

Review

Significance of apoptotic cell death in systemic complications with severe acute pancreatitis

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In severe acute pancreatitis, multiple organ failure in the early stage after onset, and sepsis in the late stage, due to infection of pancreatic or peripancreatic devitalized tissue, contribute to its high mortality. In analogy with sepsis, evidence has accumulated of the significance of apoptotic cell death in the systemic manifestations associated with acute pancreatitis. Since we identified apoptosis-inducing activity in pancreatitis-associated ascitic fluid in 1995, a number of investigators, including our group, have reported, through animal experiments, that apoptosis occurred in the parenchymal cells constituting organs, such as alveolar epithelial cells in the lung, renal tubular cells in the kidney, and hepatocytes in the liver, and this apoptosis was involved in organ dysfunction with severe acute pancreatitis. Moreover, through clinical and experimental investigations, apoptosis has been revealed to be involved in the mechanism of infectious complications in acute pancreatitis. Namely, apoptosis in lymphatic tissues and peripherally circulating lymphocytes is involved in the impairment of cellular immunity, and apoptosis in gut epithelial cells is implicated in bacterial translocation. These results suggest that apoptotic cell death may play a considerable role in affecting mortality and morbidity in severe acute pancreatitis. Control of apoptosis could be a potent strategy for improvement of the clinical outcome in severe acute pancreatitis.

Key words: severe acute pancreatitis, apoptosis, multiple organ failure, immunosuppression, bacterial translocation

Introduction

Despite recent advances in critical care management, the mortality rate of severe acute pancreatitis is still high. Multiple organ failure in the early phase (including cardiovascular, pulmonary, renal, and hepatic failures) and sepsis in the late phase (due to infection of devitalized pancreatic and peripancreatic tissues) contribute to the high mortality of severe acute pancreatitis. Therefore, it is critically important to clarify the mechanism of such systemic complications to improve clinical outcomes in patients with severe acute pancreatitis.

“Apoptosis” is one of the most frequently used words in biology and medicine today. It was introduced into modern scientific writing by Kerr, and colleagues in 1972 to describe a form of cell death distinct from necrosis.¹ Apoptosis was initially recognized as “programmed cell death,” a type of cell death that serves to eliminate excessive or unwanted cells, in the course of organ development. Upon receiving specific signals instructing the cells to undergo apoptosis, a number of distinctive biochemical and morphological changes occur in the cell. A family of proteins known as caspases is typically activated in the early stages of apoptosis. These proteins break down or cleave key cellular substrates that are required for normal cellular function, including structural proteins in the cytoskeleton and nuclear proteins, such as DNA repair enzymes. The caspases can also activate other degradative enzymes such as DNases, which begin to cleave the DNA in the nucleus. These biochemical changes result in morphological changes which are characterized by the condensation of nuclear chromatin in the nuclear periphery, cell membrane blebbing, and the formation of apoptotic bodies.^{2,3}

Meanwhile, apoptotic cell death has been reported to be involved not only in the course of organ development but also in cellular injury such as that occurring in

association with the use inflammatory disease or of antitumor chemotherapeutic agents.^{4,5} Moreover, it has been reported that many cytokines, such as interleukin-1 (IL-1),⁶ tumor necrosis factor- α (TNF- α),⁷ and transforming growth factor β (TGF- β),⁸ and free radicals, such as nitric oxide,⁹ induce apoptotic cell death in various cell systems.

Severe acute pancreatitis is recognized as a typical pathological condition complicating a systemic inflammatory response with a "cytokine storm."¹⁰ Sepsis is another typical condition associated with the activation of the cytokine cascade, and it is widely accepted that apoptotic cell death is involved in the multiple organ dysfunction and immunological impairment associated with sepsis.^{11,12} Thus, it is logical to assume that apoptotic cell death is involved in the systemic manifestations of severe acute pancreatitis. Since we reported, in 1995, that pancreatitis-associated ascitic fluid (PAAF) induced apoptosis in Madin-Darby canine kidney (MDCK) cells, evidence has accumulated to support the significance of apoptosis in severe acute pancreatitis.

As for apoptosis in pancreatitis, the significance of apoptosis in the normal pancreas is another crucial issue, and an initial report by Kaiser et al., in 1995,¹³ was followed by a number of articles on this issue. Most of the reports support the idea that pancreatic acinar cell apoptosis acts as a self-defense mechanism against the development of pancreatic necrosis. Recently, Bhatia^{14,15} reviewed this issue extensively. Thus, in this review, I will concentrate on the significance of apoptosis in the systemic manifestations of severe acute pancreatitis, rather than reviewing apoptosis in the pancreas itself.

Multiple organ failure in the early stage of severe acute pancreatitis

In multiple organ failure associated with severe acute pancreatitis, the cardiovascular system, lungs, kidney, liver, and the blood coagulation system are involved. In severe acute pancreatitis, apoptosis has been observed in these systems, as itemized in Table 1 (showing

data for experimental acute pancreatitis). Previous reports concerning apoptosis involved in organ failure in the early stage of acute pancreatitis are reviewed below.

Apoptosis in lung

In severe acute pancreatitis, pulmonary failure is characterized by hypoxia. Approximately 50% of patients with acute pancreatitis have arterial PO_2 levels of less than 60 mmHg at some time in the first few days after the disease onset. The respiratory dysfunction ranges from mild hypoxia to an adult respiratory distress syndrome (ARDS)-like pulmonary injury, and a PaO_2 level of less than 52.5 mmHg was reported to be associated with mortality of over 30%.¹⁶

As pancreatitis-associated apoptosis in the lung, apoptosis in type II pneumocytes was first observed by Wang et al. in 1998.¹⁷ Type II pneumocytes have two major functions: differentiation as precursors of type I pneumocytes for the repair of pulmonary barrier integrity, and the production and release of surfactant, maintaining the surface tension of the pulmonary alveoli. Wang et al.¹⁷ found morphological alterations characteristic of apoptosis in type II pneumocytes 12 h and 24 h after disease onset in rats with necrotizing hemorrhagic pancreatitis induced by intraductal injection of 5% sodium taurodeoxycholate. They confirmed dysfunction of the pulmonary endothelial barrier and assumed the involvement of TNF, because of elevated levels of TNF in bronchoalveolar lavage fluid during the initial phase (3 and 6 h) after onset.

The observation by Wang and colleagues was followed by a report by Yuan et al.¹⁸ in 2000. They used a model comparable to that of Wang et al.,¹⁷ and demonstrated that apoptosis was induced in alveolar epithelial cells 5 h after the induction of pancreatitis.¹⁸ They detected apoptosis by the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end labeling (TUNEL) method. Moreover, they detected the expression of bax protein (one of the members of a family of dominant inhibitors of bcl-2 that promotes apoptosis) in parallel to apoptosis induction in alveolar epithelial cells.

Table 1. Apoptosis relevant to organ failure in experimental acute pancreatitis

Organ	Type of cell	Species	Model	Reference nos.
Lung	Type II pneumocytes	Rat	Bile acid infusion	17
	Alveolar epithelial cells	Rat	Bile acid infusion	18
	Alveolar and vascular epithelial cells	Mouse	CDE diet	19
	Alveolar and bronchiolar cells	Rat	Bile acid infusion	20
Kidney	Renal tubules	Rat	Bile acid infusion	25
Liver	Hepatocytes	Rat	Bile acid infusion	32

More recently, Nakamura et al.¹⁹ reported that a rat counterpart of a 19-kD interacting protein-3 protein, which is a mitochondrial protein inducing apoptosis, increased its expression in rat lung 3 days after the onset of pancreatitis induced by retrograde intraductal infusion of 4% sodium taurocholate. In their animal model, significant apoptosis was detected not only in the alveoli but also in the bronchioles 3 days after induction of pancreatitis. Although their model of pancreatitis was similar to that in the preceding reports, the induction of apoptosis was significantly delayed (3 to 5 days after disease onset), and they detected apoptosis even in the bronchioles. The reasons for these discrepancies in time course and region are unclear.

Callicutt and colleagues²⁰ induced severe acute pancreatitis in mice by feeding them a choline-deficient ethionine (CDE) supplemented diet, and found apoptosis in pulmonary epithelial and vascular endothelial cells, by the TUNEL method, 6 days after the beginning of the feeding. The blockade of vascular adhesion molecule-1 (VCAM-1) by the administration of a monoclonal anti-VCAM-1 receptor antibody diminished both the histological lung injury and the induction of apoptosis. Therefore, they assumed the possible involvement of pulmonary leukocyte sequestration in the lung injury due to apoptosis.

Kidney

Renal failure is a major prognostic factor in acute pancreatitis, and is known to occur within a few days of onset, in spite of adequate fluid resuscitation, suggesting that direct renal cell injury occurs in the early stage of this disease. Levy et al.²¹ concluded that renal failure in acute pancreatitis was only the result of systemic hypovolemia; however Satake et al.²² contradicted this by demonstrating the existence of nephrotoxic substance(s) other than vasoactive substances or proteases.

As for the renal failure associated with sepsis, throughout the past half century, the basic understanding of this pathologic status has been that it involved acute tubular necrosis due to renal ischemia. Recently, however, accumulating evidence has suggested that septic renal failure is due to acute tubular apoptosis brought about by immunological or toxic stress rather than simply being caused by a hemodynamic condition.²³

As early as 1995, we found apoptosis-inducing activity in PAAF in MDCK cells through analyzing the toxicity of PAAF.²⁴ Subsequently, we found apoptosis in the kidneys of Wistar rats with necrotizing pancreatitis that had been induced by intraductal injection of 30% sodium deoxycholate. We confirmed apoptotic changes in renal tubules by the TUNEL method 6h after the induction of pancreatitis. In addition, PAAF collected

from the same animal models induced apoptosis *in vivo* in rat renal tubules when administered into the peritoneal cavity of a healthy rat, and this PAAF also induced apoptosis *in vitro*, in a dose- and time-dependent manner, in isolated rat renal tubules and in the normal rat kidney cell line NEK52E, as well as in MDCK cells.²⁵

Apoptosis in liver

Although liver failure is less frequent than lung or kidney failure in pancreatitis, it was reported that the frequency of liver failure in pancreatitis was 5%, and liver failure invariably led to death.²⁶ The increase of serum lactate dehydrogenase in severe acute pancreatitis suggests latent liver damage and was reported to correlate with mortality.²⁷ Hepatocyte apoptosis has been observed in various disease conditions, such as viral or chemical hepatitis,²⁸ allograft rejection,²⁹ portal vein ligation,³⁰ and ischemic-reperfusion injury.³¹ In particular, Bohlinger et al.¹¹ reported that hepatocyte apoptosis was induced in mice with endotoxin shock.

In acute pancreatitis, we reported that hepatocyte apoptosis was induced 6h after the induction of necrotizing pancreatitis in Wistar rats by retrograde injection of sodium deoxycholate.³² In addition, PAAF induced rat hepatocyte apoptosis when administered intraperitoneally to healthy rats *in vivo*, and it also induced *in vitro* apoptosis in rat primary cultures of hepatocytes in a dose- and time-dependent manner. We also found that macrophage-derived TGF- β 1 was partly involved in hepatocyte apoptosis in rat deoxycholate-induced pancreatitis.³³ During this investigation, we depleted macrophages, including Kupffer cells and peritoneal macrophages, by the administration of liposome-encapsulated dichloromethylene diphosphonate, which induces apoptosis in macrophages. The depletion of macrophages significantly attenuated liver injury and hepatocyte apoptosis.

Subsequently, Murr et al.³⁴ reported that PAAF induced hepatocyte apoptosis without activating Kupffer cells when rat liver was perfused with PAAF. They concluded that PAAF induced direct hepatocyte injury and death by heat-stable factors other than pancreatic enzymes, but not via the local production of Kupffer-cell-derived cytokines. A group from the same laboratory investigated the molecular mechanism of hepatocyte apoptosis *in vitro*, using human cultured hepatocytes, CCL-13. They demonstrated that PAAF-derived hepatocyte apoptosis occurred via the activation of p38-mitogen activated protein kinase (MAPK) and caspase-3-dependent pro-apoptotic pathways.³⁵ This group also proposed another concept, that pancreatic elastase upregulates Fas ligand within Kupffer cells, and the upregulated Fas ligand induces hepatocyte apoptosis.³⁶

Table 2. Apoptosis relevant to subsequent infection in severe acute pancreatitis

Cells	Species	Model	Reference nos.
Thymocytes	Rat	Bile acid infusion	43
Peripheral lymphocytes	Human	—	44
	Rat	Bile acid infusion	44, 46
Peripheral mononuclear cells	Human	—	45
Peripheral neutrophils ^a	Human	—	49, 50
Intestinal epithelial cells	Rat	Bile acid infusion	54, 56
Lymphocytes in Peyer's patches	Rat	Bile acid infusion	Unpublished data
Lymphocytes in MLNs	Rat	Bile acid infusion	Unpublished data

MLNs, mesenteric lymph nodes

^aDelay of spontaneous apoptosis

On the other hand, we reported that the intracellular Ca^{2+} concentration in primary cultures of rat hepatocytes significantly increased from 1 min after the addition of PAAF, in a dose-dependent manner.³⁷ In that report, we also showed that PAAF evoked the influx of extracellular Ca^{2+} across the plasma membrane by a mechanism other than via the voltage-dependent Ca^{2+} channel, and that TCV-309, a platelet-activating factor antagonist, blocked the PAAF-elicited elevation of intracellular Ca^{2+} concentration. However, to date no linkage of this phenomenon with hepatocyte apoptosis has been established.

Aside from these lines of inquiry, we have proposed hematin as a specific toxic substance that induces apoptosis.³⁸ In acute hemorrhagic pancreatitis, it was reported that hemoglobin was converted to hematin (a hem oxidative product), which binds with albumin to form metahemalbumin in the pancreas and peritoneal cavity. We detected a considerable amount of hematin in human clinical and rat experimental PAAF, and found that hematin induced hepatocyte apoptosis when injected into the peritoneal cavity of a healthy rat at a dose comparable to that determined in the PAAF. According to findings in an in vitro experiment using rat primary cultures of hepatocytes and HuH-7, a human hepatoma cell line, we assume that the apoptosis-inducing activity exerted by PAAF in hepatocytes occurs partly via hematin.

Apoptosis relevant to infectious complications in the late phase

Sepsis due to infection in devitalized pancreatic and peripancreatic tissues has become the most critical cause of death from acute pancreatitis. The mechanism of infection is now understood according to the concept of bacterial translocation—the movement of gut-origin microbes across the intact gastrointestinal tract into tissues that are normally sterile, where the organisms may then directly cause infection. Failure of intestinal

barrier function, together with bacterial overgrowth due to dysmotility and immunosuppression, constitute the pathways of pancreatic or peripancreatic contamination from bacterial translocation in patients with severe acute pancreatitis.³⁹ In reference to bacterial translocation or immunosuppression, several reports have been made, as itemized in Table 2. Reports concerning apoptosis involved in the the development of infectious complications in the early stage of acute pancreatitis will be reviewed in detail below.

Apoptosis of lymphocytes and lymphatic tissues

Antal et al.,⁴⁰ in 1978, reported the impairment of systemic cellular immunity in pancreatitis. They reported that a significant reduction in the number of peripheral T-lymphocytes was found in patients with acute pancreatitis, and the number returned to normal after recovery. In 1985, Christophi and colleagues⁴¹ reported that the absolute lymphocyte count, measured within 48h after admission, predicted the severity of the disease, and, in 1993, Curley et al.⁴² reported a significant decrease in the proportion of T-helper lymphocytes and a significant increase in the levels of IL-6 and C-reactive protein in severe pancreatitis compared to mild pancreatitis.

Meanwhile, abnormalities in cellular immunity due to apoptosis have been investigated by a number of investigators, and it is well recognized that apoptosis occurs in lymphoid tissues during sepsis or thermal injury.

Along these lines, it is very possible that the decrease in peripheral lymphocytes during pancreatitis is caused by apoptosis. Firstly, we found that significant thymic atrophy occurred, due to thymocyte apoptosis, during experimental severe acute pancreatitis in rats.⁴³ Moreover, we clarified that peripheral lymphocyte numbers on admission were significantly lower in patients with subsequent infectious complications than in the patients without these complications, and we found that the reduction in lymphocytes from the peripheral circulation was due to apoptosis.⁴⁴ Although the ratio of apoptotic

lymphocytes at collection did not differ between pancreatitis patients and normal subjects, the ratio increased significantly after 24-h incubation only in the lymphocytes collected from the pancreatitis patients. This phenomenon, i.e., the *ex vivo* acceleration of apoptosis, suggests the existence of a mechanism for the rapid clearance of apoptotic lymphocytes from the systemic circulation. In addition, similar changes in peripheral lymphocytes were observed in a rat experimental model of severe acute pancreatitis, but not in rat caerulein-induced pancreatitis, which is a model of mild acute pancreatitis.

Salomone et al.⁴⁵ also reported significant reductions of peripheral lymphocyte counts in pancreatitis patients with complications compared with findings in those without complications. They evaluated apoptosis in peripheral mononuclear cells, and reported that the ratio of apoptotic cells was lower in the early stage and higher in the late stage in severe pancreatitis compared with mild pancreatitis. Although they assumed that apoptosis in peripheral blood cells was a mechanism for the self-limitation of the progress of this disease, such an assumption is difficult to test, because the significance and dynamics of apoptosis are quite different among cell species.

The spleen, as well as the thymus, is a major immune organ, and is involved not only in the clearance of particulate antigens and injured or old cells within the host but also in the regulation of cell-mediated immune processes and the production of opsonins. Indeed, it has been well recognized that splenectomized hosts show increased susceptibility to bacterial infection and sometimes have overwhelming sepsis. Therefore, we also examined splenic involvement in acute pancreatitis.

In rats 12 and 24h after the induction of pancreatitis by intraductal infusion of 3% sodium deoxycholate, splenic weights were significantly lower than those of sham-operated rats. Numbers of splenocytes were also significantly reduced along with the reduction in splenic weight. Differently from the thymus, however, no apoptosis was detected in the splenocytes from the rats with pancreatitis.⁴⁶ Peripheral lymphocytes in the rats with pancreatitis were significantly decreased due to apoptosis compared with those in sham-operated rats. With antecedent splenectomy, peripheral lymphocyte counts were significantly lower than those in rats without splenectomy. It was suggested that splenocytes were recruited into the systemic circulation in response to peripheral lymphocyte reduction as a result of apoptosis.

As immune function is assumed to be altered in pancreatitis, we examined functional alterations of splenocytes in the same rat model of severe acute pancreatitis as that used by us previously.⁴⁶ In splenocytes harvested 24h after the induction of pancreatitis, proliferative

activity was lowered, and IL-2 secretion in response to concanavalin A was also depressed. The results obtained suggested that the Th1/Th2 balance tended toward Th1 suppression as a whole in severe pancreatitis.⁴⁷ In another study, Uehara et al.⁴⁸ reported the results of clinical observations. They found that both CD4-positive and CD8-positive T-lymphocytes from the peripheral blood in patients with pancreatitis were reduced in number, and that in severe disease, the reduction in the former cells was more pronounced. A significant positive correlation between soluble (s)CD4 and sFas was noted, and during the early stage of acute pancreatitis, the concentrations of sCD4, sCD8, sIL-2-R, IL-12, and IFN- γ increased more in the patients with severe disease than in the patients with milder disease. According to their clinical results, Th1 type CD4+ T cells were reduced in number, but they were activated and elicited proinflammatory reactions during the early stage of acute pancreatitis. However, this discrepancy was left unexplained.

Recently, in rat deoxycholate-induced pancreatitis, we observed that lymphocyte apoptosis was accelerated in mesenteric lymph nodes and Peyer's patches 6 and 24h, respectively, after the disease onset (Fig. 1; unpublished data) These observations suggest that apoptosis also occurs in gut-associated lymphatic tissues and may contribute to gut-associated immunosuppression.

Delay of neutrophil apoptosis

Neutrophil-mediated inflammation is terminated by apoptosis of the neutrophils. Neutrophil apoptosis is inhibited by a variety of inflammatory stimuli; neutrophils isolated from critically ill septic patients show profoundly delayed rates of apoptosis *in vitro*. So a delay in neutrophil apoptosis is considered to be implicated in the maintenance of inflammation. To confirm this, O'Neill et al.⁴⁹ clearly demonstrated that neutrophils isolated from patients with acute pancreatitis showed a significant delay in spontaneous apoptosis. This delay was more prominent in severe pancreatitis than in mild disease. They also found marked resistance to Fas antibody-induced apoptosis, possibly derived from the downregulation of procaspase-3, in the neutrophils from pancreatitis patients.

More recently, Chiu et al.⁵⁰ demonstrated a correlation of neutrophil apoptosis with the severity of pancreatitis, after treatment with gabexate mesilate. They evaluated patient condition and *ex vivo* neutrophil apoptosis after 1-week treatment with gabexate mesilate, and found that *ex vivo* apoptosis was delayed significantly in the neutrophils collected from patients with more than two complications compared with those collected from patients with fewer than two complications. They assumed that the delay in neutrophil

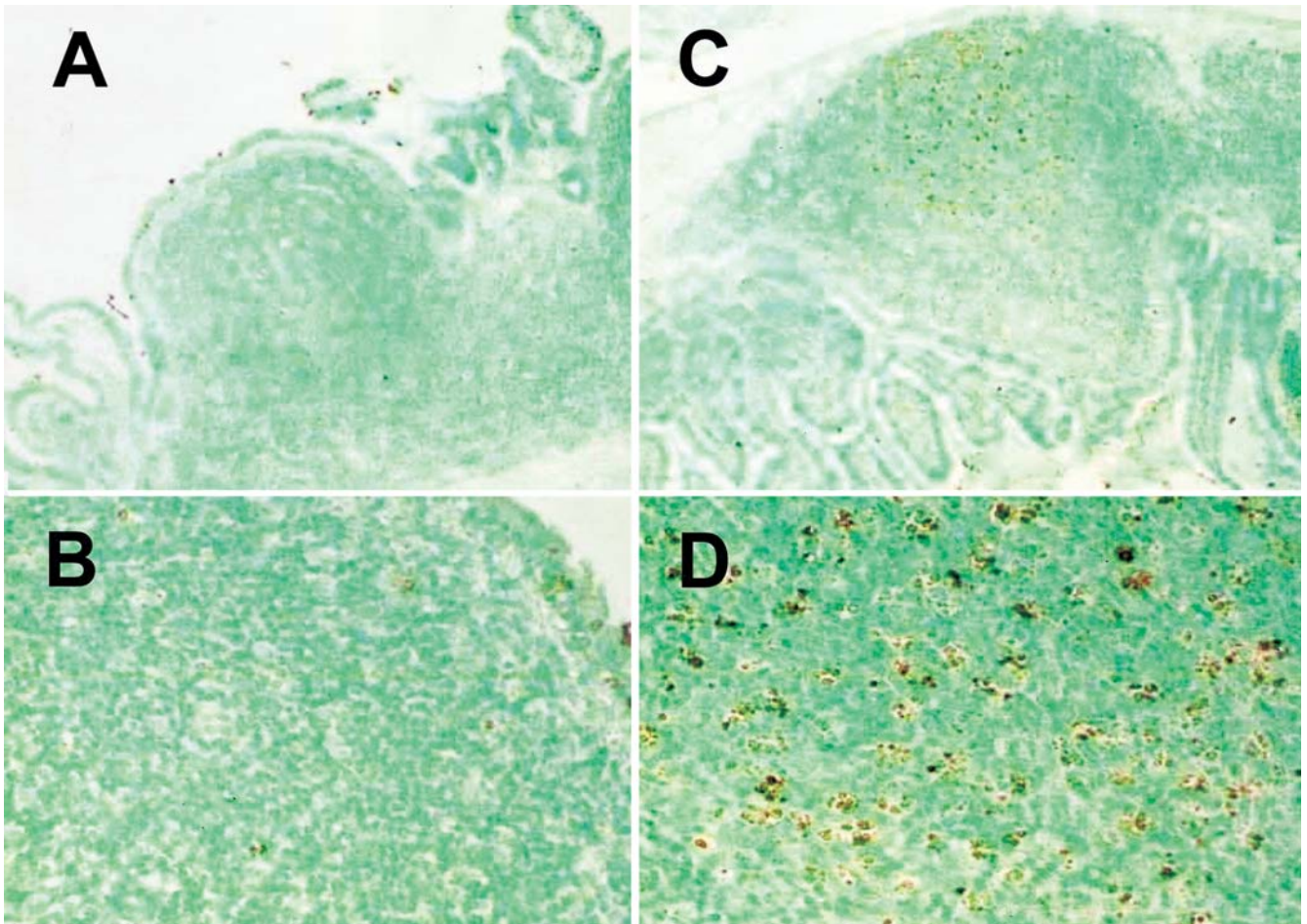


Fig. 1A–D. Apoptosis in Peyer's patch of rat ileum associated with necrotizing pancreatitis. Necrotizing pancreatitis was induced by intraductal injection of 3% sodium deoxycholate in male Wistar rats. The ileum was harvested 24 h after the induction of pancreatitis, and Peyer's patches in the ileum were stained by the terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick and labeling (TUNEL) method. Counterstaining was done with methylene green. **A,B** Sham operation; **C,D** pancreatitis. **A,C** $\times 100$; **B,D** $\times 400$

apoptosis was associated with the severity of organ dysfunction in pancreatitis.

Apoptosis of intestinal epithelial cells

Apoptosis in intestinal epithelial cells has been reported to be increased in pathological situations, such as ischemia/reperfusion, radiation-induced intestinal injury, and obstructive jaundice.^{51–53} With regard to pancreatitis, Wang et al.⁵⁴ reported that apoptosis in the intestinal epithelium was accelerated in rats with experimental pancreatitis induced by intraductal injection of 5% sodium taurocholate. In an earlier article,⁵⁵ they reported that the administration of growth hormone prevented pancreatitis-induced intestinal injury. The administration of growth hormone reduced the rate of bacterial translocation and preserved gut mucosal morphology and function.⁵⁵ In a later article, they evaluated

apoptosis by carrying out DNA agarose gel electrophoresis of DNA extracted from the entire intestinal mucosa, using fluorescein isothiocyanate-labeled annexin V and propidium iodide staining with flow cytometry of detached epithelial cells, and by TUNEL staining of transmural specimens of the ileum, and found a significant increase of apoptosis 6 h after the induction of pancreatitis. They also found that growth hormone downregulated the accelerated apoptosis in the intestinal epithelium, and they assumed that the inhibition of apoptosis was involved in the mechanism underlying the protective effects of growth hormone on intestinal barrier integrity in acute pancreatitis.

Subsequently, we confirmed significant acceleration of apoptosis in rat ileal epithelium 8 h after the induction of pancreatitis by intraductal injection of 3% sodium deoxycholate.⁵⁶ We also found that oxygenation, done by the intraperitoneal administration of oxygen-

Table 3. Apoptosis-inducing activity of PAAF

System utilized	Apoptotic cells		Reference nos.	
In vitro	Hepatocytes	Rat primary culture	32, 33	
		HuH7 (human)	37	
		CCL-13 (human)	34, 35	
	Renal tubules	MDCK cells (canine)	24	
		Rat primary culture NRK 52E (rat)	25 25	
In vivo	Intraperitoneal	Hepatocytes	Rat hepatocytes Mouse hepatocytes	32 35
		Renal tubules	Rat renal tubules	25
	In situ perfusion	Rat hepatocytes		34

ated perfluorochemical, inhibited bacterial translocation and apoptosis in the ileal epithelium. These observations indicate that apoptosis in the intestinal epithelium occurs in the early phase of severe acute pancreatitis and contributes to the development of bacterial translocation.

Factors influencing apoptosis in acute pancreatitis

As noted already in this review, apoptotic cell death occurs in a number of organs and systems, and is very possibly involved in the systemic manifestations of severe acute pancreatitis. Although a number of cells and molecules, including cytokines, have been proposed to be responsible for apoptosis in various organs and systems, no one factor has yet been found to be dominant, and a conclusion regarding this cannot be made at present.

Significance of PAAF

Among the factors assumed to induce apoptosis, however, PAAF is the strongest candidate as an apoptosis inducer. As reviewed above, PAAF has been clarified to have apoptosis-inducing activity in several cell systems in vitro and in vivo (Table 3). In 1999, we identified the importance of peritoneal macrophages as a source of apoptosis-inducing factor(s) in PAAF. We demonstrated that peritoneal macrophage depletion by peritoneal lavage deleted apoptosis-inducing activity in PAAF collected from rats with deoxycholate-induced pancreatitis.⁵⁷ Furthermore, the expression of TGF- β 1 in peritoneal macrophages and the elevation of TGF- β 1 in PAAF suggested the involvement of peritoneal macrophage-derived TGF- β 1. As a matter of fact, neutralization of TGF- β 1 by its antibody partly blocked its apoptosis-inducing activity in vitro.³² Other than TGF-

β 1, we also found that PAAF evoked marked elevation of the intracellular Ca^{2+} concentration in various cell systems, possibly via the action of platelet-activating factor.³⁷ Moreover, hematin was clarified to be partly responsible for the apoptosis-inducing activity of PAAF.³⁸

Norman and colleagues^{34,35} found multiple pathways for PAAF-induced apoptosis in hepatocytes. They identified the existence of heat-stable factor(s) in PAAF that elicited apoptosis in cultured hepatocytes, and they found that the p38-MAPK and caspase-3 dependent pathway was involved in PAAF-induced hepatocyte apoptosis.³⁵

Fas and fas ligand

Fas is a cell-surface protein belonging to the TNF receptor family, whereas Fas ligand is a member of the TNF family. Fas ligand is mainly produced by activated lymphocytes and works as an effector to remove cells infected by virus or cancer cells. Fas-induced apoptosis is reported to be involved in the parenchymal cell damage in injuries to various organs, such as liver injury and acute renal failure. Endo et al.⁵⁸ measured serum levels of soluble Fas and soluble Fas ligand in patients with pancreatitis. They observed a greater increase of soluble Fas and greater decrease of soluble Fas ligand in patients with multiple organ dysfunction syndrome than in patients without the syndrome. Their observation suggests that the Fas-Fas ligand system is involved in the apoptosis-associated systemic manifestations of severe acute pancreatitis.

Recently, Norman and colleagues³⁶ have proposed that Fas ligand, which is derived from Kupffer cells activated by pancreatic enzymes, induces the hepatocyte apoptosis in liver injury associated with pancreatitis.

Antiapoptotic factors

Hepatocyte growth factor (HGF), which was found to be a potent mitogen for parenchymal liver cells, and which functions as a hepatotrophic factor for liver regeneration after hepatic injury, is now considered to play crucial multifunctional roles in tissue repair and organogenesis. We demonstrated that serum HGF levels were elevated in patients with acute pancreatitis, and that elevation of serum HGF was closely related to the organ dysfunction in clinical acute pancreatitis.^{59,60} In rat experimental necrotizing pancreatitis, HGF was increased in both serum and PAAF, and the expression of HGF protein and HGF mRNA was enhanced in remote organs, including liver, kidney, and lung. Interestingly, neutralization of HGF by antibody administration enhanced apoptosis in renal tubules, and worsened liver function in rats with necrotizing pancreatitis. In contrast, recombinant HGF prevented PAAF-induced apoptotic cell death in MDCK cells. These observations strongly suggest that HGF is produced in injured organs and functions as an antiapoptotic factor against the organ injury in pancreatitis.⁶¹

Other than HGF, Fiedler et al.⁶² proposed the involvement of *PC3/TIS/BTG2* gene expression in a protective mechanism against apoptosis in pancreatitis. They reported that *PC3/TIS/BTG2* mRNA was overexpressed in the livers and kidneys of rats with necrotizing pancreatitis induced by the intraductal injection of 1% taurocholate 24h after induction. As *PC3/TIS/BTG2* protein is known to be a potent antiapoptotic factor, they speculated that this protein may play a protective role against the progression of apoptosis in pancreatitis.

Future perspectives

In the light of the above-reviewed lines of evidence, apoptotic cell death can be seen to be involved in the systemic manifestations of severe acute pancreatitis. Differently from apoptosis in the pancreas itself, apoptosis in remote organs or tissues is assumed to promote organ injury in pancreatitis, except in neutrophils. Based upon this concept, there are some possible future directions to improve clinical outcomes in the treatment of severe acute pancreatitis.

The first direction is to explore other organs or systems affected by apoptotic cell death in severe acute pancreatitis. Among several attractive possibilities, I can designate the possible involvement of endothelial cell apoptosis in the increased permeability of systemic capillary vessels in acute pancreatitis. Endothelial cell apoptosis has been proven to participate in the mechanism of systemic capillary leak syndrome, a rare disorder

of unknown etiology that is characterized by acute recurrent attacks of hypovolemic shock.⁶³ Thus, I suppose that endothelial cell apoptosis can occur in pancreatitis, and that it may be partially responsible for the increased permeability of systemic capillary vessels in acute pancreatitis.

The second direction is to utilize apoptosis as a prognostic marker to detect latent organ injury. For example, Endo et al.⁶⁴ measured the serum levels of nuclear matrix protein, an index of apoptosis, and found that the serum level of this molecule was significantly related to the existence of organ failure and mortality. For this direction, the exploration of feasible markers of apoptosis is essential.

The last direction is to develop a novel approach to treat severe acute pancreatitis by the control of apoptosis. According to our basic concept, control of apoptosis should be beneficial. However, it is easy to imagine that this concept cannot be simple, because the mechanism responsible for apoptosis is multifactorial and differs among various organs and tissues, as has been reviewed. So, we should target certain cells or systems exclusively, and one feasible target cell may be the gut epithelial cell. Recently, maintenance of the gut environment has been noted to be important to prevent bacterial translocation in critically ill patients, and the development of specific measures, such as the oral administration of synbiotics, has been proposed to maintain the gut environment.⁶⁵ The administration of synbiotics has proven to be beneficial for severe acute pancreatitis by preventing infectious complications.⁶⁶ I believe that the development of novel gut therapy, aiming to protect the gut epithelium from apoptosis, will work for the maintenance of the gut environment, to prevent bacterial translocation in patients with severe acute pancreatitis.

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