Trypsin and activation of circulating trypsinogen contribute to pancreatitis-associated lung injury

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Hartwig, Werner, Jens Werner, Ramon E. Jimenez, Kaspar Z’graggen, Jörg Weimann, Kent B. Lewandrowski, Andrew L. Warshaw, and Carlos Fernández-del Castillo. Trypsin and activation of circulating trypsinogen contribute pancreatitis-associated lung injury. Am. J. Physiol. 277 (Gastrointest. Liver Physiol. 40): G1008–G1016, 1999.—Pancreatic proteases are secreted in acute pancreatitis, but their contribution to associated lung injury is unclear. Applying models of mild edematous (intrahepatic) and severe necrotizing (intraductal glycodeoxycholic acid) pancreatitis in rats, we showed that both trypsinogen and trypsin concentrations in peripheral blood, as well as lung injury, correlate with the severity of the disease. To isolate the potential contribution of proteases to lung injury, trypsin or trypsinogen was injected into healthy rats or trypsinogen secreted in caerulein pancreatitis was activated by intravenous enterokinase. Pulmonary injury induced by protease infusions was dose dependent and was ameliorated by neutrophil depletion. Trypsinogen activation worsened lung injury in mild pancreatitis. In vitro incubation of leukocytes with trypsinogen showed that stimulated leukocytes can convert trypsinogen to trypsin. In conclusion, this study demonstrates that the occurrence and severity of pancreatitis-associated lung injury (PALI) correspond to the levels of circulating trypsinogen and its activation to trypsin. Neutrophils are involved in both protease activation and development of pulmonary injury.

Acute pancreatitis; proteases; leukocytes

Respiratory failure is a frequent systemic complication of acute pancreatitis. Symptoms varying from mild hypoxia to severe adult respiratory distress syndrome (ARDS) develop in up to 75% of patients with acute pancreatitis (17). Pancreatitis-associated lung injury (PALI) has many clinical and pathological features in common with ARDS seen in other circumstances, such as sepsis or trauma (26). However, the early mechanisms that promote inflammation of the lung and pulmonary microvascular injury in acute pancreatitis remain poorly understood.

Pancreatic proteases and a variety of proinflammatory mediators have been shown to be released into the systemic circulation in acute pancreatitis (33). Both phospholipase A2 (3, 13) and elastase (22) have been implicated in the genesis of lung injury in acute pancreatitis, and there is also experimental evidence from a lung lymph fistula model in sheep that trypsin is capable of inducing pulmonary vascular permeability and leukocyte margination (35). Trypsin may induce pulmonary vascular injury by activating the complement system and by generating leukocyte activation (14). Activated leukocytes and their secretory products, including oxygen radicals, proteases, and cytokines, may subsequently be critical in tissue destruction (12, 27).

Previously, we have shown that not only trypsin but also a large pool of its inactive precursor trypsinogen accumulate in the extracellular pancreatic interstitium in mild caerulein-induced pancreatitis (25). Trypsinogen may be activated in large quantities within the pancreas, thus playing a central role in the progression from mild edematous to severe hemorrhagic pancreatitis (7), but may also be released into the systemic circulation. In addition to the classic activation of trypsinogen by enterokinase, trypsinogen is capable of self activation (19, 28) and can also be activated by lysosomal enzymes (8). We hypothesize that trypsinogen may be activated in the systemic circulation or in distant organs during acute pancreatitis and that this activation increases the systemic and local concentrations of trypsin, thereby intensifying its deleterious effects.

The purpose of this study was to investigate whether the release of trypsinogen and trypsin correlates with the severity of acute experimental pancreatitis and its associated lung injury and whether active enzyme and intrasystemic activation of the proenzyme contribute to the development of PALI. Both trypsinogen and its activation to trypsin were determined by a well-established ELISA, specifically measuring the small and stable trypsinogen activation peptide (TAP) that is cleaved in equimolar amounts during conversion of trypsinogen to active trypsin (7, 24).

Materials and Methods

Animals

Experiments were performed in male Sprague-Dawley rats weighing 300–350 g. Care was provided in accordance with the procedures outlined in the Guide for Care and Use of Laboratory Animals, published by the National Institutes of Health. The study was approved by the subcommittee on animal research at our institution. Animals were fasted overnight before the experiments but were allowed free access to water.

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Anesthesia and Catheter Placement

Surgical anesthesia was induced with vaporized ether and maintained by an intramuscular injection of pentobarbital sodium (20 mg/kg; Anthony Products, Arcadia, CA) and ketamine (40 mg/kg; Parke-Davis, Morris Plains, NJ). Two polyethylene catheters (0.5 mm ID) were inserted into the left carotid artery and the right internal jugular vein, respectively, tunneled subcutaneously to the supraclavicular area, and exited through a steel tether that allowed the animals free movement.

Study Design

Association of trypsinogen release and activation with PALI. Animals were randomly allocated to a control group (n = 6) or to one of two groups of pancreatitis: mild edematous (n = 24) or severe necrotizing disease (n = 24). Mild pancreatitis was induced by intravenous infusion of the synthetic CCK analog caerulein (5 µg·kg
\(^{-1}·h
\(-1\)) Takus, Farmitalia, Carlo Erba, Freiburg, Germany) over 6 h (20). Caerulein was reconstituted in normal saline and infused at 3 ml·kg
\(^{-1}·h
\(-1\)). Necrotizing pancreatitis was induced as follows and is described in detail elsewhere (31). Briefly, the biliopancreatic duct was cannulated with a 24-gauge Teflon catheter (Critek, Tampa, FL), and bile as well as pancreatic juice were drained by gravity for 5 min with the common hepatic duct clamped at the porta hepatitis. Glycodeoxycholic acid (GDOC; Sigma, St. Louis, MO) in glycyglycine-NaOH buffered solution (pH 8.0, room temperature) was infused retrograde into the biliopancreatic duct at a concentration of 10 mmol/l in a volume (1.2 ml/kg)-time (10 min)- and pressure (30 mmHg)-controlled fashion, followed by an infusion of caerulein over 6 h. Control animals received saline infusions alone (3 ml·kg
\(^{-1}·h
\(-1\)).

Arterial blood was collected at baseline and at 1, 2, 3, 6, 12, and 24 h. Blood from the portal vein was collected before death. Trypsinogen and trypsin, assessed by TAP, were measured in all samples. To determine the time course of pancreatic and pulmonary injury in mild and severe pancreatitis, animals were killed at 3, 6, 12, and 24 h (n = 6 per group, respectively). The pancreas and lungs were excised after death to assess injury as determined by wet-to-dry weight ratio, MPO activity, and histology.

Isolation of the contribution of proteases to lung injury. To isolate the potential contributions of pancreatic proteases to lung injury, we infused trypsin or trypsinogen intravenously into rats. Bovine trypsin (Sigma) was infused at 0.75, 1.5, and 3.0 mg·kg
\(^{-1}·h
\(-1\)) for 6 h or until death (n = 6 per group). The infusions were divided into 3 aliquots/h, and each aliquot was freshly reconstituted in saline before injection to minimize autodeactivation of trypsin. One group of animals was injected with a combination of trypsin (3.0 mg·kg
\(^{-1}·h
\(-1\)) and soybean trypsin inhibitor (3.0 mg·kg
\(^{-1}·h
\(-1\)) and served as an additional control group. Bovine trypsinogen (Sigma) was infused at 4.5, 9.0, and 27 mg·kg
\(^{-1}·h
\(-1\) for 6 h (n = 6 per group) and was also divided into 3 injections/h to minimize autoactivation before injections. Trypsin activity was determined in injection solutions to control for impurity or autoactivation of trypsinogen. Saline alone was injected in controls (n = 6). In all animals, TAP concentrations were measured in arterial blood collected at 3 and 6 h. The pancreas and lungs were removed after animals died or were killed, and pancreatic and pulmonary injury were assessed by wet-to-dry weight ratio, MPO activity, and histology.

One group of animals was rendered neutropenic to investigate if the pathogenesis of protease-induced lung injury is neutrophil dependent. To induce neutropenia, antiserum to rat polymorphonuclear neutrophils (250 µl; Accurate Chemical and Scientific, Westbury, NY) was injected intravenously 24 h before trypsin infusions. Neutrophil depletion was confirmed by blood neutrophil count. Trypsin was infused at 3.0 mg·kg
\(^{-1}·h
\(-1\)) and, animals were killed at 5 h. This allowed comparison of tissue damage in neutrophil-depleted and -nondepleted animals with equal trypsin infusions.

To further characterize the potential deleterious effects of systemic trypsinogen activation in acute pancreatitis, we activated trypsinogen released during caerulein-induced pancreatitis by simultaneous intravenous infusion of enterokinase (Sigma). Enterokinase infusions (30 U·kg
\(^{-1}·h
\(-1\)) were started 2 h after the start of caerulein infusions, when significantly increased trypsinogen levels in blood are known to be present. Enterokinase or caerulein alone was infused in control groups. Blood and tissue parameters, as described in isolation of the contribution of proteases to lung injury, were measured.

Trypsinogen activation by leukocytes. Leukocytes isolated from rat blood were incubated with Hanks’ balanced salt solution (HBSS, control group), phorbol myristate acetate (PMA, 10
\(^{-6}\) mol/l), trypsinogen (10
\(^{-6}\) mol/l), and the combination of PMA and trypsinogen (all from Sigma). PMA was used for leukocyte activation. All substances were dissolved in HBSS. Leukocytes were incubated for 20 min at room temperature and subsequently chilled on ice. After centrifugation of samples (500 g, 5 min, 4°C), the supernatant was removed and stored at −20°C until assayed for TAP.

TAP and Trypsinogen Measurements

TAP specifically reflects the conversion of trypsinogen into trypsin by cleavage of the TAP fragment. Blood samples (1.0 ml) for the measurement of TAP and trypsinogen concentrations were collected in 0.2 mol/l EDTA to inactivate peptides. After centrifugation of samples (500 g, 10 min, 4°C), the remaining serum was divided in half (for TAP or trypsinogen measurement), coded, and stored at −20°C until assayed. TAP concentrations were measured in a blinded fashion by ELISA (7). Synthetic TAP (YD4K), a conjugate of rabbit serum albumin with YD4K, and rabbit anti-TAP antiserum containing calcium-independent anti-TAP antibodies were generously provided by Prof. J. Hermon-Taylor (St. George’s Hospital Medical School, London, UK). Biotin-conjugated goat anti-rabbit IgG antibody and alkaline phosphatase-labeled streptavidin were purchased from Sigma. This assay has a very close correlation to the previously reported radioimmunoassay described by Hurley et al. (16).

Trypsinogen concentrations were measured by assaying TAP after complete activation of trypsinogen by enterokinase as described elsewhere (24). Briefly, a sample of serum was incubated with chromatographically purified porcine enterokinase (Sigma) at a final concentration of 10 U/ml for 45 min at room temperature. The polypeptide tetra-L-aspartyl-L-lysine (TAP), which is released in equimolar quantities when enterokinase cleaves trypsinogen, was then measured by ELISA. Trypsinogen concentrations were calculated as the difference between the TAP concentrations in the enterokinase-incubated sample and the nonincubated sample. This indirect measurement of trypsinogen has been shown to represent a specific and sensitive method for quantitation of trypsinogen (24). TAP and trypsinogen concentrations in blood are expressed as nanomoles per liter.

Measurement of Trypsin Activity

Trypsin activity in trypsinogen and trypsin solutions used for injections were measured spectrophotometrically using...
p-toluenesulfonyl-L-arginine methyl ester (Sigma) as substrate (15).

**Edema Assessment**

Pancreatic and pulmonary edema were evaluated by measuring the wet-to-dry weight ratio. A portion of the pancreatic tail and the right upper lobe of the lungs were removed immediately after death, trimmed of fat, and weighed. The water content was determined from the initial weight (wet weight) and its weight after incubating at 160°C for 24 h (dry weight).

**MPO Activity**

Excised pancreatic and pulmonary tissues were rinsed with saline, blotted dry, shock frozen in liquid nitrogen, and stored at −80°C until thawing for determination of MPO activity. MPO activity was measured as previously described (40) and is expressed as units per milligram of wet weight of tissue.

**Histopathological Evaluation**

Histopathological evaluation of pancreatic and pulmonary injury was performed by a pathologist in a blinded fashion. Briefly, the head of the pancreas was removed, fixed in 10% phosphate-buffered formalin, and embedded in paraffin. One coronal section was made in the plane of the flattened pancreas and stained with hematoxylin and eosin. Edema, hemorrhage, inflammation, and acinar necrosis were evaluated using a scoring system (degree of injury: 0–3, 0 = no injury and 3 = worst injury) (30).

Histopathological evaluation of lung injury was performed in the formalin-inflated left upper lobe of lung. After pneumectomy, the bronchus was cannulated using a polyethylene catheter (1.0 mm ID), and 10% phosphate-buffered formalin was infused at a pressure of 30 cmH₂O until the lung distended homogeneously. After fixation for at least 36 h, the lung was dissected sagitally in the plane of the main bronchus, and both parts were embedded in paraffin. Histological sections for evaluation were made in such a way that one central and one peripheral section of the lung were obtained. Sections were stained with hematoxylin and eosin.

**Leukocyte Separation**

Leukocytes for in vitro experiments were isolated from blood of Sprague-Dawley rats. Blood was collected by cardiac puncture and mixed with 6% dextran. The mixture was kept at room temperature for 45 min to allow sedimentation of erythrocytes. The leukocyte-rich supernatant was separated and centrifuged (400 g, 5 min, room temperature). The resulting pellet was resuspended in lysing solution (FACS lysing solution; Becton Dickinson Immunocytometry Systems, San Jose, CA) to clear out remaining erythrocytes. Leukocytes were then washed and resuspended in HBSS. A final leukocyte concentration of 15–20 x 10⁶/ml was obtained. Trypan blue exclusion showed a viability of >95%.

**Statistical Analysis**

Data are presented as means ± SE. Statistical analysis was performed by using ANOVA to show an overall difference between the groups. The paired and unpaired Student’s t-tests were used to make pairwise comparisons of normal distributed parameters, and the Mann-Whitney rank sum test was used for parameters without normal distribution. A 5% probability of type I experimental error (P < 0.05) was considered statistically significant.

**RESULTS**

**Association of Trypsinogen Release and Activation with PALI**

Trypsinogen and TAP in serum. Trypsinogen and TAP concentrations in arterial blood increased early in edematous and necrotizing pancreatitis, with their maximal values at 3–6 h (Fig. 1). Both trypsinogen and TAP were higher in severe than in mild disease (both P < 0.001 by ANOVA). In severe pancreatitis, trypsinogen and TAP concentrations were significantly higher in portal vein blood than in arterial blood (P = 0.02 and P = 0.05, respectively, by ANOVA; data not shown).

Pancreatic and pulmonary edema. Confirming published results (40), pancreatic edema as assessed by wet-to-dry weight ratio was significantly greater in caerulein than in GDOC pancreatitis (P = 0.005 by ANOVA; Fig. 2A). Pancreatic edema was maximal in both models at 6 h. In contrast, significant pulmonary edema was present in severe pancreatitis (Fig. 2B) but not in mild pancreatitis. Development of pulmonary edema occurred at a later time point than peak pancreatic edema.

![Fig. 1. Trypsinogen (A) and trypsinogen activation peptide (TAP; B) in arterial blood of animals with mild (caerulein-induced) and severe (glycodeoxycholic acid (GDOC)-induced) pancreatitis. *P < 0.05, †P < 0.01, and ‡P < 0.001 vs. caerulein.](http://aiapl.physiology.org/)}
Pancreatic and pulmonary MPO activity. Pancreatic leukocyte sequestration, as assessed by MPO activity, was increased in mild and severe pancreatitis as a function of time, with maximal levels at 24 h (Fig. 3A). MPO activity in the lungs increased significantly in mild pancreatitis at 6 and 12 h compared with controls, whereas it increased in severe pancreatitis from 3–24 h. Levels of pancreatic and pulmonary MPO activity were higher in severe pancreatitis than in mild pancreatitis (both \( P < 0.001 \) by ANOVA; Fig. 3).

Histopathological evaluation. Pancreatic specimens of animals with caerulein-induced pancreatitis showed marked edema and moderate interstitial inflammation. Only minor cellular necrosis was observed. Severe pancreatitis showed marked necrosis, leukocyte infiltration, and hemorrhage. Necrosis and inflammation were significantly higher in GDOC-induced than in caerulein-induced pancreatitis (both \( P < 0.001 \) and \( P = 0.04 \), respectively; data not shown). Control animals showed normal pancreatic morphology.

Lungs of animals with mild pancreatitis showed only minor morphological evidence of injury compared with controls (Fig. 4A). In contrast, lungs of animals with severe pancreatitis showed focal atelectasis, perivascular edema, interstitial neutrophil infiltration (Fig. 4B), and proteinaceous debris in the alveolar space.

Isolation of the Contribution of Proteases to Lung Injury

Trypsin infusions. In both pancreas and lungs, wet-to-dry weight ratios and leukocyte sequestration increased with higher amounts of trypsin infused (Table 1). Histopathological evaluation of the pancreas showed moderate edema but no significant leukocyte infiltration, hemorrhage, or necrosis. Pulmonary edema was localized mainly in the perivascular space, but hemorrhage occurred interstitially and intra-alveolarly (Fig. 4C). Pathological changes also increased in severity with trypsin infusions of higher concentrations.

When soybean trypsin inhibitor was injected in combination with trypsin, the degree of injury in both pancreas and lungs was reduced compared with trypsin infusions alone (Table 1).

Animals rendered neutropenic showed a decrease of blood neutrophils to \(<150/\text{mm}^3\). During trypsin infu-
sions, none of the neutrophil-depleted animals died before they were killed at 5 h. Pulmonary edema was significantly reduced in depleted animals compared with nondepleted animals with equivalent amounts of trypsin infusions (Table 1). As expected, leukocyte sequestration was decreased due to neutrophil depletion.

Trypsinogen infusions. Only minimal trypsin activity was present in trypsinogen solutions used for injections. Trypsin activity was <2% (1.3 ± 0.3%) of those with equivalent amounts of trypsin. Similar to trypsin-infused animals, MPO activity in the lungs increased as a function of infused trypsinogen concentrations (Table 1). Likewise, pulmonary edema was increased, particularly with high trypsinogen infusions. Although pancreatic edema was present only in the group with infusion of 27.0 mg·kg\(^{-1}\)·h\(^{-1}\) trypsinogen, MPO activity in the pancreas was increased in all groups (Table 1). Representing trypsinogen activation, TAP levels in serum were increased in a dose-dependent manner and were higher at 6 h than at 3 h (Table 1). Histopathological findings in pancreata and lungs resembled those in trypsin infusions.

Comparison of trypsin and trypsinogen-infused animals indicates that the small amount of active trypsin in trypsinogen preparations is insufficient to explain the induction of pancreatic and pulmonary injury (Table 1). At <2% impurity, five times as much trypsinogen as that used would be required to provide enough trypsin to cause the observed changes.
Caerulein/enterokinase infusions. Trypsinogen released in mild caerulein-induced pancreatitis was activated by simultaneous intravenous enterokinase infusion. This resulted in markedly increased TAP concentrations in blood compared with caerulein or enterokinase infusions alone (Table 2). Also, pulmonary edema and MPO activity were increased in animals receiving combined caerulein-enterokinase infusions compared with those receiving caerulein or enterokinase alone. In the pancreas, MPO activity increased significantly with caerulein-enterokinase, but wet-to-dry weight ratio was maximal with caerulein infusions alone (Table 2). Histology showed moderate hemorrhage and acinar cell necrosis in pancreata of animals with combined caerulein-enterokinase. Pancreatic edema and leukocyte infiltration were similar to animals with caerulein infusions alone. In the lungs, marked perivascular edema and neutrophil infiltration were present with caerulein-enterokinase infusions (Fig. 4D) but not with caerulein or enterokinase alone.

**Trypsinogen Activation by Leukocytes**

Leukocytes incubated with HBSS or PMA alone showed no significant TAP generation (Fig. 5). Minimal amounts of TAP were generated when leukocytes were incubated with trypsinogen alone. Significantly increased concentrations of TAP were detected in incubations of PMA-activated leukocytes with trypsinogen (Fig. 5).

### DISCUSSION

Pancreatic proteases are among the multiple mediators that have been implicated in the pathogenesis of PALI, a common complication of severe pancreatitis that may progress to respiratory failure and ARDS (13, 22, 35). Neutrophil infiltration and loss of integrity of the alveolocapillary membrane with resulting pulmonary edema are characteristic of this injury (37, 38), and strong evidence has been found that the secretory products of neutrophils play an essential role in its evolution (12, 27). However, evidence for an initiating or dominant event in the development of remote organ injury in acute pancreatitis is still lacking.

Trypsin, the main pancreatic protease, may contribute to the systemic inflammatory response by activating the complement system (1, 14). As shown in the present study, release of both active and precursor pancreatic proteases into the systemic circulation is an early event in the course of acute pancreatitis, and both activated trypsin and uncleaved trypsinogen are released in proportion to the severity of pancreatitis. Similar findings have been described in human acute pancreatitis, and therefore these parameters may have value in determining the severity of the disease (11, 18, 29). By sampling blood at different locations, we showed higher trypsinogen and TAP concentrations in portal vein blood than in arterial blood, demonstrating that the portal vein is a major pathway by which trypsin and its derivatives enter the systemic circulation.

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### Table 1. Survival time, pancreatic/pulmonary edema and myeloperoxidase activity, and trypsinogen activation peptide in blood of animals with trypsin and trypsinogen infusions

<table>
<thead>
<tr>
<th>Survival Time</th>
<th>Edema, Wet-to-Dry Weight Ratio</th>
<th>Myeloperoxidase Activity, U/mg</th>
<th>TAP Serum, nmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pancreas</td>
<td>Lung</td>
<td>3 h</td>
</tr>
<tr>
<td>Control</td>
<td>&gt;6</td>
<td>3.9 ± 0.2</td>
<td>5.2 ± 0.1</td>
</tr>
<tr>
<td>Trypsin</td>
<td>&gt;6</td>
<td>3.8 ± 0.2</td>
<td>5.7 ± 0.2</td>
</tr>
<tr>
<td>0.75 mg·kg⁻¹·h⁻¹</td>
<td>&gt;6</td>
<td>3.6 ± 0.5</td>
<td>7.0 ± 0.4†</td>
</tr>
<tr>
<td>1.5 mg·kg⁻¹·h⁻¹</td>
<td>&gt;6</td>
<td>4.9 ± 0.2</td>
<td>6.9 ± 0.5†</td>
</tr>
<tr>
<td>3.0 mg·kg⁻¹·h⁻¹ + STI</td>
<td>5.0 (killed)</td>
<td>6.1 ± 0.2</td>
<td>6.6 ± 1.6</td>
</tr>
<tr>
<td>3.0 mg·kg⁻¹·h⁻¹ + neutrophil depletion</td>
<td>5.0 (killed)</td>
<td>5.3 ± 0.7</td>
<td>5.9 ± 0.25</td>
</tr>
<tr>
<td>Trypsinogen</td>
<td>&gt;6</td>
<td>3.9 ± 0.2</td>
<td>5.8 ± 0.2</td>
</tr>
<tr>
<td>4.5 mg·kg⁻¹·h⁻¹</td>
<td>&gt;6</td>
<td>4.3 ± 0.2</td>
<td>6.7 ± 0.1†</td>
</tr>
<tr>
<td>9.0 mg·kg⁻¹·h⁻¹</td>
<td>&gt;6</td>
<td>5.7 ± 0.6†</td>
<td>6.4 ± 0.1*</td>
</tr>
<tr>
<td>27.0 mg·kg⁻¹·h⁻¹</td>
<td>&gt;6</td>
<td>3.2 ± 0.3</td>
<td>5.1 ± 0.1</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 and †P < 0.001 compared with control. §P < 0.05 compared with control.}

### Table 2. Pancreatic/pulmonary edema, myeloperoxidase activity, and TAP in blood of animals with caerulein, enterokinase, and combined caerulein-enterokinase infusions

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Edema, Wet-to-Dry Weight Ratio</th>
<th>Myeloperoxidase Activity, U/mg</th>
<th>TAP Serum, nmol/l</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Pancreas</td>
<td>Lung</td>
<td>3 h</td>
</tr>
<tr>
<td>Caerulein</td>
<td>14.0 ± 1.8</td>
<td>5.2 ± 0.1</td>
<td>11.2 ± 2.1</td>
</tr>
<tr>
<td>Enterokinase</td>
<td>3.2 ± 0.3</td>
<td>5.1 ± 0.1</td>
<td>12.4 ± 2.9</td>
</tr>
<tr>
<td>Caerulein + Enterokinase</td>
<td>11.5 ± 1.6</td>
<td>6.0 ± 0.2*</td>
<td>18.1 ± 3.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE. *P < 0.05 and †P < 0.001 compared with caerulein.
zymogen are released into the systemic and eventually into the pulmonary circulation.

In the present study, measurement of TAP by a well-established ELISA was used to determine both trypsinogen release and trypsin generation (16, 24). Other currently available methods for measurement of concentration and/or activity of either trypsinogen or trypsin in vivo are inaccurate and erratic. Immunoassays based on specifically raised antisera cannot distinguish between proenzyme, active trypsin, and antiprotease-bound trypsin (2, 4, 6, 10, 18), and enzymatic assays for trypsin determination are affected by antiproteases present in biological systems (15, 36). Measurement of TAP by ELISA does not have these shortcomings and thus is preferable for assessing both trypsinogen and its activation to trypsin.

Systemic trypsinogen and TAP concentrations not only correlated with the severity of pancreatitis but also with the severity of lung injury. In caerulein pancreatitis, pathological findings in the lungs were minimal. MPO activity was only temporarily increased, and pulmonary edema and histopathologically assessed neutrophil infiltration or hemorrhage were absent, which is in agreement with published reports (1). However, pulmonary edema and ARDS-type changes like atelectasis, neutrophil infiltration, and proteinaceous debris in the intra-alveolar space were found in severe necrotizing pancreatitis.

To investigate if pancreatic proteases are mediators critically contributing to the development of this injury, we isolated their effects from other unknown agents of injury released in acute pancreatitis by infusing trypsin or trypsinogen. Both trypsin and trypsinogen caused dose-dependent pulmonary and pancreatic injury, which was reduced by antiprotease treatment. In agreement with our results using rats, previous studies using a lung lymph fistula model in sheep showed that infusion of trypsin is followed by increased pulmonary endothelial permeability and leukocyte margination (9, 35). Because trypsinogen injections, which possessed only minor inherent proteolytic activity, caused similar pulmonary and pancreatic injury to that of trypsin, we assume it must be activated to contribute to the development of pulmonary and pancreatic injury. Supporting this hypothesis, we found that concentrations of TAP increased in proportion to the dose of trypsinogen administered.

When we activated trypsinogen released during mild caerulein-induced pancreatitis by simultaneous intravenous infusion of enterokinase, pancreatic and pulmonary injury were markedly increased compared with animals infused with caerulein or enterokinase alone. This observation provides further evidence that activation of circulating trypsinogen becomes a major contributor to the development of PALI as well as to the progression of pancreatitis itself. Mechanisms by which trypsinogen may be activated systemically include autoactivation or activation by trypsin (19, 28). Trypsinogen activation has also been shown by lysosomal hydrolases (8). Because hydrolases and proteases are secretory products of neutrophils and macrophages (32), we hypothesized that sequestrated activated leukocytes may account for intrapulmonary trypsinogen activation in severe pancreatitis. By incubating leukocytes with trypsinogen in vitro, we showed higher trypsin generation using activated as opposed to nonactivated leukocytes. To our knowledge, this observation has not been described before. The exact mechanisms involved in trypsinogen activation by leukocytes and in severe pancreatitis will require further investigation.

Neutrophils have been implicated in the pathogenesis of ARDS (27). However, it is unclear whether the interactions between pancreatic proteases and leukocytes play an essential role in the development of PALI or if other proinflammatory mediators are critically involved. In this study, pulmonary neutrophil sequestration occurred both in animals with acute pancreatitis and in animals infused with trypsin or trypsinogen, and neutrophil depletion ameliorated lung injury from trypsin infusion. This finding is consistent with a synergistic interaction between trypsin and neutrophils in the pathogenesis of PALI. Although the contribution of other inflammatory mediators cannot be excluded, this model indicates that trypsin and neutrophils play a significant and perhaps sufficient role. Both events appear to occur early in the course of the disease and may be responsible for the progression to pancreatic necrosis as well as triggering the systemic complications in acute pancreatitis.

Possible mechanisms by which trypsin generates tissue injury may involve the complement system. Trypsin has been demonstrated to cleave complement components (41), generating peptides with chemotactic and neutrophil-activating characteristics. Proteases and oxygen metabolites derived from activated neutrophils may subvert tissue-protecting protease inhibitors, allowing proteolytic enzymes to exert their destructive effects (39). This loop is manifested by systemically reduced antiprotease levels in acute pancreatitis (21, 23), increased quantities of hydrogen peroxide in the breath of ARDS patients (34), and above-normal amounts of oxidized antiproteases in their lung lavage fluids (5).
In summary, we have shown that trypsinogen and trypsin are released from the pancreas in direct correlation to the severity of pancreatitis and that the development and severity of pancreatitis-associated lung injury correspond to the protease levels in the systemic circulation. Both the critical role for trypsin and systemically activated trypsinogen in the pathogenesis of PALI and the interaction between proteases and neutrophils in its manifestation have been demonstrated. Although multiple proinflammatory mediators acting in a synergistic fashion likely contribute to the development of lung injury associated with acute pancreatitis or other disease processes, pancreatic proteases may be one of the critical factors leading to the early development and high incidence of respiratory failure frequently observed in acute pancreatitis in humans.

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REFERENCES


