

The Role of Neutrophils, Oxidants, and Proteases in the Pathogenesis of Acid Pulmonary Injury

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We recently reported a biphasic injury pattern of nonlethal acid aspiration pneumonitis in rats. The first phase consisted of the immediate effects of the direct tissue injury, and the second phase was associated with a neutrophilic inflammatory response. Using this model, the present report examines the possible role of neutrophils, oxidants, and proteases in the pathogenesis of the second phase of this lung injury. Acid aspiration injury was induced by instillation of saline/HCl, pH = 1.25, into the trachea of rats. Lung injury was assessed by measuring the degree of alveolar capillary permeability to ¹²⁵I-labeled albumin (permeability index [PI]). Rats made neutropenic with polyclonal antineutrophil antibody had a lower PI (0.44 ± 0.07 , $P < 0.05$) 6 h after acid aspiration than similarly injured animals with normal whole blood neutrophil counts ($PI = 0.85 \pm 0.03$). Even though neutrophils appeared necessary for the full development of the lung injury in this model, the administration of different intravenous and/or intratracheal concentrations of either deferoxamine or catalase offered no protection against injury. This suggests that neutrophil oxidants were minimally involved in the injury. Large increases in leukocyte-free serine protease activity ($1,477 \pm 438$ u/ml, $P < 0.05$) were detected in the bronchoalveolar lavage fluid from the saline/HCl, pH = 1.25, injured rats at 6 h postinjury, as compared to saline/HCl, pH = 5.3, treated control animals (2.7 ± 0.2 u/ml). This study supports the hypothesis that neutrophils are necessary for the full expression of acid-induced lung injury and that the generation of leukocyte-derived oxidants does not appear to be the primary mechanism involved in this injury. Indeed, the presence of high levels of serine proteases in the bronchoalveolar lavage fluid is strong presumptive evidence that these enzymes play an important role in this injury. Results from this study suggest the possibility of new treatment strategies aimed at decreasing the lung injury from this potentially lethal complication. (Key words: Complications, pulmonary: aspiration pneumonitis. Inflammation; neutrophils; oxygen radicals; proteases. Lung(s): adult respiratory distress syndrome.)

ASPIRATION of acid gastric contents into the lungs is one of the most feared intraoperative complications associated with general anesthesia.^{1-4,¶} In addition, this type of pulmonary injury is a leading cause of adult respiratory dis-

stress syndrome (ARDS) after routine surgery.⁵ The nature of this type of injury is particularly devastating because it occurs in otherwise healthy individuals and is a leading cause of morbidity and mortality in obstetric patients.^{3,4,6,7}

Measures to reduce the risk of pulmonary aspiration of acidic gastric contents have primarily been aimed at decreasing gastric fluid volume, increasing the pH of stomach contents, and using intraoperative techniques to protect the airway of the anesthetized patient. Although these measures have been somewhat successful, they have not completely prevented the occurrence of this complication. In addition, deaths secondary to aspiration still occur despite administration of oral antacids.⁷

One of the difficulties in developing treatment strategies for this type of lung injury has been a lack of understanding of the mechanisms by which acidic gastric contents damage the alveolar capillary wall. We do know that acid aspiration in humans and experimental animals is associated with an early neutrophil infiltrate and in many cases leads to ARDS with interstitial pneumonitis and a chronic inflammatory infiltrate.^{8,9} In a previous article we reported on the development of a model to facilitate investigation into the mechanisms of pulmonary acid aspiration injury.¹⁰ This model, involving a nonlethal, quantifiable, acid-induced pulmonary injury in rats, permits evaluation of the pathogenesis of this intraoperative complication. Our previous studies on acid aspiration in rats demonstrated a biphasic pattern of injury, with the initial injury appearing to be the result of the direct chemical effect of the acid. Neutrophils appeared after 2-3 h and preceded a more severe second phase of injury characterized by an inflammatory pneumonitis.¹⁰

It is important, therefore, to determine if in fact neutrophils are required for the full expression of the injury, particularly with respect to the second-phase inflammatory response. The current report examines some of the potential mechanisms in the development of lung injury in this model. The effectors studied here include neutrophils and their inflammatory mediators, oxidants, and proteases, all of which are known to be involved in the evolution of most models of ARDS.^{11,12} Based on our previous results, the association of aspiration pneumonitis with ARDS, and results obtained in other experimental models of ARDS, we hypothesize that the resultant lung injury is caused by recruitment and activation of neutrophils in the lung and the subsequent formation and release of oxidants and proteases from these effector cells.

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Materials and Methods

ANIMAL MODEL OF ASPIRATION PNEUMONITIS

The experimental protocol was approved by the Institutional Review Committee for the use and care of laboratory animals. The animal model of aspiration pneumonia is detailed in our recent article.¹⁰ Briefly, male pathogen-free Long-Evans (Charles River, Portage, MI) rats weighing 250–300 g were anesthetized with ketamine (Parke-Davis, Morris Plains, NJ) 100 mg/kg intraperitoneally. The trachea was exposed surgically and a 16-G Teflon catheter inserted. Animals maintained spontaneous respiration throughout the procedure except for a brief period of apnea that occurred after the initial instillation of the acid into the trachea. The experimental group received 1.2 ml/kg of a normal saline/HCL solution, pH = 1.25, instilled into the trachea. The animals were placed in a semiupright position (60° from horizontal) to facilitate the distribution of the acid into the lungs. From our preliminary data, we determined that saline/HCL, pH = 1.25, at a volume of 1.2 ml/kg, generated the greatest degree of acute lung injury consistent with acute survival (24 h) of 90% of the animals. In these studies the peak of the acute injury occurred by 4–6 h after instillation of the acidic saline. Therefore, unless specified otherwise, comparisons were made by killing rats 6 h after acid instillation and quantitating the degree of lung injury at that time. Animals in the control group were treated similarly except that the pH of the saline/HCL solution instilled into the trachea was 5.3. After instillation of the aspirates into the animals' lungs, the trachea and skin were sutured with 4-0 silk. The lung injury was quantitated as described below.

ASSESSMENT OF INJURY

After instillation of the saline/HCL (acidified or control) and while the rats were still anesthetized, ¹²⁵I-albumin (100,000 cpm) was injected intravenously and the animals allowed to recover. Six hours after injury the animals were again anesthetized with ketamine (100 mg/kg intraperitoneally). A midline abdominal incision was performed, and 1 ml blood was removed from the vena cava. The vena cava was then transected and the heart and lungs removed *en bloc*. Ten milliliters saline was infused into the right ventricle to remove the blood and radiolabeled albumin from the pulmonary vasculature. The lungs were then dissected from the heart, and the amount of radiolabeled protein in both the lungs and the blood was measured using a γ counter (TM Analytic, Elk Grove, IL). A permeability index (PI) then was calculated as a ratio between the amount of ¹²⁵I-albumin that had leaked through the alveolar capillary boundary into the lungs and the concentration of radioactivity in 1 ml blood. Previously,

using morphometric analysis and transmission electron microscopy, we demonstrated a good correlation between PI measurements and histopathology.^{10,13,14}

NEUTROPHIL DEPLETION

The role of neutrophils in acid aspiration-induced lung injury was assessed by the use of a specific rabbit antirat neutrophil antibody. This antibody was prepared in our laboratory by injecting rabbits three or four times with purified rat neutrophils in incomplete Freund's adjuvant. This antibody selectively depletes circulating neutrophils in 90% of the treated animals without any effect on the other formed elements in the blood and has been used in similar studies in our laboratories for several years.^{13–15} One milliliter of this antibody was injected into nine animals intraperitoneally 12 h before the lung injury. Eight animals developed significant neutrophil depletion (< 500 polymorphonuclear leukocytes per milliliter blood, as compared to normal rats with 3,800 \pm 200 polymorphonuclear leukocytes per milliliter blood); these eight animals were used in this study. Blood monocyte counts were normal in these neutrophil-depleted animals. These rats then underwent intratracheal instillation of acidified saline, as described above, and were compared to similarly injured animals with normal neutrophil counts 6 h postinjury. One of the neutropenic animals died during instillation of the acidic saline and therefore was not included in the study.

ROLE OF LEUKOCYTE OXYGEN RADICALS

To examine the role of oxidants in the rat aspiration pneumonia model, the following interventions were performed. Rats were pretreated 2 h before injury with deferoxamine mesylate (Ciba-Geigy, Summit, NJ) 15 mg/kg (n = 8) or 30 mg/kg (n = 8) intravenously, alone or in conjunction with 5 mg deferoxamine mesylate instilled directly into the trachea at the time of acid injury to the lungs (n = 8). Deferoxamine is an iron chelator that prevents the formation of the hydroxyl radical. These doses of antioxidants were used successfully in previous studies.¹⁶

In a separate set of experiments, catalase, at a dose of 10,000 u (0.4 ml) (Sigma, St. Louis, MO) was administered intravenously to rats either at the time of the acidic lung injury (n = 10) or 2 h before the injury (n = 13). Catalase converts the strong oxidant hydrogen peroxide to water and molecular oxygen.¹⁴ Catalase (0.8 ml/kg) was also instilled directly into the trachea in doses of 6,500 u (n = 12) or 1,000 u (n = 13) at the time of the acid pulmonary injury or 6,500 u (n = 8) 30 min after instillation of the acid into the trachea to avoid any interaction of the enzyme and the acid. This time was selected based on experiments showing that the pH of tracheal aspirates re-

turns to normal 30 min after the instillation of acid into the trachea.¹⁷

LUNG LEUKOCYTE PROTEASE ACTIVITY

Bronchoalveolar lavage fluid from control ($n = 8$) and acid-treated animals ($n = 8$), at 6 h postinjury, was assessed for serine protease activity as described by Varani *et al.* using hemoglobin hydrolysis as a measure of proteolytic activity.¹⁸ Serine proteases are released primarily by neutrophils as opposed to monocytes and parenchymal cells. Serine protease activity in the bronchoalveolar lavage 6 h after acidic injury was analyzed because this was associated with the period of maximal lung injury. This proteolytic activity was compared to trypsin activity using a standard curve of dose-dependent trypsin hemoglobin hydrolysis.

STATISTICS

Data are presented as the mean \pm standard error of the mean. Statistical inference comparing two variables was determined using Student's unpaired *t* test. Analytical variables were analyzed using analysis of variance with Scheffé's correction for multiple comparisons. Significance was achieved at the 5% level ($P < 0.05$). A power analysis was performed to calculate the sample size for each of the treatment groups.

Results

ROLE OF NEUTROPHILS IN ACID PULMONARY INJURY

To examine the role of neutrophils in the lung injury produced 6 h after the instillation of acid into the lungs of rats, the leakage of protein into the alveoli, as determined by the PI, was measured in neutrophil-depleted animals. The results are described in figure 1. Rats ($n = 7$) made neutropenic by the specific antineutrophil antibody had a significant decrease in lung injury ($PI = 0.44 \pm 0.07$) 6 h after the instillation of saline/HCl, $pH = 1.25$, as compared to similarly injured animals ($n = 52$) with normal neutrophil counts ($PI = 0.85 \pm 0.03$, $P < 0.05$). Both of these groups had a significantly greater lung injury than rats that received saline/HCl, $pH = 5.3$ ($PI = 0.23 \pm 0.03$, $P < 0.05$). These permeability values paralleled the decreased lung inflammatory response as assessed morphologically in the neutrophil-depleted animals (histopathology not shown).

ROLE OF LEUKOCYTE-DERIVED OXYGEN FREE RADICALS IN PULMONARY ACID ASPIRATION

As seen in figure 2, deferoxamine had no significant effect on lung injury at either the 15- or 30-mg/kg in-

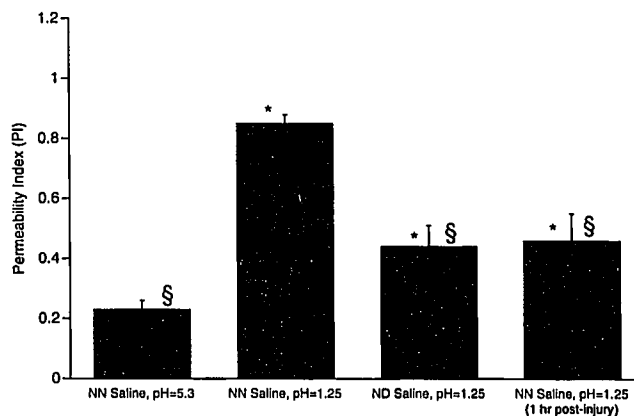


FIG. 1. Effect of neutrophil depletion on pulmonary acid injury. Comparison of the 6-h postaspiration lung injury permeability indices between rats with normal blood neutrophil counts (NN) receiving either 1.2 ml/kg saline, $pH = 5.3$ or $pH = 1.25$, and neutrophil depleted rats (ND) receiving 1.2 ml/kg saline, $pH = 1.25$. The 1-h permeability index for NN rats that received 1.2 ml/kg of saline, $pH = 1.25$, was also included as a comparison. * $P < 0.05$ vs. NN saline, $pH = 5.3$. § $P < 0.05$ vs. NN saline, $pH = 1.25$, 6-h postaspiration lung injury.

travenous dose ($PI = 0.93 \pm 0.06$). In an attempt to increase the efficacy of the deferoxamine, a 5-mg intratracheal treatment was given in conjunction with the 30-mg intravenous dose of the drug. Addition of this regimen, however, was without effect ($PI = 0.92 \pm 0.10$).

Similarly, as seen in figure 3, intravenous catalase 10,000 μ given to rats 2 h before the pulmonary acid instillation into the lung did not inhibit the lung injury ($PI = 0.69 \pm 0.05$). Direct instillation of catalase, either 1,000 or 6,500 u, into the trachea of rats at the same time as the acid did not decrease alveolar capillary leak ($PI = 0.86 \pm 0.11$ and 1.02 ± 0.07 , respectively.) When 6,500 u catalase was instilled 30 min after acid injury, however, there was a significant increase in pulmonary injury ($PI = 1.30 \pm 0.12$, $P < 0.05$) compared to the untreated injured group.

ROLE OF PROTEASES IN ACID INJURY OF THE LUNG

Figure 4 summarizes the results obtained in examining leukocyte protease activity in the bronchoalveolar lavage fluid after acidic saline injury. Rats that underwent anesthesia without aspiration of any substance had no detectable serine protease activity in the bronchoalveolar lavage fluid. Animals given saline/HCl, $pH = 5.3$, intratracheally also had virtually no free serine protease activity in their lavage fluid (2.7 ± 0.2 u/ml.) This low level of protease activity is expected, in that the lungs contain α_1 protease inhibitor as well as other locally produced protease inhibitors.¹⁹ In contrast, animals injured by the saline/HCl, $pH 1.25$, acidic solution had very high levels of free protease activity in their lavage fluid ($1,477 \pm 438$ u/ml, $P < 0.05$).

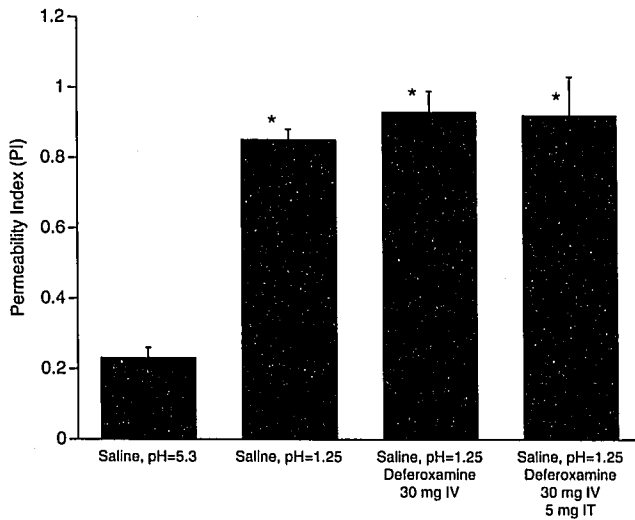


FIG. 2. Effect of deferoxamine on pulmonary acid injury. Comparison of the 6-h postaspiration lung injury permeability indices between rats receiving 1.2 ml/kg saline, pH = 5.3 or pH = 1.25, and rats receiving 1.2 ml/kg saline, pH = 1.25, in combination with either deferoxamine 30 mg iv given 2 h before aspiration injury or deferoxamine 30 mg iv given 2 h before aspiration injury and 5 mg it given at the time aspiration injury. * $P < 0.05$ vs. saline, pH = 5.3.

Discussion

In a previous report we described a nonlethal rat model of aspiration pneumonitis.¹⁰ Lung injury in this model was biphasic in nature and consisted of an initial lung injury phase that was characterized by leakage of protein

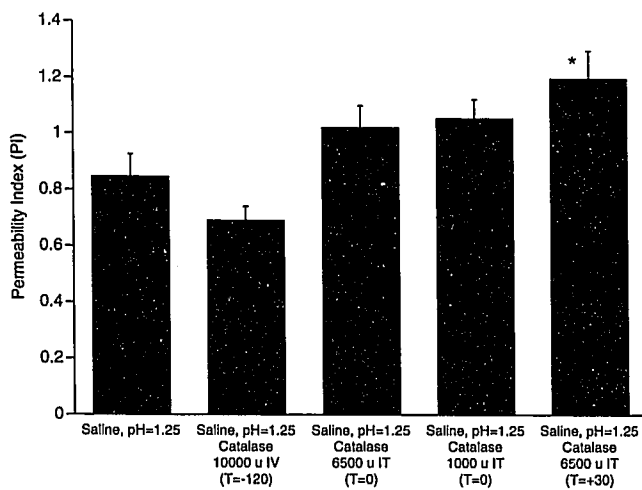


FIG. 3. Effect of catalase on pulmonary acid injury. Comparison of the 6-h postaspiration lung injury permeability indices between rats receiving 1.2 ml/kg saline, pH = 1.25, alone and rats receiving 1.2 ml/kg saline, pH = 1.25, in combination with different doses of catalase, given by different routes of administration, at different time intervals. T = -120: 10,000 units of catalase given 120 min iv before aspiration injury. T = 0: 6,500 or 1,000 units of catalase given it at the time aspiration injury. T = +30: 6,500 units of catalase given it 30 min after aspiration injury. * $P < 0.05$ vs. saline, pH = 1.25, alone.

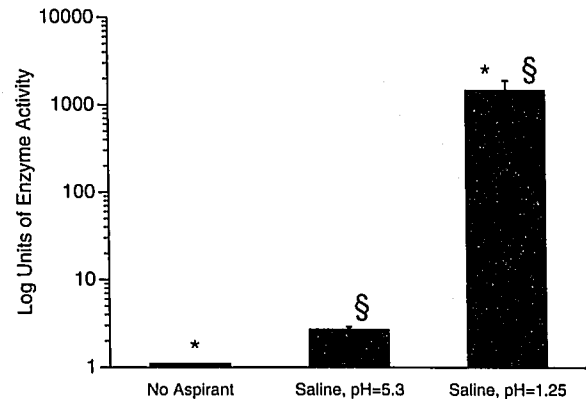


FIG. 4. Protease activity in lung lavage after acid injury. Comparison of the 6-h postaspiration lung injury serine protease activity from bronchoalveolar lavage fluid between rats receiving 1.2 ml/kg saline, pH = 5.3 or pH = 1.25, it and rats receiving no aspirant. * $P < 0.05$ vs. saline, pH = 5.3. § $P < 0.05$ vs. no aspirant.

across the alveolar-capillary border. However, this initial phase was not associated with an increase in inflammatory cell infiltration into the lung. A second phase, which was heralded at 2-3 h by the beginning of neutrophil infiltration, was maximal 5-6 h after aspiration of acidic saline. This phase was characterized by neutrophilic infiltration and a further increase in protein leakage from the pulmonary vasculature. We hypothesized that in the early phase, the leakage of protein into the alveoli was either the result of a direct chemical burn and/or a result of stimulation of afferent (capsaicin-sensitive) nerves.^{11,20} Furthermore, we hypothesized that the second phase of the injury, which was maximal at 5-6 h postaspiration, was induced by a neutrophil-mediated inflammatory response augmented by neutrophil-derived oxidants and proteases. This hypothesis was supported by correlating the leakage of protein into the alveoli with the quantitation of neutrophil infiltration into the lung by morphometric analysis.

Aspiration pneumonitis is known to be an inducer of ARDS. In ARDS induced by shock, thermal injury, hypoxia, and/or traumatic lung injury, there generally is clear evidence implicating neutrophils in the development of the injury.²¹ Several studies demonstrate neutrophil sequestration in the lungs and show that neutrophils obtained from the blood of these patients are in an activated state as assessed by increased chemotactic activity, spontaneous oxidant generation, and enzyme release *in vitro*.^{12,22-24} Thus, in patients with ARDS, neutrophil recruitment and activation in the lung appears to be involved in the development of the lung injury.

Other experimental studies also support these findings. For example, in several models of acute lung injury, including those that histologically mimic ARDS, neutrophil sequestration and activation are present. Much of the re-

sulting lung injury can be blocked by selective depletion of neutrophils. Therefore, as in human ARDS, the experimental models support the concept that neutrophils are critical effector cells in acute lung injury.^{23,25}

To examine the pathogenesis of acid aspiration-induced pulmonary injury we investigated the role of neutrophils and neutrophil-derived mediators on the late developing second phase of injury after the instillation of acidic saline into the trachea of rats. As stated above, previous histologic studies have demonstrated that this second phase of lung injury is maximal 5–6 h after acidic saline instillation and is paralleled by neutrophil infiltration. Based on these observations, we hypothesized that as with other models of ARDS, neutrophils were an important effector cell in the pathogenesis of the lung injury. The neutrophil depletion studies presented in this paper strongly support the hypothesis that neutrophils are important effectors in the pathogenesis of acid aspiration injury. The ability to decrease the second phase of lung injury, after acid aspiration in rats made selectively neutropenic, to a level similar to the PI observed in the early phase of the injury (1 h postinjury) in rats with normal neutrophil counts, argues for an important role for this effector cell in producing the lung tissue damage seen 5–6 h after acidic saline intratracheal instillation. The decrease in lung injury 6 h after acidic saline instillation, after neutrophil depletion to levels seen before infiltration of the inflammatory cells into the lung (*i.e.*, 1 h after acid instillation), suggests that neutrophils are both necessary and sufficient to account for the secondary, nonchemical, phase of lung injury in the rat model of pulmonary acid aspiration. Thus, neutrophils appear to be critically involved in the secondary or inflammatory stage of acid-induced lung injury but not the initial phase.

Activated neutrophils produce tissue damage primarily by the generation of oxygen radicals or by the release of proteolytic enzymes.^{16,26,27} During neutrophil activation there is an initial increase in the uptake and utilization of oxygen by these cells, the “respiratory burst,” which is associated with the formation of oxidants (hydroxyl radicals and hydrogen peroxide and other powerful oxidizing species). We²⁸ have found that the tissue injury caused by neutrophil-derived oxygen radical generation may be reduced if the formation of these toxic oxygen species is inhibited in another model of acute lung injury, including other types of experimental ARDS. To examine the role of oxidants in the pathogenesis of aspiration pneumonitis, rats were treated with various regimens of deferoxamine mesylate or catalase and the severity of the lung injury compared 6 h after acid injury, *i.e.*, the time of maximal neutrophil infiltration and lung injury. Treatment of the animals with deferoxamine mesylate results in the chelation of iron, which is necessary for the production of hydroxyl radicals *via* the Fenton reaction, while catalase

promotes the enzymatic breakdown of hydrogen peroxide.¹⁶

Deferoxamine has been efficacious in the treatment of other types of acute lung injury, including immune complexes, complement activation, and oxidant enzyme substrate-induced injuries.²⁴ In the experiments presented here, in doses used previously to suppress other types of oxidant-dependent lung injury, intravenous deferoxamine mesylate alone, or in conjunction with intratracheal administration of deferoxamine mesylate, did not inhibit the 6-h pulmonary injury. Therefore, the hydroxyl radical was not involved in this particular injury to the extent seen in other acute lung injury models.¹⁷

Catalase, which has the ability to decrease the hydrogen peroxide levels in inflamed tissues and thereby decrease free oxygen radical mediated damage, also did not inhibit the 6-h pulmonary injury in the acid aspiration model despite both intravenous or intratracheal administration of the enzyme.^{13,14} In summary, neither the catalase nor the deferoxamine treatment reduced the degree of lung injury, suggesting that the generation of oxidants by neutrophils is not the primary mechanism in the pathogenesis of the second, or inflammatory, phase. Tissue damage secondary to the generation of oxidants in this model is not ruled out and could still be a minor event in the development of this type of pulmonary injury. That these studies do not demonstrate a primary role for oxidants is unusual but not unreasonable. Other antioxidants, not examined in this study, may be protective. Acute lung injury induced by oleic acid and monocrotaline pyrrole also are associated with neutrophil dependence, but oxidants have not been demonstrated to play an important role in these models.^{26,29}

The negative results on the role of oxidants in the pathogenesis of the lung injury in this model, in conjunction with the apparent requirement for neutrophils in the second phase of the lung injury, suggests that other neutrophil-derived mediators such as proteases may be necessary for the development of the injury. Proteases have long been implicated as important agents in tissue injury and have been associated with the development of ARDS. In patients with ARDS, oxidative inactivation of α_1 protease inhibitor occurs, and free protease activity is present in the lungs.^{27,30–32} A neutrophil-derived serine protease, elastase, has been implicated in the development of chronic lung injury.^{33,34} Experimentally, proteases are also generated in the lungs of animals with acute injury, and direct instillation of serine proteases at a level of activity lower than was demonstrated in our experiments into the lung will induce injury.^{35–38} In these experimental lung injury models, protease inhibitors have been shown to be partially protective.^{39–41}

The release of serine proteases is intimately associated with the activation of neutrophils. Monocytes, alveolar

type-2 cells, and other lung parenchymal cells release very low levels of this type of protease. Therefore, we determined if these chemical effectors of inflammatory damage were present and could in part account for the neutrophil dependence in this model of pulmonary injury. In fact, there was an enormous amount of free serine protease activity in the lungs of these animals, which indicates that the antiprotease shield of α_1 protease inhibitor, normally present in bronchoalveolar lavage fluid, had clearly been overcome.³¹ The demonstration of elevated serine protease activity in the bronchoalveolar lavage fluid of the injured lungs 6 h after instillation of the acid supports the hypothesis that the second, or inflammatory, phase of pulmonary acid damage may be mediated primarily through the release of these enzymes. This would not be surprising in that leukocytic proteases have long been implicated in tissue injury occurring secondary to the inflammatory response.^{27,31} However, definitive proof that serine proteases, released from neutrophils, are primarily responsible for the lung injury in the second inflammatory phase of acid aspiration injury depends on future studies using specific protease inhibitors.

Neutrophil oxidants may also play a role in this model of lung injury despite the apparent lack of benefit from the antioxidants used in this study. A recent study from our laboratory suggests that oxidants and proteases work synergistically in the development of the lung injury.⁴² Much of the evolution of inflammatory injury appears to be due to a complex interaction between leukocyte oxidants and proteases.⁴³ Oxidants inactivate α_1 protease inhibitor and the oxidant hypochlorous acid activates latent metalloproteinases. These side effects of oxidants, which increase protease activity, may be extremely difficult to inhibit with the antioxidant agents used in this study, but would help explain the finding of high serine protease levels in the lavage fluid from these injured animals.

Because the injury described above is inflammatory in nature, antiinflammatory therapy should be beneficial. However, the only antiinflammatory agents tried to date are corticosteroids. Controversy still exists regarding the use of systemic steroids to reduce bronchial edema and inhibit the inflammatory response. A 24-h course of steroids is still recommended by some authorities. The primary antiinflammatory effect of steroids is inhibition of leukocyte activation, with inhibition in cytokine and prostaglandin synthesis and decreased ability of the leukocytes to respond to bacteria or other injurious agents.⁴⁴ Experimental evidence in animals does not support the efficacy of such treatment. Furthermore, the indiscriminate use of steroids may promote the growth of bacteria and further complicate the clinical picture of lung injury.⁴⁵ As suggested by these studies, perhaps the use of agents that are more specific to the neutrophilic response and/or serine protease activation may be more efficacious.

In summary, we have demonstrated that neutrophils contribute to the development of lung injury in a non-lethal acute acid pneumonitis model in the rat. This neutrophil-mediated injury is associated with the secondary inflammatory phase of the pulmonary tissue damage. Furthermore, unlike most models of acute inflammatory lung disease, in which the neutrophil is also a primary cellular effector of injury, damage to the alveolar capillary interface secondary to the generation of oxygen free radicals does not appear to be a major component of this injury. Rather, the results suggest that proteases, presumably from the activated neutrophils, are likely candidates for producing tissue damage in the acid type of pulmonary injury, although a minor role involving the generation of oxygen free radicals cannot be ruled out.

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