Viral respiratory infection increases susceptibility of young rats to hypoxia-induced pulmonary edema

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Carpenter, Todd C., John T. Reeves, and Anthony G. Durmowicz. Viral respiratory infection increases susceptibility of young rats to hypoxia-induced pulmonary edema. J. Appl. Physiol. 84(3): 1048–1054, 1998.—Recent clinical observations of a high incidence of preexisting respiratory infections in pediatric cases of high-altitude pulmonary edema prompted us to ask whether such infections would increase the susceptibility of hypoxia-induced pulmonary edema in young rats. We infected weanling rats with Sendai virus, thus causing a mild respiratory infection. Within 7 days of infection, Sendai virus was essentially undetectable by using viral culture and immunohistochemical techniques. Animals at day 7 of Sendai virus infection were then exposed to normobaric hypoxia (fraction of inspired $O_2 = 0.1$) for 24 h and examined for increases in gravimetric lung water and in vascular permeability, as well as for histological evidence of increased lung water. Bronchoalveolar lavage was performed on a separate series of animals. Compared with control groups, infected hypoxic animals showed significant increases in perivascular cuffing, gravimetric lung water, and lung protein leak. In addition, infected hypoxic animals had increases in lavage fluid cell counts and protein content compared with controls. We conclude that young rats, exposed to moderate hypoxia while recovering from a mild viral respiratory infection, may demonstrate evidence of early pulmonary edema formation, a finding of potential relevance to human high-altitude pulmonary edema.

High-altitude pulmonary edema; Sendai virus; lung water

HIGH-ALTITUDE PULMONARY EDEMA (HAPE) is a relatively rare condition, characterized by the development of noncardiogenic pulmonary edema and pulmonary hypertension on ascent to high altitude in susceptible individuals. Despite decades of research into the effects of hypoxia on the pulmonary circulation, the mechanisms underlying HAPE are still unclear. Animal studies have clearly demonstrated increases in pulmonary vascular permeability associated with exposure to hypoxia, although with considerable interspecies variation (2, 3, 15, 20, 21, 23). Human studies of these issues, however, have generated conflicting results and have not been able to identify definitively the physiological bases for the development of HAPE (8–11, 17–19, 22). Furthermore, although HAPE may occur repeatedly in certain susceptible individuals, many cases occur in individuals who have previously been at similar altitudes under similar conditions without having developed pulmonary edema. This finding raises the question of an acquired as well as a genetic susceptibility to this illness.

Respiratory infections may be a potential source of acquired susceptibility to pulmonary edema associated with altitude exposure. Although most authors have been careful to discount the possibility of an etiological role for infectious agents in HAPE, a recent retrospective study of HAPE patients in Colorado found that 79% of pediatric cases reported symptoms of an upper respiratory tract infection in the 1–2 wk before ascent and illness (6). In fact, earlier authors had also noted this association in children (7), but little experimental work has examined the finding.

These findings led us to hypothesize that preexisting viral respiratory tract infections may increase the susceptibility of young animals to hypoxia-induced pulmonary edema. We studied this hypothesis by infecting weanling rats with a murine parainfluenza virus (Sendai virus) and then exposing them to normobaric hypoxia for 24 h. Animals were then examined for changes in lung water and in pulmonary vascular permeability. In addition, bronchoalveolar lavage was performed and the lavage fluid was examined for changes in cell populations and protein content.

METHODS

Animals. Experimental animals were male, pathogen-free, weanling Sprague-Dawley rats purchased from a commercial vendor (Harlan Sprague Dawley, Indianapolis, IN). Animals arrived in Denver at age 22–24 days; they weighed 50–60 g each. Animals in the infected experimental groups were inoculated with virus 24–48 h after arrival and then housed in a separate facility from the noninfected animals. All animals were allowed free access to food and water and were subjected to a similar day-night light cycle. Animals were housed at Denver altitude (1,600 m) at all times.

Inoculation with Sendai virus. An isolate of Sendai virus was obtained from a commercial source (American Type Culture Collection, Rockville, MD). The lyophilized virus was resuspended and inoculated into tubes of rhesus monkey kidney (RMK) cell culture and incubated at 37°C in viral culture medium. After 72 h, the tubes were scraped, passed through one freeze-thaw cycle to cause cell lysis, and this crude extract was then centrifuged to remove cellular debris. The supernatant was diluted to yield a virus concentration of $\sim 1 \times 10^5$ TCID$_{50}$/ml, aliquoted, and stored frozen at $-70^\circ$C until use, where TCID$_{50}$ is 50% tissue culture infective dose.

To be inoculated with virus, animals were lightly sedated with methoxyflurane, and 50 µl of the virus stock solution ($5 \times 10^5$ TCID$_{50}$) were then instilled intranasally. Animals for uninfected experimental groups were treated in a similar manner and inoculated with a sham solution that was prepared in the same manner as the virus stock solution but from RMK cell cultures not inoculated with Sendai virus.

Characterization of Sendai virus infection. To characterize the course and severity of a Sendai virus infection in weanling rats in the experiment, a preliminary series of six animals was infected with Sendai virus, as described above, and two animals each were killed at postinoculation days 3, 5, and 7. At the time these animals were killed, nasal washings were performed with 0.5 ml sterile saline in each nare. The nasal...
washes and sections of trachea and the left lung were sent for qualitative viral culture (inoculation into RMK cell culture with virus growth detected by hemagglutination assay). When the animals were killed, serum was obtained via cardiac puncture and saved for enzyme-linked immunosorbent assay (ELISA) testing for antibody to Sendai virus. The right lung was fixed as described below and used for histological examination. Sections of lung tissue and trachea from each animal were stained to determine the presence of Sendai virus antigen by using standard immunohistochemical techniques. The primary antibody was a monoclonal mouse antibody against the nucleocapsid protein of Sendai virus (generously provided by Dr. Allen Portner at St. Jude's Children's Research Center, Nashville, TN). The antibody was detected by using a commercial kit (Vectastain; Vector Laboratories, Burlingame, CA) containing a biotinylated horse anti-mouse secondary antibody and an avidin-biotin complex-linked alkaline phosphatase enzyme. The enzyme substrate used was the Vector-Red substrate kit (Vector Labs).

Exposure to hypoxia. Experimental animals were divided into four groups: control (normoxic, uninfected), hypoxia only, virus only, and virus + hypoxia. Animals in the hypoxic test groups were weighed and then exposed to normobaric hypoxia (fraction of inspired O_2 = 0.21) for 24 h. Exposure to hypoxia was timed so that all animals were killed on day 7 postinoculation (virus or sham) to compare animals at the same age and time point in the course of their Sendai infection. One hour before they were killed, a small number of animals in each experimental group were injected via a tail vein with 1 ml/kg of Monastral blue B suspension (Sigma Chemical, St. Louis, MO), a compound used to label sites of vascular leak (1). Animals were killed by deep anesthesia (pentobarbital sodium, 80 mg/kg ip). A midline thoracotomy was then performed, and the animal was exsanguinated via cardiac puncture. Serum was saved from these samples of blood for later serological testing. The lungs and mainstem bronchi to the carina were excised and weighed. The left lung was then tied off, removed, weighed separately, and dried in an oven at 55°C until a stable weight was obtained. Total lung weight-to-prehypoxia body weight ratios, expressed as milligrams of lung tissue per gram of body weight, and wet-to-dry lung ratios were then calculated. Although Monastral blue dye is not known to increase vascular permeability in and of itself in rats, animals receiving that compound were excluded from the gravimetric lung water analysis.

The right lungs of animals that were killed as described above were fixed in 10% buffered Formalin by tracheal perfusion at 30 cmH_2O pressure for 24 h, then embedded in paraffin and sectioned for histological studies. Measurements of perivascular cuff areas were made on hematoxylin and eosin-stained sections of lung tissue by using a computer image-analysis system [Nikon Optiphot 2 microscope, Apple Macintosh Power Mac 7200 computer, and NIH Image (a public-domain image-analysis program developed at the US National Institutes of Health and available on the Internet at http://rsb.info.nih.gov/NIH-Image)]. Sections from three animals in each group were analyzed; from each section, every vessel not contained in a bronchovascular sheath was measured. To minimize the confounding effects of distorted and collapsed vessels, the circularity of each vessel was also measured, and those vessels with a circularity of <0.6 were excluded. Results were expressed as the ratio of the perivascular cuff area to the area of the vessel.

Measurement of transvascular protein escape. To confirm and extend our initial gravimetric and histological findings, we used a previously published method (19) to study additional groups of control and experimental rats (n = 6 per group) for transvascular protein escape. This technique measures the accumulation of intravascularly administered radio-labeled albumin in the extravascular space of the lung over a 1-h period. The extravascular accumulation of protein is dependent not only on vascular permeability but also on the driving concentration of the tracer protein and the surface area available for exchange. The calculations are thus corrected for these two variables.

Animals were handled as described above until 1 h before they were killed. Blood was collected in a heparinized syringe from a donor rat, then spun at 800 g for 5 min, and the plasma was decanted. The red blood cells (RBC) were labeled by gentle mixing with 51Cr for 15 min; they were then washed twice with the previously decanted plasma to remove unbound Cr. 125I-labeled human albumin (Nuclear Pharmacy, Denver, CO) was then added to the RBC. One hour before they were killed, the rats were lightly anesthetized with methoxyflurane and injected via a tail vein with 0.15 ml of the mixture of 51Cr-labeled RBC (2–5 µCi/rat) and 125I-labeled albumin (0.5 µCi/rat). One hour after injection, the animals were anesthetized with pentobarbital sodium (80 mg/kg ip). Their chests were opened, and a blood sample was obtained by cardiac puncture before removal of the lungs. The lungs and blood were then weighed and counted in a gamma scintillation counter. Extravascular protein accumulation was calculated as follows: extravascular lung 125I counts = whole lung 125I counts − intravascular lung 125I counts; intravascular lung 125I counts = blood 125I activity × lung 51Cr counts/blood 51Cr activity; blood 125I activity = blood 125I counts/g blood; blood 51Cr activity = blood 51Cr counts/g blood; lung blood weight = lung 51Cr counts/blood 51Cr activity. The protein leak index (PLI) was derived by dividing the extravascular lung 125I counts by the blood 125I activity (to correct for variations in intravascular protein concentration) and the body weight (to correct for differences in lung mass and exchange surface area).

Bronchoalveolar lavage studies. Bronchoalveolar lavage was performed in 16 additional rats divided into the same four experimental groups (n = 4 per group). After rats were anesthetized with pentobarbital sodium (80 mg/kg ip), a tracheal cutdown was performed. Lavage was performed via a tracheotomy with sterile phosphate-buffered saline, 9 ml total, lavaged in 3-ml aliquots. Returned lavage fluid was pooled and immediately placed on ice. Lavage fluid recovered from each animal was 7–8 ml. Cell counts were performed with a hemacytometer on the pooled lavage fluid, and a sample was removed for cytospin to obtain a differential WBC count. The remaining fluid was centrifuged to remove cells and debris, and the supernatant was aliquoted and frozen at −70°C. The total protein content of the supernatant was measured by the Bradford method (Bio-Rad Laboratories, Hercules, CA) by using bovine albumin standards. After the lavage, blood was obtained from all animals via cardiac puncture for Sendai virus serology as noted above.

Confirmation of viral infection. Serum obtained from all animals when they were killed, except those used for PLI determinations, was tested for seroconversion to Sendai virus by using an ELISA kit (Cappel-Organon-Teknika, Raleigh-Durham, NC). ELISA testing was performed at a serum dilution of 1:40. Results were interpreted by the difference in absorbance units at 405 nm between positive and negative test wells as follows: >150, positive; 150–75, weakly positive; <50, negative.

Statistics. For all groups, the mean values ± SE are shown. Comparative results were analyzed by one-way analysis of variance (ANOVA) and Fisher’s protected least-squares method posttesting or by the Kruskal-Wallis nonparametric
Correlation was assessed by simple linear-regression analysis. Statistical analyses were done by using Statview SE (Abacus Concepts, Berkeley, CA) and Super ANOVA (Abacus Concepts) statistical analysis software on an Apple Macintosh computer. Differences were considered significant when \( P < 0.05 \).

RESULTS

Characterization of Sendai virus infection. These preliminary studies were performed in six animals to characterize the course and severity of a Sendai virus infection in weanling rats. Infected animals all tolerated the viral infection well. No infected animal died before it was killed. Indeed, most were asymptomatic. Symptoms such as decreased activity, sneezing, and mild tachypnea were noted in some but not all animals.

Viral cultures were obtained from the nasopharynx, trachea, and lung tissue to document the location of the infection and its time course. Cultures from all three sites from the animals that were killed at postinoculation days 3 and 5 were positive for virus. None of the cultures from the animals examined at postinoculation day 7 was positive for virus.

Histological examination of lung and tracheal tissue from virally infected animals revealed changes consistent with those previously reported for Sendai virus infection in young rats (4, 5). Only mild lymphocytic infiltration around some airways was present at day 3 after infection. By day 5, more obvious evidence of a patchy bronchiolitis was present, along with significant leukocytic infiltration around many airways, epithelial sloughing and necrotic debris in the lumens of some airways and in the trachea, and scattered patches of pneumonía. The majority of the lung parenchyma, however, appeared essentially normal. Tissues from the animals at day 7 after inoculation still showed patchy areas of leukocyte infiltration around airways and vessels, but the lung parenchyma and airway epithelium appeared essentially normal.

Immunohistochemistry revealed patchy staining for Sendai virus antigen in the epithelium of both large and small airways at postinoculation day 3. By day 5, the staining was more intense and more diffuse in the epithelium of the large and small airways, and staining was also visible in areas of pneumonic lung parenchyma. At day 7, Sendai virus antigen was largely undetectable, with staining found only in widely scattered individual cells located in the bronchial submucosa or bronchovascular sheaths (Fig. 1).

Gravimetric measures of lung water. Having established the extent of a Sendai virus infection in our experimental system, we studied the effects of hypoxia and viral infection on lung weight-to-body weight ratio (Fig. 2) and lung wet weight-to-dry weight ratio (Fig. 3). Lung weight-to-body weight ratios were significantly different among groups (\( P < 0.001 \)). Normobaric hypoxia alone for 24 h \((n = 11)\) caused a small but significant increase in lung weight-to-body weight ratio compared with normal control animals \((n = 8, P = 0.02)\). Also, virus-infected but normoxic animals \((n = 9)\) had a nonsignificant trend toward increased lung weight-to-body weight ratios compared with normal control animals \((P = 0.13)\). The combination of hypoxia and viral infection \((n = 9)\), however, produced lung weight-to-body weight ratios significantly higher than all other groups \((P < 0.001)\). Lung wet-to-dry weight ratios showed a similar trend overall \((P = 0.07)\). Multiple-comparison posttesting showed that the lung wet-to-dry weight ratios of the hypoxic infected animals were significantly higher than those of the animals with hypoxia alone \((P = 0.04)\) and with virus alone \((P = 0.01)\), although the difference was not significant compared with normal controls \((P = 0.15)\).

![Fig. 1. Immunostaining of rat lung for Sendai virus. Viral nucleocapsid protein stains bright red. A: section from animal at day 5 of Sendai infection. B: section from animal at day 7 of Sendai infection. Original magnification, ×200.](http://jap.physiology.org/)

![Fig. 2. Lung weight-to-body weight ratios (LW/BW) in 4 conditions. *P = 0.02 vs. control; †P < 0.001 vs. all other groups.](http://jap.physiology.org/)
Histology. Consistent with the findings in our preliminary studies characterizing this experimental system, no persisting areas of significant parenchymal damage or pneumonia were seen in any of the animals studied at day 7 after viral inoculation, and immunostaining showed only widely scattered cells near the airways contained persistent viral nucleocapsid protein. Animals receiving Monastral blue before they were killed showed only widely scattered cells near the airways, or in the lumen of pulmonary vessels. No gross alveolar flooding or hemorrhage was noted in any of the animals studied. Patchy areas of alveolar septal thickening were more prominent in the hypoxic infected animals, although scattered areas of septal thickening were noted in the hypoxia alone and virus alone animals. Perivascular cuffing was also significantly increased in the hypoxic infected animals, whereas no significant differences were detectable between the other three groups (Table 1). The increase in cuffing was most distinctive in vessels 50–150 μm in diameter, although including the small number of vessels measured above and below that size range did not significantly alter the results. Also of note, the degree of perivascular cuffing, as measured by the average cuff area, showed a strong positive correlation with the lung weight-to-body weight ratio from the same animals (r = 0.90, P = 0.001, Fig. 5), as did the average cuff-to-vessel area ratio (r = 0.73, P = 0.01). Average cuff area also had a significant positive correlation with lung wet-to-dry weight ratio (r = 0.64, P = 0.04).

Transvascular protein escape. We sought further confirmation of our findings in a separate group of 24 animals studied for transvascular escape of protein as described above. Measurements of the PLI also revealed significant differences (P = 0.02, Kruskal-Wallis test) but with considerable scatter in the data. The PLI for control animals (n = 6) was 2.06 ± 0.11, with increases seen in the virus alone group (n = 6, PLI = 2.62 ± 0.18) and hypoxia alone group (n = 6, PLI = 2.88 ± 0.29); the largest increase was found in the hypoxic infected animals (n = 6, PLI = 3.21 ± 0.30).

Bronchoalveolar lavage studies. Bronchoalveolar lavage fluid from a separate series of 16 animals in the same experimental groups was examined for total WBC and RBC counts, differential WBC counts, and total protein content. There was not a significant difference in the total WBC and RBC numbers in the bronchoalveolar lavage fluid between any of the groups. The differential cell counts, however, revealed a significantly higher percentage of neutrophils in the infected groups and a significantly higher percentage of lymphocytes in the infected hypoxic group compared with the control groups (Table 2). The total protein content of the lavage fluid was also significantly higher in the hypoxic infected animals than in the normal control animals, the normoxic infected animals, and the hypoxic uninfected animals (Table 2).

Table 1. Effect of infection and hypoxia on perivascular cuff area, vessels 50–150 μm in diameter

<table>
<thead>
<tr>
<th>Group</th>
<th>n</th>
<th>Cuff/Vessel Ratio</th>
<th>Cuff Area, μm²</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>107</td>
<td>0.37 ± 0.04</td>
<td>1,168 ± 181</td>
</tr>
<tr>
<td>Virus</td>
<td>119</td>
<td>0.40 ± 0.06</td>
<td>1,572 ± 304</td>
</tr>
<tr>
<td>Hypoxia</td>
<td>107</td>
<td>0.42 ± 0.07</td>
<td>1,415 ± 265</td>
</tr>
<tr>
<td>Hypoxia + virus</td>
<td>109</td>
<td>0.72 ± 0.04</td>
<td>2,507 ± 414†</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of vessels measured. *P < 0.001 vs. all other groups; †P = 0.01 vs. all other groups.
Serological testing. Serological testing performed to document infection with Sendai virus was unable to detect circulating antibody to Sendai virus in animals examined before postinoculation day 7, but animals tested at day 7 had positive or weakly positive results at a 1:40 dilution. All uninfected animals had unequivocally negative results by ELISA, and all infected animals tested at day 7 had either positive or weakly positive results.

DISCUSSION

These results demonstrate that a preexisting viral respiratory infection may augment the susceptibility of young rats to increases in lung water and in pulmonary vascular permeability associated with exposure to hypoxia. These changes in lung water and permeability occurred despite the fact that the virus itself was no longer detectable in the host at the time of exposure to hypoxia and that the clinical and histological effects of the viral infection in the lung were mild. Moreover, these changes in lung water and permeability were associated with increases in bronchoalveolar lavage fluid protein content and with changes in the cell populations detectable in the lavage fluid.

The course of the Sendai virus infection in these young rats was consistent with previous reports (4, 5). The animals were not overtly ill, although cultures showed them to have live virus in the nasopharynx, trachea, and lung as long as day 5 after infection. Histological studies of the lungs showed a mild bronchiolitis, with occasional patches of pneumonia, that peaked at day 5 after infection and were largely resolved 2 days later. The plain histology was corroborated by immunostains showing the presence of the viral nucleocapsid protein in the airways through day 5 and its virtual disappearance by day 7; this finding is also in agreement with previous reports (13). The alterations in lung water that we observed with hypoxic exposure, then, came at a point when the acute infection had mostly been resolved, and ongoing lung injury from the virus would be expected to have largely ceased. This observation parallels the recently published finding that children diagnosed with HAPE frequently report having had symptoms of a respiratory infection before altitude exposure, as opposed to developing such symptoms while at altitude (6).

The mechanisms by which a viral infection of the respiratory epithelium might produce changes in lung water are not immediately apparent. Parainfluenza viruses are not known to infect blood vessels directly, and the effects of viral respiratory infections on the pulmonary vasculature per se are not well studied. Evidence does exist, however, that Sendai virus infections can cause exaggerated increases in the permeability of tracheal mucosal vessels in response to certain chemical or neurological stimuli (12, 16). In addition, the present lavage fluid and histological findings suggest ongoing, though mild, inflammation in the lungs of the infected animals. Whether the increases in lung water and vascular permeability found in this study were mediated by the host immune system or by a persistent effect of the virus itself is a question that requires further study.

Consistent with previous studies of the effects of hypoxia in rodent species, we did not find overt alveolar flooding in our experimental animals (15, 20, 23). Despite this fact, the evidence for early pulmonary edema formation in the hypoxic infected animals is convincing. Those animals showed increases in lung weight-to-body weight ratio (Fig. 2), in perivascular cuffing (Table 1), in transvascular protein escape, and a trend toward increased lung wet weight-to-dry weight ratios (Fig. 3). The fact that the increases in lung weight-to-body weight ratio in this study were matched

Table 2. Effect of hypoxia and viral infection on lavage fluid cell counts

<table>
<thead>
<tr>
<th>Group</th>
<th>WBC, n cells/mm³</th>
<th>Neutrophils, % of total</th>
<th>Lymphocytes, % of total</th>
<th>Protein, mg/l</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>64±14</td>
<td>1.5±0.7</td>
<td>3.0±1.2</td>
<td>50.8±5.9</td>
</tr>
<tr>
<td>Virus alone</td>
<td>69±15</td>
<td>9.3±1.9†</td>
<td>0.5±0.5</td>
<td>67.5±3.7†</td>
</tr>
<tr>
<td>Hypoxia alone</td>
<td>50±9</td>
<td>1.3±2.5</td>
<td>0.3±0.3</td>
<td>47.5±3.2</td>
</tr>
<tr>
<td>Virus + hypoxia</td>
<td>48±10</td>
<td>13.5±2.7†</td>
<td>10.5±2.3†</td>
<td>84.0±7.8†‡</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. WBC, white blood cells.
*P < 0.05 from control; † P < 0.05 from hypoxia alone; ‡ P < 0.05 from virus alone.

Fig. 5. Photomicrographs of thickened alveolar septa. A: lung from animal exposed to virus alone; B: lung from animal exposed to virus + hypoxia. Original magnification, ×400.
as a trend, but not proportionally, by increases in lung wet-to-dry weight ratios does bring into question the validity of the lung weight-to-body weight ratio results. This blunting of the increase in lung wet-to-dry weight ratios, however, is consistent with the idea that edema formation leads to an accumulation of water as well as protein, which decreases the sensitivity of the lung wet-to-dry weight ratio, and it is consistent with previous results from similar studies (2, 20). In addition, the decrease in lung wet-to-dry weight ratios in the hypoxia alone and virus alone groups compared with the normal controls is largely caused by a single, outlying low value in each of those two groups. Although the reasons for the low wet-to-dry lung weight ratios in these two animals are not clear, these outlying results have not been excluded from the analysis because none of the other data from those rats appeared aberrant. More importantly, the increases seen in perivascular cuffing and the high correlation between cuff area and lung weight-to-body weight ratios corroborate the gravimetric measures that show increased lung water in the hypoxic infected animals (Fig. 6).

These findings of increases in gravimetric measures of lung water as well as in vascular permeability in the hypoxic infected and, to a lesser extent, in the hypoxic only animals are consistent with the concept that inflammation may alter host responses to hypoxia. Hypoxia alone has previously been shown to cause increases in lung water and vascular permeability in adult rats, an effect that was reversible by treatment with glucocorticoids (20). In addition, priming of the immune system with small doses of endotoxin (15) has been shown to exaggerate hypoxia-associated increases in vascular permeability, an effect that was blunted in neutropenic animals. Human studies have also found evidence of systemic and pulmonary inflammation during acute episodes of HAPE, although whether this is a causative or secondary phenomenon is unclear (9, 18, 19).

As we did not measure pulmonary vascular pressures, the relative contributions of hydrostatic forces and permeability changes to the accumulation of lung water noted with hypoxia and infection in this rat model are not addressed by this study. Clearly, alterations in vascular tone can lead to increased filtration forces favoring edema formation, and such alterations may contribute to the differences between the present experimental groups, as well as to the variability seen within the groups. The increases found in Monastral blue staining and in extravascular protein accumulation, however, support the idea that an increase in vascular permeability is a contributing factor to the hypoxia-associated edema formation found in the hypoxic infected animals. The PLI results are weakened somewhat by the wide scatter in the data and the relatively small number of animals, but overall those results are also consistent with our other findings. In addition, the location of the Monastral blue staining in the alveolar septa and the interstitial thickening suggest a predominantly microvascular location for the apparent vascular leak, although leakage at other sites is obviously not excluded by these results. Finally, although we did not detect any significant amount of alveolar hemorrhage to support the concept of alveolar capillary rupture leading to edema fluid accumulation, a contribution by such a process cannot be ruled out.

The increase in neutrophil counts in the bronchoalveolar lavage fluid of both infected groups, although not of great magnitude, also suggests that even mild viral respiratory infections can lead to pulmonary inflammatory processes that may then affect the host response to hypoxia. The increase in lavage fluid lymphocyte counts noted in only the hypoxic infected animals further supports this idea. In addition, the finding of an increase in lavage fluid protein content in the infected hypoxic animals suggests a leak of fluid not only across the endothelial barrier but also across the epithelial barrier into the air spaces, despite the absence of alveolar flooding on histological examination. These findings corroborate earlier work showing increases in lavage fluid neutrophil counts and total protein in association with increases in lung water after endotoxin priming and hypoxic exposure (15), and they are consistent with human data showing increases in lavage fluid protein and in inflammatory cell populations in cases of HAPE (18, 19).

An important difference between this study and previous work is that the inflammatory stimulus in our model is directed initially at the respiratory epithelium. Whereas both cell culture and animal data support the concept of endothelial leak in the setting of hypoxia (14, 20), fluid accumulation in the air spaces requires an epithelial leak as well, and a minor increase in the permeability of the vascular endothelium is unlikely to lead to frank alveolar flooding. Our findings, particularly that of increased protein in the air spaces of the hypoxic infected animals, suggest that respiratory viral infections have the potential not only to trigger a systemic inflammatory response to which the vascular endothelium is exposed but also to alter the permeability of the epithelial barrier, thus increasing the susceptibility of an animal to pulmonary edema formation.
In conclusion, we found that young rats recovering from a mild viral respiratory infection, when exposed to moderate hypoxia for 24 h, developed signs of early pulmonary edema formation including increases in lung water, increases in inflammatory cells recovered in bronchoalveolar lavage fluid, and increases in lavage fluid protein content. Although we did not demonstrate frank alveolar flooding in these animals, evidence is accumulating that humans at high altitude develop subclinical accumulation of lung fluid as well (8, 17). Finally, while evidence implicating respiratory infections in the pathogenesis of HAPE in human adults is lacking, children may have different risk factors than adults have for this illness (6). Further investigation seems justified, then, into the interactions in the lung of viral infection and hypoxia and into their relevance to human, and in particular, pediatric, illness.

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