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Gut Origin Sepsis, Macrophage Function, and Oxygen Extraction Associated with Acute Pancreatitis in the Rat

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Abstract. It has been suggested that the gut plays a role in the development of bacterial complications, which are important contributors to morbidity and mortality in patients with acute pancreatitis. The present study evaluated the enteric bacterial translocation, bacterial homeostasis, and reticuloendothelial system function in experimental acute pancreatitis induced by intraductal injection of 5% sodium taurodeoxycholate in the rat. The incidence of bacterial translocation from the gut to mesenteric lymph nodes (MLNs) and lungs significantly increased after 12 hours and to the systemic circulation, ascites, and pancreas at 24 hours. The number of anaerobic bacteria and lactobacilli decreased in the colon and distal ileum from 6 or 12 hours, whereas the number of *Escherichia coli* increased from 12 hours. The systemic uptake rate of radiolabeled bacteria decreased from 6 hours after induction of acute pancreatitis. The uptake of radiolabeled bacteria by Kupffer cells decreased from 6 hours, whereas the uptake by macrophages from blood, lungs, and the intestine increased. A decrease in macrophage killing capacity was noted, reflected by an increase in the number of cultured viable bacteria from isolated macrophages. The whole-body oxygen extraction rate increased 4 to 24 hours after induction of pancreatitis, whereas the gut oxygen extraction rate decreased at 2 and 4 hours, followed by an increase at 12 to 24 hours. These data show that translocation of enteric bacteria occurs during the early stage of acute pancreatitis and that the MLN–thoracic duct–circulation may be a major route of bacterial dissemination. Compromised gut oxygen metabolism, overexaggerated intestinal macrophages, and impaired host immune function may be involved in the development of infectious complications associated with acute pancreatitis.

Bacterial infections are serious complications responsible for up to 80% of deaths in patients with acute pancreatitis [1, 2]. The fact that most bacteria associated with pancreatic and peripancreatic infections are of enteric origin implies that the gut plays a role in the pathogenesis of pancreatic infection [2–4]. Experimental studies have demonstrated that failure of the gastrointestinal tract occurs with acute pancreatitis induced by the continuous intravenous infusion of cerulein, obstruction of the common biliopancreatic duct, or intraductal injection of sodium taurodeoxycholate [5–7], resulting in translocation of enteric bacteria into extraintestinal sites and the systemic circulation.

Bacterial translocation from the gut has been studied in several other experimental situations associated with shock or sepsis [8–10]. Possible underlying mechanisms include compromised host immune function, insufficient blood supply, disturbed enteric bacterial ecology, and injured intestinal barrier function. Bacterial translocation in experimental acute pancreatitis has been associated with cecal bacterial overgrowth, mucosal injury, and decreased gut motility [5–7]. One aim of the present study was to assess the extent and role of bacterial translocation in the early stage after induction of acute pancreatitis in rats. Furthermore, macrophages were harvested from the liver, lungs, intestine, and blood in order to evaluate the capacity of uptake and killing of radiolabeled, alive bacteria. Finally, possible concomitant changes in body and gut oxygen extraction were assessed.

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Methods

Animals

Adult male Sprague-Dawley rats, weighing 250 to 300 g, were fed standard rat chow (R_3; Astra-Ewos, Södertälje, Sweden) and water ad libitum. The rats were allowed to acclimatize to our laboratory conditions for 4 to 6 days and were subjected to a regimen of 12-hour day–night cycle living in mesh stainless steel cages (three rats per cage) at constant temperature (22°C).

Induction of Acute Pancreatitis

The rats were operated on under aseptic conditions using light ether anesthesia. Acute pancreatitis was induced by intraductal infusion of 0.2 ml 0.025 M glycylglycin-NaOH buffer, pH 8.0, containing 5% sodium taurodeoxycholate by an infusion pump at a speed of 0.04 ml/min after clamping the proximal end of the common bile duct and puncture through the duodenum into the biliary-pancreatic duct. The sodium taurodeoxycholate solution was sterilized at 100°C for 20 minutes. Sham operation (controls) was performed as above without the bile infusion.

Determination of Bacterial Translocation

A midline incision was made using sterile technique. A 0.2 ml sample of blood was taken from the portal and caval veins, and

samples from ascites (0.2 ml), mesenteric lymph nodes (MLNs), liver, pancreas, lungs, and parts of the distal ileum (10–12 cm from the cecum) and transverse colon were harvested and weighed prior to determination of bacterial growth. Samples from blood and ascites were immediately inoculated into 3 ml of blood culture medium. The harvested organs were homogenized in test tubes containing 3 ml Brain Heart Infusion Broth (Difco Laboratories, Detroit, MI, USA), from which 0.2 ml of the supernatant was taken for culture of aerobic, microaerophilic, and anaerobic bacteria. All media for aerobic culture were incubated at 37°C for at least 3 to 5 days; organ samples for anaerobic bacteria were cultured at 37°C for 7 days before being regarded as negative. Enteric gram-negative bacteria were identified by API 20 system (BioMeriux SA, Marcy-1'Etoile, France) and *Lactobacillus acidophilus* by API 50 CH (Analytab Products, Plainview, NY, USA). All other aerobic, microaerophilic, and anaerobic microbes isolated were identified by standard procedures [11]. The number of living bacteria were calculated and expressed as number of living organisms (colony-forming units, CFU) per gram of organ tissue.

Labeling of Bacteria

Escherichia coli (serotype 046:KI:H31), a facultative anaerobe with agglutination with collagen type I, fibronectin, vitronectin, and laminin, lacking hemolysin activity, was cultured overnight in Todd-Hewitt broth. 1-Carbon 14 oleic acid 25 Ci (New England Nuclear, Boston, MA, USA) was extracted by N_2 dry purification and resuspended using 200 μl of 0.02 N NaOH solution with 0.5 ml of alcohol. Purified oleic acid saline was added to 0.2 ml of hot, distilled water and 4 ml of a stirring broth (Brain Heart Infusion Broth), supplemented by 20% bovine serum albumin to 100 ml. *E. coli* was incubated in the nutrient broth containing 20% bovine serum albumin and the isotopic material for 4 hours. Labeled bacteria were harvested after centrifugation, washed, and resuspended with normal saline three times. Free radioactive fatty acids were removed by repeated washings, and the number of radiolabeled bacteria was estimated by observation of the optical density at 550 nm. The resuspended bacteria were then diluted to a final concentration of approximately 10^6 *E. coli* (cpm)/ml bacterial suspension [12].

Evaluation of Host RES Function

Total host reticuloendothelial system (RES) function was studied by measuring the whole-body uptake rate as described previously [13]. Three main RES organs (liver, spleen, lungs) were harvested 15 minutes after injection of the radiolabeled bacteria, weighed, cut in small species, and put into glass scintillation vials with 4 ml aqueous solubilizer, followed by gentle agitation of the samples. The samples were then incubated at 50°C for 4 hours, and the radioactivity was measured by a β-counter (LS 1800; Beckman, New York, NY, USA). A theoretic standard uptake value for the rat was defined by the mean values of the total uptake in the three RES organs studied (liver, spleen, lungs) in each rat subjected to the sham operation. The body uptake rate was defined as the total uptake in the three organs in the experimental animals compared with the theoretic standard uptake value.

Isolation of Macrophages

Blood Macrophages. Blood obtained by puncture of the aorta was placed in heparinized tubes. White blood cells separated by centrifugation were layered on lymphocyte separation medium (Sigma) and then centrifugated at 400× *g* for 20 minutes. The mononuclear cells were harvested from the interface and washed three times using Hanks' balanced salt solution (HBSS, Sigma). An average yield of $1.0–2.0 \times 10^6$ mononuclear cells per milliliter of blood was obtained. Separation on gelatin-plasma-coated flasks (2% gelatin, BDH Chemicals, Poole, UK) was used to purify isolated macrophages from blood [12]. The cellular suspension containing 3×10^6 mononuclear cells in 10 ml of RPMI supplemented by 10% (v/v) rat serum was introduced into each flask, followed by incubation for 2 hours at 37°C (pH 7.4) with 95% O_2/5% CO_2, after which nonadherent cells were removed and the adherent cells were washed three times using RPMI 1640 supplemented with 10% rat serum prewarmed at 37°C. The adherent cells were detached by incubation for 30 minutes with 10 ml of RPMI 1640 supplemented with 5 mM ethylenediaminetetraacetic acid (EDTA, Sigma) and harvested in plastic tubes to a final concentration of 10^6 adhered macrophages/ml followed by three washings.

Isolation of Kupffer Cells. The portal vein was cannulated with a 0.15 cm (outside diameter) cannula (Hythe) and was perfused with HBSS supplemented with 25 mM sodium bicarbonate and 2 mM EDTA in situ under light ether anesthesia at 20 to 25 ml/min by a peristaltic pump (Watson-Marlow, Falmouth, Cornwall, UK) [14]. The cell suspension was filtered after excising the perfused liver and cutting it into small pieces. Kupffer cells were harvested by centrifugation with stractan (Sigma) gradients as described by Friedman and Roll [15]. The cells present in the interface between 8% and 12% stractan were collected and washed with Eagle's minimal essential medium without Ca^{2+} (Sigma) three times. Kupffer cells were placed in culture plates and incubated at 37°C for 1 hour; adherent cells were resuspended at a concentration of 10^6 cells/ml for measurement.

Collection of Alveolar Macrophages. After exposing the trachea, alveolar macrophages were harvested by cannulating the tracheobronchial tree and repeatedly lavaging with HBSS supplemented with 25 mM sodium bicarbonate and 1% EDTA [16]. The cells obtained were washed three times in RPMI 1640 and centrifugated with lymphocyte separation medium at 400× *g* for 20 minutes. After 2 hours of incubation at 37°C, nonadherent cells were removed by washing, and adherent cells were harvested at a concentration of 10^6 cells/ml.

Isolation of Intestinal Macrophages. The intestinal specimens were washed with HBSS supplemented with 25 mM sodium bicarbonate without Ca^{2+} and Mg^{2+}. The mucosa from the distal ileum was dissected from the muscularis mucosa, and fragments were incubated with 1 mM dithiothreitol (Sigma) at 20°C for 15 minutes. Epithelial cells were removed by 3×30-minute incubations in 5 mM EDTA at 37°C during shaking after washing with HBSS three times [17]. The mucosa was subsequently cut into small pieces and digested using collagenase (Sigma) at a concentration of 1 mg/ml RPMI 1640 for 3 hours. Mononuclear cells were centrifugated with lymphocyte separation medium (Sigma).

After removing nonadherent cells by incubating for 2 hours followed by washing three times, adherent cells were harvested at 10^6 cells/ml for further experiments.

Cellular viability and identification were determined by the trypan blue exclusion test and staining with nonspecific esterase. All media used contained penicillin 100 U/ml, gentamicin 5 μg/ml, and streptomycin 100 μg/ml.

Assays on Macrophage Uptake and Killing Capacity

Animals subjected to either sham operation or acute pancreatitis were challenged with ^{14}C-labeled live *E. coli* through a femoral vein catheter (O.D. 0.94 mm; Dow Corning, Detroit, MI, USA). At 6, 12, and 24 hours after operation, 1 ml of radiolabeled bacteria, containing about 6×10^7 bacteria per rat was injected intravenously 15 minutes prior to sampling. Macrophages were then isolated from each organ or tissue. The capacity of macrophage uptake was expressed by the radioactivity (counts per minute, cpm) in harvested macrophages as measured by a β-counter (LS 1800, Beckman). The capacity to kill macrophages was reflected in the bacterial growth (*E. coli* serotype: 046:KI: H31) from isolated macrophages, expressed as colony forming units (CFU)/10^6 macrophages.

Oxygen Extraction

Hemoglobin concentration and oxygen and carbon dioxide tensions were measured in blood obtained from the abdominal aorta, inferior vena cava, and portal vein, respectively, by a hemoglobin analyzer (Vitratron, HBF 200, Amsterdam, The Netherlands) and an acid-base analyzer (ABL 30, Radiometer A/S, Copenhagen, Denmark). The oxygen content in systemic arterial and portal venous blood was calculated by the formula:

$$O_2 \text{ content} = (\text{hemoglobin} \times \% \text{ saturation} \times 1.34) + (PO_2 \times 0.003)$$

Whole-body oxygen extraction was deduced from the difference in oxygen content between the abdominal aorta (A) and inferior vena cava (SV):

$$\text{Body oxygen extraction} = (\text{A oxygen content} - \text{SV oxygen content})/\text{A oxygen content}$$

Oxygen extraction by the gastrointestinal tract was calculated from the difference in oxygen content between the abdominal aorta and the portal vein (PV):

$$\text{Gut oxygen extraction} = (\text{A oxygen content} - \text{PV oxygen content})/\text{A oxygen content}$$

Experimental Design

The animals were randomly divided into two groups: sham operation ($n = 38$) and acute pancreatitis ($n = 30$, surviving animals) and enteric bacterial translocation was studied 2, 4, 6, 12, and 24 hours after operation (6 or 10 rats per time point). At laparotomy samples were taken for bacterial culture, and blood from the caval and portal veins and the abdominal aorta was obtained for blood gas analysis and hemoglobin determination. Another 60 rats were used to evaluate macrophage uptake and killing capacity after intravenous injection of radiolabeled bacteria (30 rats per group, 6 rats per time point). All studies were carried out in surviving animals during the experimental period. The mortality rate within 24 hours was assessed in two additional groups (12 per group) not subjected to any other measurements.

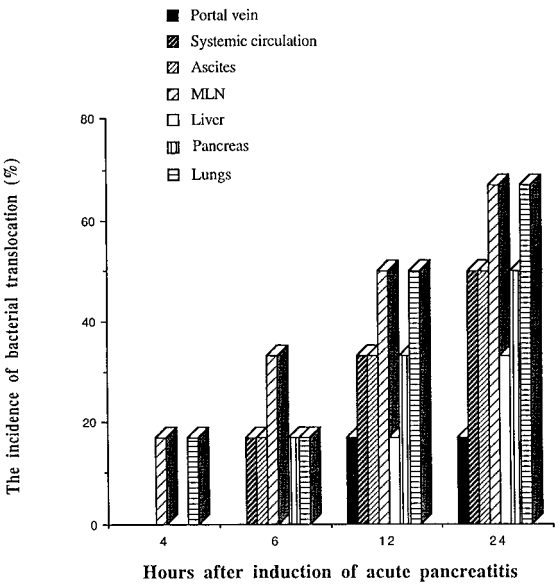

Fig. 1. Incidence of bacterial translocation from the gut to the portal vein, systemic circulation, ascites, mesenteric lymph nodes (MLN), liver, pancreas, and lungs in rats with sham operation (controls) or acute pancreatitis within 24 hours after operation.

Statistics

Parametric data are reported as the mean ± standard error of the mean (SEM). Comparisons of the incidence of bacterial translocation and mortality rate after operation were made by using Fisher's exact test. Student's *t*-test was used to evaluate bacterial overgrowth, radioactivity levels of ^{14}C-labeled bacteria, and oxygen extraction rate. Statistical significance was considered at a probability level of < 0.05.

Results

All operations were completed within 5 to 7 minutes, and the animals woke up within 5 minutes after the end of the operative procedure. The mortality rate within the initial 24 postoperative hours among animals with acute pancreatitis was 50% (6 of 12; $p < 0.01$ versus controls). Ascites formation and pancreatic edema, bleeding, and necrotic spots were seen from 2 hours onward after induction of pancreatitis.

Samples taken from control animals harbored no bacteria. Positive bacterial cultures from MLNs and lungs were demonstrated 4 hours after induction of acute pancreatitis, significantly increasing at 12 and 24 hours, compared to controls ($p < 0.05$ and 0.01, respectively) (Fig. 1). The incidence of bacterial translocation from the gut to the systemic circulation, ascites, and pancreas significantly increased in pancreatitis animals after 24 hours (50%, $p < 0.05$) (Fig. 1). The total incidence of bacterial translocation in all animals to the systemic circulation was 20% ($n = 6$ of 30), to ascites 20% (6 of 30), to MLNs 33% (10 of 30), to liver 13% (4 of

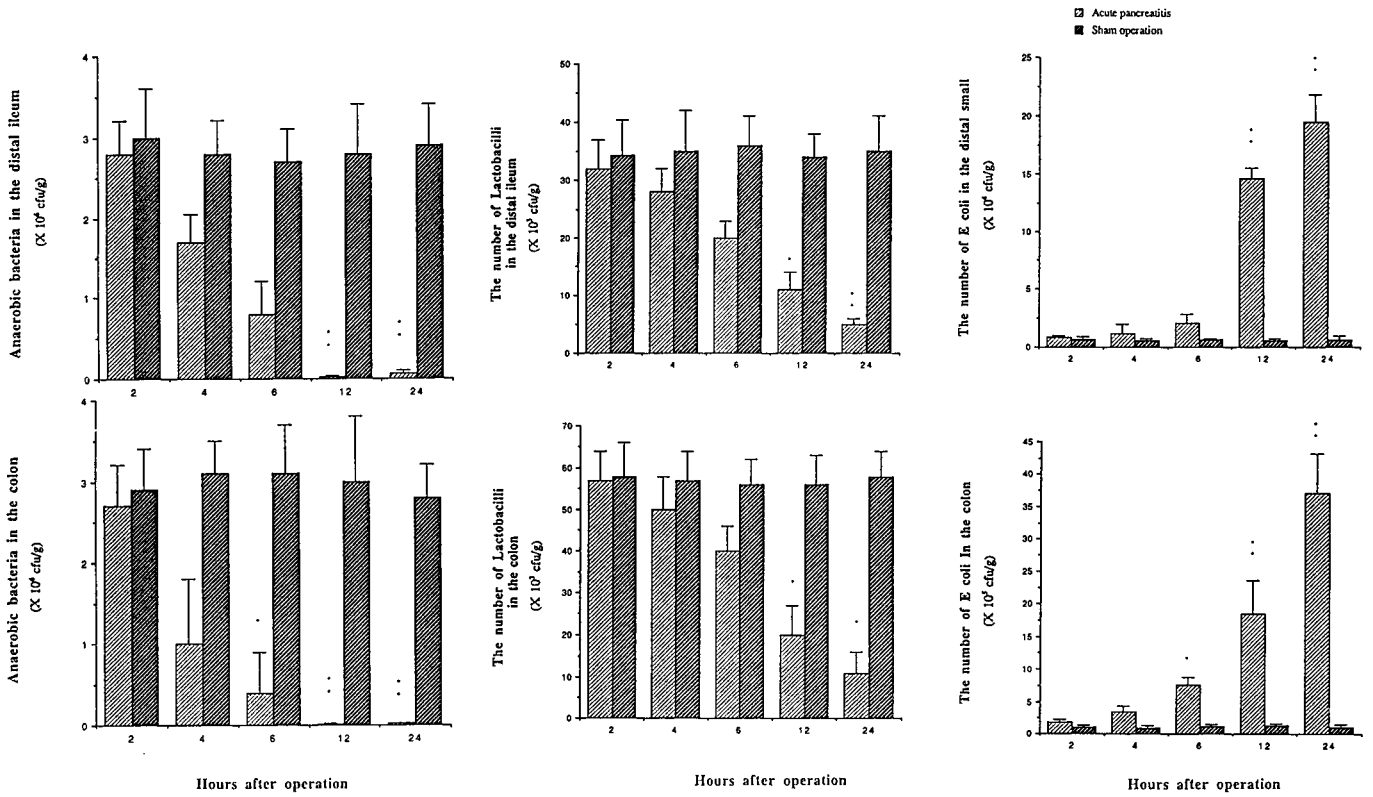

Fig. 2. Number of anaerobic bacteria, *Lactobacillus*, and *Escherichia coli* in the distal ileum and colon 2, 4, 6, 12, and 24 hours after sham operation or induction of acute pancreatitis. * and **: probability levels of < 0.05 and < 0.01, respectively, compared with sham operation.

30), to pancreas 20% (6 of 30), and to lungs 30% (9 of 30). Translocation to these sites was significantly higher in animals with pancreatitis than in the controls ($p < 0.05$). However, only 7% (2 of 30) of portal vein samples from pancreatitis animals were positive for bacterial growth.

A significant decrease in the number of anaerobic bacteria was seen in the distal ileum at 12 and 24 hours and in the colon 6, 12, and 24 hours after induction of acute pancreatitis ($p < 0.05$ versus controls) (Fig. 2). The number of lactobacilli in both the distal ileum and colon decreased significantly at 12 and 24 hours in animals with pancreatitis ($p < 0.05$) (Fig. 2). However, the number of *E. coli* increased gradually and significantly in the distal ileum at 12 and 24 hours and in the colon 6, 12, and 24 hours after induction of acute pancreatitis ($p < 0.05$) (Fig. 2) compared to controls.

Alterations in systemic host RES function are depicted in Figure 3. The whole-body uptake of radiolabeled bacteria gradually decreased after induction of acute pancreatitis, reaching significant differences at 6, 12, and 24 hours in rats with pancreatitis compared to the controls ($p < 0.01$).

During macrophage preparation, cellular viability and purification were 94.7 ± 1.3 and 91.4 ± 1.9, as assessed by trypan blue exclusion and nonspecific esterase staining, respectively. No significant difference in macrophage uptake was noted within the first 4 hours after induction of pancreatitis. Uptake of radiolabeled bacteria by Kupffer cells significantly decreased 6, 12, and 24 hours after induction of pancreatitis, whereas the uptake by

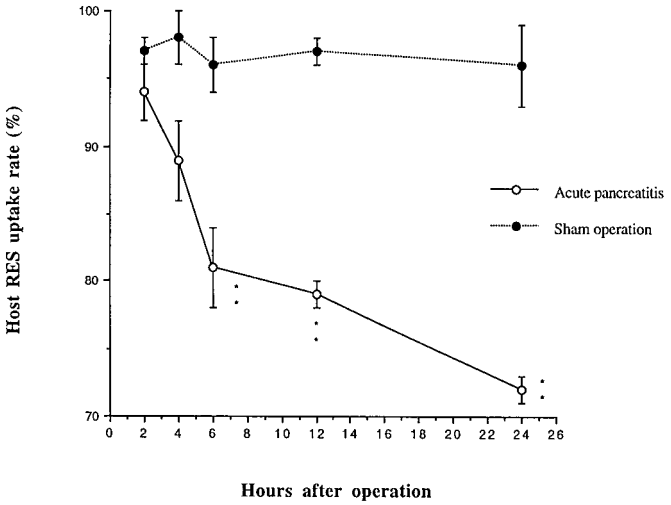

Fig. 3. Host RES uptake rate in rats 2, 4, 6, 12, and 24 hours after sham operation or induction of acute pancreatitis. * and **: probability levels of < 0.05 and < 0.01, respectively, compared with sham operation.

macrophages in blood, lungs, and intestine increased ($p < 0.05$) (Fig. 4). A decrease in macrophage killing capacity was seen with acute pancreatitis, reflected by an increase in the number of cultured bacteria from isolated macrophages (Fig. 5). The number of bacteria cultured from Kupffer cells thus significantly increased

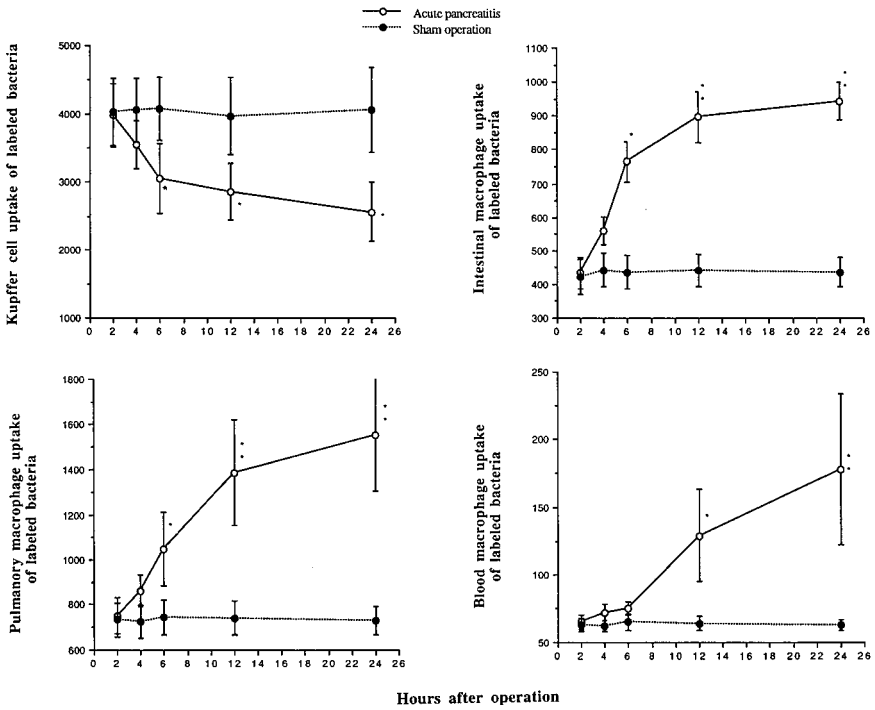

Fig. 4. Cellular uptake (cpm/10^6 cells) of radiolabeled, live bacteria by macrophages obtained from blood, liver, lungs, and intestine 2, 4, 6, 12, and 24 hours after sham operation or induction of acute pancreatitis. * and **: probability levels of < 0.05 and < 0.01, respectively, compared with sham operation.

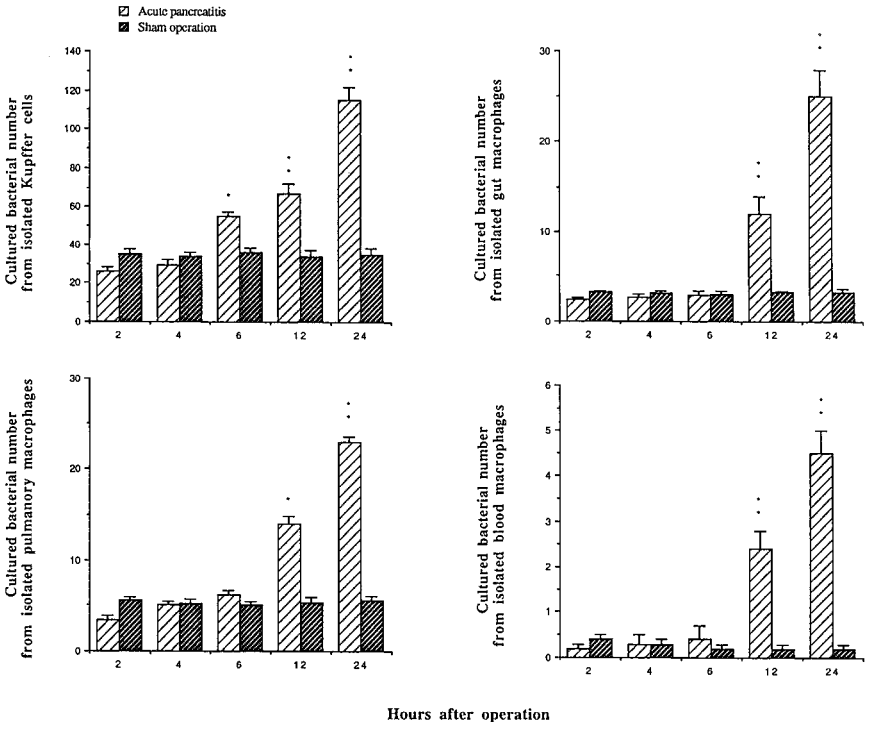

Fig. 5. Number of cultured, live *Escherichia coli* from isolated macrophages (cfu/10^5 cells) from blood, liver, lungs, and intestine 2, 4, 6, 12, and 24 hours after sham operation or induction of acute pancreatitis. * and **: probability levels of < 0.05 and < 0.01, respectively, compared with sham operation.

from 6 hours onward, and in monocytes and pulmonary and intestinal macrophages from 12 hours onward ($p < 0.05$) (Fig. 5) compared to controls.

A significant increase in the whole-body oxygen extraction rate was seen 4, 6, and 24 hours after induction of acute pancreatitis ($p < 0.05$ versus controls) (Fig. 6), whereas the gut oxygen extraction rate significantly decreased after 2 and 4 hours followed by an increase at 12 and 24 hours ($p < 0.05$ versus controls) (Fig. 6).

Discussion

At least 10% of patients with acute pancreatitis develop a severe form of the disease associated with high morbidity and mortality

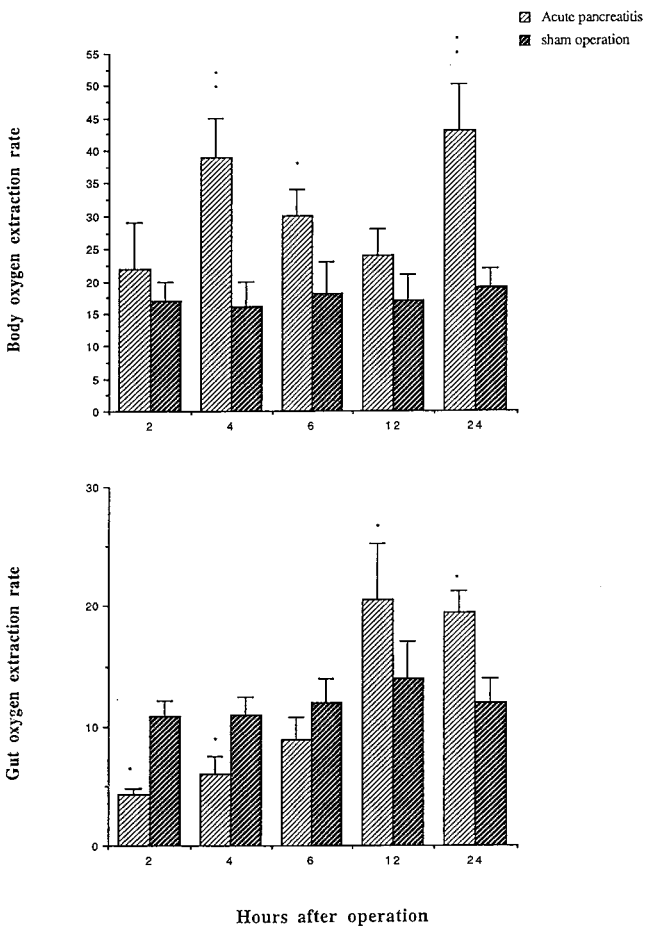

Fig. 6. Whole-body and gut oxygen extraction rate (%) in rats 2, 4, 6, 12, and 24 hours after sham operation or induction of acute pancreatitis. * and **: probability levels of < 0.05 and < 0.01, respectively, compared with sham operation.

and frequent bacterial infections [18–20]. Bacteria normally present in the gastrointestinal tract (e.g., *E. coli, Enterobacter aerogenes*, enterococci, *Pseudomonas aeruginosa, Proteus, Bacteroides*, and *Klebsiella* [3, 19]) are the ones most frequently isolated in acute pancreatitis, indicating that the gut may play an important role in the pathogenesis of these pancreatitis-related infections. In experimental acute pancreatitis induced by biliopancreatic obstruction alone, all MLN samples were positive for bacterial growth after 48 and 96 hours [6]. With acute, nonlethal pancreatitis induced by a 12-hour continuous intravenous infusion of cerulein, the incidence of bacterial translocation from the gut to MLNs and pancreas was 17% (2 of 12) and 33% (4 of 12) respectively, and the translocation rate of fluorescent latex beads from the gut to the pancreas was 91% (10 of 11) [5]. With acute pancreatitis induced by intraductal injection of bile, associated with 25% mortality within 48 hours, enteric bacteria translocated from the gut to the MLNs (100%), pancreas (89%), peritoneal fluid (78%), and blood (56%) in nine surviving rats 2 days after induction of acute pancreatitis [7]. In the present study, acute pancreatitis induced by retrograde intraductal injection of 5% sodium taurocholate, a model with about 50% 24-hour mortality, consistent with a previous report [21], was used. Our data show that bacterial translocation from the gut to MLNs occurred 4 hours after induction, significantly increasing from 12 hours onward, mainly to MLNs and lungs. These results support the hypothesis that enteric bacterial invasion and dissemination may contribute to the pathogenesis of pancreatitis-related infections and complications [5, 6, 22].

The route of bacterial translocation from the gut in those with acute pancreatitis has not been clarified and may depend on the underlying pathogenetic cause. In several experimental models (e.g., burn injury, major liver resection, acute liver failure) the MLN–thoracic duct route has been reported to dominate [10, 12, 23–26], although Mainous et al. [27] reported that the portal vein constituted a major transport route in a model of systemic inflammation induced by zymosan. They proposed that the route and extent of enteric bacterial translocation might be associated with the magnitude of the insult and that the portal–systemic circulation axis might act as a major route for systemic dissemination of bacterial translocation in serious situations. Studies on bile reflux pancreatitis, with or without primarily infected bile, suggested that enteric bacteria translocated to MLNs or spread directly along the pancreatic duct [22]. In the present study, the MLN was the first and most frequent site of enteric bacterial growth, whereas only two (7%) samples from the portal vein were bacteriologically positive. This finding suggests that the MLN–thoracic duct–systemic circulation axis may be the predominant route of enteric bacterial invasion in the presence of acute experimental pancreatitis.

Intestinal bacterial overgrowth is accompanied by changes in intestinal microbial ecology, which is associated with bacterial translocation in a variety of conditions [7–10, 12, 28]. Reported alterations in enteric bacteria associated with acute experimental pancreatitis have been contradictory. A moderate rate of bacterial translocation to MLNs and the pancreas occurred with nonlethal acute pancreatitis induced by ligation of the common bile duct, without causing alterations in enteric bacterial ecology or bacterial overgrowth [5]. However, a disturbed enteric bacterial balance and bacterial overgrowth in pancreatitis with increases in both the percentage of the total bacterial count and the actual number of gram-negative bacteria have been reported [6]. In the present study, the number of *E. coli* was determined, as this species is of importance both as a pathogen and as a representative of the intestinal ecosystem. Overgrowth of *E. coli* occurred in the colon and distal ileum 6 and 12 hours after induction of pancreatitis. The mechanisms of bacterial overgrowth in acute pancreatitis have been suggested to be associated with reduced intestinal motility, leading to failure of the normal peristaltic clearance [6], absence of pancreatic juice in the gut resulting in decreased antibacterial activity within the intestinal lumen [29], and altered intestinal microcirculation and abnormal oxygen delivery and extraction resulting in intestinal barrier failure as indicated by the present data. Our findings also imply that interactive enteric bacteria may fail to maintain a normal intestinal bacterial ecology in experimental acute pancreatitis. Obligate anaerobic bacteria are responsible for enteric bacterial antagonism and colonization resistance, as they closely associate with mucosal epithelial cells and form a barrier serving as the first line of defense to limit direct attachment or intimate association of pathogenic bacteria to the mucosa [30]. The number of anaerobic bacteria significantly decreased in both the colon and distal ileum in acute pancreatitis, probably contributing to failure of bacterial antagonism and *E.*

coli overgrowth. Furthermore, a decrease in the number of lactobacilli, followed by an increase in the number of *E. coli*, was seen. Lactobacilli possess antimicrobial activities (e.g., bacteriocins, low-molecular-weight antibiotic substances, organic acids, yellow pigments, and other metabolic end-products), contributing to enteric bacterial homeostasis and stimulating the systemic immune function [31–33].

Impairment of host immune defense has been suggested as essential for the induction of enteric bacterial translocation in experimental studies. This hypothesis is also supported by the fact that enteric bacteria frequently infect immunocompromised patients [34]. With experimental severe acute pancreatitis there is a decreased systemic clearance rate of *E. coli* [35]. In the present study, the systemic uptake rate of labeled bacteria significantly decreased during the early stage of acute pancreatitis. A decrease in Kupffer cell uptake contributed to the reduced host phagocytosis. The impaired RES function could be at least partly due to release of large amounts of pancreatic proteases into the portal circulation, which might interfere with the phagocytosis by Kupffer cells [35]. Macrophage uptake capacity in various organs (e.g., lungs, intestine, blood) increased in animals with acute pancreatitis, perhaps in an attempt to compensate for the decrease in Kupffer cell phagocytosis. In a previous study, we showed that a nonlethal septic challenge stimulates phagocytic activity [36]. However, the increased phagocytosis in extrahepatic organs seems insufficient to improve the impaired RES function induced by acute pancreatitis. Release of tissue-destructive agents, mediators, or cytokines by these activated phagocytic cells could probably result in tissue injury and eventually lead to multiple organ failure [37]. So might these overexaggerated pulmonary and intestinal macrophages, as shown in the present study, be responsible for initiating pulmonary complications and failure of intestinal function in patients with acute pancreatitis. Activated intestinal macrophages in acute pancreatitis may, alternatively, "carry" translocating bacteria from the gut through the mucosal barrier and deliver them into MLNs. When evaluating the role of the macrophage in translocation of enteric bacteria in immunocompetent and macrophage-defective mice "monoassociated" with a streptomycin-resistant strain of *E. coli*, the incidence of bacterial translocation from the gut to MLNs was 100% in immunocompetent animals but only 40% in macrophage-defective animals [38]. Blocking these activated macrophages by intravenous injection (acting systemically) or oral administration (blocking intestinal macrophages) of a water-soluble macromolecule reduced the occurrence of bacterial translocation from the gut induced by major liver resection in the rat [12]. These experimental studies imply that overactivated macrophages, especially intestinal macrophages, participate in the pathogenesis of bacterial translocation, probably by the action of released mediators, mechanical phagocytosis and transport, or lytic enzymes. In the present study, experimental acute pancreatitis led to a decrease in killing capacity of macrophages, which implies that these macrophages fail to clean translocating bacteria.

Circulatory alterations or failure of oxygen metabolism after induction of acute pancreatitis may also correlate with the development of enteric bacterial translocation. The gastrointestinal tract is one of the initial organs with compromised tissue oxygenation during shock, and the superficial layers of the villi are in the first part of the intestine, where a decrease in oxygenation is noted [39]. Hypovolemia and shock are common with severe acute pancreatitis and probably a major contributory factor for pancreatitis-induced multiple organ system failure [40]. The present model of inducing acute pancreatitis results in a decrease in both systemic and intestinal blood flow [41]. This finding gives reason to expect some ischemia-reperfusion injury with production of reactive oxygen metabolites and membrane lipid peroxidation [42, 43]. Potential oxidative stress may at least partly explain the occurrence of intestinal barrier failure and translocation, as we previously showed that intestinal barrier permeability increased in both in vivo and in vitro experimental acute pancreatitis, findings that were reversed by treatment with the hydroxyl radical scavenger dimethylsulfoxide [44]. In the present study the whole-body oxygen extraction rate significantly increased from 2 hours onward after induction of acute pancreatitis, whereas gut oxygen extraction rate initially decreased followed by an increase at 24 hours. The increase in the oxygen extraction probably represents the effect of defective tissue oxygenation due to hypovolemia or an increased metabolic demand for oxygen exceeding that which can be delivered. Decreased gut oxygen extraction following acute pancreatitis reflects an inability to extract and utilize available oxygen or the existence of intestinal microcirculatory shunting. A decrease in gut oxygen extraction could also be due to an increase in the hemoglobin oxygen affinity and a shift in the hemoglobin dissociation curve to the left, which may impair tissue oxygen extraction [45]. Because intermediary metabolism and energy production have an absolute oxygen dependence and oxygen cannot be stored intracellularly, an inadequate oxygen supply rapidly leads to intestinal cellular dysfunction, injury, and ultimately cell death, resulting for example in failure of the intestinal barrier against enteric bacterial invasion. The fact that the enteric bacterial ecology was disturbed with bacterial overgrowth and increasing numbers of aerobic and decreasing numbers of anaerobic bacteria implies that the impaired tissue oxygenation and microenvironment are not less important than other factors, such as decreased intestinal motility and clearance, increased adherence and colonization abilities of potential pathogens, and other factors.

In conclusion, translocation of enteric bacteria occurred during the early stage of acute pancreatitis in the rat. The MLN–thoracic duct–circulation axis is suggested as a major route for bacterial dissemination. Compromised gut oxygen extraction and overactivated intestinal macrophages probably play an important role in the pathogenesis of intestinal barrier failure. Impaired host immune function, reflected in a decrease in the systemic uptake rate of radiolabeled bacteria and impaired macrophage killing capacity, provides possibilities for enteric bacteria to invade and disseminate into extraintestinal sites and the systemic circulation.

Résumé

On suggère que l'intestin joue un rôle dans le développement des complications infectieuses, contribuant de façon significative à la morbidité et à la mortalité des patients atteints de pancréatite aiguë. Les buts de cette étude ont été d'évaluer la translocation bactérienne intestinale, l'homéostasie bactérienne et la fonction réticuloendothéliale dans la pancréatite aiguë expérimentale induite par l'injection intracanalaire de taurodesoxycholate de sodium à 5% chez le rat. L'incidence des translocations à partir de l'intestin jusqu'aux ganglions mésentériques et aux poumons a augmenté de façon significative après 12 heures et, après 24

heures, dans la circulation systémique, dans l'ascite et au niveau du pancréas. Le nombre de bactéries anaérobies et de *lactobacille* a diminué dans le côlon et dans l'iléon en 6 à 12 heures, alors que le nombre de *E coli* a augmenté en 12 heures. La présence de bactéries marqués dans la circulation systémique a diminué 6 heures après l'induction de la pancréatite aiguë. La captation de bactéries marquées dans les cellules de Kuppfer a diminué à 6 heures alors que la captation de bactéries marquées par les macrophages à partir du sang, des poumons et de l'intestin, a augmenté. On a mis en évidence une baisse de la capacité « tueuse » des macrophages, témoignant de l'augmentation du nombre de bactéries viables en culture à partir des macrophages isolés. L'extraction d'oxygène du corps a diminué 4 à 24 heures après l'induction de la pancréatite alors que l'extraction de l'oxygène de l'intestin a diminué à 2 et à 4 heures, suivie par une augmentation. Ces données montrent que la translocation des bactéries intestinales a lieu au début de la pancréatite aiguë et que la circulation entre les ganglions mésentériques et le canal thoracique peut être une voie de dissémination majeure. L'altération du métabolisme intestinal de l'oxygène, l'exagération de l'activité des macrophages intestinaux et la baisse de l'activité immunologique de l'hôte peuvent être à l'origine des infections dans la pancréatite aiguë.

Resumen

Se ha sugerido que el intestino juega un papel de importancia en el desarrollo de complicaciones bacterianas, las cuales son factores que contribuyen a la morbilidad y mortalidad en pacientes con pancreatitis aguda. El presente estudio se propuso evaluar la translocación entérica bacteriana, la homeostasis bacteriana y la función del sistema reticuloendotelial en la pancreatitis aguda experimental inducida por la inyección intraductal de taurodesoxicolato al 5% en la rata. La incidencia de translocación bacteriana del intestino a los ganglios linfáticos mesentéricos (GLM) y los pulmones se incrementó significativamente luego de 12 horas, y a la circulación sistémica, ascitis y páncreas a las 24 horas. El número de bacterias anaeróbicas y de *Lactobacilli* disminuyó en el colon y en el íleon distal a partir de las 6 o 12 horas, en tanto que el número de *E. coli* aumentó a partir de las 12 horas. La captación sistémica de bacterias radiomarcadas disminuyó a partir de 6 horas luego de la inducción de la pancreatitis aguda. La captación de bacterias radiomarcadas por parte de las células de Kupffer disminuyó a partir de 6 horas, en tanto que la captación por la sangre, pulmones y el intestino, disminuyó. Se observó una disminución en la capacidad letal de macrófagos aislados. La extracción corporal total de oxígeno aumentó 4–24 horas luego de la inducción de la pancreatitis, en tanto que la tasa intestinal de extracción de oxígeno disminuyó a las 2 y 4 horas, lo cual fue seguido de un incremento a las 12–24 horas. Estos hallazgos muestran que la translocación de bacterias entéricas ocurre en una fase temprana de la pancreatitis aguda y la circulación GLM-canal torácico puede ser una ruta principal para la diseminación bacteriana. Es posible que factores tales como un metabolismo oxidativo intestinal afectado, sobrexageración de los macrófagos intestinales y alteración de la función inmunitaria del huésped, se hallen involucrados en el desarrollo de las complicaciones sépticas de la pancreatitis aguda.

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References

1. McClave, S.A., McAllister, E.W., Karl, R.C., et al.: Pancreatic abscess: ten year experience at the University of South Florida. Am. J. Gastroenterol. *81*:180, 1986
2. Lumsden, A., Bradley III, E.L.: Secondary pancreatic infections. Surg. Gynecol. Obstet. *170*:459, 1990
3. Beger, H.G., Bittner, R., Block, S., Buchler, M.: Bacterial contamination of pancreatic necrosis: a prospective clinical study. Gastroenterology *91*:433, 1986
4. Bradley, E.L., Aller, K.: A prospective longitudinal study of obstruction versus surgical intervention in the management of necrotizing pancreatitis. Am. J. Surg. *161*:19, 1991
5. Medich, D.S., Lee, T.K., Melhem, M.F., Rowe, M.I., Schraut, W.H., Lee, K.K.W.: Pathogenesis of pancreatic sepsis. Am. J. Surg. *165*:46, 1993
6. Runkel, N.F., Moody, F.G., Smith, G.S., Rodriguez, L.F., LaRocco, M.T., Miller, T.A.: The role of the gut in the development of sepsis in acute pancreatitis. J. Surg. Res. *51*:18, 1991
7. Tarpila, E., Nyström, P.O., Franzén, L., Ihse, I.: Bacterial translocation during acute pancreatitis in the rat. Eur. J. Surg. *159*:109, 1993
8. Berg, R.D.: Inhibition of *Escherichia coli* translocation from the gastrointestinal tract by normal cecal flora in gnotobiotic or antibiotic-decontaminated mice. Infect. Immun. *29*:1073, 1980
9. Deitch, E.A., Taylor, M., Grisham, M., Ma, L., Bridges, W., Berg, R.: Endotoxin induces bacterial translocation and increases xanthine oxidase activity. J. Trauma *29*:1679, 1989
10. Wang, X.D., Soltesz, V., Andersson, R., Bengmark, S.: Bacterial translocation in surgically acute liver failure induced by 90% hepatectomy in the rat. Br. J. Surg. *80*:72, 1993
11. Lennette, E.H.: Manual of Clinical Microbiology. Washington, D.C., American Society for Microbiology, 1985
12. Wang, X.D., Andersson, R., Soltesz, V., Guo, W., Bengmark, S.: Water-soluble ethylhydroxyethyl cellulose prevents bacterial translocation induced by major liver resection in the rat. Ann. Surg. *217*:155, 1993
13. Wang, X.D., Andersson, R., Ding, J.W., Norgren, L., Bengmark, S.: Reticuloendothelial system function following acute liver failure induced by 90% hepatectomy in the rat. HPB Surg. *6*:151, 1993
14. Wang, X.D., Ar'Rajab, A., Ahrén, B., Anderson, R., Bengmark, S.: Improvement of the effects of intrasplenic transplantation of hepatocytes after 90% hepatectomy in the rat by cotransplantation with pancreatic islets. Transplantation *52*:462, 1990
15. Friedman, S.L., Roll, F.J.: Isolation and culture of hepatic lipocytes, Kupffer cells, and sinusoidal endothelial cells by density gradient centrifugation with stractan. Ann. Biochem. *161*:207, 1987
16. Callery, M.P., Kamei, T., Mangino, M.J., et al.: Organ interactions in sepsis. Arch. Surg. *126*:28, 1991
17. Mahida, Y.R., Patel, S., Gionchetti, P., Vaux, D., Jewell, D.P.: Macrophage subpopulations in lamina propria of normal and inflamed colon and terminal ileum. Gut *30*:826, 1989
18. Frey, C.F., Bradley, E.L., Beger, H.G.: Progress in acute pancreatitis. Surg. Gynecol. Obstet. *167*:282, 1988
19. Holden, J.L., Berne, T.V., Rosoff, L.: Pancreatic abscess following acute pancreatitis. Arch. Surg. *111*:858, 1976
20. Renner, I.G., Savage II., W.T., Pantoja, J.L., Renner, V.J.: Death due to acute pancreatitis: a retrospective analysis of 405 autopsy cases. Dig. Dis. Sci. *30*:1005, 1985
21. Dabrowski, A., Gabryelewicz, A.: Oxidative stress: an early phenomenon characteristic of acute experimental pancreatitis. Int. J. Pancreatol. *12*:193, 1992
22. Runkel, N.S., Rodriguez, L.F., LaRocco, M.T., Moody, F.G.: Mechanisms of pancreatic infection in acute pancreatitis in opossums. Curr. Surg. *Nov/Dec*:460, 1990

23. Maejima, K., Deitch, E.A., Berg, R.: Promotion by burn stress of the translocation of bacteria from the gastrointestinal tract of mice. Arch. Surg. *119*:166, 1984
24. Tokyay, R., Zeigler, S.T., Loick, H.M., et al.: Mesenteric lymphadenectomy prevents postburn systemic spread of translocated bacteria. Arch. Surg. *127*:384, 1992
25. Wang, X.D., Andersson, R., Soltesz, V., Wang, L., Bengmark, S.: Effect of portal hypertension on bacterial translocation induced by major liver resection in rats. Eur. J. Surg. *159*:343, 1993
26. Wang, X.D., Pärsson, H., Andersson, R., Soltesz, V., Johansson, K., Bengmark, S.: Bacterial translocation, intestinal ultrastructure and cell membrane permeability early after major liver resection in the rat. Br. J. Surg. *81*:579, 1994
27. Mainous, M., Tso, P., Berg, R.D., Deitch, E.A.: Studies of the route, magnitude, and time course of bacterial translocation in a model of systemic inflammation. Arch. Surg. *126*:33, 1991
28. Berg, R.D., Owens, W.E.: Inhibition of translocation of viable E. coli from the gastrointestinal tract of mice by bacterial antagonism. Infect. Immunol. *25*:820, 1979
29. Pierzynowski, S., Sharma, P., Sobczyk, J., Garwacki, S., Barej, W.: Influence of feeding regimen and postnatal developmental stages on antibacterial activity of pancreatic juice. Int. J. Pancreatol. *12*:121, 1992
30. Van der Waaij, D., Berghuid-de Vries, J.M., Lekkerkerk-van der Wees, J.E.C.: Colonization resistance of the digestive tracts and the spread of bacteria to the lymphatic organs in mice. J. Hyg. *69*:405, 1971
31. Fernandes, C.I., Shahani, K.M., Amer, M.A.: Therapeutic role of dietary lactobacilli and lactobacillic fermented dietary products. FEMS Microbiol. Rev. *46*:343, 1987
32. Axelsson, L.Y., Chung, T.C., Dobrogosz, W.J., Lindgren, S.E.: Production of a broad spectrum antimicrobial substance by Lactobacillus reuteri. Microb. Ecol. Health Dis. *2*:131, 1989
33. Fuller, R.: Probiotics in human medicine. Gut *32*:439, 1991
34. Berg, R., Wommack, E., Deitch, E.A.: Immunosuppression and intestinal bacterial overgrowth synergistically promote bacterial translocation from the GI tract. Arch. Surg. *123*:1359, 1988
35. Widdison, A.L., Karanjia, N.D., Alvarez, C., Reber, H.A.: Influence of levamisole on pancreatic infection in acute pancreatitis. Am. J. Surg. *163*:100, 1992
36. Andersson, R., Srinivas, V.: Peritoneal phagocyte activity during non-lethal E. coli abdominal sepsis in the rat. Surg. Res. Commun. *11*:119, 1991
37. Border, J.R.: Multiple systemic organ failure. Ann. Surg. *216*:111, 1992
38. Wells, C.L., Maddaus, A., Simmons, R.L.: Role of the macrophage in the translocation of intestinal bacteria. Arch. Surg. *122*:48, 1987
39. Antonsson, J.B., Fiddian-Green, R.G.: The role of the gut in shock and multiple system organ failure. Eur. J. Surg. *157*:3, 1991
40. Mcfadden, D.W.: Organ failure and multiple organ system failure in pancreatitis. Pancreas *6*:S37, 1991
41. Andersson, R., Wang, X.D., Ihse, I.: The influence of abdominal sepsis on acute pancreatitis in rats: a study on motality, permeability, arterial pressure, and intestinal blood flow. Pancreas 365:11, 1995
42. Halliwell, B.: Oxygen radicals: a commonsense look at their nature and medical importance. Med. Biol. *62*:71, 1994
43. Czyrko, C., Steigman, C., Turley, D.L., Drott, H.R., Ziegler, M.M.: The role of reperfusion injury in occlusive intestinal ischemia of the neonate: malonaldehyde-derived fluorescent products and correlation of histology. J. Surg. Res. *51*:1, 1991
44. Wang, X.D., Wang, Q., Andersson, R., Ihse, I.: Alterations in intestinal function in acute pancreatitis in the rat. Br. J. Surg. (in press, 1996)
45. Cain, S.M., Bradley, W.E.: Critical O_2 transport at lowered body temperature in rats. J. Appl. Physiol. *55*:1713, 1987

Invited Commentary

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Migration of intraluminal pathogenic bacteria outside the protected boundaries of the intestinal tract has been demonstrated to occur in a host of experimental preparations involving shock or sepsis. In a typically elegant experimental study from Professor Ihse's department, Wang and his coworkers collate much of what is known about bacterial translocation. In keeping with the results of previous investigators, they have shown a significant increase of gut-originated bacteria in mesenteric lymph nodes and in pulmonary tissue 12 hours after induction of severe acute pancreatitis. Intestinal bacteria in blood, ascites, and pancreatic tissue became significantly increased at 24 hours compared to controls. Moreover, during the period of bacterial translocation there was an alteration of intestinal bacterial homeostasis; the number of anaerobes significantly decreased and *Escherichia coli* significantly increased.

Importantly, this study also documented significant changes in the reticuloendothelial system during acute pancreatitis: reduced bacterial uptake by fixed macrophages (Kupffer cells), increased bacterial uptake by wandering macrophages, and an overall decrease in macrophage killing capacity.

In summary, these authors demonstrate that experimental severe acute pancreatitis results in increased bacterial access via widespread dissemination of intestinal bacteria to other organs and tissues, combined with simultaneous suppression of host bacterial defense mechanisms. This "double hit" could well account for the high incidence of secondary infections known to occur in patients with necrotizing pancreatitis.

Because the earliest appearance of intestinal bacteria occurred in mesenteric lymph nodes and lung at 4 hours, Wang and his associates hypothesize that the lymphatic system is the predominant pathway for bacterial translocation in experimental pancreatitis. Although not stated by the authors, early involvement of the lung might occur via transdiaphragmatic lymphatics, as significant bacteremia did not appear until 24 hours.

Despite concern in some quarters that bacterial translocation principally represents a laboratory phenomenon and is of little clinical consequence, recent studies involving patients with acute pancreatitis provide strong support that this process is both operational and important in humans. Significant reductions in overall mortality and the incidence of infection have been observed in patients with severe acute pancreatitis during prospective controlled studies employing prophylactic antibiotics [1, 2]. Agents in these studies were specifically chosen to produce selective intestinal decontamination or to combat translocated intestinal bacteria by penetration into pancreatic and peripancre-

atic tissue. On the basis of these and other similar observations, it is not unreasonable to predict that antibiotic-induced reduction of translocated bacteria will ultimately achieve an equal clinical footing with stress ulcer prophylaxis in appropriate patient populations.

Although the data from this study confirm and enhance our knowledge of the pathophysiology of bacterial translocation, a number of important clinical concerns remain: (1) At what level of illness severity is prophylactic antibiotic therapy justified? (2) For maximal effects, should antibiotic prophylaxis be administered orally, parenterally, or in combination? (3) Which antibiotics are preferred? Answers to these questions will go a long way toward improving survival after infection in severely ill and injured patients.

References

1. Luiten, E.J.T., Hop, W.C.J., Lange, J.F., Bruining, H.A.: Controlled clinical trial of selective decontamination for the treatment of severe acute pancreatitis. Ann. Surg. *222*:57, 1995
2. Pederzoli, P., Bassi, C., Vesentini, S., Campedelli, A.: A randomized multicenter clinical trial of antibiotic prophylaxis of septic complications in acute necrotizing pancreatitis with imipenem. Surg. Gynecol. Obstet. *176*:480, 1993