Biochemical detection of type I cell damage after nitrogen dioxide-induced lung injury in rats

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McElroy, Mary C., Jean-François Pittet, Lennell Allen, Jeanine P. Wiener-Kronish, and Leland G. Dobbs. Biochemical detection of type I cell damage after nitrogen dioxide-induced lung injury in rats. Am. J. Physiol. 273 (Lung Cell. Mol. Physiol. 17): L1228–L1234, 1997.—We have previously shown that injury to lung epithelial type I cells can be detected biochemically by measuring the airway fluid content of a type I cell-specific protein, rTI40, in a model of severe acute lung injury [M. C. McElroy, J.-F. Pittet, S. Hashimoto, L. Allen, J. P. Wiener-Kronish, and L. G. Dobbs. Am. J. Physiol. 268 (Lung Cell. Mol. Physiol. 12): L181–L186, 1995]. The first objective of the present study was to evaluate the utility of rTI40 in the assessment of alveolar injury in a model of milder acute lung injury. Rats were exposed to 18 parts/million NO2 for 12 h; control rats received filtered air for 12 h. In NO2-exposed rats, the total amount of rTI40 in bronchoalveolar fluid was elevated 2-fold compared with control values (P < 0.001); protein concentration was 8.5-fold of control values (P < 0.001). The increase in rTI40 was associated with morphological evidence of injury to type I cells limited to the proximal alveolar regions of the lung. The second objective was to correlate the severity of alveolar type I cell injury with functional measurements of lung epithelial barrier integrity. NO2 inhalation stimulated distal air space fluid clearance despite a significant increase in lung endothelial and epithelial permeability to protein. These data demonstrate that rTI40 is a useful biochemical marker for mild focal injury and that exposure to NO2 alters lung barrier function. Taken together with our earlier studies, these results suggest that the quantity of recoverable rTI40 can be used as an index of the severity of damage to the alveolar epithelium.

rTI40; rat type I cells; lung barrier function

ALVEOLAR EPITHELIAL TYPE I cells are large squamous cells that cover > 95% of the surface area of the lungs. Although morphological studies demonstrate that type I cells are injured by a diverse array of agents (20), until recently there were no methods to detect and quantify injury to these cells. rTI40 is a novel protein that is localized exclusively to rat alveolar epithelial type I cells within the lung (7, 18, 23). rTI40 was identified by a monoclonal antibody raised against partially purified type I cells (7). This monoclonal antibody was subsequently used to clone the cDNA from an expression library (23) and to purify the protein from rat lung (9). The cDNA for rTI40 is identical to OTS-8 (9, 23), a cDNA initially cloned from mouse osteoblast cell lines (16). The function of rTI40 in type I cells is unknown.

In previous studies, we demonstrated that the content of rTI40 in alveolar fluid is associated with the extent of damage to alveolar type I cells (14). In a model of acute lung injury caused by Pseudomonas aerugi-

nosa, the rTI40 content of lung fluid was increased by ~80-fold above control values (14). This increase in rTI40 was associated with severe morphological injury to alveolar type I cells as judged by electron microscopy (14). However, in a rat model of hyperoxic lung injury (>95% O2 for 60 h), bronchoalveolar lavage (BAL) fluid levels of rTI40 were elevated by only twofold (15). These studies suggested that BAL fluid levels of rTI40 are a sensitive marker for alveolar epithelial damage.

NO2 inhalation is known to cause injury to alveolar epithelial type I cells in a focal region of the lung (1, 8, 21, 22). Cabral-Anderson et al. (1) and Stephens et al. (22) demonstrated that exposure to sublethal NO2 concentrations [i.e., 17 parts/million (ppm) NO2] injures alveolar type I cells in alveoli proximal to the terminal bronchioles; type I cells in more distal locations are morphologically unchanged (22). The first objective of this study was to confirm that rTI40 is a sensitive marker of alveolar epithelial injury by determining levels in BAL fluid from NO2-exposed rats. The second objective of this study was to compare BAL fluid levels of rTI40 with physiological measurements of lung injury (e.g., protein fluxes across the air spaces, rate of fluid clearance) to determine how lung function is affected after NO2 exposure.

METHODS

Animals and Exposure Chamber

Sprague-Dawley adult male rats (300–350 g) were housed in specially designed environmental Plexiglas chambers. During the study period, the chambers were housed in a room kept at constant temperature (20°C). The airflow through the chamber was filtered (Balston air filter, Lexington, MA) and was sufficient to allow 25 chamber volumes change per hour. The dimensions of the chambers were 60 × 20 × 20 cm. The NO2 flow was controlled by a stainless steel variable area flowmeter (Fisher and Proctor, Warminster, MA). The concentration of NO2 in the chamber was monitored continuously with an NO2 analyzer (Thermo Electron, Waltham, MA; kindly loaned by Drs. Fading and Adams, University of California, Davis). The analyzer was periodically checked with NO2 span gas/calibration gas (Scott-Marrin). Control rats were housed under identical conditions but were exposed to filtered air at the same flow rates as rats exposed to NO2. All studies were approved by the University of California, San Francisco Animal Care Committee. The mean ± SD of the NO2 concentration for the course of the experiments was 18.0 ± 0.5 ppm.

BAL Studies

Time course. Rats were exposed to either filtered air or 18 ppm NO2 for 1, 3, 6, 9, 12, or 24 h; n = 3 for all times except 12 h where n = 6. At the end of the exposure period, rats were
exsanguinated, and their lungs were lavaged through a tracheotomy with a 10.3 mM N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid-2.6 mM phosphate buffer (pH 7.4) containing (in mM) 133 NaCl, 5.2 KCl, 1.9 CaCl2, and 1.3 MgSO4 (6). Lavage samples were centrifuged at 80 g for 10 min to remove cells, and the supernatant liquid was frozen at −80°C for total protein and rTI40 determinations. No rats died during exposure to either filtered air or 18 ppm NO2.

rTI40. BAL fluid samples were diluted 1:10 with 20 mM bicarbonate buffer (pH 9.2), and then 100 µl were applied to Immobilon-P membranes (Millipore, Bedford, MA) using a dot-blot apparatus. Membranes were blocked for 2 h at room temperature with 0.4% nonfat milk in tris(hydroxymethyl)- aminomethane (Tris)-buffered saline (TBS; 20 mM Tris base and 154 mM NaCl; pH 8.2), incubated with anti-rTI40 hybridoma supernatant for 30 min (6, 7), washed with TBS containing 0.05% Tween 20 (TBS-T), and incubated with sheep anti-mouse immunoglobulin conjugated to horseradish peroxidase (1:250,000 dilution; Amersham). Blots were washed 10 times with TBS-T before being developed with enhanced chemiluminescence reagents (Amersham) and were exposed to film (Hyperfilm-MP; Amersham) for 2.5 min. A control blot without primary antibody was performed in each assay. Semicontinuous data from the dot blots was obtained by densitometry (GS 300 transmittance/reflectance scanning densitometer; Hoeffer Scientific Instruments, San Francisco, CA). To ensure that the readings obtained were in a linear region, a standard curve with normal rat lung homogenate was also run with each assay (14).

Western blots. Concentrated BAL fluid samples were diluted in sample buffer containing 10% mercaptoethanol. Samples were electrophoresed on a 10% sodium dodecyl sulfate-polyacrylamide gel with a 4% acrylamide stacking gel (11). Proteins were then transferred to nitrocellulose paper by electrophoresis, and rTI40 was detected using the same protocol as for dot blots. Control Western blots without primary antibody were routinely performed as previously described (14).

Protein assay. Protein concentrations were determined using the bicinchoninic acid method (BCA protein assay kit).

Morphological Study

Rats were exposed to filtered air (n = 3) or 18 ppm NO2 (n = 3) for 12 h. The rats were killed by exsanguination, and the lungs were inflated to total lung capacity with a solution of 1% paraformaldehyde and 2% glutaraldehyde in 0.1 M phosphate buffer (pH 7.4). The proximal alveolar regions were isolated (21), trimmed, and postfixed in 1.5% osmium tetroxide in veronal acetate buffer (pH 7.4). Tissue blocks were then processed for ultrastructural analysis at the electron microscope level (14).

Lung Barrier Function Studies

Surgical preparation and ventilation. At the end of the exposure period, rats were anesthetized with 60 mg/kg ip pentobarbital sodium, and anesthesia was maintained with one-half of this dose of pentobarbital sodium every 2 h. A vascular catheter was inserted in the carotid artery in the neck to monitor systemic arterial pressure and to obtain blood samples. Pancuronium bromide (0.3 mg·kg−1·h−1) was given intravenously for neuromuscular blockade. An endotracheal tube (PE-220) was inserted through a tracheotomy. The animals were ventilated with a constant-volume pump (Harvard Apparatus, Millis, MA) with an inspired O2 fraction (FiO2) of 1.0, peak airway pressures of 8–12 cmH2O, and a positive end-expiratory pressure of 3 cmH2O. The respiratory rate was adjusted to maintain the arterial Pco2 between 35 and 45 mmHg. A heated blanket (37°C) was used to maintain the temperature of the rats during the experimental period.

Preparation of instillate. A 5% rat albumin solution was prepared in lactated Ringer solution and was adjusted with NaCl to be isosmolar with plasma, as we have previously described (12, 14, 17). The solution was filtered through a 0.2-µm filter (Nalge, Rochester, NY), and 0.5 mg of anhydrous Evans blue (to confirm location of the instillate at the end of the study) and 1 µCi of 125I-labeled human serum albumin (Merck-Frosst) were added to the bovine albumin solution. A sample of the instilled solution was saved for total protein measurement, measurement of radioactivity, and wet-to-dry weight ratio measurements so that the dry weight of the protein solution could be subtracted from the final lung water calculation.

General protocol. For all lung barrier function studies, the following general protocol was used. After surgery, a 1-h baseline period of stable heart rate and blood pressure preceded intravenous administration of 1 µCi of 131I-labeled human albumin (vascular tracer). This vascular tracer was used to calculate the flux of plasma protein into the lung interstitium (see below). Then the rat was placed in the left lateral decubitus position to facilitate deposition of the air space protein tracer into the left lung. This instillate (3 ml/kg of 5% rat albumin solution with 125I-albumin) was delivered into the left lung over 30 min using a 1-ml syringe and a polypropylene tube [0.5 mm inner diameter (ID)]. After 4 h, the abdomen was opened, and the animal was exsanguinated. Urine was sampled for radioactivity. The lungs were removed through a sternotomy, and liquid from the distal air spaces (0.1–0.2 ml) was obtained by passing a propylene tube (0.5 mm ID) into a wedged position in the left lower lobe. After centrifugation, the total protein concentration and the radioactivity of the liquid sample were measured. Right and left lungs were homogenized separately for total protein measurement, wet-to-dry weight ratio measurement, and radioactivity counts.

Specific protocols: Control rats exposed to filtered air and rats exposed to 18 ppm NO2. Rats were exposed to filtered air (n = 6) or 18 ppm NO2 (n = 6) for 12 h. Then the rats were anesthetized and were mechanically ventilated at an FiO2 of 1.0 for the remainder of the experiment. After a 1-h base line period, 3 ml/kg of a 5% rat albumin solution were instilled into the left lower lobe over 30 min. The rats were studied for the subsequent 4 h. After this time interval, the rats were exsanguinated and were prepared as described above.

Measurements. Hemodynamics, pulmonary gas exchange, and protein concentration. During the acute experimental studies, systemic arterial and airway pressures were continuously measured, and arterial blood gases were measured at 30-min intervals over the 4-h study period.

Lung endothelial and epithelial barrier protein permeability. Two different methods were used to measure the flux of albumin across the lung endothelial and epithelial barriers as has been done previously (12, 14, 17). The first method requires measurement of residual 125I-albumin (the air space protein tracer) in the lung as well as of accumulation of 125I-albumin in the plasma. The second method requires the measurement of 131I-albumin (the vascular protein tracer) in the air space and in the extravascular space of the lung.

The total quantity of 125I-albumin (the air space protein tracer) instilled into the lung was determined by measuring duplicate samples of the instilled solution for radioactivity (disintegrations·min−1·g−1) and then multiplying this data by the total volume instilled into the lung. To calculate the residual 125I-albumin in the lung at the end of the studies, the
average radioactivity of two 0.5-g samples obtained from the lung homogenate (see below) was multiplied by the total volume of lung homogenate. The $^{125}$I-albumin in the lung homogenate data was added to the recovered counts in the final aspirate from the distal air space fluid (DAF) to calculate the amount of instilled $^{125}$I-albumin that remained in the lung at the end of the studies. The $^{125}$I-albumin in the circulating plasma was measured from a sample of plasma obtained at the end of the experiment. The plasma fraction was estimated by multiplying the disintegrations per minute per gram by the plasma volume (body wt × 0.07 (1− hematocrit)).

The second method requires measurement of the vascular protein tracer $^{131}$I-albumin in the final alveolar sample and in the extravascular compartments of the lung. We performed this according to previously described methods (12, 17). Briefly, the amount of radioactivity in the circulating plasma measured during the experimental period was averaged, and the radioactivity in the final air space sample was expressed as a ratio of the plasma radioactivity. The extravascular $^{131}$I-albumin accumulation in the lungs was calculated by taking the total lung $^{131}$I-albumin measured in lung homogenate and subtracting the vascular space $^{131}$I-albumin. The $^{131}$I-albumin in the vascular space was calculated by multiplying the radioactivity in the final plasma sample by the calculated plasma volume in the lungs based on gravimetric methods. The extravascular accumulation of $^{131}$I-albumin in the lung is expressed as plasma equivalents or milliliters of plasma that would account for the radioactivity in the lung.

**FLUID CLEARANCE ACROSS THE AIRWAY AND ALVEOLAR EPITHELIA.** Changes in the concentration of nonlabeled bovine albumin and instilled $^{125}$I-labeled human albumin were used to measure liquid clearance from the air spaces (12, 17). Air space fluid clearance was estimated by the measurement of the increase in final alveolar fluid protein concentration compared with initial instilled alveolar protein concentration. DAF clearance was calculated as follows:

$$DAF = \frac{(V_i \times F_{wi} - V_f \times F_{wi})}{(V_i \times F_{wi})} \times 100$$

where $V_i$ is the volume of initial instilled alveolar fluid, $V_f$ is the volume of final alveolar fluid, $F_{wi}$ is the water fraction of the initial instilled alveolar fluid, and $F_{wi}$ is the water fraction of the final alveolar fluid. The water fraction is the volume of water per volume of solution measured by the gravimetric method. The volume of cleared fluid ($V_c$; in ml) was estimated as follows:

$$V_c = \frac{(V_i \times TP_i \times F_i)}{TP_f}$$

where $TP_i$ is the total protein concentration of initial instilled alveolar fluid, $TP_f$ is the total protein concentration of final alveolar fluid, and $F_f$ is the fraction of alveolar tracer protein that remained in the lung at the end of the experiment.

**LUNG WET-TO-DRY WEIGHT RATIO.** We estimated the extravascular water in the lung after 4 h by previously described methods (12). Each lung was homogenized separately, and the extravascular lung water was determined by calculating the wet-to-dry weight ratio. Changes in the wet-to-dry weight ratio of the contralateral (noninstilled) lung was used as an index of lung endothelial injury because the noninstilled lung did not have the confounding presence of the instilled protein solution in its air spaces.

**MEASUREMENT OF RADIOACTIVITY NOT BOUND TO ALBUMIN (FREE $^{125}$I).** To determine the $^{125}$I binding to albumin, 20% trichloroacetic acid (TCA) was added to all tubes that were then centrifuged to obtain the supernatant for measurement of free $^{125}$I radioactivity. The results are expressed as a percentage of the unbound $^{125}$I radioactivity to the total amount of $^{125}$I-albumin radioactivity instilled. Prior studies in our laboratories have shown that these fluid samples contain <0.6% of radioactivity in the supernatant, i.e., not precipitable by TCA and therefore presumably not bound to protein (12).

**Statistics**

The data are expressed as means ± SD. One-way analysis of variance and Fisher’s exact test were used to compare the different rat groups. $P < 0.05$ was considered significantly different (24).

**RESULTS**

**BAL rTI$_{40}$ and Protein Concentration**

A time-course study demonstrated that the total amount of protein recovered in BAL fluid increased between 1 and 12 h of exposure to 18 ppm NO$_2$. BAL proteins were still elevated above control values after 24 h of continuous exposure to 18 ppm NO$_2$ (Fig. 1). The largest increase in amount of protein recovered in BAL fluid, with respect to control values, was observed at 12 h (i.e., an 8.5-fold increase) (Fig. 1, Table 1). In contrast, the total amount of rTI$_{40}$ recovered in BAL fluid from NO$_2$-exposed rats was only elevated above control values after 12 h of continuous exposure to NO$_2$ (Table 1); the amount of rTI$_{40}$ in BAL fluid was

**Table 1. Effect of NO$_2$ exposure on recovery of rTI$_{40}$ and protein in BAL fluid**

<table>
<thead>
<tr>
<th>Time (h)</th>
<th>BAL rTI$_{40}$ (mg)</th>
<th>BAL Protein (mg)</th>
<th>Volume of BAL Fluid Recovered (ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0.03 ± 0.01</td>
<td>0.06 ± 0.02</td>
<td>0.3 ± 0.1</td>
</tr>
<tr>
<td>NO$_2$</td>
<td>0.1 ± 0.02</td>
<td>0.08 ± 0.03</td>
<td>0.4 ± 0.2</td>
</tr>
</tbody>
</table>

Values are means ± SD; n is no. of rats. Rats were exposed to either filtered air (controls) or 18 parts/million (ppm) NO$_2$ for 12 h. rtTI$_{40}$ and total protein concentrations were subsequently measured in bronchoalveolar lavage (BAL) fluid. dlu, Relative desintegations values. *P < 0.001 vs. control.
two times that found in BAL fluid from rats exposed to filtered air (Table 1; $P < 0.001$). No differences were found in the BAL fluid content of rTI40 between control and NO2-exposed rats at other time points studied (i.e., 1, 3, 6, 9, and 24 h; data not shown). Additionally, there was no significant difference in the amount of BAL fluid recovered between NO2-exposed and control rats (Table 1). The molecular mass of rTI40 in BAL fluid from both control and exposed rats was 40–42 kDa by Western blot analysis (Fig. 2).

Additional studies were performed on rats exposed to 18 ppm NO2 for 12 h to characterize further alveolar-capillary barrier damage.

**Morphology**

Exposure of rats to NO2 for 12 h damaged alveolar epithelial type I cells localized in the proximal alveolar region of the lung (Fig. 3B), as reported in previous studies (1, 22). The cytoplasm of type I cells was swollen, and in some places the plasma membrane was ruptured and fragmented. Type I cells in adjacent alveoli often appeared normal (Fig. 3B).

### Table 2. Effect of exposure of rats to NO2 for 12 h on changes in distal air space protein concentration

<table>
<thead>
<tr>
<th></th>
<th>Alveolar Total Protein, g/100 ml</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Initial</td>
</tr>
<tr>
<td>Filtered air (controls)</td>
<td>6</td>
</tr>
<tr>
<td>Filtered air + NO2 (18 ppm)</td>
<td>6</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of rats. *P < 0.05 vs. initial values; †P < 0.05 vs. controls.

### Effect of NO2 on Lung Barrier Function

Rats exposed to NO2 (18 ppm for 12 h) and then mechanically ventilated for 4 h at an FiO2 of 1.0 showed an upregulation of the fluid transport across the lung epithelium. The protein concentration of the final sample of the air space instillate was higher in rats exposed to NO2 than in air-exposed controls (Table 2); an increase in air space protein concentration occurs if there is upregulation of alveolar liquid clearance. There was a significant increase in alveolar liquid clearance in rats exposed to 18 ppm NO2 compared with air-exposed controls (62 ± 16 vs. 39 ± 3%; $P < 0.05$). Similar results were obtained when $^{125}$I-albumin was used instead of nonlabeled albumin to calculate the alveolar liquid clearance (61 ± 10 vs. 40 ± 7%; $P < 0.05$).

This upregulation of the alveolar epithelial fluid clearance occurred despite a significant increase in the bidirectional protein permeability across the alveolar epithelium (Table 3). The amount of airway tracer ($^{129}$I-albumin) recovered in blood was 8.6-fold greater in NO2-exposed rats compared with control values (Table 3). Similarly, the amount of vascular tracer ($^{131}$I-albumin) found in air space fluid was elevated by 4.7-fold in NO2-exposed rats compared with control rats (Table 3).

NO2 exposure also increased the accumulation of fluid and protein in the extravascular spaces of the lungs (Table 4). There was a significant increase in the wet-to-dry weight ratio of the noninstilled lung in rats exposed to NO2 (18 ppm for 12 h) compared with...
Table 3. Effect of exposure of rats to NO2 for 12 h on protein flux across the epithelium

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Alveolar Protein Tracer, %</th>
<th>Vascular Protein Tracer Ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Filtered air (controls)</td>
<td>6.96 ± 0.37</td>
<td>0.07 ± 0.02</td>
</tr>
<tr>
<td>Filtered air + NO2 (18 ppm)</td>
<td>85.8 ± 1.7*</td>
<td>6.0 ± 2.4* 0.33 ± 0.18*</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no of rats. *P < 0.05 vs. controls.

DISCUSSION

rTI40 is the first protein found to localize to type I cells in the lung (7, 18). Because there were no direct methods to measure alveolar type I cell lung injury, we performed a number of studies to determine whether the levels of rTI40 in BAL fluid could be used as a quantitative measure of injury. In previous studies, we demonstrated that BAL fluid levels of rTI40 are elevated >80-fold above control values in a rat model of severe epithelial damage caused by P. aeruginosa (14). In contrast, in a rat model of hyperoxic injury, which was shown previously by Harris et al. (10) to cause subtle structural alterations to type I cells, we found only a twofold increase in BAL fluid levels of rTI40 (15). Taken together, these data demonstrate that BAL fluid content of rTI40 measures injury to the alveolar epithelium caused by different agents and suggests that rTI40 is a sensitive marker of type I cell damage.

The first objective of our current study was to confirm the sensitivity of rTI40 as a quantitative marker of damage by measuring BAL fluid levels in a model of NO2-induced injury. We selected NO2 inhalation as a model of injury because Evans et al. (8) had previously shown that sublethal concentrations of NO2 (~17 ppm) caused sloughing of type I cells from their basement membranes in alveoli proximal to the terminal bronchioles. More distal type I cells remained intact (1, 8, 22). They also showed that focal injury to type I cells occurred within a 24-h period of continuous exposure to NO2 (depending on the age of the rats) and that basal basement membranes in the alveolar region were replaced by cuboidal epithelial cells after 24–48 h of continuous exposure (1, 22). In a similar NO2 model, we detected a twofold increase in BAL fluid levels of rTI40 after 12 h of exposure to 18 ppm NO2. We also confirmed the focal nature of type I cell injury by morphological examination of lung at the electron microscope level (Fig. 3). We did not detect changes in BAL fluid levels of rTI40 at earlier or later time points (data not shown). At the present time, we do not know whether this is because our assay was insufficiently sensitive to detect smaller changes or because injury occurred at time points we did not sample. Nonetheless, our current study demonstrates that relatively small amounts of alveolar epithelial injury (limited in both extent and in anatomic localization) can be detected by the measurement of rTI40. These data extend our previous studies by confirming that rTI40 is a sensitive marker of type I cell injury.

The second objective of our current study was to determine the effect of 18 ppm NO2 on changes in the function of the alveolar-capillary barrier. Our data demonstrate that 18 ppm NO2 increased the protein permeability of the alveolar-capillary barrier to a maximum value at 12 h of exposure (Table 1). The 8.4-fold increase in total protein recovered in BAL fluid after 12 h of exposure to NO2 fluid is similar to results reported by others (Table 1; see Ref. 4). In addition, we demonstrated that this general increase in alveolar-capillary protein permeability was due to an increase in the bidirectional flux of protein tracers between the air and vascular spaces of the lung (Table 2). In our previous study with a rat model of hyperoxic lung injury (>95% O2, 60 h; see Ref. 15), we found a similar increase in BAL protein concentration (7-fold); however, this increase was associated with a unidirectional flux of protein (131I-albumin) from the vascular space to the air spaces (15). Hyperoxic exposure did not affect the flux of protein (125I-albumin) from the air spaces into vascular compartment (15). These data demonstrate that oxidant gases may have different effects on flux of proteins between the air space and the vascular compartments of the lung.

In contrast to the large differences in the magnitude of protein flux across the epithelium in the NO2 and hyperoxic injury models (i.e., 8.5-fold and no change), the amount of rTI40 in BAL fluid was similar (i.e., a 2-fold increase after NO2 exposure [Table 1] and a 1.7-fold increase after hyperoxia [15]). It would seem unlikely, however, that type I cell injury after hyperoxia and NO2 exposure are the same. From the morphologic evidence available, we suspect that we are detecting rTI40 as a consequence of nonlethal plasma membrane blebbing after hyperoxic exposure (10); however, after NO2 exposure, we are detecting rTI40 as a result of lethal injury to a few type I cells (Fig. 3; see Refs. 1 and 22). At the present time, we do not know whether the difference in protein flux out of the air spaces (125I-albumin) in the two models is a consequence of the location of type I cell injury (i.e., alveoli near the terminal bronchioles vs. widespread mild damage), or, alternatively, injury to other components of the lung barrier...
Upregulation of distal air space liquid clearance above control values may be an adaptive response by the alveolar epithelium to clear fluid during the early phase of acute lung injury (reviewed in Ref. 13). The rate of distal airway fluid clearance increases 1.8- to 2-fold in BAL fluid content of rTI40 was associated with the complete absence of alveolar-liquid clearance (unpublished observations). In our current NO2 study and previous studies on both hyperoxic injury (15) and septic shock (17), no changes or small increases (e.g., ~2-fold) in BAL fluid rTI40 content were associated with an increase in fluid movement out of the air spaces (Table 2; see Refs. 15 and 17). Although these observations are preliminary, they demonstrate that only large increases (i.e., 80-fold) in BAL fluid levels of rTI40 are associated with impaired ability of the distal airway epithelium to clear fluid in the models we have so far studied.

In summary, we have shown that rTI40, an integral membrane protein localized to the apical plasma membrane of alveolar type I cells, can be used as a sensitive biochemical marker for the focal alveolar epithelial injury. Our combined studies also demonstrate that BAL fluid levels of rTI40 are not necessarily associated with the extent of protein fluid flux from the air spaces but may be associated with the ability of the alveolar epithelium to clear excess fluid. These data support the belief that different causative agents of acute lung injury may damage individual components of the alveolar-capillary barrier to differing extents.

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