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THORACIC DUCT LIGATION IN THE RAT ATTENUATES LUNG INJURIES IN ACUTE PANCREATITIS

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ABSTRACT

In acute pancreatitis (AP), inflammatory cells and products disseminated in abdominal lymph and blood induce systemic inflammation. Interruption of abdominal lymph flow, and thereby reduction of lymphatic dissemination, could alter the course of the disease. Therefore, we investigated whether thoracic duct ligation (TDL) in a rat model of cerulein-induced AP results in reduced lung damage as a marker for reduction of systemic dissemination through the lymphatic system. Thirty-four male rats were assigned to TDL (TDL-rats, n=8), AP (AP-rats, n=8), TDL+AP (TDL+AP-rats, n=9) or sham TDL (Ctr-rats, n=9) groups. TDL and sham TDL were established first. Two days later, AP was induced in AP- and TDL+AP-rats by a series of subcutaneous injections of cerulein. Vehicle was injected in the same manner in Ctr- and TDL-rats as controls. Rats were sacrificed six hours after the end of the serial injections. Histological examination showed that AP-induced damage to the pancreas and ileum were similar in AP- and TDL+AP-rats whereas lung damage was less severe in TDL+AP-rats than in AP-rats. Assays demonstrated that: hepatic and pulmonary myeloperoxidase activities were increased in AP-rats but not in the TDL+AP-rats; more Il-6 was found in AP-rat than TDL+AP-rat lungs; and lung-lavage fluid from AP-rats yielded more angiopoietin-2 than TDL+AP-rats. In conclusion, prior TDL in the rat attenuates lung damage in acute pancreatitis.

Keywords: acute pancreatitis, lung injury, rat, cerulean, thoracic duct ligation, lymph

In acute pancreatitis (AP), pancreas-derived inflammatory cells and products disseminated in abdominal lymph and blood induce systemic inflammatory response syndrome (1,2). As this syndrome involves the intestines, bacteria residing there are also transported to other organs by abdominal lymph and blood (3,4). Thus, interruption of abdominal lymph flow may change the course of AP. In fact, this possibility has been tested in previous studies. For instance, when the thoracic duct was drained in patients with AP, beneficial effects were seen in some, but not all, of the subjects (5,6). In the rat, abdominal lymph flow can be disrupted by thoracic duct ligation (TDL) or by ligation of the mesenteric lymphatic duct (7,8). In previous studies, these procedures either caused AP or attenuated AP that was induced by other pathogens (7,8). Thus, whether abdominal lymph modifies AP remains unresolved. In the present study, we revisited this question in the rat model focusing on the disease in the early stages.

METHODS

In Vivo Experiments

This study was approved by the Committee for Animal Ethics in our institution (2009/11). Male Wistar rats (250±50 g) were obtained from the Chinese Academy of Medical Sciences (Beijing, China). After acclimation, rats were randomly assigned to TDL (TDL-rats, n=8), AP (AP-rats, n=8), TDL+AP (TDL+AP-rats, n=9) or sham TDL (Ctr-rats, n=9) groups. TDL was performed in rats anesthetized by chloral hydrate (300 mg/kg, i.p.) following the methods described by Müller et al (7). In brief, a midline incision was made to open the abdominal cavity. Following a medial visceral rotation, part of the parietal peritoneum covering the left kidney was exposed and a small incision was made in the peritoneum under which the thoracic duct was identified and ligated cephalad to the cisterna chyli. In Ctr- and AP-rats, laparotomy was performed as sham TDL.

Two days after TDL and sham TDL, cerulein (Sigma, Shanghai, China) was dissolved in 0.9% NaCl and injected subcutaneously in AP- and TDL+AP-rats at 50 µg per kilogram of body weight. The injections were administered hourly for five hours. In addition, vehicle was injected in Ctr- and TDL-rats for controls. Six hours after the last serial injection, rats were anesthetized and sacrificed by exsanguination. A silicon tube was then placed into the right bronchus through which 2 milliliters of normal saline were injected into the right lung. The fluid was retained for five minutes and withdrawn through the same tube. The lavage was repeated and the total lavage fluid was pooled (4 ml/rat). Pancreas, liver, terminal ileum, and the left lung were removed, organs cut in half, and either stored at -70°C or fixed in 5% formaldehyde.

Assays and Statistics

Formaldehyde-fixed organs were embedded in paraffin, sectioned (5 µm thick), and stained with hematoxylin and eosin (H&E). Total protein concentrations were determined using a kit (A045) from Jiancheng Bioengineering Institute (Nanjing, China). To measure myeloperoxidase (MPO) activity, frozen liver and lung specimens were homogenized in ice-cold buffer and centrifuged (3,000 g, 30 min). Pellets were suspended in phosphate-buffered saline with 0.5% hexadecyl trimethyl ammonium bromide, incubated at 60°C for 60 min., centrifuged again (3,000 g, 10 min), and finally the supernatants collected. MPO activities were determined using a kit from Jiancheng Bioengineering Institute (A044). Samples were incubated in a 96-well plate with kit reagents for 30 min. Then the chromogen was added, incubated for 10 min., and read with a spectrophotometer at 460 nm. MPO values were normalized against protein content of the same samples. Interleukin-6 (Il-6) and tumor necrosis factor-α (TNF-α) were determined in lung homogenates using ELISA kits from Cusabio Biotech (Wuhan, China). Protein content in lung-lavage fluids was determined using the Jiancheng Bioengineering Institute kit. Interleukin-1 (Il-1), angiopoietin-2 (Ang-2), and intercellular adhesion molecule-1 (ICAM-1) were determined in lung-lavage fluids using ELISA kits from Cusabio Biotech (Wuhan, China).

Data were expressed as means ± SEM. Statistical differences were tested using analysis of variance followed with the Tukey HSD post-hoc test with p values less than 0.05 regarded as significant.

RESULTS

Compared to the normal pancreas in Ctr-rats (*Fig.1a*), pancreas of TDL-rats showed both normal tissue (*Fig. 1b*, upper) and tissue with moderate edema (*Fig. 1b*, lower). AP induced similar changes in pancreas of AP- and TDL+AP-rats. These alterations included severe edema and infiltration of white blood cells (*Fig. 1c*).

The ileum of TDL-rats showed dilated lymphatic vessels and thickened villi *(Fig. 2b*)

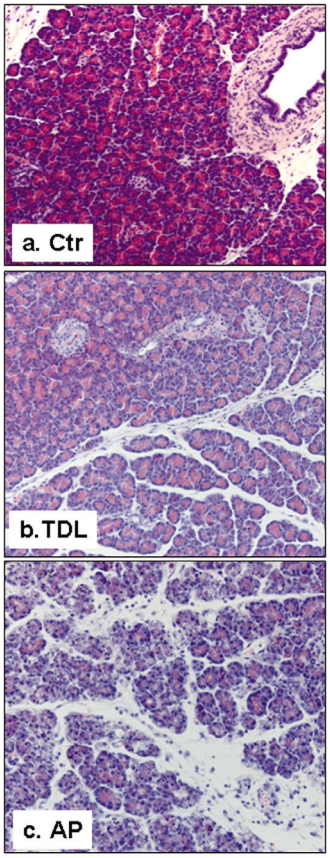

Fig. 1. **Pancreas Histology.** *Male rats were subject to thoracic duct ligation (TDL), sham TDL (Ctr), cerulein-induced acute pancreatitis (AP), and TDL + AP (L+P). Rats in a control group (Ctr) were subject to sham TDL. Pancreatic sections were prepared and stained with H&E. Compared to the normal pancreas in Ctr-rats (a), pancreas of TDL-rats showed both normal tissue (b, upper) and tissue with moderate edema (b, lower). AP induced severe edema and infiltration of white blood cells in pancreas of AP-rats (c) and TDL+AP-rats. Original magnification: 100 x.*

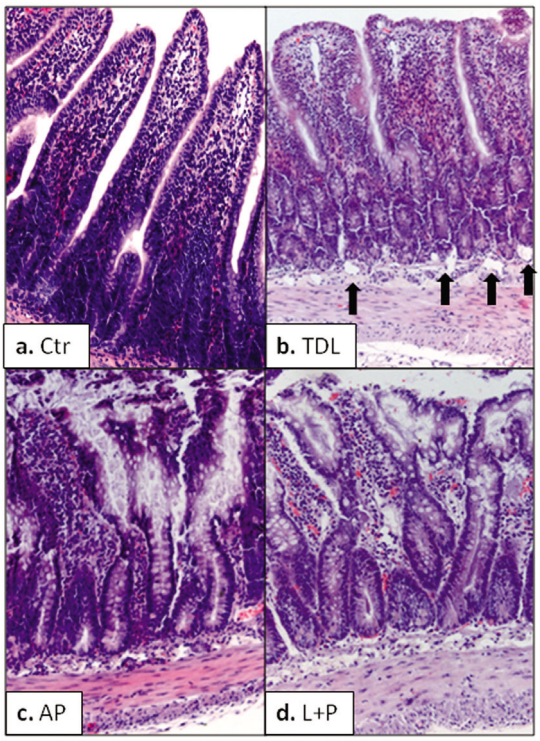

Fig. 2. **Ileum Histology.** *Male rats were subject to thoracic duct ligation (TDL), sham TDL (Ctr), cerulein-induced acute pancreatitis (AP), and TDL + AP (L+P). Ileum sections were prepared and stained with H&E. The ileum of TDL-rats (b) showed thickened villi and dilated lymphatic vessels (indicated by the arrows) compared to Ctr-rats (a). The ileum villi were damaged similarly in AP-rats (c) and L+P rats (d). Original magnification: 100 x.*

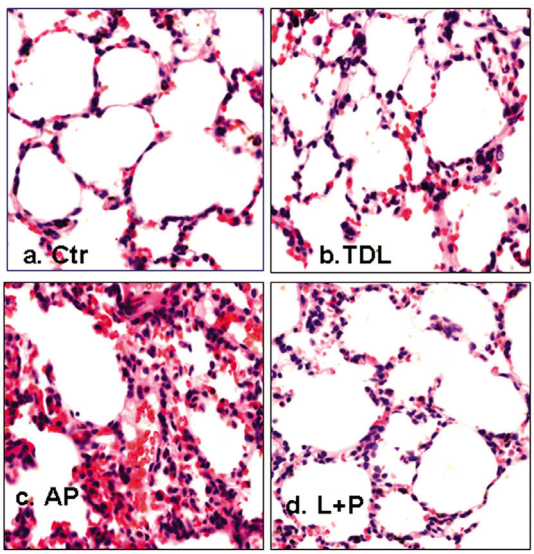

Fig. 3. **Lung Histology.** *Male rats were subject to thoracic duct ligation (TDL), sham TDL (Ctr), cerulein-induced acute pancreatitis (AP), and TDL + AP (L+P). Lung sections were prepared and stained with H&E. In the lungs of TDL-rats, the walls of alveolar sacs were thicker (b) than those of Ctr-rats (a). In the lungs of AP-rats, the walls of alveolar sacs were markedly thickened (c). Alterations in TDL+AP-rat lungs (d) were less severe than that in the AP-rat lungs. Original magnification: 400 x.*

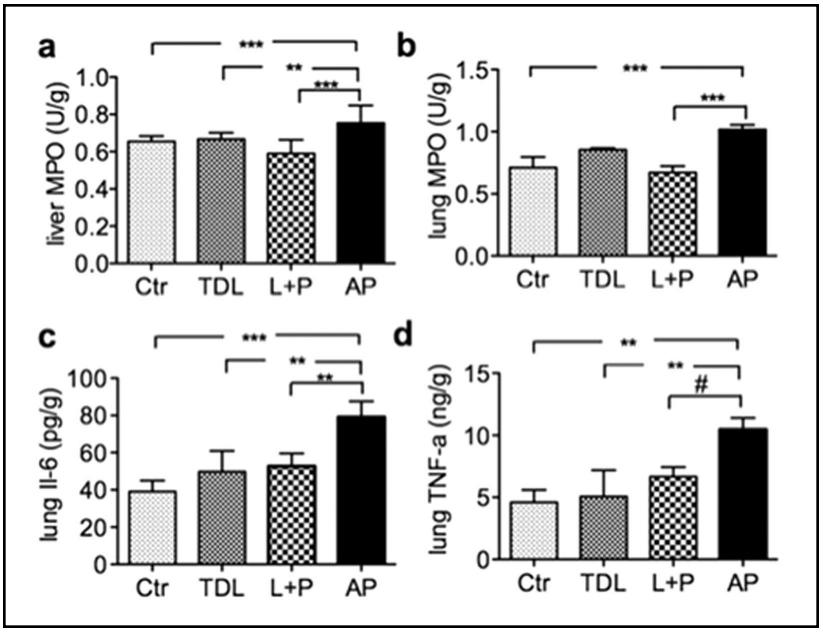

Fig. 4. **Liver and Lung Inflammatory Analysis.** *Male rats were subject to thoracic duct ligation (TDL, n=8), sham TDL (Ctr, n=9), cerulein-induced acute pancreatitis (AP, n=8), and TDL + AP (L+P, n=9). Myeloperoxidase activities in the liver (a) and lungs (b) were determined. Lung contents of Il-6 (c) and TNF-α (d) were also examined. # P = 0.09, * P < 0.05, ** P < 0.01, and *** P < 0.001.*

compared to Ctr-rats (*Fig. 2a*), suggesting that the TDL-rat ileum was edematous after AP. The ileum villi were damaged similarly in AP-rats (*Fig. 2c*) and TDL+AP rats (*Fig. 2d*).

In the lungs of TDL-rats, the walls of alveolar sacs were thicker (*Fig. 3b*) than those in the lungs of Ctr-rats (*Fig. 3a*), likely reflecting edema secondary to TDL. In the lungs of AP-rats, the walls of alveolar sacs were markedly thickened (*Fig. 3c*). Alterations in TDL+AP-rat lungs (*Fig. 3d*) were similar to that in the TDL-rat lungs and were less severe than the AP-rat lungs.

MPO activity is an index of neutrophil granulocyte infiltration (8). In the present study, AP-rats had greater hepatic MPO activity than Ctr-, TDL-, and TDL+AP-rats (*Fig. 4a*). AP-rats also had greater pulmonary MPO activity than Ctr- and TDL+AP-rats (*Fig. 4b*). In contrast, hepatic and pulmonary MPO activities were normal in TDL+AP-rats (*Figs. 4a and 4b*). These findings suggest that TDL inhibited neutrophil granulocyte infiltration. Il-6 was increased in the lungs of AP-rats but not TDL- and TDL+AP-rats (*Fig. 4c*). AP-rats also had higher TNF-α levels than Ctr- and TDL-rats with only a marginal increase compared to the TDL+AP-rats (*Fig. 4d*).

Protein content in lung-lavage fluid serves as an index of capillary permeability. Lung-lavage fluids from TDL-, AP-, and TDL+AP-rats all contained more protein than the Ctr-rats (*Fig. 5a*). Lung-lavage fluid from AP-rats also had more protein than the TDL+AP-rats (*Fig. 5a*), suggesting that TDL protected against increased capillary permeability during AP. In addition, more Il-1 was washed out from AP- and TDL+AP-rats than from Ctr- and TDL-rats (*Fig. 5b*), more Ang-2 was washed out from AP-rats than from TDL+AP-rats (*Fig. 5c*), and ICAM-1 from AP-rats was marginally increased compared to TDL+AP-rats (*Fig. 5d*).

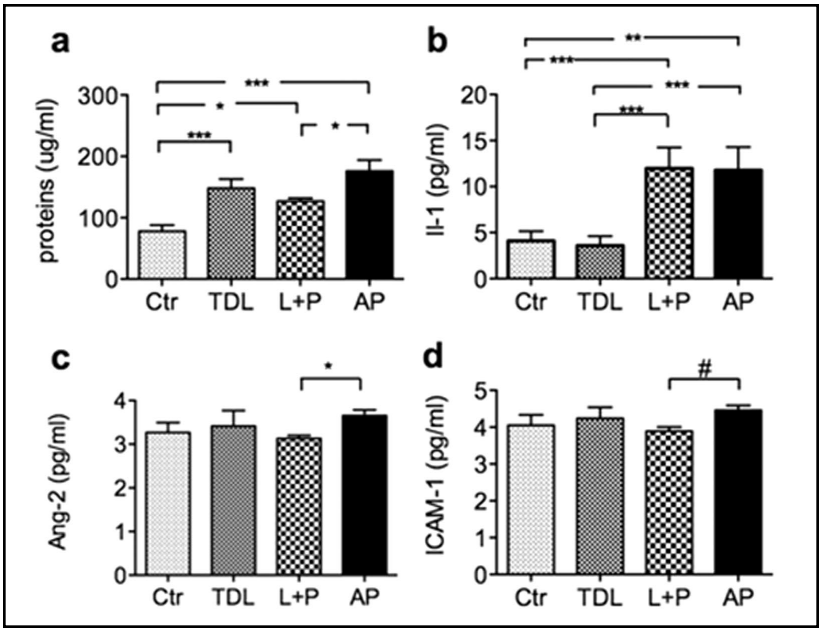

Fig. 5. **Lung Lavage Analysis.** *Male rats were subject to thoracic duct ligation (TDL, n=8), sham TDL (Ctr, n=9), cerulein-induced acute pancreatitis (AP, n=8), and TDL + AP (L+P, n=9). Total protein (a), Il-1 (b), Ang-2 (c), and ICAM-1 (d) concentrations were determined in lung-lavage fluids from the rats. # P = 0.06, * P < 0.05, ** P < 0.01, and *** P < 0.001.*

DISCUSSION

Dumont et al performed thoracic duct cannulation in human patients with or without pancreatic disease and showed that there is a direct functional lymphatic pathway for pancreatic secretion (9). In the present study, moderate edema was seen in the pancreas and ileum of TDL-rats. This feature may be attributed to overload and increased pressure in abdominal lymphatic vessels after thoracic duct ligation. Anatomically, TDL did not interrupt lymph drainage in the lungs (10). Interestingly, TDL-rats also showed mild edema in their lungs. In addition, lung-lavage fluid from TDL-rats contained more protein than Ctr-rats. Mechanisms underlying the TDL-induced lung edema are unclear and require further investigation.

Prior TDL attenuated AP-induced lung injuries although it also may have induced lung edema by itself. In a previous study, mesenteric lymphatic duct ligation (MLDL) attenuated AP in the rat (8). When thoracic duct drainage was seen to decrease AP severity in humans, the beneficial effect also manifested as an improvement in lung function (5). Taken together, interruption of abdominal lymph flow appears to inhibit progression of AP in both humans and laboratory animals.

Il-1, Il-6, TNF-α, Ang-2, and ICAM-1 are inflammatory cytokines that are implicated in the progress of AP (1,11,12). In the present study, these inflammatory factors were measured in lung tissue and/or in lung-lavage fluids. Results from these assays suggest that reduced Il-6 and Ang-2 were associated with TDL-induced lung protection in TDL+AP-rats during AP. A prior study reported that lungs were damaged eight hours after AP was induced in rats (13). Our study results demonstrate that lung damage occurs even earlier after AP is induced.

In conclusion, abdominal lymph flow and transport appears to play a role in the development of lung injury during acute pancreatitis, and interruption of abdominal lymph flow inhibits the progression of acute pancreatitis and systemic inflammatory sequelae in this model.

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