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EXPERIMENTAL ACUTE PANCREATITIS: NEW INSIGHTS INTO THE PATHOPHYSIOLOGY

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1. ABSTRACT

Acute pancreatitis is a disease of variable severity in which patients can experience mild or severe attacks. Most observers believe that acute pancreatitis results from an early intra-acinar cell activation of inactive zymogens into their active forms. Following this early activation, a trypsin cascade occurs in the gland which leads to the auto-digestion of acinar cells. Recent experimental data indicate that synthesis and release of pro-inflammatory cytokines and chemokines are also responsible for local injury and systemic dispersion of the inflammatory mediators. Experimental studies also provide evidence for the involvement of the immune system in the development of pancreatitis, including lymphocyte and neutrophil activation. However, the factors that will dictate the ultimate severity of the attack are still unknown. Following an attack, the pancreas completely recovers or becomes fibrotic through the action of newly described mediators within the pancreas such as TGF-beta and IGF-1 and the presence of pancreatic stellate cell that is known to play a crucial role in the fibrogenesis.

2. INTRODUCTION

Acute pancreatitis is an inflammatory process which usually occurs in a normal organ and which is diagnosed mainly by acute abdominal pain associated with a concomitant rise of serum amylase and lipase concentrations (1-3). Gallstone migration into the common bile duct and alcohol abuse account for most of the etiologies of the disease in western countries (4-7). The injury is usually mild in 70 to 80% of cases, but 20% of the patients have a severe injury and, among them, 15 to 25% will die (4, 8).

The pathophysiology of the disease includes the activation and release of pancreatic enzymes in the interstitium, the autodigestion of the pancreas, and a multiple organ dysfunction following the release of these enzymes into the systemic circulation (9-12). Moreover,

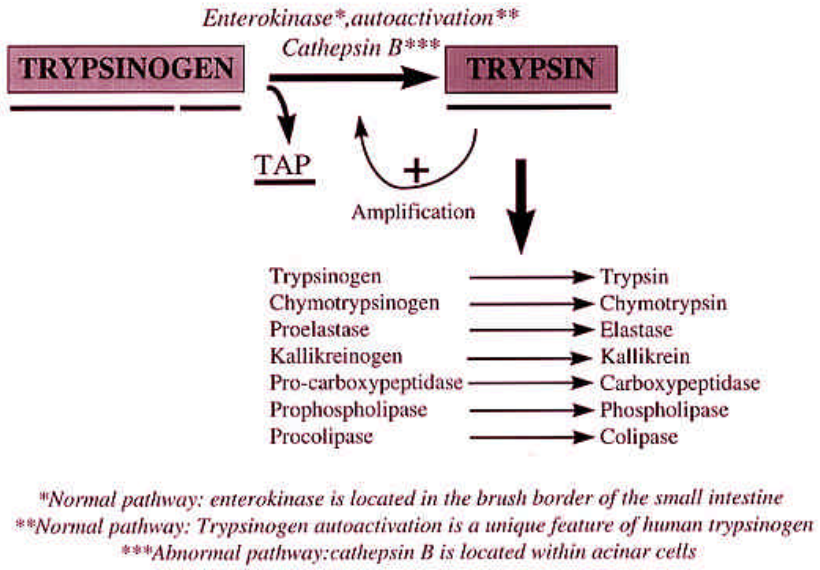

Figure 1. Trypsinogen activation: Trypsinogen can be either activated into active trypsin by enterokinase or by the lysosomal enzyme cathepsin B. Once trypsin is activated, it can catalyze the activation of other digestive enzymes, initiating the auto-digestion of the gland. (reproduced with permission from reference 21).

significant evidence has accumulated that synthesis and release of pro-inflammatory cytokines and chemokines is also responsible for local injury and systemic dispersion of the inflammatory mediators (13, 14). Thus, inflammatory mediators produced within the gland increase pancreatic injury and radiate to distant organs. The effects of these inflammatory mediators on the pancreas and remote organs have recently been evaluated using genetically modified mice or blocking experiments (15). However, the factors responsible for the ultimate severity of the attack are still unknown and many experimental and clinical studies are conducted to determine these factors (16).

Because of the inaccessibility of human pancreas during the early stages of pancreatitis, most of our knowledge rely on various experimental models of acute pancreatitis in rodents (17-20). Despite tremendous recent progress as a result of these experiments, a complete and definitive explanation of the mechanisms leading to acute pancreatitis is lacking. This review will summarize the emerging concepts in the pathophysiology of acute pancreatitis with a special emphasis on the distinction between the early and the late events of the disease.

3. BIOLOGY OF THE EXOCRINE PANCREAS

The pancreas is an important organ of the digestive process because it produces a broad spectrum of enzymes (digestive enzymes or zymogens) that breakdown all categories of nutrients. The exocrine product or pancreatic juice flows from the pancreas to the duodenum through the main pancreatic duct. Acini are clusters of secretory (or acinar) cells that produce abundant digestive enzymes. The pancreatic juice has also an aqueous component that is rich in $HCO3^-$ and Cl^-.

The major proteases of the pancreatic juice are trypsin, chymotrypsin, and carboxypeptidase. In the acinar cells, these proteases are produced in inactive form and isolated from the rest of the cells in granules, preventing the pancreas from self-digestion. Additionally, the presence of protease inhibitors such as Protease Trypsinogen Inhibitor (PSTI) synthesized along with the zymogens prevent premature activation in response to any minor pancreatic insult (21). Within the duodenum, trypsinogen is activated into trypsin by enterokinase, an intestinal brush border enzyme. Trypsin, in turn, activates two other pancreatic proteases (chymotrypsinogen, and procarboxypeptidase) to chymotrypsin and carboxypeptidase (Figure 1). Other pancreatic enzymes (amylase, lipases, and nucleases) are secreted in active form but require ions or bile to be present in the intestinal lumen for an optimal activity. Among the major pancreatic lipases are triacyglycerol hydrolase and Phospholipase A2.

The pancreatic exocrine secretion is controlled by both neural and hormonal signals elicited by the presence of acid and digestion products in the duodenum. Secretin elicits the ductular secretion of the aqueous component and cholecystokinin stimulates the acinar secretion of pancreatic enzymes. Both of these hormones are released by the duodenal mucosa. Other agonists that elicit secretions from pancreatic acinar cells are acetylcholine, gastrin, substance P, secretin, and vasointestinal peptide (VIP).

Similar to digestive enzymes, lysosomal enzymes are synthesized on ribosomes attached to the Golgi system. They both contain signal sequence that mediate their transport to the Golgi where they are sorted and packaged : lysosomal enzymes in lysosomes and digestive enzymes or zymogens in zymogen granules. Stimulation of the pancreas with both

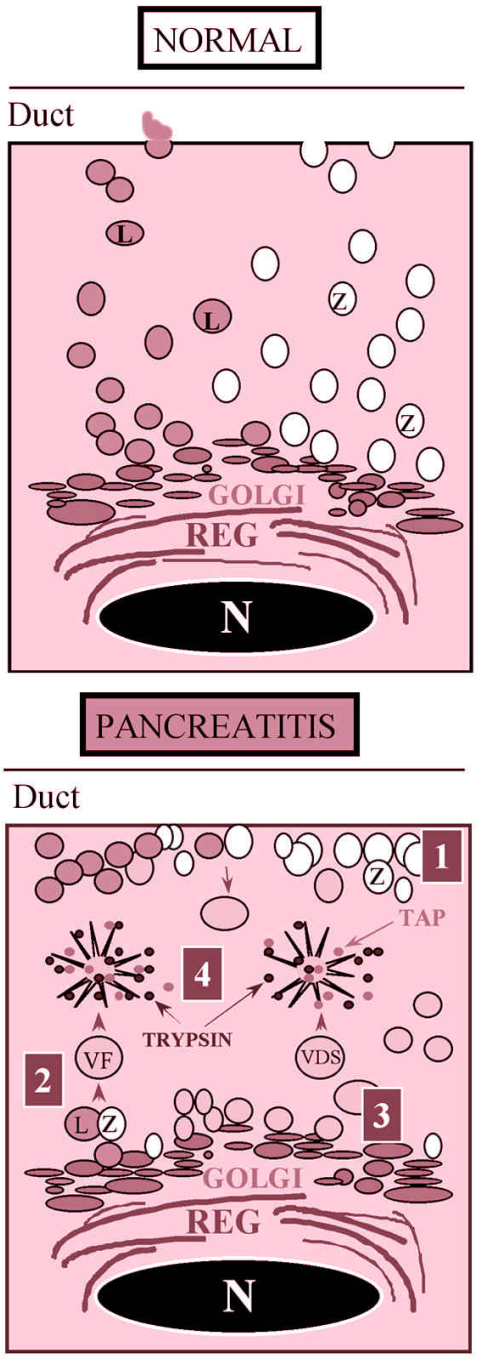

Figure 2. Co-localization theory : Blockage of acinar secretion is a common feature of cerulein-induced pancreatitis and bile duct ligation-induced pancreatitis (Step 1). Zymogen granules accumulate at the apex of the cell. Co-localization results either from direct fusion of zymogen with lysosomal granules (Step 2) or from derangement in the intracellular sorting events of zymogen and lysosomal proteins (Step 3). Colocalization of trypsinogen with cathepsin B in the same compartment results in the activation of trypsinogen into active trypsin (Step 4) and leads to the activation cascade of other pancreatic pro-enzymes within the acinar cell. (Adapted from reference 11 with permission).

secretin and cholecystokinin release in parallel digestive and lysosomal enzymes. The activation of most enzymes occurs in acid solution and as the pancreatic juice is alkaline, they are inactive in the pancreas. Lysosomal enzymes have no direct action on the food digestion but have a role in intracellular digestion and in the elimination of degraded cellular constituents.

4. EARLY EVENTS IN ACUTE PANCREATITIS

4.1. Cellular localization of the early events

The location of the earliest lesions during acute pancreatitis was identified by Steer and his colleagues who examined pancreatic tissue removed from opossum shortly after a pancreatic duct obstruction (22). Acinar necrosis is detected within 3-6 hours after the duct obstruction and the magnitude of the necrosis increased over time. Patchy acinar necrosis is observed after 12 hours but perilobular and periductal alterations are observed by 24 hours. Thus, the earliest events in acute pancreatitis are likely to occur within acinar cells (22).

4.2. Co-localization theory

The normal secretion of digestive enzymes is likely to be modified during pancreatitis (Figure 2). While the synthesis and the early phase of the intracellular transport are not modified, the enzyme segregation is altered (10, 23). Digestive enzymes, which are exported outside the cells and lysosomal hydrolases, which are transported into lysosomes, are normally separated from each other while they pass through the Golgi system (24, 25). Lysosomal hydrolases are glycosylated, 6-mannose phosphorylated, bind to mannose 6-phosphate receptors, and are transported to lysosomes. In contrast, digestive enzymes are not 6-mannose phosphorylated, pass through the Golgi complex, and accumulate into condensing vacuoles, which in turn mature into dense granules as they migrate from the Golgi system to the apical membrane of the acinar cell (zymogen granules). In experimental pancreatitis, segregation or separation of digestive enzymes from lysosomal hydrolases is defective and, as a result, both types of enzymes become co-localized within intracellular vacuoles (24). Steer and his colleagues were the first to suggest that this co-localization phenomenon may result in premature activation of digestive enzymes. Subsequent rupture of these vacuoles liberates the activated digestive enzymes in the cytoplasmic space, followed by a cascade of events that finally results in acute pancreatitis (Figure 2).

4.3. Trypsinogen activation by cathepsin B

In rats, the perfusion of a supramaximally stimulating dose of cerulein for 1 to 3 hours induces an interstitial edematous pancreatitis with local inflammation and patchy necrosis. The trypsinogen activation can be detected within the pancreas before any evidence of biochemical or morphological abnormalities. An experimental in vivo model confirms the rapid activation of trypsinogen (26). Trypsin activity induced by a 30-minutes exposure of isolated acini to cerulein is similar to the one reported in *in vivo* models (26).

The fact that the lysosomal hydrolase cathepsin B activates trypsinogen while trypsin activates the remaining digestive enzymes clearly support the hypothesis of the co-localization of both the zymogens and lysosomal enzymes within the same subcellular compartment (27, 28). However, the finding that E64 (a cathepsin B inhibitor) does not prevent experimental pancreatitis is puzzling (26). This might be explained in part because E64 incompletely inhibits the cathepsin B activity, and E64d, which is more cell permeable than E64, completely prevents the cathepsin B activity and the cerulein-induced activation of trypsinogen (26).

Recently, Halangk *et al.* (29) developed cathepsin B-deficient mice. After induction of pancreatitis, the pancreatic trypsin activity in cathepsin B-deficient mice was lower (-80%) than in wild-type mice. However, because the pancreatic injury was only decreased by 50% in cathepsin B-deficient mice, cathepsin B is not the only pathway involved in the premature intra-acinar activation of trypsinogen. Additional mechanisms such as the trypsinogen activation by other lysosomal enzymes must be considered. Moreover, the systemic inflammation as well as the degree of pancreatic leukocyte infiltration are not modified, indicating that these events are not cathepsin B-mediated.

The hypothesis of an intra-acinar premature activation of trypsinogen is also supported by recent clinical studies demonstrating that a mutation in the trypsinogen gene is responsible for the human hereditary pancreatitis (30, 31) Hereditary pancreatitis results from mutation of the cationic form of the trypsinogen gene with expression of trypsinogen that, once activated, is resistant to inactivation. Individuals affected with this genetic disorder may suffer from recurrent acute pancreatitis which progress to chronic pancreatitis and have a high risk of developing a pancreatic cancer (40%) (31).

4.4. Subcellular localization of the early events

After the activation of trypsinogen by cerulein, an increase in trypsin and trypsinogen activation peptide (TAP) is detected in the pancreas within 15-30 minutes (26, 27, 32). TAP is a small peptide (5 amino acids) which is cleaved when trypsinogen is activated into trypsin. After 30 minutes of cerulein stimulation and up to 3 hours, a small amount of the trypsin activity is measured in the lysosome/mitochondria-enriched fraction but the major part of the trypsin activity is detected in the zymogen granule-enriched fraction. With sustained stimulation (up to 3 hours), trypsin activity gradually falls in the zymogen granule-enriched fraction, and rises in the soluble fraction or cytoplasm. Concomitantly, TAP is also measured in the zymogen granule-enriched fraction. With prolonged cerulein stimulation, TAP concentrations decrease in this fraction and become detectable (35%) in the soluble fraction. By 3 hours after stimulation, all TAP (measured by Elisa technique) is found in the soluble compartment. *Cathepsin B* is mainly found in the zymogen granule-enriched fraction while a small amount is measured in the lysosome/mitochondria-enriched fraction. The redistribution of cathepsin B from the lysosome/mitochondria-enriched fraction to the zymogen granule-enriched fraction following cerulein administration is detected within 15-30 minutes. This finding suggests the formation of cytoplasmic vacuoles containing both digestive enzymes and lysosomal hydrolases.

Immunolocalization of TAP at the electron microscopic level is possible by immunogold labeling of pancreatic cryosections. When anti-TAP antibodies are used in normal pancreas, only a weak background of gold decoration is observed. After a 30-minutes cerulein stimulation, the cytoplasmic vacuoles become the main TAP-containing compartments and neither zymogen granules nor the acinar lumen are labeled with the antibodies. After 3.5 hours of cerulein infusion, the TAP-specific labeling becomes less apparent, and the surrounding cytoplasm close to the cytoplasmic vacuoles becomes strongly TAP positive. Light microscopy immunolocalization with antibody directed against TAP and cathepsin B also shows that both TAP and cathepsin B are localized in cytoplasmic vacuoles (32).

4.5. Co-localization theory and premature intra-acinar activation of trypsinogen: a synthesis

Many researchers agree that acute pancreatitis is initiated by the deleterious co-localization of digestive enzyme zymogens and lysosomal hydrolases in acinar cells. Evidences supporting this theory include: 1) the earliest morphological alterations are observed in acinar cells; 2) digestive enzyme zymogens and lysosomal hydrolases are co-localized in cytoplasmic vacuoles that appear before any evidence of cell injury; 3) the lysosomal hydrolase enzyme cathepsin B can activate trypsinogen in vitro; 4) activated trypsin is detected before any evidence of acinar cell injury; 5) the activation of trypsinogen by cerulein in isolated acinar cells can be prevented by the cell-permeant, specific cathepsin B inhibitor E64d and finally, the trypsin activity in the pancreas of cathepsin B deficient mice is more than 80% lower than in cathepsin sufficient mice.

Following cerulein administration, a premature activation of digestive enzymes occurs which is initially confined to the cytoplasmic vacuoles in acinar cells. Later, because TAP is found as a free compound in the cytosol, cytoplasmic vacuoles, which are unstable cellular organelles, might disintegrate and their content including TAP and trypsin, is released into the cellular cytosol. Only limited areas containing cytoplasmic vacuoles are positive for both TAP and cathepsin B. The origin of these cytoplasmic vacuoles needs to be determined.

4.6. Injury in acinar cells

Pancreatic injury is evidenced by the morphologic alterations of acinar cells and the enzyme release. The cell injury is detected within 30 min following cerulein exposure and its severity increases over time (33). The cellular injury is prevented (or markedly reduced) when the cholecystokinin-A receptors are blocked by the antagonist L-364,718 or when Ca^{2+} is withdrawn from the cell medium (33). Protease inhibitors such as pefabloc or

benzamidine also prevent the cellular injury induced by cerulein (33). Under these protective conditions, no lactate dehydrogenase release from acinar cells is detectable after cerulein administration. Injury is then the consequence of the release of proteolytic activity within the cytoplasm of acinar cells.

4.7. Does trypsinogen activation lead to acinar injury?

Interestingly, the trypsinogen activation which is detected within minutes after cerulein administration, depends on the presence of Ca^{2+} in the medium, and can be prevented by L-364,718 addition, suggesting that trypsinogen activation and cellular injury are closely related. Trypsinogen activation is likely to occur prior to cellular injury. Thus, acute pancreatitis might be the consequence of trypsinogen activation which leads to the cellular injury mediated by various digestive proteases.

4.8. Role of phospholipase A2

Among the various digestive enzymes released during acute pancreatitis, Phospholipase A2 is of great importance. Two forms of the enzyme exist: the type I originates from the pancreas whereas the type II is a mediator of the acute phase response. In the pancreas, Phospholipase A2 induces cell necrosis by converting the lecithin of cellular membranes into the more toxic compound lysolecithin (34). Thus, the Phospholipase A2 might also play a role in lung injury associated with acute pancreatitis by altering pulmonary surfactant since surfactant contains phospholipids which are substrates for the Phospholipase A2 (35)

4.9. Role of reactive oxygen species

The role of reactive oxygen species was initially reported by Sanfey (36) and Guice (37). Administration of reactive oxygen species scavengers diminishes the pancreatic edema and the increased serum amylase concentrations induced by ischemic pancreatitis. In contrast, Wisner *et al.* (38) were unable to detect any protective effect of reactive oxygen species scavengers in the cerulein model of pancreatitis. Reactive oxygen species are potent oxidizing and reducing agents that directly damage cellular membranes by lipid peroxidation. Thus, increased lipid peroxidation within the pancreas in the course of cerulein-induced pancreatitis was recently shown (39). The peroxidation products rapidly increase by 30 min after the administration of cerulein. Lipid peroxidation modifies cytoskeleton function leading to alterations in the intracellular transport of digestive enzymes and the premature activation of these enzymes (40, 41). In acinar cells, the high production of both free radicals and activated digestive enzymes increases cell permeability and attracts chemotactic factors into the interstitial and vascular spaces. Although these experimental findings provide new insights in the pathogenesis of acute pancreatitis, clinical trials do not find any benefit of free radical scavenger treatment in patients with acute pancreatitis (42).

4.10. Abnormalities of the microcirculation

Vascular injuries such as vasculitis, atherosclerotic embolization, and hypoperfusion can induce acute pancreatitis (5, 43). Considering the high susceptibility of the pancreas to hypoperfusion and ischemic injury, abnormalities of the microcirculation are in turn likely to be observed during pancreatitis (44). Thus, a spatial heterogeneity of the microcirculation is described during experimental pancreatitis with a hypoperfusion in severely injured areas while moderately injured areas remain well-perfused (45, 46). Ischemia-reperfusion following pancreas transplantation is another pathology associated with pancreatitis (47). In a bile salt-induced model of pancreatitis, Kusterer et al. (48) shows that pancreatitis is associated with an early arteriolar vasoconstriction (and hypoperfusion of the microcirculation), followed by a late arteriolar vasodilatation with re-establishment of the perfusion of the capillaries. Interestingly, an increased leukocyte-endothelial cell interactions is observed in postcapillary venules during vasodilation but is not detected during vasoconstriction.

Moreover, by treating animals previously subjected to cerulein with phenylephrine, the edematous form of pancreatitis is converted to an hemorrhagic form associated with parenchymal necrosis (49). All these findings confirm that abnormalities of the microcirculation participate in the pathophysiology of the disease.

4.11. Necrosis versus apoptosis

Necrotic and apoptotic cells have an opposite behavior. While necrotic cells release their cytosolic contents into the extracellular space and elicit an inflammatory response, apoptotic cells are rapidly phagocytosed by macrophages (or neighboring cells) and do not release cytosolic components and no inflammation is observed (50, 51). In experimental models, mild pancreatitis is associated with extensive apoptotic acinar cells while more severe forms of the disease involve more necrosis than apoptosis (52). These observations led to the hypothesis that apoptosis might be a more favorable response to acinar cell injury. Thus, interventions that favor induction of apoptosis might reduce the severity of an attack of pancreatitis (53). Bhatia *et al.* (54) show that prior administration of crambene (an *in vivo* inducer of apoptosis) in mice subjected to cerulein administration reduces the severity of pancreatitis.

Apoptosis is also involved in the recovery from pancreatitis because the injured pancreatic cells are eliminated by this regulatory mechanism. Tumor-necrosis factor-alpha (TNF-alpha) which has proapoptotic properties has a key role in the recovery of the disease (55).

4.12. Nuclear factor-kappaB (NF-kappaB) activation

NF-kappaB is a pleiotropic regulator of many genes involved in the stress and inflammatory responses (56). Many stimuli activate NF-kappaB, including cytokines, activators of protein kinase C, viruses and oxidants. NF-kappaB is also involved in the control of apoptosis, immune functions as well as embryonic development (57). During cerulein-induced acute pancreatitis, Steinle *et al.* (58) and Gukovsky *et al.* (59) show that NF-kappaB is rapidly activated.

In unstimulated cells, NF-kappaB is found within the cytoplasm bound to its inhibitory protein, I-kappaB. Upon activation of the cells, I-kappaB is degraded allowing the release of NF-kappaB which migrates to the nucleus where its binds to specific sequences of the promoter regions of the target genes, mostly genes coding for pro-inflammatory proteins. Pharmacological inhibition of NF-kappaB inhibits the mob-1 gene, a member of the α-chemokine (CXC family) and decreased the severity of experimental acute pancreatitis. Studying the protection caused by a short hyperthermia in an experimental model of acute pancreatitis, we also demonstrated that the decreased severity of the disease was attributed to a delayed activation of NF-kappaB associated with a reduced pancreatic expression of various cytokines (60).

In summary, the initial phase of the disease originates from the activation of trypsinogen into active trypsin within acinar cells, which in turn activates various enzymes such as elastase and Phospholipase A2 and the complement and the kinin systems. In experimental and human pancreatitis TAP is increased in the pancreas. The higher the peptide concentration is in plasma, urine and ascites, the greater the severity of the disease. In normal conditions, to prevent a premature activation, the potential harmful digestive enzymes are synthesized in acinar cells and released as inactive precursors. When passing through the Golgi system, these digestive enzymes are separated from other lysosomal enzymes which may activate them. During pancreatic injury, the *intra-acinar co-localization* of the digestive and lysosomal enzymes is an important mechanism although the clinical relevance of this concept remains unclear (32, 61). Another feature observed in this initial phase is the disruption of the paracellular membranes of acinar cells and intralobular pancreatic duct cells with extravasation of pancreatic enzymes into the interstitium (62).

5. LATE EVENTS IN ACUTE PANCREATITIS

Following pancreatic edema and inflammation, acute pancreatitis may progress to necrosis and hemorrhage. Presumably, alterations of microcirculation leading to pancreatic ischemia favor the progression from mild to severe form of the disease (49). Alternatively, the severity of the injury may be established at the beginning of the disease. Thus, Ranson *et al.* (63, 64) show that the severity of the disease (mortality and subsequent complications) can be evaluated with clinical and biological scores that are available within 48 hours after onset of the disease. These observations suggest that the severity of the lesion is established early in the course of pancreatitis and argue against the evolution of a mild to a more severe form of the disease. Thus, factors that initiate the evolution towards a severe disease remain unknown.

5.1. Local complications

Pancreatitis is frequently associated with three local manifestations that are fat necrosis, pseudocyst, and pancreatic abscess (9, 65). Each of these complications are related to the release of activated digestive enzymes. Release of lipase leads to adipose tissue injury and fat necrosis that usually takes place in peripancreatic tissue (as well as in remote organs). The extraductal collection of pancreatic juice, resulting from ductal rupture, forms pseudocyst. The more severe complication of acute pancreatitis is abscess because it is associated with a substantial morbidity and mortality (66). Pancreatic abscess includes peripancreatic necrosis of connective tissue and contains both activated digestive enzymes and a mixed flora of bacteria (67).

5.2. Complications in remote organs

The disease is frequently associated with an hypovolemic shock, resulting from transudation and exsudation of intravascular fluid into the peripancreatic retroperitoneum (65). In addition, diffuse capillary leakage in remote organs, including the peritoneal cavity and the pulmonary interstitium aggravate the hypovolemia. Hypoalbuminemia decreases the intravascular colloid osmotic pressure and contributes to the fluid exsudation (68). In addition, vasoactive agents (69) and pro-inflammatory cytokines (16, 70, 71) participate in the hyperdynamic syndrome which is similar to the one described in cirrhotic and septic patients (72).

Severe attacks of pancreatitis are also associated with pulmonary and/or renal injury (73). The mechanisms by which the lungs and/or kidneys fail is controversial. Initially the injury was related to sepsis and/or hypotension but experimental studies clearly show that neutrophils and proinflammatory factors such as TNF-α play a key role (74). Although the pulmonary failure during pancreatitis is similar to the one described in adult respiratory distress syndrome a specific injury is related to the release of activated digestive enzymes or vasoactive agents (75). Experimental studies clearly demonstrate the deleterious effects of Phospholipase A2 on lung surfactant (76). Renal lesions result from the injury of the basement membrane by circulating enzyme/enzyme-inhibitor complexes or additional proteins specific for the disease (77).

5.3. Role of inflammatory cells

Macrophages are normal resident cells in the pancreas but during acute pancreatitis, they contribute to the spreading of the local inflammation to remote organs. Indeed, studies blocking macrophage activity by gadolinium showed that the severity of pancreatitis was not modified, whereas the severity of the pancreatitis-associated lung injury was reduced (78).

Lymphocytes isolated from patients with pancreatitis usually show impaired proliferative response to mitogens in comparison to volunteers or to patients with other intra-abdominal diseases (79). Moreover, persistent alterations in lymphocyte function have been reported in patients with acute pancreatitis as late as one month after the clinical event (79). In experimental studies, lymphocytes play a central role in the development of acute pancreatitis. In control mice, CD4 cells are present in the pancreas and are recruited during cerulein-induced acute pancreatitis, but in nude mice, histological lesions and serum amylase levels are significantly decreased (80). T-lymphocyte transfert into nude mice partially restores the severity of acute pancreatitis. Furthermore, the severity of acute pancreatitis is also reduced by in vivo CD4 T-cell depletion.

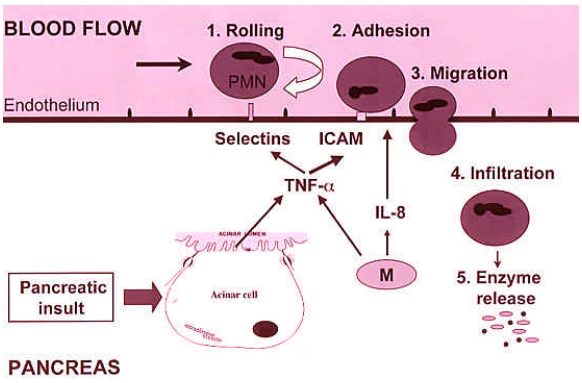

Figure 3. Role of intercellular molecule adhesion-1 (ICAM-1) : Leukocyte migration within the pancreas is a multistep process. Pancreatic insult is characterized by the release of local inflammatory mediators such as TNF-alpha and interleukin-8 (IL-8). Circulating leukocytes are attracted by IL-8 and will subsequently adhere to the endothelial wall via the endothelial expression of intercellular molecule adhesion-1 (ICAM-1). The emigration of leukocyte to inflammatory sites requires leukocyte rolling (1. Rolling) via the endothelial expression of selectins and intercellular molecule adhesion-1 (ICAM-1) (2. Adhesion) and the trans-endothelial migration into the injured tissue (3. Migration). Activated leukocytes infiltrate the pancreas and release their deleterous enzymes (4. Infiltration). Of note, ICAM-1 is upregulated by TNF-alpha.

In experimental pancreatitis induced by a supramaximal cerulein stimulation, leukocytes appear in the pancreas within 1 hour and peaks by 4-12 hours. Sequestration of inflammatory cells is a multistep process that begins with leukocyte activation, involves the adhesion of circulating inflammatory cells to the activated endothelium of the microcirculation, and culminates in the transmigration of these cells across the endothelial barrier into the parenchyma (Figure 3). Recently, considerable research has focused on 1) the chemoattractants responsible for the leukocyte sequestration within injured tissues; and 2) the factors released from inflammatory cells that contribute to the evolution of a local inflammation into a systemic disease (Figure 4).

5.4. Role of cytokines

Over the last decade, acute pancreatitis has been identified as an inappropriate inflammatory response (14). Thus, the disease exhibits many of the features observed in the systemic inflammatory response syndrome (SIRS) which is defined as a multiple organ failure following multiple trauma or burns (81). Similarly to other causes of multiple organ failure, cytokines have been extensively evaluated using either blocking experiments or transgenic animals (15, 74, 82-86) (Figure 5).

The use of transgenic and knock-out mice for pro- and anti-inflammatory mediators are useful tools to examine the effect of specific factors without any drawback induced by pharmacological agents. In acute pancreatitis, experimental studies clearly identify interleukin (IL)-1, IL-6, TNF-α, ICAM-1, and CD40 ligand as pro-inflammatory mediators whereas IL-10 has an anti-inflammatory role. However, a single genetic deletion cannot completely prevent the occurrence of pancreas and distant organ injury, emphasizing the concept of a "cytokine thunderstorm" which characterizes the course of the disease.

5.5. Role of platelet activating factor: a new therapeutic target?

Activated neutrophils found in the pancreas as well as injured acinar cells contain Phospholipase A2 which catalyze the release of platelet activating factor (PAF) from membrane phospholipids (87, 88). PAF is involved in the local as well as in the systemic complications of the disease (89, 90). In experimental models, PAF is found within the pancreas, in the serum, and in remote organs and PAF antagonists diminish the severity of the disease (91, 92). Consequently, clinical studies included patients treated with PAF antagonists with promising results. The PAF antagonism, Lexipafant, decreased the number of complications and the severity of the disease (93, 94). Unfortunately, the last European multicenter study failed to prove any benefit in patients with acute pancreatitis (95).

6. REGENERATION FOLLOWING ACUTE PANCREATITIS

The mechanisms leading to a complete regeneration of the organ after an acute attack of pancreatitis are mostly unknown, but recent studies have focused on the role of growth factors, such as transforming growth factor-beta (TGF-beta) and insulin-like growth factor (IGF-1) in this process. Pancreatic regeneration after cerulein administration is characterized by a transient fibroblast proliferation followed by the replication of acinar cells. Ludwig *et al.* (96) found that pancreatic IGF-1 concentrations increase over 50-fold during regeneration. Interestingly, IGF-1 also increases the number of acinar cells in a dose-dependent manner. In a similar experimental model of pancreatitis, Muller-Pillasch *et al.* (97) found that pancreatic TGF-beta concentrations increase by 24 hours after cerulein administration and return to control values after 48 hours. The largest amount of TGF-beta mRNA is found in pancreatic acinar cells and in stromal cells. In rats receiving cerulein and antibodies directed against TGF-beta, the pancreatic hydroxyproline content and the expression of collagen and TGF-beta are significantly reduced (97) .

7. EVOLUTION TO CHRONIC PANCREATITIS

Ten years ago, Kloppel *et al.* (98) postulated that chronic alcoholic pancreatitis results from the recurrence of

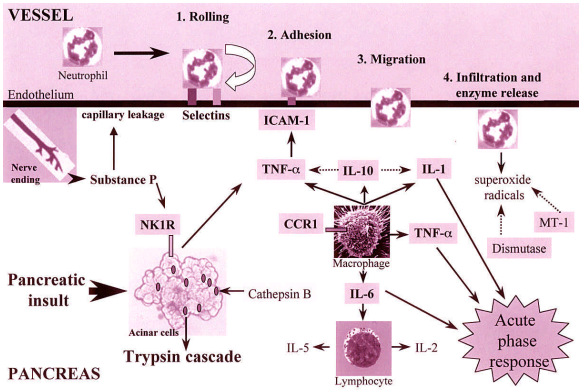

Figure 4. Genetically modified mice and the pathophysiology of acute pancreatitis. After a pancreatic insult, trypsinogen becomes activated to trypsin in the presence of cathepsin B. Then, trypsin leads to the activation cascade of other zymogens within the pancreas. The genetic deletion of cathepsin B reduces the intrapancreatic concentrations of trypsin. Substance P, which is a mediator produced by nerve ending and which is responsible for capillary leakage, is up-regulated during acute pancreatitis, as well as the neurokinin1 receptor (NK1R) on endothelial and acinar cells. Deletion of the NK1R gene does not modify the pancreatic cell responses but reduces the lung injury associated with the disease. During acute pancreatitis, acinar cells and macrophages produce tumor necrosis factor-α (TNF-α), which in turn upregulates the expression of intercellular adhesion molecules-1 (ICAM-1), an adhesion molecule responsible for the firm attachment of neutrophils on endothelial cells. Deletion of TNF-α protects mice with pancreatitis. After tissue transmigration, neutrophils release their deleterious enzymes and superoxide radicals which are partly scavenged by the superoxide dismutase and the metallothionein-1 (MT-1). Mice overexpressing MT-1 have less severe pancreatitis. TNF-α, interleukin-1 (IL-1), and IL-6 are released by activated macrophages primed by chemokines such as the chemoattractant cytokine receptor-1 (CCR-1). Deletion of CCR-1 has no effect on the severity of the pancreatic disease but significantly reduces lung injury. Additionally, TNF-α, IL-1, and IL-6 enhance the synthesis of the acute phase proteins and activate neutrophils and lymphocytes. Finally, mice overexpressing IL-10 have a less severe pancreatic injury. Genetically modified genes are in grey boxes; arrows are dotted when the mediator is beneficial for the outcome of the pancreatic disease; arrows are plain when the mediator is deleterious (reproduced with permission from reference 15).

severe acute pancreatitis. The disappearance of large areas of fat and hemorrhagic necrosis is followed by the appearance of fibrosis, possibly through the action of mediators, such as TGF-beta, epidermal growth factor (EGF), fibroblast growth factor (FGF) and IGF-1. The fibrosis develops primarily in the perilobular space which was the main location of necrosis. This hypothesis might explain the patchy distribution of fibrosis and the late occurrence of calculi in the pancreatic ducts of patients with chronic pancreatitis. However, we do not know why biliary pancreatitis, which may be as severe as alcoholic pancreatitis, never progresses to chronic pancreatitis. A second hypothesis suggest that long term ethanol consumption increases the protein content in the pancreatic juice, causing protein plugs within the ducts, which will later calcify. Lithostatin A is a protein detectable in the pancreatic juice that prevents CaCO3 precipitation (99). It is likely that the decreased secretion of lithostatin caused be either an acquired or inherited defect in its biosynthesis contributes to the calcification of protein plugs in the pancreatic ducts (100). The formation of stones obstruct the pancreatic duct and damage the duct epithelium with subsequent acinar atrophy and fibrosis upstream of the obstruction.

7.1. Role of stellate cells in pancreatic fibrosis

In the liver, stellate cells play an important role in fibrogenesis. Similar cells have recently been isolated from the pancreas. Pancreatic stellate cell activation was associated with fibrosis in both human and experimental chronic pancreatitis (101). The highest concentrations of TGF-beta are observed in acinar cells adjacent to fibrotic areas containing stellate cells. Apte *et al.* (102) also showed that pancreatic stellate cells produce collagen and other extracellular matrix proteins. Moreover, exposure of stellate cells to ethanol or acetaldehyde led to cell activation and intracellular lipid peroxidation (103).

8. CONCLUSIONS

In summary, the pathophysiology of acute pancreatitis includes the activation and the release of pancreatic enzymes in the interstitium, the autodigestion of the pancreas and the development of a multiple organ dysfunction following the release of pancreatic enzymes and other mediators into the circulation. The early phase of the disease originates from the premature intra-acinar activation of trypsinogen into active trypsin via the co-localization of trypsinogen and cathepsin B. The premature activation of trypsin activates in turn various enzymes such as elastase, Phospholipase A2, and the complement and kinin systems. Although intra-acinar co-localization of digestive and lysosomal enzymes appears important in experimental pancreatitis, its relevance in patients remains unclear. Other early events are also involved such as the production of reactive oxygen species and modifications of the microcirculation.

The late events of the disease include the synthesis and release of pro-inflammatory mediators which in turn amplifies a local inflammation into a systemic one. Injury in the pancreas and remote organs originates from the release of pro-inflammatory mediators such as IL-1, IL-6, interleukin-8, and TNF-alpha by activated inflammatory cells such as neutrophils and macrophages.

The ratio between the apoptosis and necrosis is an important factor and treatments which favor the induction of apoptosis are effective in reducing the severity. Finally, the ultimate severity of pancreatitis results from a balance between pro- and

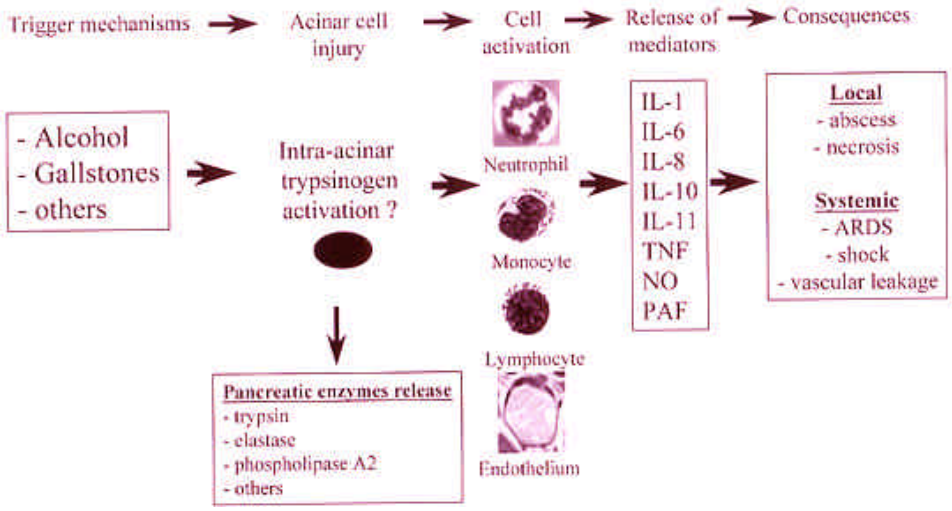

Figure 5. Pathophysiology of acute pancreatitis (reproduced with permission from reference 12).

anti-inflammatory mediators as well as between necrosis and apoptosis.

Following an attack, the gland completely recovers, but if the injury is severe, the healing process may be altered. Recent studies emphasize the role of growth factors in this regeneration process. Pancreatic regeneration is characterized by a transient proliferation of fibroblasts followed by the replication of acinar cells. The extent and the nature of this fibroblast proliferation influence the degree of fibrosis and the evolution towards chronic pancreatitis.

Following the activation of pancreatic enzymes, a local production of inflammatory mediators within the pancreas (late events) occurs (16, 23). Finally, the synthesis and release of these inflammatory mediators from the pancreas may in some severe forms of the acute pancreatitis transform a local inflammation into a systemic disease.

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Abbreviations: L: lysosomes; Z: zymogen granules; N: nucleus; REG: endoplasmic reticulum; VF: vacuoles resulting from the fusion of zymogen with lysosomal granules; VDS: vacuoles resulting from derangement in the intracellular sorting events of zymogen and lysosomal proteins; TAP: trypsinogen activation peptide

Key Words: Acute Pancreatitis, Early Events, Late Events, Cytokines, Apoptosis, Necrosis, Lung Injury, Fibrosis, Review

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