Neutrophil-Dependent, Oxygen-Radical Mediated Lung Injury Associated with Acute Pancreatitis

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Cerulein-induced acute pancreatitis in rats is associated with a reversible lung injury that is characterized by alveolar capillary endothelial-cell injury, increased microvascular permeability, interstitial edema formation, and intraalveolar hemorrhage and fibrin deposition. The role of mediators in this injury was analyzed using gravimetric data, microvascular permeability indices, electron microscopy, and a quantitative morphometric analysis. Neutrophil depletion induced by a specific antibody was highly protective against lung injury. Interruption of the complement pathway (using low dose *Naja naja* cobra venom factor) also protected against lung injury. Catalase and superoxide dismutase were also protective. The iron chelator deferoxamine and the hydroxyl radical scavenger, dimethylsulfoxide, were not protective against acute lung injury. These data suggest that complement, neutrophils, and neutrophil-derived (H_2O_2-dependent) oxygen products mediate lung injury that occurs secondary to cerulein-induced pancreatitis. In contrast to other models of neutrophil-dependent, oxygen-radical-mediated lung injury, this lung injury does not appear to be an iron-dependent and hydroxyl-radical mediated injury. We postulate that the process of acute pancreatitis leads to complement activation followed by neutrophil recruitment, sequestration, and adherence to alveolar capillary endothelial cells. Ultimately lung injury appears to result from local endothelial-cell injury secondary to neutrophil-generated oxygen products that may be myeloperoxidase dependent.

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PULMONARY DYSFUNCTION ASSOCIATED with acute pancreatitis occurs in as many as 50% to 70% of human patients.[1,2] Symptoms are most frequently tachypnea or mild hypoxemia and the underlying lung injury appears mild and generally reversible.[3] In approximately one third of patients the lung injury is progressive and may be associated with acute respiratory failure and fully developed Adult Respiratory Distress Syndrome (ARDS).[1] The events that discriminate between these outcomes are not fully understood. However it is probable that a variety of host responses and external events determine the development of this lung injury.

We have recently characterized in rats a reversible acute lung injury occurring secondary to acute pancreatitis.[4] The model uses cerulein-induced acute pancreatitis and is associated with an acute alveolar capillary endothelial-cell injury. The injury is not attributable to cerulein itself and appears to share some of the features of human ARDS, including increased microvascular permeability in the lung, interstitial edema formation, infiltration of neutrophils and prominence of macrophages, intraalveolar hemorrhage and fibrin deposition, and endothelial-cell injury. At the ultrastructural level separation of the endothelial cell from the basement membrane occurs and even cell death is observed.

The features of increased microvascular permeability and morphologic evidence of injury are shared with a number of other experimental animal models of acute lung injury[5-7] and with human ARDS associated with pancreatitis.[8-10] This series of experiments was designed to evaluate the role of the complement system, neutrophils, and neutrophil-derived oxygen-free radicals in the pathogenesis of acute lung injury after cerulein-induced pancreatitis.

Materials and Methods

Animal Model

Male pathogen-free Sprague-Dawley rats (200 to 250 gm; Charles River, Portage, MI) were used for all experiments. Protocols were approved by the University of Michigan Animal Subjects Review Committee. Anesthesia was induced with subcutaneous administration of

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ketamine hydrochloride (100 mg/kg body weight). Silastic (TM) (Dow Corning, Midland, MI) catheters (0.20 mm internal diameter) were placed by cutdown into the jugular vein under sterile conditions. The catheters were tunneled subcutaneously to exit in the dorsal cervical region of each rat. Each catheter was secured, flushed with sterile physiologic saline, and capped. The rats were allowed to recover overnight in individual cages and had free access to food and water. Infusion of cerulein (Sigma Chemical, St. Louis, MO) or physiologic saline was performed the following day. Sterile physiologic saline was used for the preparation of the cerulein infusate as well as for infusion of control animals. The dose of cerulein was 5 μg/kg/hr as described by Lampel and Kern.[11] The infusion apparatus consisted of stainless steel wrapped intravenous (IV) tubing connected to 20-mL syringes. Before infusion catheters were flushed with sterile physiologic saline (0.5 to 1.0 mL) to insure patency. Continuous individual intravenous infusions were given using infusion pumps (Harvard, Pump 22, South Natick, MA) at an infusion rate of 2 mL per hour for three hours. Immediately before infusion an intravenous bolus of ^{125}I-bovine serum albumin (BSA) (800,000 cpm) was given to allow assessment of microvascular permeability changes. After infusion the rats were sedated with ketamine hydrochloride (50 mg/kg body weight, i.p.).

At sacrifice laparotomy was performed on anesthetized rats, 1 mL of blood was aspirated from the inferior vena cava and then transected, resulting in exsanguination. The heart and lungs were removed *en bloc* and 10 mL of sterile saline was infused into the right ventricle to wash residual blood and ^{125}I-BSA from the pulmonary vascular bed. When done rapidly a spontaneous heart beat persists and the lung parenchyma develops a typical white blanched appearance when bloodless. The whole lungs were then weighed and evaluated in a gamma scintillation counter with the 1-mL blood sample. The ratio of lung-to-blood counts was taken as a measure of microvascular permeability and has been shown to be a reliable, quantifiable marker of lung injury in this as well as other lung-injury models.[5,6] This aspect of the permeability assessment is described in detail in several recent publications.[4–6]

In addition the pancreatic capillary permeability changes were determined. The pancreas was quickly dissected, blotted, weighed, and counted along with each animal's lungs and blood in a gamma scintillation counter. The ratio of pancreas-to-blood counts in this case represents pancreatic microvascular permeability and reflects one component of acute pancreatitis edema formation.

Interventions

Specific interventions were designed to assess the involvement of complement, neutrophils, and oxygen-derived free radicals.

(1) Complement depletion was accomplished by intraperitoneal injection of cobra venom factor (purified from *Naja naja* venom[12]) at a dose of 50 units per rat for each of 2 days before the experiment. This treatment regimen results in nearly complete depletion of C3 and does not, *per se*, produce acute lung injury.[5]

(2) Neutrophil depletion was achieved by intraperitoneal injection of rabbit anti-rat neutrophil antiserum (0.5 mL/100 gm body weight) 24 hours before infusion with cerulein or normal saline. Within 18 hours this specific antiserum results in a drastic reduction in peripheral blood neutrophil counts (less than 250 neutrophils/mL). Before infusion, blood samples from each rat were obtained and total neutrophil counts were done. Only those rats with counts of less than 250 neutrophils/mL were used for subsequent experiments.

(3) Superoxide dismutase (SOD; Sigma, St. Louis, MO) was given as an intravenous bolus (20 mg) before each experiment and was followed by a continuous infusion (10 mg/hr) given simultaneously with the cerulein or normal saline infusion.

(4) Polyethylene glycol conjugated SOD (PEG-SOD; Enzon, Fairlawn, NJ) was given as a single intravenous bolus (25,000 μ) before infusion with cerulein or normal saline.

(5) Catalase (CAT; Sigma, St. Louis, MO) was given in a manner similar to SOD (20 mg IV bolus followed by continuous infusion of 10 mg/hr).

(6) PEG-catalase (PEG-CAT; Enzon, Fairlawn, NJ) was given as a single bolus of 25,000 μ IV before infusion with cerulein or normal saline.

(7) Deferoxamine mesylate (Ciba-Geigy, Summit, NJ) was given as a single IV bolus of 5 mg/kg before infusion with cerulein or normal saline.

TABLE 1. *Wet Lung Weights in Rats with Cerulein-Induced Pancreatitis*

Intervention	Wet Lung Weights	p value*
None	0.51 ± 0.01 (32)	—
SOD	0.34 ± 0.01 (5)	$<10^{-6}$
PEG-SOD	0.36 ± 0.01 (6)	$<10^{-6}$
CAT	0.44 ± 0.01 (6)	0.006
PEG-CAT	0.47 ± 0.01 (7)	0.078
Neutrophil depletion	0.43 ± 0.01 (6)	0.002
Deferoxamine	0.55 ± 0.05 (8)	0.209
Dimethylsulfoxide	0.59 ± 0.06 (7)	0.025

Saline-infused control weights = 0.35 ± 0.02.
Wet lung weights expressed as % total body weight (mean ± SEM).
* Statistical analysis by Student's t test; animals with cerulein-induced pancreatitis and antioxidant intervention compared to cerulein-induced pancreatitis with no antioxidant intervention.
SOD, superoxide dismutase.
PEG-SOD, polyethylene glycol conjugated SOD.
CAT, catalase.
PEG-CAT, PEG-catalase.

(8) Dimethylsulfoxide (DMSO; Fisher, Fairlawn, NJ) was given as an intraperitoneal bolus of 1.5 mL per kg before cerulein or normal saline infusion.

Morphologic Studies

For histologic study rats were prepared identically with either cerulein or saline infusion. ^{125}I-BSA injections were omitted. The pancreas was quickly dissected and placed in 4% glutaraldehyde in cacodylate buffer. At sacrifice the lungs were inflated *via* the trachea with 4% glutaraldehyde in cacodylate buffer. At each time point evaluated, at least five animals per group were assessed histologically. After fixation biopsies were taken from each lobe of the lung and embedded in epon. For light microscopy studies 1-μm sections were taken from the plastic blocks and stained with toluidine blue. For electron microscopy, ultrathin sections were cut and processed for ultrastructural analysis using a Philips 401 transmission electron microscope (Philips Co., Amsterdam, The Netherlands). For morphometric analysis, plastic embedded sections (1 μm thick) of lung tissue stained with toluidine blue were prepared as described above. A minimum of 60 different interstitial capillaries and alveoli from each of five rats per group were examined by morphometric techniques using an Olympus microscope with a 40x objective (Olympus, Tokyo, Japan). The numbers of neutrophils per 40x power field were determined for each section. Details of these techniques have been previously published.[7]

Amylase/Lipase Measurement

Serum amylase and lipase were assessed by the clinical chemistry laboratory in the Department of Pathology using the DuPont aca analyzer. Amylase is measured by the hydrolysis of the substrate maltopentose.[13] Lipase is determined by turbidity measurements using triolein as the substrate.[14,15]

Myeloperoxidase Assay

A time-course study to determine the amount of myeloperoxidase in lung tissue was done by infusing rats with cerulein or normal saline for 30 minutes, 3 hours, and 6 hours. At sacrifice washed lung parenchyma was weighed and then homogenized in phosphate buffer. The amount of myeloperoxidase present was determined spectrophotometrically using O-dianisidine dihydrochloride.[16,17]

Results

The body, wet lung, and wet pancreas weights for each rat were obtained. Wet lung and wet pancreas weights

TABLE 2. *Pancreas Microvascular Permeability Indices and Weights, Serum Amylase, and Lipase in Rats with Cerulein-Induced Pancreatitis*

Intervention	Saline-Infused Controls					Cerulein-Induced Pancreatitis				
	PI	Wt	Amylase	Lipase	(n)	PI	Wt	Amylase	Lipase	(n)
None	0.13 ± 0.02	0.30 ± 0.01	549 ± 40	<1	(6)	0.92 ± 0.12†	0.84 ± 0.10†	3516 ± 364†	467 ± 49†	(18)
SOD	0.12 ± 0.01	0.21 ± 0.02	533 ± 107	<1	(6)	0.86 ± 0.17†	0.47 ± 0.05†	7465 ± 959†	816 ± 69*†	(5)
PEG-SOD	0.13 ± 0.02	0.20 ± 0.02	799 ± 112	<1	(5)	0.88 ± 0.21†	0.50 ± 0.04†	2724 ± 158†	346 ± 42†	(5)
CAT	0.11 ± 0.02	0.29 ± 0.01	516 ± 44	<1	(5)	0.23 ± 0.04*	0.35 ± 0.04*	4957 ± 610*†	605 ± 37†	(9)
PEG-CAT	0.11 ± 0.03	0.32 ± 0.01	657 ± 45	<1	(9)	0.93 ± 0.28†	0.90 ± 0.14†	2644 ± 371†	358 ± 37†	(7)
Neutrophil depleted	0.11 ± 0.01	0.31 ± 0.02	942 ± 114	<1	(4)	0.41 ± 0.07*†	0.48 ± 0.01†	4355 ± 414†	306 ± 42†	(6)
Complement depleted	0.17 ± 0.01	—	815 ± 80	<1	(8)	0.28 ± 0.03*†	—	5679 ± 663*†	520 ± 57†	(16)
Deferoxamine	0.07 ± 0.01	0.28 ± 0.01	—	—	(3)	1.02 ± 0.29†	0.96 ± 0.15†	—	—	(8)
Dimethylsulfoxide	0.10 ± 0.01	0.32 ± 0.02	—	—	(12)	0.92 ± 0.08†	0.77 ± 0.07†	—	—	(8)

Wt, pancreatic wet weight expressed as percent total body weight.
PI, Microvascular Permeability Index expressed as pancreas/blood cpm
* $p < 0.05$, comparing groups with cerulein-induced pancreatitis and antioxidant interventions to similar animals with no antioxidant intervention; Student's t test (data expressed as means ± SEM).
† $p < 0.05$, comparing groups with cerulein-induced pancreatitis to appropriate saline controls; Student's t test (data expressed as means ± SEM).
SOD, superoxide dismutase.
PEG-SOD, polyethylene glycol SOD.
CAT, catalase.
PEG-CAT, PEG catalase.

TABLE 3. *Lung Microvascular Permeability Indices in Rats with Cerulein-Induced Pancreatitis*

Intervention	Lung Permeability Index*	p value†
None	0.24 ± 0.04 (32)	—
SOD	0.02 ± 0.05 (5)	0.030
PEG-SOD	0.03 ± 0.04 (5)	0.040
CAT	0.07 ± 0.02 (16)	0.004
PEG-CAT	0.07 ± 0.01 (7)	0.051
Neutrophil depletion	0.02 ± 0.03 (6)	0.001
Complement depletion	0.02 ± 0.04 (15)	0.020
Deferoxamine	0.11 ± 0.06 (8)	0.138
Dimethylsulfoxide	0.35 ± 0.09 (7)	0.221

* Permeability indices reported as normalized values (experimental minus paired saline-infused control). Index calculated as lung/blood cpm (see text for description) (mean ± SEM).

† Statistical analysis by Student's t test, animals with cerulein-induced pancreatitis and antioxidant intervention compared to no antioxidant intervention.

SOD, superoxide dismutase.
PEG-SOD, polyethylene glycol conjugated SOD.
CAT, catalase.
PEG-CAT, PEG catalase.

were expressed as a percentage of total body weight and are summarized for each treatment group. Lung and pancreas gravimetric data are included in Tables 1 and 2.

Microvascular permeability indices are presented in Tables 2 and 3.. The data are expressed as the ratio of lung counts or pancreas counts compared to a reference of counts per 1 mL of blood. This eliminates the variability inherent in injecting small volumes of a tracer and provides data that are intuitively interpretable. A ratio of 0.20, for example, indicates that an equivalent of 20% of the intravascular ^{125}I label in 1.0 mL blood has been sequestered in the extravascular compartment of lung or pancreas. For reference purposes the permeability values obtained in normal, saline-infused, intact rat lungs and pancreas are less than 0.1 to 0.2.[4,18]

Requirement for Neutrophils in Acute Lung Injury After Pancreatitis

Rats not subjected to neutrophil depletion procedures developed significant increases in lung wet weights (Table 1; 0.35% ± 0.02% *versus* 0.51% ± 0.01% of total body weight; $p < 0.05$ compared to saline-infused controls) and in lung microvascular permeability indices (0.30% ± 0.02% *versus* 0.54% ± 0.04%; $p < 0.003$ compared to controls) after cerulein production of acute pancreatitis. Neutrophil depletion was protective against permeability changes and increases in wet weights as shown in Tables 1 and 3. The wet lung weight was significantly lower (0.43% ± .01%, $p = 0.002$) in neutrophil-depleted animals than in neutrophil-intact animals, and the increase in the microvascular permeability index was significantly less (0.02% ± 0.03%, $p = 0.001$) in neutrophil-depleted animals with pancreatitis than in neutrophil-intact animals.

It is important to note that cerulein infusion did result in the development of acute pancreatitis in neutrophil-depleted animals as defined by increases in serum enzyme content, although there was some attenuation in the permeability index in the pancreas (Table 2). Although neutrophil-depleted animals had less edema formation in the pancreas after infusion of cerulein, pancreatic weight and ^{125}I BSA leak were significantly elevated compared to neutrophil-depleted saline-infused controls. The observations that neutrophil depletion prevents lung injury but not the development of acute pancreatitis suggest that the protection against lung injury in the depleted animals results from lack of availability of neutrophils rather than prevention of pancreatic injury.

Evidence for the sequestration of neutrophils in the injured lung after induction of acute pancreatitis by infusion of cerulein is suggested by myeloperoxidase measurements in lung tissue (Fig. 1). Myeloperoxidase is an unequivocal measurement of neutrophil accumulation in tissue. There was a twofold increase in myeloperoxidase extractable from the lung tissue after the induction of acute pancreatitis. It can be calculated on the basis of myeloperoxidase content in rat neutrophils that this represents (as a minimal estimate) the presence in lung of 6.23×10^6 neutrophils per gram of tissue (compared to 2.68×10^6 neutrophils/gram in saline-infused lungs). These findings confirm the presence of neutrophils in injured lungs and support the concept that their presence is related to the induction of lung injury.

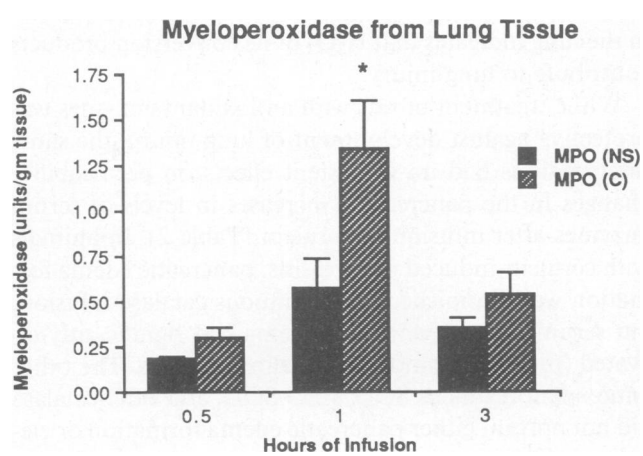


FIG. 1. Lung-tissue myeloperoxidase, a marker for neutrophil accumulation in alveolar capillaries, is illustrated. A significant accumulation of neutrophils occurs after one hour of cerulein infusion. (* $p < 0.05$, Students t test, compared to paired saline control.)

Complement Requirement for Lung Injury After Induction of Acute Pancreatitis

In rats with an intact complement system, cerulein-induced pancreatitis generated an increase in the lung microvascular permeability index (Table 3; 0.24% ± 0.04%) that was significantly different ($p < 0.05$) from values obtained in saline-infused animals. Rats depleted of complement exhibited little evidence of alveolar endothelial-cell injury and microvascular leak after induction of pancreatitis (microvascular permeability index increase 0.02 ± 0.14, $p = 0.02$) compared to complement intact animals with pancreatitis.

It is important to emphasize that pancreatic edema formation (permeability index) was diminished in cerulein-treated animals that were complement depleted (Table 2), but ^{125}I BSA sequestration was still significantly ($p<0.05$) elevated and serum lipase and amylase levels were not reduced as a result of complement depletion in cerulein-infused rats (Table 2). Therefore complement depletion appears protective against lung injury but does not protect against pancreatic acinar-cell injury.

Evidence that Lung Injury Is Dependent on Formation of H_2O_2

Both SOD and PEG-SOD pretreatment prevented lung weight increases and microvascular permeability increases in the lung after cerulein-induced acute pancreatitis (Tables 1 and 3). A similar protective effect was evident for CAT- and PEG-CAT-treated animals, although in PEG-CAT-treated animals the degree of protection was of borderline statistical significance ($p = 0.051$ for lung permeability index, $p = 0.078$ for wet lung weights). Figure 2 illustrates the proposed chemical pathways involved and the points for antioxidant interventions. The observation that catalase protects against interstitial edema formation in the lung indicates that H_2O_2 or its conversion products contribute to lung injury.

While treatment of rats with antioxidant enzymes was protective against development of lung injury, the same interventions had no consistent effects on permeability changes in the pancreas or increases in levels of serum enzymes after infusion of cerulein (Table 2). In animals with cerulein-induced pancreatitis, pancreatic edema formation was diminished by continuous catalase infusion, but serum amylase and lipase remained significantly elevated ($p <0.05$) compared to saline controls. The other antioxidant regimens SOD, PEG-SOD, and PEG-catalase did not prevent either pancreatic edema formation or elevation of serum amylase and lipase but were protective against the lung injury. We have recently inferred that the effects of PEG-conjugated antioxidants are restricted to the intravascular compartment as a result of their large

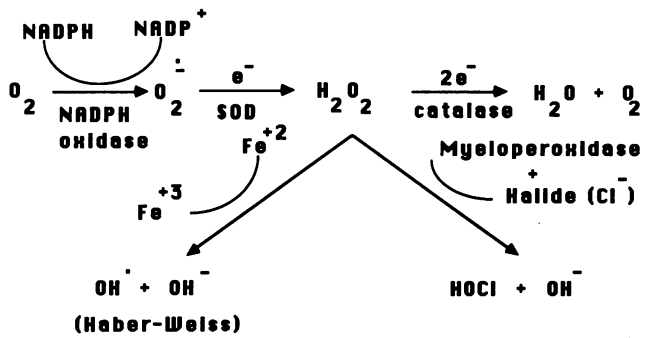


FIG. 2. The sequential reduction of molecular oxygen to H_2O_2 and ultimately $H_2O + O_2$ is illustrated together with the short-lived toxic oxygen-radical intermediates coincidently generated. Sites of action are shown for SOD, catalase, and iron. Note particularly that the hydroxyl radical ($OH^\cdot$) results from the iron catalyzed Haber-Weiss reaction.

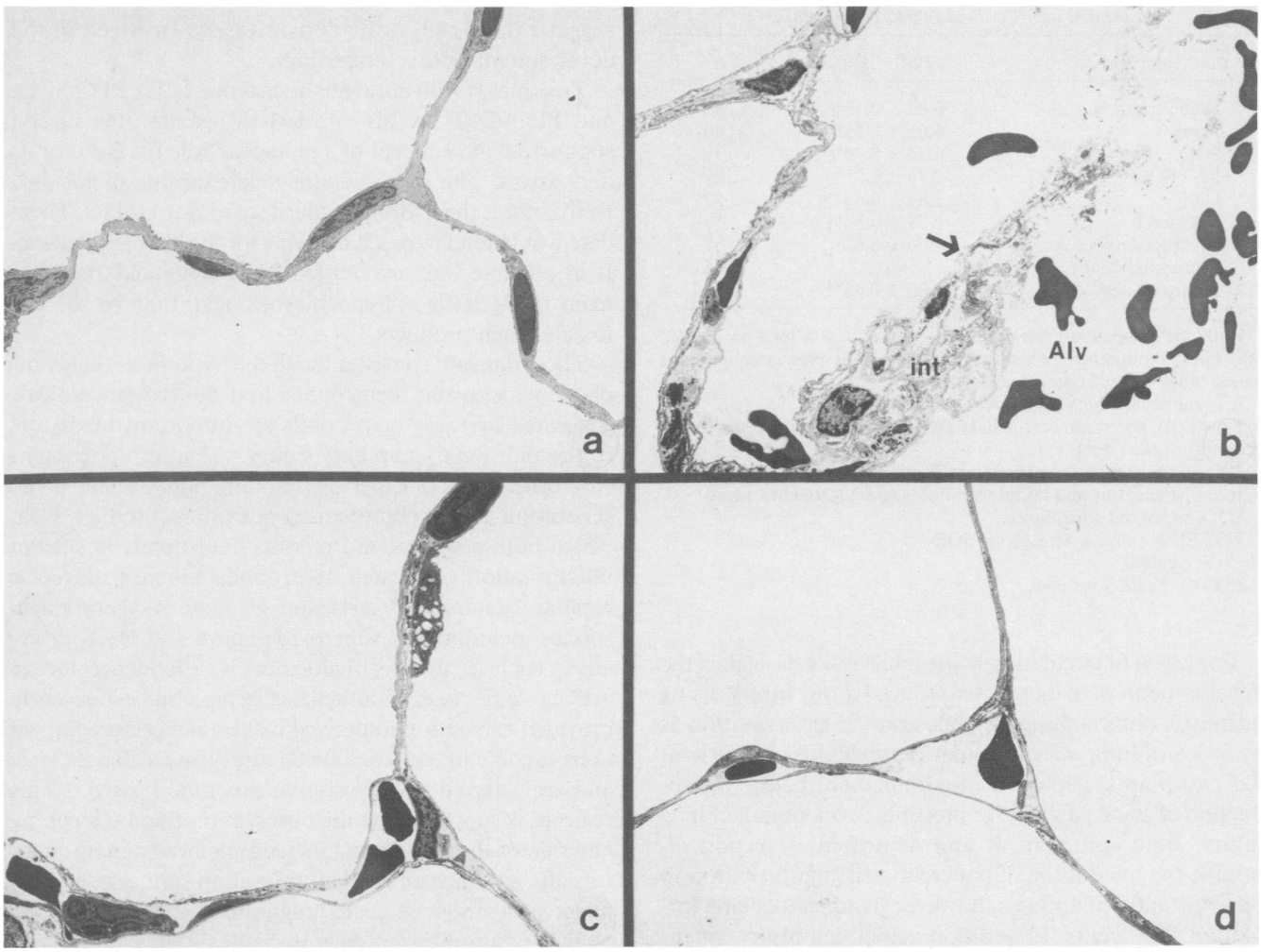
molecular size.[18] Because neutrophils are sequested along interstitial microvascular capillaries of the lung and the damage of endothelial cells is neutrophil dependent, it is possible that in this situation PEG-derived antioxidant enzymes have ready access to oxidant products of neutrophils, whereas in the pancreas the enzymes cannot gain sufficient access to pancreatic acinar cells, which are external to the vascular compartment. These data taken together support the hypothesis that the mediators of lung injury include H_2O_2 or H_2O_2-derived products.

Lack of Iron in Acute Lung Injury

The iron chelator deferoxamine was not protective against the development of acute lung injury or acute pancreatitis. The data in Tables 1 and 3 show that this intervention fails to prevent increased lung weight or microvascular permeability. Furthermore pancreatic edema formation (Table 2) was at least as great in animals treated with deferoxamine. That the scavenger DMSO was not protective against either pancreatitis or acute lung injury is consistent with the failure of deferoxamine to protect against injury if $OH^\cdot$ were involved in this type of injury. These data suggest that microvascular injury to both the pancreas as well as the lung after infusion of cerulein is dependent on H_2O_2, which is presumably derived from neutrophils activated by products of the complement system. The failure of deferoxamine and DMSO to protect implies that the oxygen-dependent species responsible for the injury could be either H_2O_2 or a product of H_2O_2 such as HOCl, which can be generated by neutrophil-derived myleoperoxidase. It is not possible to answer this question by *in vivo* interventions because there are no known methods to inactivate myeloperoxidase *in vivo*.

Morphometric Analysis

Ultrastructural features of the acute lung injury associated with pancreatitis are illustrated in Figure 3. We



FIGS. 3A–D. Photomicrographs (X2200) are shown from a normal saline-infused rat lung (A) compared to a lung from an animal with cerulein-induced pancreatitis (B). (C) and (D) are lung samples taken from rats with cerulein-induced pancreatitis treated with SOD or neutrophil depletion, respectively. Extensive endothelial damage is present in the cerulein-infused rat lung (B, arrow), as well as intra-alveolar hemorrhage (B, Alv), and interstitial edema (B, int). These features of injury are absent in the SOD-treated, neutrophil-depleted, and normal lungs (C, D, A).

Similar attenuation of the injury was found in the other antioxidant-treated animals and complement-depleted animals, but not in deferoxamine- or DMSO-treated groups.

have previously described in nonprotected rats the features in lung of interstitial edema formation, capillary endothelial-cell injury, neutrophil sequestration, and intra-alveolar hemorrhage and fibrin deposition after cerulein-induced pancreatitis.[4] Representative photomicrographs in the neutrophil-depleted and SOD-treated animals show protection against edema formation and capillary endothelial-cell injury. Similar findings of an attenuated injury were obtained in PEG-SOD-, CAT-, and PEG-CAT-treated, and complement-depleted animals. Except in those animals that were depleted of circulating neutrophils, neutrophil sequestration in the alveolar capillaries occurred even in animals that were subjects of protective interventions (Table 4). These findings are consistent with the hypothesis that neutrophil recruitment and adherence to alveolar capillary endothelial cells occurs initially, followed by the *in situ* generation of toxic oxygen products.

Discussion

Acute lung injury associated with acute pancreatitis is a common feature of human disease and can be reproduced experimentally in rats with cerulein-induced pancreatitis. The lung injury shares some clinical and histologic features of ARDS and is believed to result, in part, from activation of the endogenous inflammatory response.[4] The experiments presented above use techniques of neutrophil depletion, complement depletion, and antioxidant treatment to examine the mechanisms by which pancreatitis-induced lung injury occurs.

TABLE 4. *Lung Morphometric Analysis of Rats with Acute Lung Injury Associated with Cerulein-Induced Pancreatitis*

Intervention	PMN/HPF	n
Saline controls	0.83 ± 0.13†	60
None	6.65 ± 0.53*	60
SOD	4.74 ± 0.74*†	38
PEG-SOD	2.75 ± 0.97*†	105
CAT	3.86 ± 0.69*†	52
PEG-CAT	3.59 ± 0.53*†	59
Complement depleted	1.60 ± 0.62†	57
Neutrophil depleted	<0.1	45
Deferoxamine	4.80 ± 0.48*†	68

PMN/HPF, mean number of PMNs observed in 40× high-power field on 1 micron toliudine blue sections (normalized as experimental minus paired saline-infused control values).

n, = number of high power fields counted (40×).

* $p < 0.05$, comparing cerulein-treated animals to saline-infused controls. (Student's t test).

† $p < 0.05$, comparing experimental groups to groups with cerulein-induced pancreatitis and no intervention (None) (Student's t test).

SOD, superoxide dismutase.

PEG-SOD, polyethylene glycol SOD.

CAT, catalase.

PEG-CAT, PEG catalase.

Depletion of circulating neutrophils protects against the development of pancreatitis-induced lung injury using histologic criteria, lung weights, and ^{125}I extravasation as an index of lung microvascular permeability. Using similar end points, depletion of complement before the induction of acute pancreatitis prevents development of lung injury. Both complement and neutrophil depletion diminish the magnitude of pancreatic edema formation in cerulein- infused animals, however significant edema formation still occurs. In addition acinar-cell injury, qualitatively estimated by enzyme (amylase and lipase) release, is not attenuated by neutrophil or complement depletion. Because a pancreatic injury is induced in these experiments but the lung injury is prevented, a role for complement products and neutrophils is inferred in the pathogenesis of the lung injury. Similarly catalase infusion protects against lung injury and attenuates pancreatic edema formation but not acinar-cell enzyme release. This suggests that H_2O_2 or its derivatives are involved in the development of this lung injury.

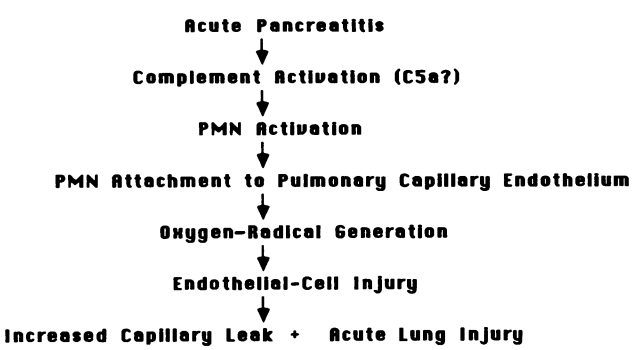


FIG. 4. Proposed sequence of pathogenic events in the development of pancreatitis-induced acute lung injury.

Treatments with antioxidant enzymes SOD, PEG-SOD, and PEG-CAT are also protective against lung injury, supporting the concept of a phlogistic role for H_2O_2 or its derivatives. The iron chelator deferoxamine is not protective nor is the hydroxyl radical scavenger DMSO. These data would tend to preclude a role for the hydroxyl radical. It is possible that myeloperoxidase-generated products from H_2O_2, such as hypochlorous acid, may be the key toxic oxygen products.

These data are consistent with the hypothesis that complement- activated neutrophils and the oxygen radicals generated by these neutrophils are important mediators of the pulmonary capillary injury. A pattern consistent with other types of experimental lung injury includes the generation of the complement activation product, C5a, which both activates and recruits neutrophils to sites of inflammation. Activated neutrophils adhere to alveolar capillary endothelial cells and generate oxygen-radical species including the superoxide anion and H_2O_2 derivatives such as the hypohalous acids.[19] Evidence for an active role of target endothelial cells has also been recently provided.[20] Neutrophil-derived oxygen radicals are known to be capable of endothelial-cell injury and killing *in vitro* and are believed to have similar potential *in vivo*.[19] This concept is supported by the observation that by blocking any one of three elements the complement system, neutrophils, or oxygen-radical formation protects against pancreatitis-induced acute lung injury. This proposed sequence is summarized diagrammatically in Figure 4.

The acute lung injury associated with cerulein-induced pancreatitis in rats appears to be complement, neutrophil, and oxygen-radical dependent. The events related to the process of complement activation and the inflammatory response within the pancreas are yet to be determined.

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