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Early events in acute pancreatitis

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The physiological function of the exocrine pancreas consists in the synthesis and secretion of digestive enzymes into the small intestine to catalyze the hydrolysis of food constituents. Many of these enzymes are proteases that are synthesized as proenzymes (zymogens) that require a proteolytic activation by cleavage of their propeptide. In the pancreatic juice entering the small intestine, the zymogen trypsinogen is activated by the brush–border endoprotease enteropeptidase (enterokinase). After this initial activation, trypsin catalyzes further activation of trypsinogen and is capable of a proteolytic conversion of other zymogens into their active forms. Therefore, under physiological conditions, trypsinogen and other pancreatic proteases remain in an inactive state during their synthesis, transport, and storage within the acinar cell and after secretion into the pancreatic duct.

Acute pancreatitis is a disease of varying severity, including pathological events in the pancreas and in other secondarily affected organs. Although the pathogenesis of acute pancreatitis is not understood fully, most hypotheses are based on the concept of a premature activation of digestive zymogens in the pancreas, leading to tissue necrosis by autodigestion. About a century ago, Chiari suggested that the pancreas of a patient who had died during episodes of a necrotizing pancreatitis was autodigested by its own digestive enzymes, and that autodigestion after premature activation of zymogens is the underlying pathogenetic mechanism of this diseases [1]. Since that time, intensive research has been undertaken to prove the role of premature, intracellular zymogen activation as an initial or an initiating

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event in the course of acute pancreatitis. The questions of why, where, and how the activation of zymogen starts within the acinar cell remain topics of research activities and scientific debate.

The mild form of acute pancreatitis, which accounts for some 75% to 80% of cases, has virtually no mortality, and patients recover more or less spontaneously. The severe form, however, is characterized by local and systemic complications and may lead to multi-organ failure and is burdened with a mortality rate between 5% and 20%. No causal treatment for pancreatitis is known. The most common etiological factors are alcohol abuse and gallstone migration, which, together, account for more than 80% of cases with acute pancreatitis in most Western countries.

Investigations that address initiating pathophysiological events in the human pancreas are few for several reasons. First, the pancreas is rather inaccessible because of its location in the retroperitoneal space. This limits the possibilities to obtain biopsies. Additionally, patients who suffer from acute pancreatitis usually contact the physician in a state in which the initial disease stages already have passed. On the other hand, much current knowledge regarding the onset of acute pancreatitis came from investigations into animal models of experimental pancreatitis or from studies on isolated cells. In particular, the issue of premature protease activation has been studied extensively in animal models of acute pancreatitis. These murine models are standardized and highly reproducible; they allow stimulation of various states of diseases severity, and they recapitulate many of the cellular events that are associated with clinical pancreatitis [2,3]. Although none of these models completely reflects the complex situation in human disease, the results give strong evidence that acute pancreatitis begins within the acinar cells, and not in the interstitium, the pancreatic duct, or the fatty tissue [4]. These findings enabled many investigators to study the cellular and molecular events occurring in the initial phase of pancreatic injury in isolated acinar cells.

Although the pathogenesis of acute pancreatitis, particularly in people, remains unclarified, some essential events could be identified by studies in experimental disease models or with isolated cells. Fig. 1 illustrates that acute pancreatitis is initiated by stimuli that alter physiological functions of acinar cells and ultimately lead to cell injury. The pathological events, however, do not remain restricted to acinar cells, because early in the time course of the disease various other cell types are affected and activated, and these can contribute to the acceleration of the disease state. This article summarizes the early intracellular events that lead to acinar cell injury and focuses on the role of Ca^{++} and of proteolytic enzymes in premature zymogen activation.

Disturbances in Ca^{++} signaling

Under physiological conditions, Ca^{++} is an essential second messenger in the stimulus–secretion coupling in exocrine pancreatic cells [4]. In response

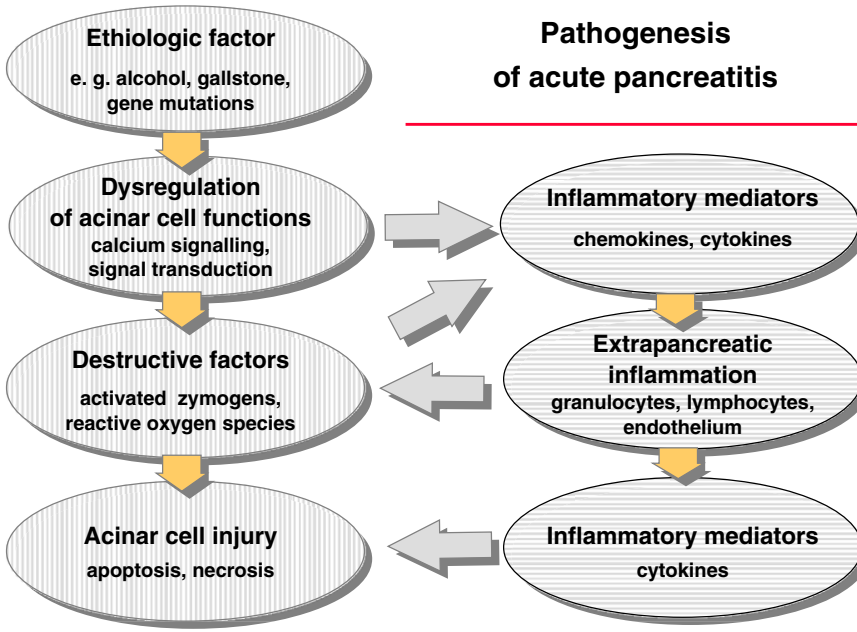

Fig. 1. Intra- and extrapancreatic events in acute pancreatitis After induction of pathophysiological events within the acinar cell (left hand sequence), signal metabolites initiate an extrapancreatic inflammatory response (right hand sequence). At various stages of the disease, these processes interact and contribute to disease progression.

to various hormonal signals, the cytosolic free Ca^{++} concentration rises and regulates the exocytosis of digestive enzymes from the apical pole of the acinar cell. A prerequisite for this physiological response is that under resting conditions the acinar cell maintains a Ca^{++} gradient across the plasma membrane with a low intracellular (nanomolar range) facing high extracellular (millimolar range) Ca^{++} concentrations. This enables a rapid Ca^{++} release from intracellular stores in response to external and internal stimuli and regulates such diverse biological events as growth and proliferation, locomotion and contraction, and the regulated secretion of exportable proteins. An impaired cellular capacity to maintain the Ca^{++} gradient across the plasma membrane has been identified as a pathophysiological characteristic in a secretagogue-induced model of acute pancreatitis [5,6]. Studies in which the effect of a disruption of intracellular Ca^{++} signaling on premature protease activation in isolated acini was studied seem to confirm the hypothesis that impaired cellular calcium signaling is causally involved in the cascade leading to cell injury. Regardless of whether intracellular Ca^{++} stores were depleted by calcium–ATP-ase inhibition, withdrawal of extracellular Ca^{++}, or complex formation with Ca^{++} chelators, intracellular protease activation in response to supramaximal hormone stimulation was reduced greatly or abolished [7,8]. Increasing intracellular Ca^{++}

concentrations with Ca^{++} ionophores or the calcium ATP-ase inhibitor thapsigargin, however, did not induce premature protease activation. These experiments indicate that high intracellular Ca^{++} concentrations are a requirement for premature protease activation but may not be sufficient to induce this process. Although the requirement for calcium in protease activation is undisputed, some authors believe that elevated intracellular calcium in general, and regardless of its subcellular site and mechanism of release, is sufficient to trigger premature protease activation [9]. The latter view remains in conflict with trials that used other lines of evidence in addition to single cell measurements [7,8]. Although all of the previously mentioned studies used hormone-induced models of intra-acinar cell protease activation, the most recent investigation could demonstrate that changes in intracellular calcium dynamics also are involved in the onset of pancreatitis in models that mimic the human disease [10]. Pancreatic duct ligation in rats and mice, a condition that simulates the situation in human gallstone-induced pancreatitis, induced leukocytosis, hyperamylasemia, pancreatic edema, and increased neutrophil accumulation in lung tissue, all of which were not observed in bile duct-ligated controls. Acini from pancreatic duct-ligated animals showed slightly elevated resting [Ca^{++}] but diminished calcium peaks after hormonal stimulation and a reduced amylase secretion. On the single cell level, pancreatic duct ligation reduced the percentage of cells in which physiological secretagogue stimulation was followed by a physiological response and increased the percentage of cells with a pathological response. In animals that were treated systemically with the intracellular calcium chelator BAPTA-AM, the parameters of pancreatitis and the intrapancreatic trypsinogen activation induced by duct ligation were found to be reduced significantly. These experiments suggest that pancreatic duct obstruction, the critical event involved in gallstone-induced pancreatitis, rapidly changes the physiological response of the exocrine pancreas to a pathological Ca^{++} signaling pattern. This pathological Ca^{++} signaling is associated with premature digestive enzyme activation and the onset of pancreatitis, both of which can be prevented by administering an intracellular calcium chelator.

Another line of evidence for the critical role of calcium in the initiation of acute pancreatitis comes from the observations that hypercalcemia in patients suffering from endocrine diseases is known to predispose to developing pancreatitis [11], and those who develop pancreatitis after extracorporeal blood circulation for major cardiac surgery are thought to develop the disease because of an exposure to supraphysiological concentrations of calcium [12]. In animal experiments, hypercalcemia was shown to either decrease the threshold level for the onset of pancreatitis or to induce morphological alterations equivalent to pancreatitis [11,13]. These clinical and experimental results favor the pathophysiological concept that an elevation of acinar cytosolic free ionized calcium should be regarded as the most probable common denominator for the onset of various clinical varieties of acute or chronic pancreatitis [14].

The mechanism and intracellular site of zymogen activation

There was a long-lasting controversy concerning where pancreatitis begins and through what mechanisms the disease is initiated. Early hypotheses based on autopsy studies of patients who died in the course of pancreatitis favored peripancreatic fat necrosis as the initial event [15]. For this hypothesis, pancreatic lipase secreted from acinar cells in an active form plays a central role. Another hypothesis suggested that periductal cells represented the site of initial damage and that pancreatic juice leaking from the pancreatic duct was responsible for the onset of pancreatitis [16]. Subsequent controlled studies performed in animal models that simulate the human disease have demonstrated that the acinar cell is the initial site of morphological damage [17]. This conclusion has been supported by genetic studies in patients with a hereditary form of pancreatitis that is linked to mutations in the trypsinogen gene [18,19]. Therefore, it is accepted widely that pancreatitis begins in exocrine acinar cells, and not in the pancreatic ducts, peripancreatic fat tissue, or the interstitium. Because of this pathobiological concept, various cell biological investigations of the underlying causes of pancreatitis are applicable to isolated acinar cells.

Trypsinogen and other pancreatic proteases are synthesized in the acinar cell as inactive precursor molecules and stored in membrane-confined zymogen granules. After secretion into the small intestine, trypsin activates other pancreatic proenzymes such as chymotrypsinogen, proelastase, and prophospholipase A_2 [20]. Several protective mechanisms normally prevent cell damage by trypsin activity that likely is generated under physiological conditions within the acinar cell. These protective mechanisms include: the presence of large amounts of pancreatic trypsin inhibitor; an acidic pH within organelles of the distal secretory pathway, including zymogen granules, that are far from optimal for enzymatic activity; and the presence of proteases that can degrade other already active proteases. Theoretically, premature activation of large amounts of trypsinogen could overwhelm these protective mechanisms and lead to damage of the zymogen-confining membranes and the release of activated proteases into the cytosol. Moreover, the release of large amounts of calcium from zymogen granules into the cytosol might activate calcium-dependent proteases such as calpains, which, in turn, could contribute to cell injury.

The suggestion that prematurely activated digestive enzymes play a central role in the pathogenesis of pancreatitis is based on the following observations:

- The activity of pancreatic trypsin and elastase increases early in the course of experimental pancreatitis [21,22].
- The activation peptides of trypsinogen and carboxypeptidase A_1 (CPA_1), which are cleaved from the respective proenzyme during the process of activation, are released into the pancreatic tissue or the serum early in the course of acute pancreatitis [20,23–27].

- Pretreatment with gabexate mesilate, a serine protease inhibitor, reduces the incidence of endoscopic retrograde cholangiopancreatography (ERCP)-induced pancreatitis [28,29].
- Serine protease inhibitors reduce injury in experimental pancreatitis [30,31].
- Hereditary pancreatitis often is associated with various mutations in the cationic trypsinogen gene that could render trypsinogen either more prone to premature activation or may render active trypsin more resistant to degradation by other proteases [32,33].
- Mutations in the serine protease inhibitor, Kazal type 1 gene that might render pancreatic secretory trypsin inhibitor (PSTI) less effective are associated with certain forms of chronic pancreatitis [34–36].

In clinical and experimental studies that investigated the time course of pancreatitis, it was found that zymogen activation occurs very early in the disease course. One study that employed the caerulein model of acute pancreatitis reported a biphasic pattern of trypsin activity that reached an early peak after 1 hour and a later second peak after several hours [27]. This observation suggests that more than one mechanism may be involved in the activation of pancreatic zymogens, and the second peak may require the infiltration of inflammatory cells into the pancreas [27]. Taken together, these observations represent compelling evidence that premature, intracellular zymogen activation plays a critical role in initiating acute pancreatitis.

The identification of the subcellular site where pancreatitis begins was addressed by three different approaches. Using a fluorogenic, cell permeant substrate specific for trypsin fluorescence microscopy could localize trypsinogen activation to the secretory compartment in acinar cells within minutes after supramaximal secretagogue stimulation [37]. When subcellular fractions containing different classes of secretory vesicles were subjected to density gradient centrifugation, it was found that trypsinogen activation initially does not arise in mature zymogen granules but in membrane confined vesicles of lesser density that most likely correspond to immature condensing secretory vacuoles [37]. In experiments in which antibodies directed against the activation peptide of trypsin (TAP) were used for ultrastructural immunocytochemistry, electron microscopy showed that TAP was found in membrane-confined secretory vesicles that were much less condensed than mature zymogen granules [38]. Taken together, these data not only confirm that digestive protease activation begins within pancreatic acinar cells, as opposed to the pancreatic ducts or the interstitial space, but they also indicate that mature zymogen granules in which digestive proteases are highly condensed are not necessarily the primary site of this activation. The first trypsin activity in acinar cells following a pathological stimulus is clearly detectable in membrane-confined secretory vesicles in which trypsinogen and lysosomal enzymes, are both physiologically present.

Cathepsin B in premature digestive protease activation

Earlier studies suggested a possible role for the lysosomal cysteine protease cathepsin B in the premature and intrapancreatic activation of digestive enzymes [39,40]. Observations that would support such a role of cathepsin B include the following:

- Cathepsin B can activate trypsinogen in vitro [41,42].
- Subcellular fractionation experiments using animal tissue from experimental pancreatitis models indicate that cathepsin B is redistributed from its lysosomal to a zymogen–granule-enriched subcellular compartment [43].
- Lysosomal enzymes colocalize with digestive zymogens in membrane-confined organelles during the early course of experimental pancreatitis [44].

Although the cathepsin hypothesis appeared attractive from a cell biological point of view, and testable alternative hypotheses were missing, it has received much criticism, because the following experimental observations appear to be incompatible with its assumptions:

- A colocalization of cathepsins with digestive zymogens has been observed not only in the initial phase of acute pancreatitis but also under physiological control conditions and in secretory vesicles that are destined for regulated secretion from healthy pancreatic acinar cells [45,46].
- A redistribution of cathepsin B into a zymogen-enriched subcellular fraction can be induced in vivo by experimental conditions that interfere with lysosomal sorting and are neither associated with, nor followed by, the development of acute pancreatitis [47].
- The administration of potent lysosomal enzyme inhibitors in vivo does not prevent the onset of acute experimental pancreatitis in some studies [4].
- Both increases and decreases in the rate of intracellular trypsinogen activation have been reported in experiments that used lysosomal protease inhibitors in vitro [48,49].
- Even a protective role against premature zymogen activation has been considered for cathepsin B [50,51].

In view of the limited specificity and bioavailability of the available inhibitors for lysosomal hydrolases, the only remaining option to address the cathepsin hypothesis conclusively was to generate cathepsin B-deficient animals. When experimental pancreatitis in a strain of mice in which the cathepsin B gene had been deleted by targeted disruption was studied, the disease course was altered in several ways [52]. The most dramatic change compared with wild-type control animals, and also the most relevant in regard to the cathepsin hypothesis of acute pancreatitis, was a reduction in premature, intrapancreatic trypsinogen activation. In terms of substrate-defined

trypsin activity, this reduction amounted to more than 80% over the course of 24 hours. When the greater pancreatic trypsinogen content of cathepsin B knock-out animals was taken into account, trypsinogen activation was reduced by 90% compared with wild-type animals during pancreatitis. This observation alone can be regarded as the first direct experimental evidence for a critical role of cathepsin B in the intracellular events that determine premature digestive protease activation during the onset of acute pancreatitis.

The decrease in trypsinogen activation was not paralleled by a dramatic prevention of pancreatic necrosis, and the systemic inflammatory response during pancreatitis was not affected at all. This observation and the fact that cathepsin B can activate pancreatic digestive zymogens other than trypsinogen [53] raises two important questions: (1) whether trypsin activation itself, which is clearly cathepsin B-dependent, is involved directly in acinar cell damage and, (2) whether cathepsin B-induced activation of other digestive proteases ultimately causes pancreatic necrosis for which trypsin is not the culprit. To study the role of cathepsin B in the human pancreas tissue, specimens and pancreatic juice from patients with hereditary and sporadic pancreatitis were investigated recently. Cathepsin B was shown to be abundantly present in the subcellular secretory compartment of the healthy human pancreas and in the pancreatic juice of controls and pancreatitis patients [54]. It also was found to be a potent activator of human trypsinogen. This observation alone indicates that a redistribution of cathepsin B into the secretory compartment of the exocrine pancreas, which was reported from various models of experimental pancreatitis [43], is not required for an interaction between trypsinogen and cathepsin B, because both classes of enzymes already are colocalized under physiological conditions in the human pancreas. The capacity of cathepsin B to activate trypsinogen, on the other hand, was not affected by the most common trypsinogen mutations found in association with hereditary pancreatitis. Although these data indicate that the onset of human pancreatitis may involve mechanisms that depend on cathepsin B-induced protease activation, the cause of hereditary pancreatitis cannot be reduced easily to an increased cathepsin-B induced activation of mutant trypsinogen.

These data suggest that cathepsin B-induced protease activation is a critical component in the onset of pancreatitis. Several issues regarding the cathepsin-hypothesis of pancreatitis remain to be addressed, however:

- Different lysosomal cathepsins (eg, B, H, L, and others) may have vastly different roles in terms of digestive protease activation or degradation.
- The conditions under which two physiologically colocalized classes of enzymes begin to activate or degrade each other remain unknown and may have important therapeutic implications.
- The cellular basis of the subcellular redistribution phenomenon of lysosomal enzymes remains poorly understood and could involve protein sorting or vesicular fusion events.

- If either the ratio of lysosomal cathepsins and digestive proteases, or the processing of lysosomal cathepsins itself, were to vary from one class of vesicular compartment to the next, or even within the same compartment, this may change the interpretation of the role of cathepsins in pancreatitis. Therefore, this needs to be explored.

Role of trypsin in premature digestive protease activation

The present understanding regarding the role of trypsin in the initial events of pancreatitis have come from two different approaches: cell biological studies on isolated rat pancreatic acini and biochemical studies on recombinant human trypsinogens with hereditary pancreatitis-associated mutations. Unfortunately, these two approaches sometimes lead to seemingly incompatible conclusions, indicating that the role of trypsin in the disease onset is more complex than has been appreciated.

No animal models exist in which wild-type rodent trypsinogens are replaced with mutant human trypsinogens. Therefore, isolated pancreatic acini and lobules offer an alternative to study the role of trypsin in pancreatitis. In a recent study that used a specific, cell permeant and reversible trypsin inhibitor, it was found that complete inhibition of trypsin activity does not prevent, or even reduce the conversion of trypsinogen to trypsin [55]. A cell permeant cathepsin B inhibitor, on the other hand, prevented trypsinogen activation completely. In inhibitor wash-out experiments, it was determined that, following hormone-induced trypsinogen activation in pancreatic acinar cells, 80% of the active trypsin is inactivated immediately and directly by trypsin itself. Taken together, these experiments suggest that trypsin activity is neither required nor involved in trypsinogen activation, that trypsinogen does not autoactivate in living pancreatic acinar cells, and that its most prominent role is in autodegradation [55]. This, in turn, suggests that intracellular trypsin activity might have a role in the defense against other, potentially more harmful digestive proteases. Consequently, structural alterations that impair the function of trypsin in hereditary pancreatitis would eliminate a protective mechanism rather than generate a triggering event for pancreatitis [56]. Whether these experimental observations obtained from rodent pancreatic acini and lobules have any relevance to human hereditary pancreatitis is unknown, because human cationic trypsinogen may have different activation and degradation characteristics in vivo. An important advantage of studies employing isolated pancreatic acini and lobules to investigate the biological role of trypsin is the fact that information is obtained on a cellular level rather than in vitro, and pancreatic enzymes can be studied in their physiological environment. A disadvantage of this approach is the difficulty involved in obtaining human material and the limitations in distinguishing between different varieties and isoforms of trypsin.

The question of why structural changes in the cationic trypsinogen gene caused by germline mutations would lead to the onset of hereditary pancreatitis also has been a matter of debate. Because trypsin is one of the oldest known digestive enzymes, and because trypsin can activate several other digestive proteases in the gut and in vitro, and because pancreatitis is regarded as a disease caused by proteolytic autodigestion of the pancreas, it would seem reasonable to assume that pancreatitis is caused by a trypsin-dependent protease cascade within the pancreas itself. If this hypothesis was correct, the trypsinogen mutations that are found in association with hereditary pancreatitis should confer a gain of enzymatic function [18,19], and mutant trypsinogen would be activated more readily inside acinar cells or, alternatively, active trypsin would be degraded less rapidly inside acinar cells. Both events would lead to a prolonged or increased enzymatic action of trypsin within the cellular environment. On the other hand, several arguments can be raised against the gain of trypsin function hypothesis of hereditary pancreatitis. Statistically, most hereditary disorders, including most autosomal dominant diseases, are associated with loss-of-function mutations that render a specific protein either defective or impair its intracellular processing and targeting [57]. Moreover, 16 amino acid mutations in the cationic trypsinogen protein, scattered over the various regions of the molecule, have been reported to be associated with pancreatitis or hereditary pancreatitis [58]. It seems unlikely that such a great number of mutations located in entirely different regions of the serine protease 1 (PRSS1) gene would have the same effect on trypsinogen and result in a gain of enzymatic function. A loss of enzymatic function in vivo would, accordingly, be a much simpler and consistent explanation for the pathophysiological role of hereditary pancreatitis mutations. To investigate the two alternative hypotheses, in vitro studies were performed that analyzed the biochemistry of recombinant human trypsinogens into which pancreatitis-associated mutations were introduced. Several studies found that either facilitated trypsinogen autoactivation or extended trypsin activity can result under defined experimental conditions [59–62]. Whether these in vitro conditions reflect the highly compartmentalized situation under which intracellular protease activation begins in vivo [7,63] is unknown, but the findings strongly favor a gain of trypsin function as a consequence of several trypsinogen mutations. Certain mutations stabilize cationic trypsin against autolysis [32,59,60,64], suggesting that autodegradation might play a role in safeguarding the human pancreas against excess intrapancreatic trypsin activity. Although experimentally not demonstrated yet, logic would suggest that other pancreatic proteases might participate in a similar protective mechanism. In humans, mesotrypsin has been discussed as a candidate for this function [65,66]. The human pancreas secretes three isoforms of trypsinogen, encoded by PRSS1, 2 and 3. On the basis of their relative electrophoretic mobility, the three trypsinogen species commonly are referred to as cationic trypsinogen, anionic trypsinogen, and mesotrypsinogen. Mesotrypsin constitutes less than 5% of

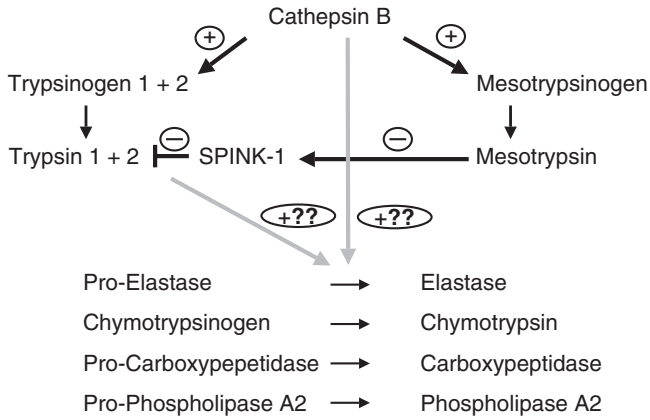

Fig. 2. Initial steps in intracellular zymogen activation Considers the possible role of mesotrypsin as a proteolytic inactivator of SPINK-1 (PSTI). An effective activation of cationic (trypsinogen 1) and anionic (trypsinogen 2) trypsinogen requires proteolytic activation by cathepsin B and a lowering of the inhibitory potential of pancreatic trypsin inhibitors by selective cleavage of PSTI by mesotrypsin. Although the role of trypsin in activating other zymogens in vitro is unequivocal, it remains unclear how trypsin is involved in zymogen activation in vivo and whether cathepsin B is the master activator of the entire activation cascade.

Symbols: $\xrightarrow{+}$ proteolytic activation; $\xrightarrow{-}$ proteolytic inactivation; $\dashv$ inhibition.

total secreted trypsinogens. Interestingly, because of a G198R substitution (G193R in chymotrypsin numbering), this isoform is inhibited poorly by PSTI, which led to the suggestion that mesotrypsin might participate in degradation of other zymogens and proteases [67]. Mesotrypsin, however, is grossly defective, not only in inhibitor binding, but also in cleaving the most protein substrates [68]. A pathophysiological role of mesotrypsin in intracellular protease degradation and a protective function in pancreatitis is therefore very unlikely. On the other hand, two remarkable properties were found for this enzyme. Cathepsin B activates mesotrypsinogen very effectively, and somewhat better than cationic or anionic trypsinogen. Because of the low affinity to PSTI, mesotrypsin is capable to proteolytically cleave and inactivate this inhibitor. On the basis of these findings, it can be hypothesized that an initiation sequence exists that includes cathepsin B as activating enzyme of cationic, anionic, and mesotrypsinogen. In this hypothesis active mesotrypsin would be an accelerating factor because of its action as PSTI inactivating enzyme (Fig. 2). Further investigations are necessary to evaluate the relevance of such a concept.

Summary

Considerable progress in the understanding of the pathogenesis of acute pancreatitis is based on the conclusive finding that the initiation of the disease occurs within the acinar cell. Two lines of evidence have contributed to the

progress in understanding the disease process: (1) the identification of patients with a hereditary form of pancreatitis as carriers of germline-mutations in the genes for cationic trypsinogen and the pancreatic secretory trypsin inhibitor and (2) the use of various transgenic and knock-out mouse strains in experimental models of acute pancreatitis. On the other hand, these studies have delivered several unexpected results that appear to be incompatible with long-standing dogmas and paradigms of pancreatic research. Further progress in knowledge will result if the well-characterized enzymatic properties of human enzymes that are involved in the initial activation cascade can be investigated under in vivo conditions in transgenic animals or in permanent acinar cell lines. Such studies will permit the development of effective strategies for the prevention and treatment of this disease.

References

[1] Chiari H. Über die Selbstverdauung des menschlichen Pankreas. Zeitschrift für Heilkunde 1896;17:69–96.
[2] Lerch MM, Adler G. Experimental pancreatitis. Curr Opin Gastroenterol 1993;9:752–9.
[3] Lerch MM, Adler G. Experimental animal models of acute pancreatitis. Int J Pancreatol 1994;15:159–70.
[4] Go VLW, DiMagno EP, Gardner JD, Lebenthal E, Reber WA, Scheele GA, editors. The pancreas: biology, pathobiology, and disease. 2nd edition. New York: Raven Press; 1993.
[5] Ward JB, Sutton R, Jenkins SA, Petersen OH. Progressive disruption of acinar cell calcium signaling is an early feature of cerulein-induced pancreatitis in mice. Gastroenterology 1996; 111:481–91.
[6] Bragado MJ, San Roman JI, Gonzalez A, Garcia LJ, Lopez MA, Calvo JJ. Impairment of intracellular calcium homoeostasis in the exocrine pancreas after caerulein-induced acute pancreatitis in the rat. Clin Sci 1996;91:365–9.
[7] Kruger B, Albrecht E, Lerch MM. The role of intracellular calcium signaling in premature protease activation and the onset of pancreatitis. Am J Pathol 2000;157:43–50.
[8] Saluja AK, Bhagat L, Lee HS, Bhatia M, Frossard JL, Steer ML. Secretagogue-induced digestive enzyme activation and cell injury in rat pancreatic acini. Am J Physiol 1999;276: G835–42.
[9] Raraty M, Ward J, Erdemli G, Vaillant C, Neoptolemos JP, Sutton R, et al. Calcium-dependent enzyme activation and vacuole formation in the apical granular region of pancreatic acinar cells. Proc Natl Acad Sci U S A 2000;97:13126–31.
[10] Mooren FC, Hlouschek V, Finkes T, Turi S, Weber IA, Singh J, et al. Early changes in pancreatic acinar cell calcium signaling after pancreatic duct obstruction. J Biol Chem 2003; 278:9361–9.
[11] Mithofer K, Fernandez-del Castillo C, Frick TW, Lewandrowski KB, Rattner DW, Warshaw AL. Acute hypercalcemia causes acute pancreatitis and ectopic trypsinogen activation in the rat. Gastroenterology 1995;109:239–46.
[12] Fernandez-del Castillo C, Harringer W, Warshaw AL, Vlahakes GJ, Koski G, Zaslavsky AM, et al. Risk factors for pancreatic cellular injury after cardiopulmonary bypass. N Engl J Med 1991;325:382–7.
[13] Frick TW, Fernandez-del Castillo C, Bimmler D, Warshaw AL. Elevated calcium and activation of trypsinogen in rat pancreatic acini. Gut 1997;41:339–43.
[14] Ward JB, Petersen OH, Jenkins SA, Sutton R. Is an elevated concentration of acinar cytosolic free ionised calcium the trigger for acute pancreatitis? Lancet 1995;346:1016–9.

[15] Kloppel G, Dreyer T, Willemer S, Kern HF, Adler G. Human acute pancreatitis: its pathogenesis in the light of immunocytochemical and ultrastructural findings in acinar cells. Virchows Archive A 1986;409:791–803.
[16] Foulis AK. Histological evidence of initiating factors in acute necrotizing pancreatitis in man. J Clin Pathol 1980;33:1125–31.
[17] Lerch MM, Saluja AK, Dawra R, Ramarao P, Saluja M, Steer ML. Acute necrotizing pancreatitis in the opossum: earliest morphological changes involve acinar cells. Gastroenterology 1992;103:205–13.
[18] Whitcomb DC, Gorry MC, Preston RA, Furey W, Sossenheimer MJ, Ulrich CD, et al. Hereditary pancreatitis is caused by a mutation on the cationic trypsinogen gene. Nat Genet 1996;14:141–5.
[19] Whitcomb DC. Genes means pancreatitis. Gut 1999;44:150.
[20] Rinderknecht H. Activation of pancreatic zymogens. Normal activation, premature intrapancreatic activation, protective mechanisms against inappropriate activation. Dig Dis Sci 1986;31:314–21.
[21] Bialek R, Willemer S, Arnold R, Adler G. Evidence of intracellular activation of serine proteases in acute cerulein-induced pancreatitis in rats. Scand J Gastroenterol 1991;26: 190–6.
[22] Luthen R, Niederau C, Grendell JH. Intrapancreatic zymogen activation and levels of ATP and glutathione during caerulein pancreatitis in rats. Am J Physiol 1995;268:G592–604.
[23] Schmidt J, Fernandez-del Castillo C, Rattner DW, Lewandrowski K, Compton CC, Warshaw AL. Trypsinogen-activation peptides in experimental rat pancreatitis: prognostic implications and histopathologic correlates. Gastroenterology 1992;103:1009–16.
[24] Appelros S, Thim L, Borgstorm A. Activation peptide of carboxypeptidase B in serum and urine in acute pancreatitis. Gut 1998;42:97–102.
[25] Gudgeon AM, Heath DI, Hurley P, Jehanli A, Patel G, Wilson C, et al. Trypsinogen activation peptides assay in the early prediction of severity of acute pancreatitis. Lancet 1990; 335:4–8.
[26] Mithofer K, Fernandez-del Castillo C, Rattner D, Warshaw AL. Subcellular kinetic of early trypsinogen activation in acute rodent pancreatitis. Am J Physiol 1998;274:G71–9.
[27] Gukovskaya AS, Vaquero E, Zaninovic V, Gorelick FS, Lusis AJ, Brennan ML, et al. Neutrophils and NADPH oxidase mediate intrapancreatic trypsin activation in murine experimental acute pancreatitis. Gastroenterology 2002;122:974–84.
[28] Tympner F, Rosch W. Effect of secretin and gabexate-mesilate (synthetic protease inhibitor) on serum amylase level after ERCP. Z Gastroenterol 1982;20:688–93.
[29] Cavallini G, Tittobello A, Frulloni L, Masci E, Mariana A, Di Francesco V. Gabexate for the prevention of pancreatic damage related to endoscopic retrograde cholaniopancreatography. N Engl J Med 1996;335:919–23.
[30] Lasson A, Ohlsson K. Protease inhibitors in acute pancreatitis: correlation between biochemical changes and clinical course. Scand J Gastroenterol 1984;19:779–86.
[31] Niederau C, Grendell JH. Intracellular vacuoles in experimental acute pancreatitis in rats and mice are an acidified compartment. J Clin Invest 1998;81:229–36.
[32] Várallyay E, Pál G, Patthy A, Szilágyi L, Gráf L. Two mutations in rat trypsin confer resistance against autolysis. Biochem Biophys Res Commun 1998;243:56–60.
[33] Gorry MC, Gabbaizedeh D, Furey W, Gates LK Jr, Preston RA, Aston CE, et al. Mutations in the cationic trypsinogen gene are associated with recurrent acute and chronic pancreatitis. Gastroenterology 1997;113:1063–8.
[34] Witt H, Luck W, Hennies HC, Classen M, Kage A, Lass U, et al. Mutations in the gene encoding the serine protease inhibitor, Kazal type 1 are associated with chronic pancreatitis. Nat Genet 2000;25:213–6.
[35] Pfützer RH, Barmada MM, Brunskill AP, Finch R, Hart PS, Neoptolemos J, et al. SPINK1/PSTI polymorphisms act as disease modifiers in familial and idiopathic chronic pancreatitis. Gastroenterology 2000;119:615–23.

[36] Threadgold J, Greenhalf W, Ellis I, Howes N, Lerch MM, Simon P, et al. The N34S mutation of SPINK1 (PSTI) is associated with a familial pattern of idiopathic chronic pancreatitis but does not cause the disease. Gut 2002;50:675–81.
[37] Kruger B, Lerch MM, Tessenow W. Direct detection of premature proteases activation in living pancreatic acinar cells. Lab Invest 1998;78:763–4.
[38] Hofbauer B, Saluja AK, Lerch MM, Bhagat L, Bhatia M, Lee HS, et al. Intra-acinar cell activation of trypsinogen during caerulein-induced pancreatitis in rats. Am J Physiol 1998; 275:G352–62.
[39] Steer ML, Meldolesi J. The cell biology of experimental pancreatitis. N Engl J Med 1987;316: 144–50.
[40] Gorelick F, Matovcik L. Lysosomal enzymes and pancreatitis. Gastroenterology 1995;109: 620–5.
[41] Figarella C, Miszczuk-Jamska B, Barrett A. Possible lysosomal activation of pancreatic zymogens: activation of both human trypsinogens by cathepsin B and spontaneous acid activation of human trypsinogen-1. Biol Chem Hoppe Seyler 1988;369:293–8.
[42] Greenbaum LA, Hirshkowitz A. Endogenous cathepsin activaties trypsinogen in extracts of dog pancreas. Proc Soc Exp Biol Med 1961;107:74–6.
[43] Saluja A, Hashimoto S, Saluja M, Powers RE, Meldolesi J, Steer ML. Subcellular redistribution of lysosomal enzymes during caerulein-induced pancreatitis. Am J Physiol 1987;253:G508–16.
[44] Watanabe O, Baccino FM, Steer ML, Meldolesi J. Supramaximal caerulein stimulation and ultrastructure of rat pancreatic acinar cell: early morphological changes during development of experimental pancreatitis. Am J Physiol 1984;246:G457–67.
[45] Tooze J, Hollinshead M, Hensel G, Kern HF, Hoflack B. Regulated secretion of mature cathepsin B from rat exocrine pancreatic cells. Eur J Cell Biol 1991;56:187–200.
[46] Willemer S, Bialek R, Adler G. Localization of lysosomal and digestive enzymes in cytoplasmic vacuoles in caerulein-pancreatitis. Histochemistry 1990;94:161–70.
[47] Lerch MM, Saluja AK, Dawra R, Saluja M, Steer ML. The effect of chloroquine administration on two experimental models of acute pancreatitis. Gastroenterology 1993; 104:1768–79.
[48] Leach SD, Modlin IM, Scheele GA, Gorelick FS. Intracellular activation of digestive zymogens in rat pancreatic acini. Stimulation by high does of cholecystokinin. J Clin Invest 1991;87:362–6.
[49] Saluja AK, Donovan EA, Yamanaka K, Yamaguchi Y, Hofbauer B, Steer ML. Cerulein-induced in vitro activation of trypsinogen in rat pancreatic acini is mediated by cathepsin B. Gastroenterology 1997;113:304–10.
[50] Gorelick FS, Modlin IM, Leach SD, Carangelo R, Katz M. Intracellular proteolysis of pancreatic zymogens. Yale J Biol Med 1992;65:407–20.
[51] Klonowski-Stumpe H, Luthen R, Han B, Sata N, Haussinger D, Niederau C. Inhibition of cathepsin B does not affect the intracellular activation of trypsinogen by cerulein hyperstimulation in isolated rat pancreatic acinar cells. Pancreas 1998;16:96–101.
[52] Halangk W, Lerch MM, Brandt-Nedelev B, Roth W, Ruthenbuerger M, Reinheckel T, et al. Role of cathepsin B in intracellular trypsinogen activation and the onset of acute pancreatitis. J Clin Invest 2000;106:773–81.
[53] Hakansson HO, Borgstrom A, Ohlsson K. Porcine pancreatic cationic proelastase. Studies on the activation, turnover and interaction with plasma proteinase inhibitors. Biol Chem Hoppe Seyler 1991;372:465–72.
[54] Kukor Z, Mayerle J, Kruger B, Tóth M, Steed PM, Halangk W, et al. Presence of cathepsin B in the human pancreatic secretory pathway and its role in trypsinogen activation during hereditary pancreatitis. J Biol Chem 2002;277:21389–96.
[55] Halangk W, Krüger B, Ruthenburger M, Sturzebecher J, Albrecht E, Lippert H, et al. Trypsin activity is not involved in premature, intrapancreatic trypsinogen activation. Am J Physiol 2002;282:G367–74.

[56] Lerch MM, Gorelick FS. Trypsinogen activation in acute pancreatitis. Med Clin North Am 2000;84:549–63.
[57] Scriver CR. Mutation analysis in metabolic (and other genetic) disease: how soon, how useful. Eur J Pediatr 2000;159:243–5.
[58] Teich N, Mössner J, Keim V. Mutations of the cationic trypsinogen in hereditary pancreatitis. Hum Mutat 1998;12:39–43.
[59] Sahin-Tóth M, Tóth M. Gain-of-function mutations associated with hereditary pancreatitis enhance autoactivation of human cationic trypsinogen. Biochem Biophys Res Commun 2000;278:286–9.
[60] Sahin-Tóth M. Human cationic trypsinogen. Role of Asn-21 in zymogen activation and implications in hereditary pancreatitis. J Biol Chem 2000;275:22750–5.
[61] Teich N, Ockenga J, Hoffmeister A, Manns M, Mössner J, Keim V. Chronic pancreatitis associated with an activation peptide mutation that facilitates trypsin activation. Gastroenterology 2000;119:461–5.
[62] Szilágyi L, Kenesi E, Katona G, Kaslik G, Juhász G, Gráf L. Comparative in vitro studies on native and recombinant human cationic trypsins. Cathepsin B is a possible pathological activator of trypsinogen in pancreatitis. J Biol Chem 2001;276:24574–80.
[63] Kruger B, Weber IA, Albrecht E, Mooren FC, Lerch MM. Effect of hyperthermia on premature intracellular trypsinogen activation in the exocrine pancreas. Biochem Biophys Res Commun 2001;282:159–65.
[64] Simon P, Weiss FU, Sahin-Tóth M, Parry M, Nayler O, Lenfers B, et al. Hereditary pancreatitis caused by a novel PRSS1 mutation (Arg-122 → Cys) that alters autoactivation and autodegradation of cationic trypsinogen. J Biol Chem 2002;277:5404–10.
[65] Rinderknecht H, Renner IG, Abramson SB, Carmack C. Mesotrypsin: a new inhibitor-resistant protease from a zymogen in human pancreatic tissue and fluid. Gastroenterology 1984;86:681–92.
[66] Nyaruhucha CN, Kito M, Fukuoka SI. Identification and expression of the cDNA-encoding human mesotrypsin(ogen), an isoform of trypsin with inhibitor resistance. J Biol Chem 1997; 272:10573–8.
[67] Katona G, Berglund GI, Hajdu J, Gráf L, Szilágyi L. Crystal structure reveals basis for the inhibitor resistance of human brain trypsin. J Mol Biol 2002;315:1209–18.
[68] Szmola R, Kukor Z, Sahin-Toth M. Human mesotrypsin is a unique digestive protease specialized for the degradation of trypsin inhibitors. J Biol Chem 2003;278:48580–9.