage.

**Changes in Alveolar-Capillary Permeability**

In our study, although both species showed evidence for transendothelial fluid extravasation, the magnitude of this phenomenon was species dependent. Whereas in the rat, the derangement of the alveolar-capillary barrier allowed transendothelial passage of serum proteins of several molecular sizes to the alveolar spaces, in hamsters, proteins of smaller molecular weight were found increased in BALF. Although the significance of this difference is not known, we expected to find differences in fluid accumulation between species. However, the lung dry-to-wet weight ratio in our model was not sensitive enough to detect changes in alveolar-capillary fluid permeability after elastase treatment in any of the species, a finding that has been reported previously (35).

Increased alveolar-capillary permeability has generally been viewed as the main marker of acute lung injury, as there is a well-known correlation between the magnitude of the alveolar-
capillary barrier derangement and the degree of impairment of gas exchange and clinical severity (52). Noteworthy, our results show that the rat, despite being the species with the largest increase in alveolar-capillary permeability develops less chronic damage than the hamster, which is the species showing less alteration of alveolar-capillary permeability but develops more severe emphysema.

In this regard, in organs other than the lungs, the increase in alveolar-capillary permeability that is characteristic of any inflammatory reaction does not have all the negative implications that it has in the lung, since it contributes to provide substances with a protective role, such as antiproteases. In the lungs, the increase in alveolar-capillary permeability results in alveolar flooding, with a negative impact on gas exchange that does not allow us to appreciate the contribution that transendothelial passage of fluid makes to the increment in alveolar antiprotease level. Thus, in the rat, the large transendothelial passage of $\alpha_1$-AT likely contributes to reduce the elastolytic attack and the risk of developing a severe emphysema. It is tempting to speculate that in the hamster, having an inadequate antiprotease level, transendothelial passage of $\alpha_1$-AT is reduced compared with the rat, with the net result that the instilled elastase is not properly counteracted and remains free to damage the extracellular matrix, thus increasing the risk of developing severe emphysema (Fig. 6).

Species Differences in Lung Hemorrhagic Changes

Although less prominent for pancreatic elastase than for leukocyte elastase, hemorrhage is a characteristic feature of lung injury by elastase (41), and changes in bleeding have been used as a method for the in vivo evaluation of several inhibitors of elastase (19). Leukocyte elastase has been shown by others to produce intense hemorrhage at skin sites of injection and lysis of vascular basement membranes (23). In our study, lung hemorrhage was significantly more prominent in the hamster than in the rat. In hamsters, BALF showed both macroscopic evidence of bleeding (not seen in rats) and a higher hemoglobin content than in rats. In addition, a larger number of red blood cells were seen in hamster lung tissue sections compared with rats.

Fig. 7. Summary of the species differences in early lung response to intratracheal elastase instillation. The duration and the relative magnitude of the changes in the parameters studied are shown for each species. The largest differences were seen in cytokine release profile, hemoglobin concentration, and $\alpha_1$-AT concentration and activity. Although the three cytokines increased significantly in rats, only IL-1$\beta$ (light gray) and TNF-\(\alpha\) (dark gray) exhibited a small increase in hamsters. No changes in IL-6 (black) were observed in hamsters.
rats. All of these findings support a larger alveolar-capillary rupture in hamsters than in rats. Although there is the possibility that species differences in lung matrix components could explain part of the observed differences in lung hemorrhage (data not available), it is likely that the more severe bleeding in the hamster reflects more severe damage to the alveolar-capillary structures caused by the noncounteracted elastase itself in this species.

Despite interspecies differences in magnitude, damage to the alveolar-capillary barrier is back to normal at 4 days in both species after elastase instillation (Figs. 2 and 7), a result that is in agreement with other studies investigating changes in alveolar-capillary permeability (39).

**Species Differences in α1-AT Concentration and Activity**

Some of the most significant species differences were those related to the behavior of α1-AT, the main lung antiprotease, which is synthesized primarily in the liver (30).

Unlike other proteins, most of the research on α1-AT has been done in humans, and very little is known about this antiprotease in other species. However, Amemiya et al. (1) have shown that rat and hamster α1-AT are not significantly different with regard to structure and function, and both have a very high association constant with pancreatic elastase.

The alveolar-capillary membrane acts as a barrier to diffusion of α1-AT, causing a gradient in α1-AT levels between the blood and alveoli (54).

In our model, BALF α1-AT levels increased significantly in the first hour after elastase treatment in rats. This increase was likely favored by the aforementioned large increase in alveolar-capillary permeability, since the time course of the changes in α1-AT concentration was similar to that of the increment in alveolar-capillary permeability (Figs. 1, 3, and 7). Additionally, the large capillary-alveolar difference in α1-AT concentration (29), likely contributes to transendothelial passage of this protein. Furthermore, in rats, of the total α1-AT present in the lung after elastase treatment, a large proportion remains active since information provided by the two independent methods used by us in this study supports this concept. On the one hand, the Western blot analysis showed that elastase was counteracted by α1-AT since α1-AT and elastase complexes and the proteolytic fragment derived from this interaction were seen in the first hours after instillation. On the other hand, the biochemical assay showed a significant increase in BALF α1-AT activity in association with the increase in α1-AT concentration. Interestingly, restitution to control values of BALF α1-AT concentration and activity was associated with restoration of alveolar-capillary permeability barrier.

In hamsters on the other hand, the comparatively smaller increase in alveolar-capillary permeability (Fig. 1) contributes less than in rats to the transendothelial passage of new α1-AT to the alveolar spaces, a phenomenon that could be related to the lower capillary-alveolar difference in α1-AT concentration in this species compared with the rat, since hamster’s α1-AT in blood is only 60% of the α1-AT concentration in rats (21), and it has been estimated that α1-AT concentration in the epithelial lining fluid is one-tenth of that in serum (29, 54). Furthermore, α1-AT present in the hamster lung after elastase treatment is largely inactive (Figs. 3C and 4B). The absence of [α1-AT-elastase] complex formation in the Western blot study and the lack of increment of α1-AT activity in BALF in the bioassay after elastase treatment both support this notion.

The lung oxidant/antioxidant imbalance in this species likely contributes to inhibit α1-AT function. The long-lasting reduction in lung total GSH content in the hamster makes this species vulnerable to functional inhibition of α1-AT by oxidants, and thus, more susceptible to injury by elastase than it would be considering only its low α1-AT level (7). Although the relevance of the increase in BALF total GSH content is unknown, a similar response (e.g., reduction in lung tissue level and increase in BALF level) has been documented in a model of severe lung damage by oxidative stress (40) and is likely related to alveolar cell rupture. An alternative mechanism likely to contribute to α1-AT inhibition could be the interaction of the antiprotease with substances released from the damaged extracellular matrix; however, no information is available about this point.

In summary, several mechanisms are responsible for a lower antielastase protection in the hamster lung compared with the rat: 1) lower serum and lung α1-AT concentration, previously shown by us and others (6, 21); 2) lower baseline BALF concentration of α1-AT with less transendothelial passage of serum α1-AT after elastase injury; and 3) functional inhibition of α1-AT allowing more free elastase to interact with extracellular matrix components.

It has been shown that the level of α1-AT does not only influence emphysema development in the model of elastase-induced emphysema, as shown in this study. It also modulates other types of lung injury such as 1) emphysema development in models of mice exposed to cigarette smoke (10, 13, 49) and 2) acute lung injury induced by bleomycin (5). Whereas bleomycin-induced lung injury is potentiated by a deficit in α1-AT (11), the administration of exogenous α1-AT reduces bleomycin-induced lung injury (34).

**Species Differences in Proinflammatory Cytokines**

Elastase is a strong inflammatory stimulus that induces the release of proinflammatory mediators, such as IL-6, IL-1β, and TNF-α, among others (9). It is not known whether species differences exist in the pattern of release of these cytokines that could influence emphysema development. In experimental animals, some cytokines stimulate the synthesis of several components of the acute phase response, among them α1-AT (2, 17). It is tempting to speculate that a particular pattern of cytokine release could increase serum α1-AT levels, thus raising the capillary-alveolar difference and favoring transendothelial passage of the antiprotease in lieu of the increase in alveolar-capillary permeability induced by elastase. Therefore, in this study, we investigated whether these two rodent species exhibit a differential pattern of lung cytokine release in early lung response to elastase and found significant differences between these species. In rats, the three cytokines studied showed a marked increase after elastase treatment, whereas very mild changes were seen for IL-1β and TNF-α without changes in IL-6 levels in hamsters. It is of interest that the hamster, the species more susceptible to the effects of elastase, is the one with less proinflammatory mediator release. This result, although not completely understood, is in agreement with that observed in different strains of mice differing in α1-AT levels when they are exposed to cigarette smoke (55).
Species differences in the pattern of cytokine release may have important effects locally in the lungs (50) and may signal systemic responses involving extrapulmonary synthesis of α1-AT differentially. Since it is known that the α1-AT gene promoter responds mainly to IL-6 (2, 16, 33) and in smaller proportion to IL-1β and TNF-α (8), the lack of an increment in lung IL-6 levels after elastase treatment in hamsters could result in an impaired synthesis of new α1-AT, thus worsening the already nonfunctional low-antiprotease defense of this animal.

Strengths and Limitations of Emphyema Models for Susceptibility Studies

Chronic obstructive pulmonary disease (COPD) is a disease with a poor understanding of susceptibility factors, and animal models play an important role in attempts to elucidate them. Notwithstanding that the model of elastase-induced lung injury is less frequently used than other models for studies of the pathogenesis of COPD, the identification of naturally occurring species with differences in susceptibility (6) prompted us to study protective mechanisms in the resistant species (rat) not present in the susceptible one (hamster). Our aim was to improve our understanding of differences in susceptibility not only to the effects of elastase but also to those of other agents that damage the lungs by mechanisms involving protease release in humans. Thus, with new approaches to an “old” model, we identified variations in the initial lung response, which although needing testing in other models, may become useful biomarkers that could predict chronic outcomes. IT elastase instillation is a good model of acute lung injury that accounts for the differences in susceptibility to elastase. We identified variations in the initial lung response, both to its low α1-AT and even more important, on the early response of the antiprotease upon cytokine release induced by the elastolytic challenge.

On the other hand, genetically manipulated, cigarette-smoke exposed mice, the most frequently used COPD model, is far from providing the cure for COPD. Perhaps the most important contribution of this model has been the understanding of some underlying susceptibility factors for the development of tobacco smoke-induced emphyema. Interestingly, α1-AT baseline levels also accounts for differences in susceptibility in this model (10).

Thus, no matter what model is used, the role of the early α1-AT response needs to be further studied.

In conclusion, our comprehensive assessment of several components of the acute lung response to elastase shows significant differences between Sprague-Dawley rats and Syrian Golden hamsters. Both the pattern of cytokine release and the behavior of α1-AT, likely modulated by changes in antioxidants, were the most significant components of the initial lung response that differed among species and that could account for the differences in susceptibility to elastase.

Perspectives and Significance

Susceptibility of hamsters to elastase-induced emphysematous development relates both to its low α1-AT concentration and to a cytokine release pattern that may not contribute to the de novo synthesis of adequate levels of α1-AT. Whether or not hamsters develop more tissue damage than rats, due to α1-AT deficiency, when exposed to injurious agents other than exogenous elastase is unknown. The possibility exists, since elastase release from inflammatory cells is involved in the lung response to many different stimuli. Further comparative studies exposing rats and hamsters, as well as different strains of mice differing in α1-AT concentration, to other injurious agents (including cigarette smoke), are required to evaluate the role of α1-AT in acute lung injury. One can also speculate that differences in the response of α1-AT may account for the wide heterogeneity of phenotypes of acute lung injury and chronic outcomes in humans. Interventional studies are needed to evaluate the role of therapies targeting cytokine effects and/or early treatment with exogenous α1-AT in acute lung injury to prevent or reduce the development of chronic lung damage.

REFERENCES

ACUTE LUNG RESPONSE AND EMPHYSEMA DEVELOPMENT AFTER ELASTASE


