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Endotoxin translocation in two models of experimental acute pancreatitis

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Abstract

To test the hypothesis that endotoxin is absorbed from the gut into the circulation in rats with experimental acute pancreatitis we studied two different animal models. In the first model necrotizing pancreatitis was induced by the ligation of the distal bilio-pancreatic duct while in the second, experimental oedematous acute pancreatitis was induced by subcutaneous injections of caerulein. In both experiments, in the colon of rats with acute pancreatitis endotoxin from *Salmonella abortus equi* was injected. Endotoxin was detected by immunohistochemistry in peripheral organs with specific antibodies. The endotoxin was found only in rats with both acute pancreatitis and endotoxin injected into the colon and not in the control groups. The distribution of endotoxin in liver at 3 and 5 days was predominantly at hepatocytes level around terminal hepatic venules, while in lung a scattered diffuse pattern at the level of alveolar macrophages was identified. A positive staining was observed after 12 hours in the liver, lung, colon and mesenteric lymph nodes of rats with both caerulein pancreatitis and endotoxin injected into the colon. We conclude that the experimental acute pancreatitis leads to early endotoxin translocation from the gut lumen in the intestinal wall and consequent access of gut-derived endotoxin to the mesenteric lymph nodes, liver and lung.

Keywords: pancreatitis • gut-derived endotoxin • caerulein

Introduction

High endotoxin concentrations were measured in the blood of patients with acute necrotizing pancreatitis. Extrapancreatic complications, such as coagulation defects or renal and pulmonary failure occurred predominantly in patients with endotoxemia and some authors concluded that endotoxemia contributes to a severe course of pancreatitis [1-3]. Endotoxin plays a pivotal role for host-derived proinflammatory mediators in generating the fever, hypotension, tissue damage and multiple system organ failure.

A local infection in necrotizing pancreatitis is considered to take place in the 2nd week of the

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attack [1]. From this point of time the infected necrotized pancreatic tissue is probably the main source of endotoxin. The severe course of the disease can be attributed to the trigger effects of endotoxin and to the bioactive compounds released by the necrotic tissue. One important event in the acute severe pancreatitis seems to be the absorption of endotoxin and bacteria from the gut.

We assumed that the early endotoxemia is the consequence of the direct translocation of endotoxin and bacteria from the gut. Several studies have demonstrated that acute pancreatitis promotes bacterial translocation from the gut. Experimental works revealed translocation of endotoxin through the damaged gut in intestinal ischemia and peritonitis [4]. Although a few studies provide indirect evidence that endotoxin translocation may be a major initiating pathophysiologic event for systemic complications in severe acute pancreatitis, direct evidence for this has not been well documented. However, intestinal endotoxin translocation is not necessarily associated with the passage of intact bacteria [4].

The purpose of this study was to test the hypothesis that endotoxin is absorbed from the gut into the circulation in experimental acute pancreatitis. Two different animal models of acute pancreatitis characterized by different degrees of severity were studied. In the first model, ligation of the distal bilio-pancreatic duct resulted in a form of necrotizing pancreatitis. The second model used the intraperitoneal infusion of caerulein to produce a self-limited acute interstitial pancreatitis.

Materials and methods

Experimental design

Animals

Female Wistar rats with the weight between 250 and 300g were used in all experiments. The animals were kept at 22 ± 1°C on a 12 hours light and dark cycle with free access to water and food. The experiment was performed according to the Guide for the Care and Use of Laboratory Animals [DHEW publication No. (NIH) 85-23, Revised 1985, Office of Science and Health Reports, DRR/NIH, Bethesda, Maryland 20892).

Experiment 1

A number of 24 animals were tailed into 4 groups:

– **group 1 (Lig),** under pentobarbital anesthesia acute pancreatitis was induced by distal ligation of the bilio-pancreatic duct,

– **group 2 (Lig+Etox)** acute pancreatitis was induced by identical surgical procedure and 5 mg of endotoxin from *Salmonella abortus equi* (COSTAR GmbH, Bodenheim) diluted in distilled water were injected into the colon,

– **group 3 (Etox 1)** endotoxin was injected into the colon of normal rats (without acute pancreatitis),

– **group 4 (C 1)** the animals were injected into the colon with an isotonic NaCl solution; this group served as control.

All animals were fed ad libitum until killing. All rats were sacrificed by decapitation. Two animals from each group were killed at 1, 3 and 5 days, respectively. At the time of sacrifice, blood was harvested by cardiac puncture to determine the serum amylase levels by a standard method using the Phedebas amylase test (Pharmacia Laboratories, N.J., USA).

Experiment 2

A group of 16 rats was used for this experimental protocol in which the acute pancreatitis was induced by four subcutaneous injections of 20 mg/kg body weight of caerulein (Serva, Feinbiochemica, GmbH Co., Heidelberg, Germany) at hourly intervals over 3 hours [5]).

As in the experiment I, four groups of animals, each with 4 rats, were prepared:

– **group 1 (Cer),** with caerulein induced acute pancreatitis,

– **group 2 (Cer+Etox),** with acute pancreatitis and endotoxin injected into the colon,

– **group 3 (Etox 2)** only with caerulein injected into the colon,

– **group 4 (C 2),** sham operated rats, which served as control animals; they received the same volume of isotonic NaCl solution in a similar manner but without endotoxin .

In this experiment two rats from each group were killed at 12 and 24 hours after the first caerulein injection. Blood samples were obtained as in experiment 1.

Table 1 Area of positive immunohistochemical staining for endotoxin in animals with acute pancreatitis induced by ligation of the main bilio-pancreatic duct and endotoxin from *Salmonella a. equi* injected into the colon (% from the total tissue area, mean value ± SD).

	Liver	Lung	Pancreas	Colon	Mesenteric lymph nodes
Day 1	6,3 ± 1,17	6,3 ± 1,3	0	28 ± 5,57	5,86 ± 2,80
Day 3	20,47 ± 3,56	9,10 ± 1,59	0	6,18 ± 2,31	8,50 ± 1,03
Day 5	40,3 ± 6,67	11,47 ± 1,67	1,91 ± 1,16	1,86 ± 0,69	7,95 ± 2,80

Histologic examination

Tissue samples were harvested from colon, liver, pancreas and lung, fixed in 4% paraformaldehyde for 12 hours at 4°C. The fragments were paraffin-embedded according to standard methods. From each organ were prepared sections 5 - 7μm thick.

Immunohistochemistry

For immunohistochemical detection of endotoxin we used specific rabbit antibody (gift from Dr. M. Freudenberg, Freiburg, Germany). The staining was accomplished with the streptavidin-biotin complex method, employing a commercially available staining kit (DAKO Corporation Carpinteria, CA). Finally the sections were counterstained with haematoxylin and eosin.

Quantitative analysis

An interactive video measuring system (Olympus Microscope BX40, with Image Analysis System, Micro Image, version 3.0) was used in which the image is recorded by a videocamera and displayed on the computer screen. The amount of positive immunohistochemical staining was quantified by analysing a number of 50 microscopic fields at a magnification of 40X for each animal. Values for each animal were expressed as a percentage of the tissue area. Data were expressed as mean value ± standard deviation of the mean.

Results

Experiment 1

Histologic alterations induced by the ligation of the biliopancreatic duct

24 hours after the ligation of the biliopancreatic duct we found interstitial edema, hemorrhagic necrosis and fat necrosis into the pancreas, the mesentery and the retroperitoneal tissue until the perirenal fatty tissue. At 5 days, the pancreatic lesions were severe and involved the entire gland. The acinar architecture was completely destroyed and the foci of fat necrosis become infiltrated by neutrophils. At 24 hours the lungs presented pathologic accumulation of neutrophils and congestion and at 3 days the hemorrhagic lesions are obvious. No liver or colon lesions were found until the end of the experiment.

Serum amylase activity

The mean serum values of amylase appear strongly increased 24 hours after the common biliopancreatic duct ligation (9246 ± 1823 I.U.) and slowly decreased after 72 hours (1835 ± 528 IU), remaining at a low range after 5 days (250 ± 114 I.U.).

Immunohistochemistry

The endotoxin was found in the Lig+Etox group as described in Table 1. No endotoxin was detected in the Lig, Etox and C groups.

No endotoxin translocation was observed in rats with obstructive jaundice induced by the proximal ligation of the biliary duct after 5 days (group Lig). In the group of animals with acute pancreatitis and

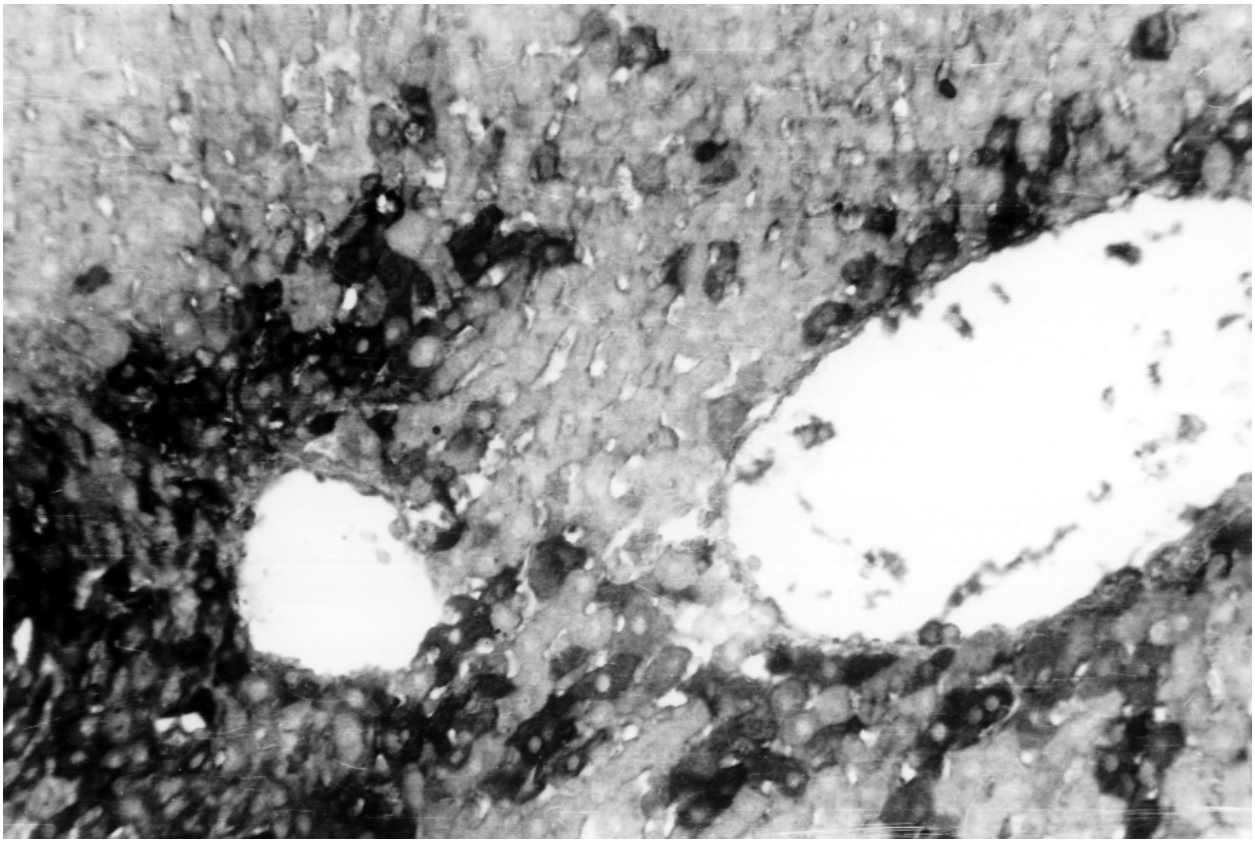

Fig. 1 Endotoxin positive staining in the liver of rats with acute pancreatitis induced by ligation of the bilio-pancreatic duct and with endotoxin from *Salmonella abortus equi* injected into the colon. Time point: 5 days after the experiment. Immunohistochemistry with anti-endotoxin antibodies and amplified with streptavidin biotin. Magnification 400X.

injection of endotoxin into the colon (Lig+Etox) the distribution of endotoxin in liver at 3 days was predominantly at hepatocytes level around terminal hepatic venules (Fig. 1). The same aspect was recorded at 5 days, but more hepatocytes containing endotoxin were identified.

On day 3 the endotoxin in the lung was scattered with diffuse pattern in the alveolar macrophages. At 5 days the same aspect was identified, but a bigger number of alveolar macrophages staining for endotoxin was recorded.

Experiment 2

Histologic alterations induced by caerulein

Four subcutaneous injections of caerulein (20 mg/kg body weight) induced marked interstitial edema of the pancreas and cytoplasm vacuolization in acinar cells. Cellular infiltration with neutrophils, as the predominant inflammatory cells, was seen in the interstitial space as well as between acinar cells. Cellular degeneration and necrosis with loss of granularities were minimally observed in some acinar cells. Light microscopic examinations did not reveal any structural histologic abnormalities of the lung, liver or colon in rats with acute pancreatitis.

Serum amylase activity

Subcutaneous administration of caerulein induced a significant increase (about 10 times) in serum amylase activity at 12 hours after the first injection (4946±1127 I.U.) as compared to the basal level (470±250 I.U.)

Immunohistochemistry

Twelve hours after caerulein injection, a distinct positive staining was observed in the liver, lung,

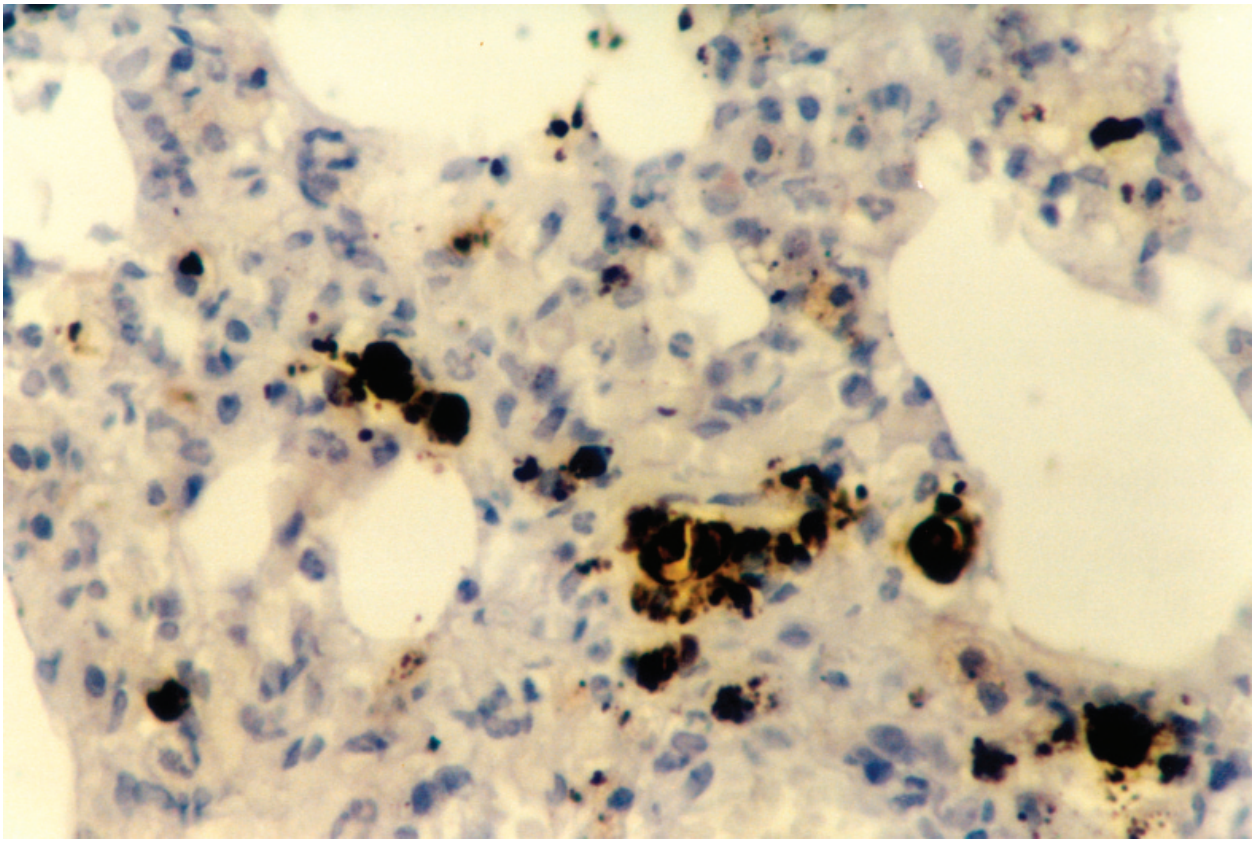

Fig. 2 Endotoxin positive staining in the lung of rats with caerulein induced acute pancreatitis and with endotoxin from *Salmonella abortus equi* injected into the colon. Time point: 12 hours after the experiment. Immunohistochemistry with anti-endotoxin antibodies and amplified with streptavidin biotin. Magnification 400X.

colon and mesenteric lymph nodes indicating the presence of endotoxin (Table 2). In the liver the endotoxin was associated primarily with sinusoidal cells. The parenchyma presented a small number of hepatocytes associated with endotoxin in a diffuse pattern. A positive immunohistochemical reaction was also observed in the lung, with a stronger activity in interstitial cells, in capillaries and especially in some alveolar and bronchiolar macrophages (Fig. 2).

At 24 hours the immunohistochemical reaction was absent.

The detection of the endotoxin positive reaction in the liver, lung, pancreas, colon and mesenteric

Table 2 Area of positive immunohistochemical staining for endotoxin in rats with acute caerulein pancreatitis and endotoxin from *Salmonella abortus equi* injected into the colon (% from the total tissue area, mean value ± SD).

	Liver	**Lung**	**Pancreas**	**Colon**	**Mesenteric lymph nodes**
12 hours	11,95 ± 3,57	6,18 ± 2,48	0	13 ± 4,85	6 ± 2,28
24 hours	0	0	0	0	0

lymph nodes of rats with caerulein induced acute pancreatitis and endotoxin injected into the colon is summarized in Table 2.

Discussion

The widely accepted model of severe acute pancreatitis is the sequential course with an early vasoactive toxic phase and a late phase dominated by septic complications [6]. Systemic complications during the initial phase are believed to be mediated by activated pancreatic enzymes and other vasoactive and toxic agents released from the pancreas [7]. Secondary infection of pancreatic necrosis is currently the most lethal complication of acute necrotizing pancreatitis particularly during later stages of the disease. Infection of sterile pancreatic necrosis is a secondary phenomenon [6]. The whole body inflammatory reaction often complicates the course of severe acute pancreatitis from the early stage and may progress to systemic effects with multiple organ failure and high mortality [8]. All these effects are largely related to the uncontrolled activation of mononuclear phagocytes and the consequent release of inflammatory mediators. Endotoxin is the most potent trigger of this mediators cascade and endotoxaemia is a feature of acute pancreatitis [9-11].

In the late stage of disease the main source of endotoxin is the infected pancreatic necrosis. In the early phase, before the bacterial colonization of the pancreatic and peripancreatic necrosis, endotoxaemia may be the consequence of the increased intestinal permeability with passage of bacteria and/or endotoxin from the gut to remote organs.

Recent work with animal models offer further support to the concept that endotoxin contributes to the development of multiple organ failure in acute pancreatitis [12-14].

In order to evaluate endotoxin translocation from the gut we used two models of acute pancreatitis characterized by histological and biochemical differences. Mild pancreatitis was induced by injecting rats with a supramaximally stimulating dose of caerulein. The resulting disease reproduces the human form of edematous pancreatitis [5, 15]. The ligation of the biliopancreatic duct induced a severe pancreatitis, with pancreatic and peripancreatic necrosis, extensive retroperitoneal adiponecrosis and lung lesions like trombosis and hemorrhage. The macroscopic and histologic findings of this model are very close to those of human necrotizing acute pancreatitis.

Microscopic examination did not reveal any structural histological abnormalities of the intestine in rats with acute pancreatitis.

Twenty-four hours after ligation of the biliopancreatic duct the animals presented higher amylase levels than those of rats with caerulein-induced pancreatitis reflecting the pathologic differences between the two experimental models. However, the amylase level decreased after 3 days and remains at a low range at 5 days after ligation of the biliopancreatic duct, probably as a consequence of the destruction of pancreatic tissue.

Although a big amount of information exists on the microbial translocation in acute pancreatitis [13, 16, 17] similar understanding of endotoxin translocation and distribution among various organs is still lacking.

Endotoxin translocation was found only in rats with acute pancreatitis and not in control animals. Endotoxin administered into the gut in concentrations of 10-100 mg/ml did not induce morphologic or functional effects on the rabbit jejunum and even ingestion of 150 mg of *E. coli* endotoxin by humans did not induce adverse reactions [4]. Several experimental models such as acute bowel ischemia or chemical peritonitis revealed transmigration of endotoxins through the damaged gut [4].

Endotoxemia can also occur in the absence of Gram negative bacteremia. Of note, obstructive jaundice consecutive to the duct ligation model of acute pancreatitis, may itself promote bacterial translocation [16]. However, our previous findings showed that no endotoxin translocation was observed in rats with obstructive jaundice induced by duct ligation after 5 days.

The data reported in the present study suggest that the degree of endotoxin translocation could be proportional to the severity of the pancreatitis model, results that are consistent with those of Gianotti *et al.* [18].

More than this, endotoxin can induce cellular events resembling acute pancreatitis by a direct effect on pancreatic acinar cells [19].

With regard to the route of endotoxin translocation, studies have focused primarily on the portal vein [20]. Some papers indicated an alternative

route of transport of intestinal endotoxins. Our data clearly showed the presence of translocated endotoxin in the mesenteric lymph nodes suggesting that the lymphatic transport of endotoxin is the alternative route to the portal one [13].

The initial effector cell of endotoxin-induced organ injury is probably the tissue macrophage [21]. Translocated endotoxin seems to enter the intestinal wall, activates the gut-associated macrophages in the lamina propria, releasing cytokines and causing rapid increase of cytokines levels in portal and systemic circulation during the early stage of the disease. Additional inflammatory mediators are produced when Kupffer cells are activated by the endotoxin transported via portal vein into the liver [20].

It is known that inflammatory cytokines generated in the lung in response to endotoxin cause pulmonary edema and hiperpermeability associated with lung sequestration of neutrophils. The identification of these cytokines (IL-1, TNF) in bronhoalveolar lavage fluid predicts mortality associated with ARDS [4].

Conclusion

Experimental acute pancreatitis leads to early endotoxin translocation from the gut in the intestinal wall and consequent access of gut-derived endotoxin to the mesenteric lymph nodes, liver and lung. The degree of translocation directly correlates with the severity of pancreatitis model. Our results support the lymphatic transport as an alternative way to the portal route for intestinal endotoxins to the remote organs. The presence of large amounts of translocated endotoxins in liver and lung suggests that the main mechanism involved in the occurrence of the whole body inflammatory reaction (systemic inflammatory response syndrome) in acute pancreatitis is probably the endotoxin stimulation of the tissue macrophage and not of the blood mononuclear cell.

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