

Pathophysiology of Acute Pancreatitis

Madhav Bhatia Fei Ling Wong Yang Cao Hon Yen Lau Jiali Huang
Padmam Puneet Lakshmi Chevali

Department of Pharmacology, National University of Singapore, Singapore, Singapore

Key Words

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Abstract

Acute pancreatitis is a common clinical condition. It is a disease of variable severity in which some patients experience mild, self-limited attacks while others manifest a severe, highly morbid, and frequently lethal attack. The exact mechanisms by which diverse etiological factors induce an attack are still unclear. It is generally believed that the earliest events in acute pancreatitis occur within acinar cells. Acinar cell injury early in acute pancreatitis leads to a local inflammatory reaction. If this inflammatory reaction is marked, it leads to a systemic inflammatory response syndrome (SIRS). An excessive SIRS leads to distant organ damage and multiple organ dysfunction syndrome (MODS). MODS associated with acute pancreatitis is the primary cause of morbidity and mortality in this condition. Recent studies have established the role played by inflammatory mediators in the pathogenesis of acute pancreatitis and the resultant MODS. At the same time, recent research has demonstrated the importance of acinar cell death in the form of apoptosis and necrosis as a determinant of pancreatitis severity. In this review, we will discuss about our current understanding of the pathophysiology of acute pancreatitis.

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Introduction

Acute pancreatitis is a common clinical condition, the incidence of which has been increasing over recent years [1]. Mild acute pancreatitis may be self-limiting and not requiring any treatment, but up to 25% of patients suffer a severe attack and between 30 and 50% of these will die [2–6]. Most cases are secondary to biliary disease or excess alcohol consumption. Acute pancreatitis is an inflammatory disorder, which develops a complex cascade of immunological events, which not only affect the pathogenesis but also the course of the disease. At present it is widely accepted that the premature activation of digestive enzymes within the pancreatic acinar cells is a critical initiating event that leads to autodigestion of pancreas [4–6]. Whatever is the initiating event, the disease progression can be viewed as a three-phase continuum: local inflammation of the pancreas, a generalized inflammatory response, and the final stage of multiorgan dysfunction [4–6].

Patients who die from acute pancreatitis can be considered in two groups [4–6]. About 50% of deaths occur within the first week; these patients suffer a severe initial attack and develop an exaggerated systemic inflammatory response syndrome with the development of multiple organ dysfunction syndrome and death. Patients with a severe attack who survive beyond this period often go on to develop extensive retroperitoneal pancreatic necrosis. Infection in necrotic tissue leads to sepsis, a persisting systemic inflammatory response and multiple organ dysfunction syndrome, and accounts for patients who die late.

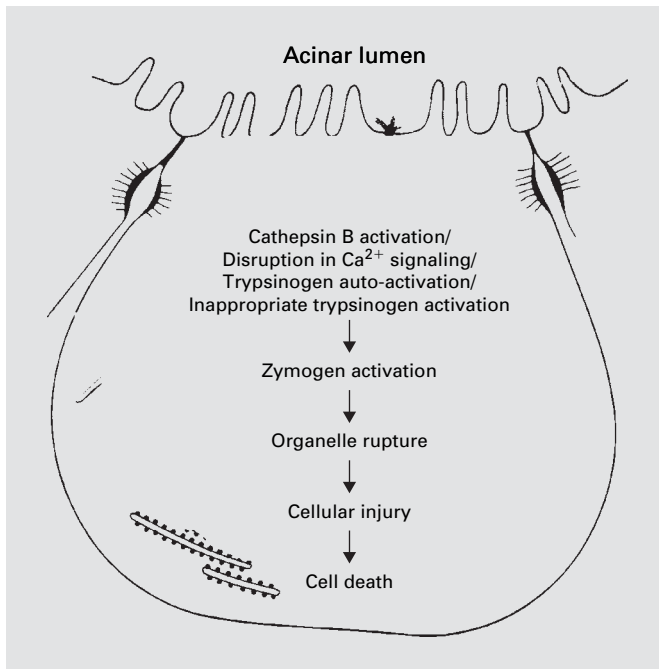


Fig. 1. Acute pancreatitis: activation of digestive enzymes within the acinar cell leads to pathological changes.

Patients with a severe attack who survive the initial inflammatory insult often die following a relatively minor second event that would not normally be life threatening. According to the two-hit hypothesis, the initial overactive systemic inflammatory response syndrome somehow primes the inflammatory response. Recovery is possible if no further insult occurs. A relatively minor secondary event such as a line or chest infection, will, however, lead to an exaggerated secondary inflammatory response and possibly death [4–6].

Development of *in vivo* experimental models of acute pancreatitis and associated lung injury has enabled us to study the pathogenesis of acute pancreatitis and associated systemic organ damage [6, 7]. For example, administration of a supramaximally stimulating dose of cholecystokinin analog cerulein to rodents results in either mild (rats) or severe (mice) acute pancreatitis which develops over hours [8]. In another model, young female mice are fed a choline-deficient diet supplemented with ethionine, the ethyl analog of methionine (CDE diet). Mice fed this diet develop severe necrotizing acute pancreatitis [9]. In the third kind of model, either retrograde injection of bile salts into the pancreatic duct of rats, or the ligation of the common biliopancreatic duct in the opossum leads to a severe acute pancreatitis [10]. In our

studies, we were able to establish evidence of lung injury associated with acute pancreatitis induced either by cerulein hyperstimulation [11], or by CDE diet administration [12], or by biliopancreatic duct ligation [13]. These models have proved to be invaluable in investigating the pathophysiology of acute pancreatitis.

Early Events in Acute Pancreatitis

Acute pancreatitis involves a complex cascade of events, which start in the pancreatic acinar cells (fig. 1). The exact mechanisms of the development of acute pancreatitis are still a subject of debate. The most common and widely accepted theory is that pancreatitis develops because of an injury or disruption of the pancreatic acini, which permit the leakage of pancreatic enzymes (trypsin, chymotrypsin and elastase) into pancreatic tissue. The leaked enzymes become activated in the tissue, initiating autodigestion and acute pancreatitis. The activated proteases (trypsin and elastase) and lipase break down tissue and cell membranes, causing edema, vascular damage, hemorrhage and necrosis.

Mechanisms of Zymogen Activation

Several pathways are likely to be involved in the intracellular conversion of pancreatic zymogens to active (mature) enzymes [14]. These include: (1) trypsinogen autoactivation to trypsin, (2) cleavage of trypsinogen to trypsin by the lysosomal hydrolase cathepsin B (CTSB), (3) diminished activity of the intracellular pancreatic trypsin inhibitor, (4) leakage of zymogens and lysosomal enzymes into the cytoplasm and subsequent proteolytic activation, (5) shunting of zymogens into membrane-bound compartments that contain active proteases, (6) uptake and processing of secreted zymogens by endocytic pathways, and (7) enhanced susceptibility of zymogens to proteolysis because of oxidation or decondensation [14]. The mechanisms that have received the most attention are trypsinogen autoactivation [15], CTSB activation of trypsinogen [14] and inappropriate activation of trypsinogen [16]. Some recent studies also point to an important role of calcium in trypsinogen activation. These mechanisms are discussed in some detail in the following pages.

Trypsinogen Autoactivation

According to this theory, trypsinogen autoactivates and, therefore, a trypsin-induced trypsinogen activation represents the triggering event for acute pancreatitis [17].

Recent findings suggest that trypsinogen activation occurs intracellularly along the normal secretory pathway within small cytoplasmic vacuoles that contain lysosomal markers but are not lysosomes and that release of trypsin into the cytoplasm occurs in a time-dependent fashion [18]. Further research is needed in order to better define the nature of these cytoplasmic vacuoles. Observations made in the secretagog model suggest that progressive disassembly of microtubules and filaments in the acinar cells causes a blockage of luminal exocytosis, with subsequent accumulation of zymogen granules [19]. In a recent study [20], however, using a cell-permeant, highly specific, and reversible trypsin inhibitor, the authors were able to completely inhibit trypsin activity in isolated rat pancreatic acini or lobules. The data indicate that the conversion of trypsinogen to trypsin in response to supra-maximal cerulein remains completely unaffected by the presence of a specific trypsin inhibitor and thus by the presence or absence of free trypsin activity within the acinar cells. Therefore, according to this study [20], auto-activation of trypsinogen is *not* an initiating factor for the intrapancreatic proteolytic cascade.

CTSB Activation of Trypsinogen

One hypothesis predicts that the lysosomal cysteine proteinase CTSB plays an essential role in the molecular mechanisms responsible for the intracellular activation of trypsinogen [21]. The largely circumstantial evidence for this 'CTSB/colocalization hypothesis' is based on the following observations: (a) CTSB was shown to activate trypsinogen *in vitro* [15]; (b) during the initial phase of acute pancreatitis, a redistribution of CTSB into a zymogen granule-containing subcellular compartment was detected by density-gradient centrifugation [22]; (c) in the experimental acute pancreatitis, lysosomal enzymes were detected by immunogold electron microscopy in secretory organelles that also contained digestive enzymes, e.g. trypsinogen [22]; (d) CTSB-deficient mice in which the *ctsb* gene had been deleted by targeted disruption was found 80% lower in the trypsin activity and 50% lower in pancreatic damage as indicated by serum activities of amylase and lipase, or by the extent of acinar tissue necrosis, after induction of experimental secretagogue-induced pancreatitis [23]. These experiments provide the evidence that CTSB may play a role in intrapancreatic trypsinogen activation and the onset of acute pancreatitis. However, a partial protection against trypsinogen activation and acute pancreatitis indicates that other, as yet unknown, factors may be involved in early trypsinogen activation in acute pancreatitis.

Inappropriate Activation of Trypsinogen

The pancreas has several safety mechanisms to cope with the problem of autoactivation of zymogens, which lead to autodigestion of the organ [16]. Normally, trypsinogen becomes active only when it is secreted into the duodenum, where the intestinal endopeptidase called enterokinase hydrolyzes the Lys²³-Ile²⁴ bond and releases the amino-terminal octapeptidase called trypsinogen-activation peptide. Trypsin can also cleave trypsinogen at this point (autoactivation). Pancreatic secretory trypsin inhibitor (PSTI) is present in secretory granules of acinar cells. It binds to the active site of trypsin in the ratio of 1:1 and inhibits trypsin activities. The molar ratio of PSTI to trypsin is estimated to be 1:10 [16]. When more than 10% of trypsinogen is activated, this inhibitory mechanism is no longer effective. Any disorders or agents that cause abnormalities in this natural protective mechanism can cause pancreatitis [16].

The Role of Calcium

Calcium (Ca²⁺) may also play an important role in early acute pancreatitis. Pancreatitis induced by cerulein hyperstimulation and by pancreatic duct obstruction has been shown to cause a rise in intracellular Ca²⁺ and a disruption of acinar cell Ca²⁺ signaling. This is associated with acinar cell vacuolization and the intracellular trypsinogen activation events that occur in early acute pancreatitis [4, 24, 25].

Although there is clear evidence that intracellular Ca²⁺ plays a very important role in intracellular trypsinogen activation, it is unclear if disruption in Ca²⁺ signaling alone is sufficient for this effect. Incubation of pancreatic acini with Ca²⁺-ATPase inhibitor thapsigargin caused trypsinogen activation in one study [24] but not in another study [26] published around the same time. Therefore, more research is needed to clearly define the role of Ca²⁺ in trypsinogen activation.

Trypsinogen autoactivation requires an acidic pH and is enhanced in the presence of Ca²⁺ [15]. The affinity of pancreatic trypsin inhibitor is greatest at a neutral pH and is reduced at an acidic pH. Therefore, the generation of low-pH compartments within the acinar cell during experimental pancreatitis may be important to trypsinogen activation [15].

Hereditary Pancreatitis

Conclusive evidence for the involvement of trypsin in acute pancreatitis has come from the study of hereditary pancreatitis, which is a rare condition in which affected individuals suffer repeated attacks of acute pancreatitis

and may eventually develop chronic pancreatitis. In the past, several theories have been proposed seeking to explain the relationship between alcohol and the development of acute and chronic pancreatitis. However, recent investigations in hereditary pancreatitis provided important insights into chronic pancreatitis pathogenesis and offer an important model for understanding pancreatic inflammation.

The majority of patients with hereditary pancreatitis express one of two mutations (R122H or N29I) in the cationic trypsinogen gene (PRSS1 gene) [17, 27, 28]. Less common mutations in this gene are A16V, R122C, N29T, D22G, and K23R [27]. Trypsin is known to lose its activity spontaneously by autolysis. The initial hydrolytic point of trypsin is Arg¹²²-Lys¹²³. Thus, the R122H mutation renders a mutant resistant to autolysis. Once an inappropriate activation of trypsinogen occurs and its levels exceed those of PSTI, the autoactivation process will not cease, which leads to the activation of other zymogens and acute pancreatitis. Several lines of evidence suggest that mutations of PSTI gene act as disease modifiers by lowering the threshold of pancreatitis or worsening the severity of pancreatitis [4, 16, 17, 28].

In light of the evidence in the literature discussed above, therefore, there are several different opinions about the early events in acute pancreatitis, although trypsinogen activation is believed to be an early critical step in the pathogenesis of acute pancreatitis. Despite disagreements over the early events in acute pancreatitis, however, it is now established that these early events lead to acinar cell injury and eventual cell death that triggers the later events in acute pancreatitis.

Pancreatic Acinar Cell Apoptosis versus Necrosis

Acinar cell death is a hallmark of both human and experimental acute pancreatitis [29]. The mechanisms of acinar cell death in pancreatitis are still poorly understood. In acute pancreatitis, acinar cell death occurs by both necrosis and apoptosis [29–32]. Recent studies noted that the form of acinar cell death itself may be an important determinant of the severity of acute pancreatitis [29–33]. Moreover, we and other investigators have shown that induction of apoptosis reduces the severity of experimental pancreatitis [30–32] while inhibition of it may worsen the disease [34]. Therefore, elucidating the molecular mechanisms of pancreatic cell death would provide valuable insights into clinical pancreatic disor-

ders and may offer potential pharmacological interventions.

Caspase Activation and Apoptosis in Acute Pancreatitis

Apoptosis was defined originally as a physiological or programmed form of cell death that affects scattered cells in a tissue, and has a characteristic and stereotypical morphology, including cell shrinkage, retention of organelles and nuclear chromatin condensation which occur in response to a variety of stress-related stimuli [29, 35]. Several protease families are implicated in apoptosis, the most prominent being caspase. Caspases are the molecular executioners of apoptosis because they bring about most of the morphological and biochemical characteristics of apoptotic cell death [29, 36]. Therefore, the caspase activation may be one of the mechanisms of apoptosis in acute pancreatitis. In fact, there are studies showing that several pathogenic factors in acute pancreatitis lead to an activation of caspases in acinar cells. According to one report, stimulation of isolated rat pancreatic acini with cholecystokinin, which serves as a model for human acute edematous pancreatitis, leads to a rapid redistribution and activation of caspase-8 [37]. Caspase-8 is regarded as an early regulator of a proteolytic cascade leading to apoptosis. Besides initiator caspase, the activation of executioner caspase is also reported during acute pancreatitis. A recent paper has reported that oxidative stress, a major pathogenic factor in acute pancreatitis, induces apoptosis of acinar cells involving activation of caspase-3, which degrades the DNA repair protein Ku70 and Ku80 [38]. Another recent report has shown that in addition to apoptosis, caspases also regulate other processes in the pancreatic acinar cell that play key roles in pancreatitis; in particular, caspases negatively regulate necrosis and intra-acinar cell activation of trypsin [39]. This may explain the inverse correlation between the extent of apoptosis on the one hand and necrosis and severity of the disease on the other hand observed in experimental models of pancreatitis.

Apoptosis-Associated Gene Expression and Apoptosis in Acute Pancreatitis

In addition to caspase, the Bcl-2 family of apoptotic regulators is another one of the functional components comprised in the apoptosis pathway [29, 40]. A current paradigm suggests that cell fate is determined, in part, by the balance between the products of anti- and proapoptotic genes of the Bcl-2 family of genes [29]. The Bcl-2 family of genes includes Bcl_{XL}, Bcl-w, A1/Bfl-1, Boo/Diva, Mcl-1, Bax, Bak, Bad, Bcl_{XL}S, and others [29, 36].

Bcl-2 and Bcl_{XL} can inhibit apoptosis; bax can dimerize with Bcl-2 or Bcl_{XL} and inhibits their function, and thereby promotes apoptosis. Recent studies have shown that acinar cell apoptosis observed in acute pancreatitis is also at least partly attributable to the greatly increased proapoptotic Bax gene expression. Gomez et al. [41] reported that acute pancreatitis induced a prompt increase in pancreatic bax mRNA levels closely followed by an increase in bax protein levels in the acinar cells and they suggested that acinar cell apoptosis observed during cerulein-induced acute pancreatitis is attributable, at least in part, to the greatly increased bax expression.

Besides Bcl-2 family, stress-induced protein gene has recently been identified, whose expression localized in acinar cell, and demonstrated that as a stress-inducible gene, it is overexpressed during pancreatitis and promoted cell apoptosis [42].

Transcription Factor Involvement in Acinar Cell Apoptosis

Transcription factors may be involved in the signal transduction pathways leading to apoptosis. For example, the transcription factor p53 plays a role in the initiation of apoptosis by inducing bax expression. A possible role of this factor in acinar cell apoptosis, however, remains unclear. In a study [41], it was reported that pancreatic p53 mRNA levels are temporally coordinated with those of bax during acute pancreatitis. An earlier in vitro study [43] has shown that supraphysiological concentrations of cerulein dose- and time dependently induce apoptosis in AR4-2J cells, and this cerulein-induced apoptosis may partly be mediated by wild-type p53. However, another study reported that the mechanisms of pancreatic acinar cell apoptosis correlated with the expression of apoptosis-regulated gene Bax, but had no relationship with the expression of p53 [44].

Other transcription factors, such as nuclear factor κ B (NF- κ B) and activator protein-1, which are involved in the control of numerous immune and inflammatory response genes (see below), may also be involved in the signal transduction pathways leading to apoptosis. Earlier studies have suggested a possible link between apoptosis and activation of NF- κ B in pancreatic acinar cells [43]. And the potential role of NF- κ B in cell death was suggested by activation of tumor necrosis factor alpha (TNF- α) transcription [45]. This was recently supported by Masamune et al. [46], who have reported that lyso-PC, which has been implicated in the pathogenesis of acute pancreatitis, activates NF- κ B and activator protein-1 and induces apoptosis.

Neutrophils and Apoptosis

Neutrophil activation is an important determinant of severity of acute pancreatitis (see below). Recently, some reports have indicated that the depletion of neutrophils results in a significant increase in acinar cells undergoing apoptosis. Sandoval et al. [47] have reported a dramatic increase in apoptotic acinar cells by administration of antineutrophil serum, which, comparable to aICAM-1 (an intercellular adhesion molecule-1, neutralizing antibody), significantly reduced the extent of necrosis and inflammatory infiltrate. Similar results were observed in another study wherein treatment with methotrexate resulted in a significant depletion of neutrophils as shown by low myeloperoxidase levels and a consecutive increase in apoptotic acinar cell death [48]. Yet another study, using taurocholate-induced severe acute pancreatitis as the experimental model, showed that neutrophils, via a TNF- α -dependent mechanism, may be involved in the development of apoptotic as well as necrotic forms of acinar cell death [49].

Cytokines and Apoptosis

In addition to their role in inducing inflammation (see below), cytokines, such as TNF- α or interleukin-1 β , which are released by neutrophils (and to a lesser extent, by acinar cells) have recently been shown to induce apoptosis in pancreas [50]. Pancreatic acinar cells have been reported to produce and release TNF- α [50]. These authors found that TNF- α was involved in the development of pancreatitis, and it mediated apoptosis in acinar cell suspension in vitro and also in vivo in the cerulein model of acute pancreatitis [50]. Another study subsequently reported that TNF- α can elicit apoptosis of pancreatic acinar cells in a time- and dose-dependent manner and it induces concomitantly proapoptotic and antiapoptotic mechanisms in pancreatic AR4-2J cells. The effect of TNF receptor (TNFR) activation by TNF- α will result in a balance between the activation of NF- κ B and the effectors of mitogen-activated protein kinases and Fas-associated death domain/caspases activity [51]. Mitochondria participate in necrotic as well as apoptotic cell death by opening the mitochondrial permeability transition pore. Indeed, despite the presumed fundamental difference between apoptosis and necrosis, growing evidence supports an essential role of the mitochondrial permeability transition in the release of cytochrome c and initiation of apoptosis in many models exposed to TNF- α [52].

As discussed above, the mechanisms of pancreatic acinar cell death – apoptosis and necrosis – in acute pancreatitis are just beginning to be identified. The vast array

of molecules involved in both the apoptotic and necrotic cell death pathways offers several potential targets for therapeutic intervention [29]. It is, therefore, reasonable to speculate that selective induction of pancreatic acinar cell apoptosis may be of value in acute pancreatitis.

Inflammation: A Critical Feature of Acute Pancreatitis

An important characteristic feature of acute pancreatitis is the pancreatic inflammation with excessive recruitment of leukocytes. Inflammatory mediators appear to play a critical role in the pathogenesis of pancreatitis and more so of the subsequent inflammatory response [4–6, 52]. It was first hypothesized in 1988 that cytokines could play an important role in acute pancreatitis and suggested that inappropriate activation of the immune system might increase the severity of the local disease and the systemic complications [53]. Proinflammatory mediators believed to participate in the pathophysiology of this condition include: TNF- α , interleukin (IL)-1 β , IL-6, platelet activating factor (PAF), ICAM-1, IL-8, growth-related oncogene- α /cytokine-induced neutrophil chemoattractant (GRO- α /CINC), monocyte chemoattractant protein-1 (MCP-1), and substance P. Anti-inflammatory mediators that play an important role in acute pancreatitis include IL-10, complement component C5a, soluble TNF receptors (sTNFR), IL-1 receptor antagonist (IL-1ra), and neutral endopeptidase (NEP). The expression of several of these mediators is regulated by transcription factors such as NF- κ B [54, 55].

NF- κ B

The exact cellular mechanism by which the cytokines are activated by NF- κ B is not completely known, although the activity of NF- κ B is mediated by the activation and translocation of NF- κ B hetero- and homodimers into the nucleus [54, 56]. Upon activation, degradation of the inhibitory element of NF- κ B releases the latter, resulting in the translocation of NF- κ B into the nucleus, where it activates gene transcription. Activation of NF- κ B and decreased expression of inhibitory element of NF- κ B have been demonstrated in cerulein-induced pancreatitis in rats [45, 57–59]. Considering the important role proinflammatory cytokines play in acute pancreatitis, NF- κ B has been investigated by researchers as a potential therapeutic target. One report [60] showed that inhibition of NF- κ B enhanced tissue injury and inflammation while others found that this inhibition attenuates severity or

even improves the survival in different experimental models of acute pancreatitis [61–63]. In a recent study, Chen et al. [64] directly activated NF- κ B within the pancreas using adenoviral-mediated transfer of an active subunit, RelA/p65, delivered by intraductal injection. In this study, activation of NF- κ B within the pancreas was sufficient for the initiation of an inflammatory response. Despite conflicting results with this transcription factor, NF- κ B remains an important eukaryotic transcription factor, whose precise function in the pathophysiology of acute pancreatitis has yet to be determined by future research.

TNF- α and IL-1 β

Levels of both these proinflammatory mediators are elevated at the onset and during the progress of acute pancreatitis [3–6, 65–67]. Naturally occurring sTNFR and IL-1ra, by neutralizing the activity of TNF- α and IL-1 β , respectively, act as anti-inflammatory mediators [3–6, 65–67]. Pancreatic acinar cells have been shown to produce and release TNF- α [50]. On induction of acute pancreatitis, knockout mice lacking receptors for IL-1 or/and TNF- α have significantly improved survival when compared to wild-type mice [68]. Moreover, the inhibition of IL-1 and TNF- α has been shown to attenuate the severity of pancreatitis in different experimental models of acute pancreatitis. Blockade of the IL-1 receptor before or soon after induction of pancreatitis is associated with decreased severity of pancreatitis and reduced intrinsic pancreatic damage. Also, neutralization of TNF- α with a polyclonal antibody significantly reduces the severity of acute pancreatitis in the rats. Strategies that interfere with TNF- α or IL-1 β translation, intracellular processing and release rather than antagonizing their effects also decrease the severity of an attack in experimental models [69]. On the other hand, combined augmentation of serum IL-1 β and TNF- α in clinical acute pancreatitis, exerts synergistic proinflammatory effects [70].

IL-6

IL-6 is a proinflammatory cytokine that is produced by a wide range of cells including monocytes/macrophages, endothelial cells, fibroblasts and smooth muscle cells in response to stimulation by endotoxin, IL-1 β and TNF- α [3–6]. It is also produced by periacinar myofibroblasts in response to TNF- α and IL-1 β [71]. IL-6 levels are raised in a number of acute conditions such as burns, major surgery, and sepsis. Plasma levels of IL-6 correlate with hemodynamic abnormalities (cardiac output, left ventricular filling) characteristic in acute pancreatitis in the rabbit [72]. Administration of IL-6 induces pyrexia.

IL-6 levels are raised in patients with acute pancreatitis and correlate with disease severity [73, 74]. Transgenic mice overexpressing human IL-6 are more susceptible to acute pancreatitis, and in these mice a monoclonal anti-IL-6 antibody has a protective effect [75].

IL-10

IL-10 is an anti-inflammatory cytokine [3–6]. In cultured monocytes, IL-10 upregulates IL-1ra and sTNFR production, reduces IL-8 and MCP-1 levels [76]. Experimentally, IL-10 was found to reduce the extent of inflammation as well as the mortality associated with acute pancreatitis [77, 78]. Employing recombinant IL-10 in experimental models of acute pancreatitis, the animals were found to be protected to a significant extent [79, 80]. Synthetic IL-10 agonist pretreatment in rabbits also lowers the extent of lung injury and mortality from this condition [81]. Similarly, IL-10 gene deletion [82], or administration of anti-IL-10 monoclonal antibody [83] makes mice more susceptible to acute pancreatitis.

In one study of clinical acute pancreatitis, serum IL-10 levels were found to be increased to a greater extent in the mild, as against severe, acute pancreatitis [84]. Yet, three other studies reported that IL-10 levels correlate with the severity of acute pancreatitis [85–87]. Two clinical trials involving IL-10 have been carried out in patients with postendoscopic retrograde cholangiopancreatography pancreatitis. In both trials, patients received either recombinant IL-10 or placebo before endoscopic retrograde cholangiopancreatography. During the course of the condition, one study reported no significant difference in clinical outcome between the two groups [88] while other reported a significant protection by IL-10 [89]. Even in the study by Deviere et al. [89], only 2 patients had severe acute pancreatitis, i.e. developed organ failure or had an in-patient stay greater than 10 days. Therefore it is at present uncertain if IL-10 will reduce the severity of acute pancreatitis in patients with severe disease from other causes.

PAF

The proinflammatory mediator PAF (1-o-alkyl-2-acetyl-sn-glycero-3-phosphocholine) is a low-molecular-weight phospholipid which acts via specific cell surface receptors that have been identified on numerous cells and tissues including platelets, leukocytes and endothelial cells [3–6]. Isolated pancreatic acini have been reported to synthesize PAF and pancreatic tissue concentrations rise during the course of an attack [13, 90]. In animal models intraperitoneal or intravascular injection can

bring about or increase the severity of acute pancreatitis [91, 92]. Blood and pulmonary tissue levels also rise coordinately indicating that PAF is a key mediator of the systemic inflammatory response [13].

Specific PAF antagonists have been evaluated in experimental models with varying success. Prophylactic treatment with these antagonists causes a reduction in local inflammation and acinar cell necrosis, in several experimental models of acute pancreatitis [93, 94]. In a recent study using a model of severe acute pancreatitis induced by infusion of bile salts into the pancreatic duct in combination with a supramaximal dose of cerulein administered intravascularly, treatment with lexipafant, a PAF antagonist had no effect on survival or local inflammation [95].

PAF is inactivated by the enzyme PAF acetylhydrolase (PAF-AH). The activity of PAF depends on the ether linkage at the sn-1 position and the acetate group at the sn-2 position. PAF-AH hydrolyzes the acetyl group, yielding lyso-PAF, which is inactive. In severe acute pancreatitis induced by the ligation of combined biliopancreatic duct in the opossum, there was significant protection against acute pancreatitis and associated lung injury when treatment with PAF-AH was started 48–96 h after the induction of acute pancreatitis [13].

Despite promising results from a phase II [96] clinical study, in a large multicenter phase III study lexipafant failed to reduce organ failure or mortality in severe acute pancreatitis [97]. Further development of lexipafant as a therapy for acute pancreatitis has since been abandoned [98]. It is noteworthy that some experimental studies [95] had also shown lack of effect of lexipafant. Nevertheless, large multicenter studies such as these demonstrate that it is possible to investigate the potential of anti-inflammatory mediator therapy for acute pancreatitis in the clinic.

ICAM-1

Leukocyte sequestration within areas of injury and inflammation is a multistep process that begins with leukocyte activation, involves the adhesion of circulating activated inflammatory cells to microvascular endothelial surfaces, and culminates in the transmigration of those cells across the endothelial barrier and into the involved tissue [4–6]. Currently available evidence indicates that the adhesion of activated leukocytes, including neutrophils, to endothelial surfaces results from the interaction of leukocyte surface proteins, such as CD11/CD18, with endothelial cell surface adhesion molecules such as ICAM-1 (CD54). ICAM-1 is an inducible protein expressed on

the surface of endothelial cells. Under physiological conditions, ICAM-1 is not constitutively expressed or is expressed at low levels in most tissues; during inflammation its levels are upregulated [4–6]. It has recently been reported that ICAM-1 is present on rat pancreatic acinar cells, is upregulated by cerulein and mediates direct binding of neutrophils to acinar cells. ICAM-1 was also upregulated in pancreas of rats with experimental pancreatitis induced by supramaximal doses of cerulein [99]. ICAM-1 knockout mice are protected against acute pancreatitis and associated lung injury, pointing to an important role for ICAM-1 in the development of pancreatitis and subsequent organ damage [100]. The protective effect of ICAM-1 gene deletion does not differ from that seen following neutrophil depletion in the CDE diet model of acute pancreatitis [12]. Indeed neutrophil depletion in ICAM-1 knockout mice affords no additional protection [100]. Blocking ICAM-1 has been shown to have a protective effect against local and systemic organ damage in different experimental models of acute pancreatitis [101]. These results suggest that ICAM-1 deficiency interferes with neutrophil recruitment and supports the concept of a therapeutic strategy directed against neutrophil migration and activation.

C5a

C5a is a potent anaphylatoxin and chemoattractant that is generated from C5 as part of both the classic and alternate pathways of complement activation. C5a, acting via C5aR on target cells, is generally believed to serve as a ‘complete’ proinflammatory mediator [4–6, 102, 103]. Recent studies using C5aR-deficient knockout mice have supported this belief by demonstrating that these mice are unable to clear intrapulmonary-instilled *Pseudomonas aeruginosa* in spite of their ability to mobilize neutrophils to the lung [102]. As a result, they succumb to overwhelming pneumonia. On the other hand, the C5aR-deficient mice are protected against a sterile form of lung injury, i.e. immune-complex-mediated pulmonary inflammation [103]. We evaluated the role of C5a in a model of acute pancreatitis and systemic organ damage after pancreatitis using two independent but complementary approaches. In the first, mice that do not express C5aR were used, whereas in the second set of experiments, mice that do not express C5 were employed. The results of both studies were similar, i.e. interruption of C5a action either by deletion of its receptor or by deletion of its parent protein resulted in a worsening of pancreatitis. The severity of pancreatitis-associated lung injury was also increased when C5a action was rendered inoperative [104]. Wheth-

er this worsening of lung injury reflects involvement of C5a as an anti-inflammatory mediator in the lung or, alternatively, merely reflects worsened pancreatitis in these animals is, however, unclear.

Chemokines – A Special Class of Inflammatory Mediators

After an initial pancreatic acinar cell injury, trafficking of leukocytes from the circulation into pancreatic acinar cells takes place. This trafficking requires communication between the circulating leukocytes and the vascular endothelial cell barrier [4–6]. As part of the migration process, the leukocytes and the inflammatory cells must first adhere to the endothelium via expression of adhesion molecules. This adhesion subsequently leads to leukocyte transendothelial migration (diapedesis) towards damaged pancreatic acinar cells. This migration of leukocytes is dependent upon several adhesive interactions and leukocyte-specific chemotactic molecules and these series of events are collectively called ‘chemotaxis’ [4–6]. Chemotaxis is the fundamental process by which the leukocytes are directed to the sites of tissue damage under the influence of a concentration gradient of the soluble chemotactic molecules or chemoattractants. It is a well-orchestrated process that involves a number of proteins, including proinflammatory cytokines, adhesion molecules, matrix metalloproteinases and the large cytokine subfamily of chemotactic cytokines – the chemokines [4–6]. Over the past 10 years, numerous chemokines have been identified as inflammatory mediators with potent leukocyte-activating properties and have been shown to be involved in the pathophysiological process of experimental acute pancreatitis.

Chemokines are a group of low-molecular-weight (8–10 kDa) polypeptides containing 78–80 residues. These are key components of immune surveillance. They can be divided into major subgroups on the basis of the position and spacing of N-terminal cysteine residues. Presently, the C, CC, CXC and CX₃C families are recognized. In CXC chemokines, the first two cysteine residues are separated by a single amino acid (X), while in the CC subfamily the first two cysteine residues are adjacent [4–6, 105–108]. Historically, CC chemokines [such as MCP-1, macrophage inflammatory protein-1 α (MIP-1 α), RANTES (Regulated upon Activation, Normal T-cell Expressed and Secreted)] have been believed to act principally on monocytes, and CXC chemokines which contain an ELR motif at the amino-terminal end (such as IL-8,

GRO- α , epithelial-derived neutrophil-activating peptide-78; ENA-78) are believed to act upon neutrophils. Recent work by us, as well as other investigators, has, however, shown that these narrow definitions are no longer valid [109–112]. Chemokines bind to a family of 7-transmembrane-domain G-coupled receptors on the surface of leukocytes. The expression of receptor types and the profile of the ligand on the surface of leukocytes at the site of inflammation determine the nature of inflammatory infiltrate, which may be site specific [105–108].

Although the importance of chemokines in inflammatory conditions is now well recognized, very little work has yet been undertaken to evaluate their role in acute pancreatitis. Mob1, a CXC rat chemokine, has been shown to be elevated within 1 h of induction of acute pancreatitis in the rat by cerulein hyperstimulation [58]. We have recently shown that pancreatic acinar cells produce the CC chemokine MCP-1 and that treatment with supramaximally stimulating doses of cerulein causes an upregulation of MCP-1 production. Cerulein-induced stimulation of chemokine production is regulated via NF- κ B and Ca²⁺ [59]. Interestingly, in this study, incubation of pancreatic acini with Ca²⁺-ATPase inhibitor thapsigargin caused upregulation of MCP-1 [59], further demonstrating the importance of Ca²⁺ in the pathogenesis of acute pancreatitis.

Levels of IL-8, GRO- α , and ENA-78 are raised in acute pancreatitis and are predictors of disease severity [113–115]. The chemokines are an ideal target for anti-inflammatory therapy. An anti-human IL-8 antibody was recently shown to reduce lung injury in a rabbit model of acute pancreatitis induced by retrograde injection of 5% chenodeoxycholic acid [116]. This, however, is not a well-established model of acute pancreatitis, and the criteria used for lung injury were only histological, which can be quite subjective.

The best characterized of the rat CXC chemokines is CINC, the homolog of the human chemokine GRO- α . Circulating levels of CINC are raised in experimental acute pancreatitis [117] and treatment with neutralizing antibody against CINC protects rats against acute pancreatitis-associated lung injury [118]. In this paper, functional as well as histological criteria of the severity of lung injury were used. We have also shown that in knockout mice, the deletion of the MIP-1 α /RANTES receptor CCR1 decreased the pulmonary damage seen in severe acute pancreatitis. There was little protection against local pancreatic damage [112]. Similarly, treatment with Met-RANTES, a CCR1 antagonist, protected mice against acute pancreatitis-associated lung injury, with lit-

tle or no protection against local pancreatic damage [110]. These studies show the critical role played by chemokines in the pathogenesis of acute pancreatitis and associated lung injury.

There are over 50 different chemokines and over 20 different receptors, with overlapping functions. Despite the complexity and apparent redundancy of this system, it is reasonable to believe that specific chemokine receptor antagonists that interfere with leukocyte migration and activation could be useful in acute pancreatitis.

Substance P and Neutral Endopeptidase – Neurogenic Inflammation in Acute Pancreatitis

Substance P is an 11-amino-acid neuropeptide that is released from nerve endings in many tissues. Subsequent to its release, substance P binds to neurokinin-1 (NK1) receptors on the surface of effector cells and in addition to being a mediator of pain it has been shown to play an important role in many inflammatory states including asthma, immune-complex-mediated lung injury, experimental arthritis, and inflammatory bowel disease. Substance P has been detected within the pancreas and may act as a neurotransmitter for sensory afferent nerves in the pancreas. Receptors for substance P have also been detected on guinea pig pancreatic acinar cells and the neuropeptide has been shown to act as a secretagogue, stimulating amylase secretion from acinar cells via a G protein-, phospholipase, inositol phosphate-, and calcium-mediated mechanism in that species [119–121]. Rat pancreatic acinar cells apparently do not express receptors for substance P and the neuropeptide does not stimulate enzyme secretion from rat acinar cells. In a recent study, we have shown the presence of substance P in the pancreas and of NK1 receptors on pancreatic acinar cells in mice [11]. On induction of pancreatitis, there is a multifold upregulation of pancreatic substance P levels and of NK1 receptors on pancreatic acinar cells [11]. Moreover, knockout mice deficient in NK1 receptors are protected against pancreatitis. Interestingly, these mice are almost completely protected against pancreatitis-associated lung injury [11]. In a recent paper, we have shown the role of preprotachykinin-A (PPT-A) gene products (e.g. substance P and neurokinin-A) in the pathogenesis of acute pancreatitis and associated lung injury [122]. NK1 receptors bind other peptides in addition to substance P, not all of which are derived from the PPT-A gene. In this study, we found that knockout mice deficient

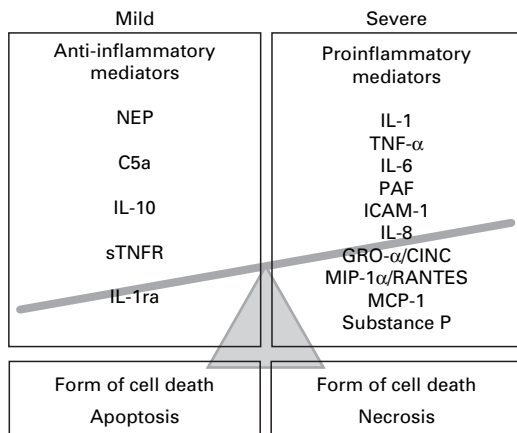


Fig. 2. Factors that determine the severity of acute pancreatitis.

in the PPT-A gene were protected against acute pancreatitis and associated lung injury. These two papers clearly show that PPT-A gene products, acting via NK1 receptors, are critical proinflammatory mediators in acute pancreatitis and the associated lung injury [11, 122]. These results are further substantiated by the observation that knockout mice deficient in NEP, the enzyme that hydrolyzes substance P, thereby terminating its action, are more susceptible to experimental acute pancreatitis and associated lung injury [123, 124]. Termination of the action of substance P by NEP can, therefore, be considered an anti-inflammatory action. Also, treatment with CP96345, an NK1 receptor antagonist, protects mice against acute pancreatitis and associated lung injury [125].

The mechanisms by which substance P acts to amplify the severity of pancreatitis are not clear. From studies probing the role of substance P in inflammatory processes involving other tissues, one might suspect that the neuropeptide acts primarily on endothelial cells to increase vascular permeability and promote edema formation. Although this explanation could account for the finding that NK1 receptor deletion lessens the increased pulmonary vascular permeability noted in pancreatitis-associated lung injury, it would not account for some of the other effects of NK1 receptor deletion that we have noted including: (1) diminished acinar-cell injury/necrosis, (2) decreased sequestration of neutrophils in the pancreas, and (3) decreased sequestration of neutrophils in the lung. The observations that acinar cell expression of NK1 receptors is increased during cerulein-induced pancreatitis and that NK1 receptor deletion does not alter the acinar

cell secretory response to the secretagog cerulein suggests that the proinflammatory effects of substance P may be directly exerted on the acinar cells themselves. It is also tempting to speculate that this phenomenon also explains the role of substance P and NK1 receptors in pancreatitis-associated lung injury, i.e. that substance P acting via NK1 receptors on acinar cells increases the severity of acinar cell injury and the subsequent increased release of proinflammatory mediators from the pancreas leads to increased lung injury. On the other hand, it remains possible that substance P, acting via NK1 receptors, has a direct injurious effect on the lung during pancreatitis.

These results demonstrate the critical role played by substance P in the pathogenesis of acute pancreatitis and point to yet another potential therapeutic approach against this clinical condition.

Conclusions

On the basis of our current knowledge, the factors that determine the severity of the disease are summarized in figure 2. Significant progress has been made in recent years in our awareness of the pathophysiology of acute pancreatitis. Trypsinogen activation that leads to acinar cell injury has been demonstrated to be a critical early event in acute pancreatitis, although the precise acinar cell mechanisms remain a subject of considerable debate amongst different investigators. The final severity of the disease is determined, to a great extent, both by the type of acinar cell death (apoptosis or necrosis) as well as by the systemic inflammatory response mediated by the pro- and anti-inflammatory mediators discussed in this review. Experimental animal models of acute pancreatitis have been of great help in this direction and early clinical studies point to the clinical relevance of these observations. We hope that an understanding of the pathophysiology of acute pancreatitis will help identify therapeutic targets and develop clinically effective therapy for this condition.

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