

Recent insights into the cellular mechanisms of acute pancreatitis

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In acute pancreatitis, initiating cellular events causing acinar cell injury includes co-localization of zymogens with lysosomal hydrolases, leading to premature enzyme activation and pathological exocytosis of zymogens into the interstitial space. This is followed by processes that accentuate cell injury; triggering acute inflammatory mediators, intensifying oxidative stress, compromising the microcirculation and activating a neurogenic feedback. Such localized events then progress to a systemic inflammatory response leading to multiorgan dysfunction syndrome with resulting high morbidity and mortality. The present review discusses some of the most recent insights into each of these cellular processes postulated to cause or propagate the process of acute pancreatitis, and also the role of alcohol and genetics.

Key Words: *Alcoholic pancreatitis; Cholecystokinin; Exocytosis; Pancreatitis*

PREMATURE ACTIVATION OF PANCREATIC ENZYMES WITHIN THE PANCREATIC ACINAR CELL

The main role of the exocrine pancreas is to synthesize and secrete digestive enzymes, such as trypsinogen, into the intestinal lumen. In the lumen, trypsinogen is activated by duodenal enterokinase into trypsin, which is then capable of activating other pancreatic enzymes to collectively perform nutrient digestion (1). However, in acinar cells, these enzymes are maintained in inactive proforms (ie, trypsinogen) within the zymogen granules (ZGs) by inhibitors such as serine protease inhibitor, Kazal type 1 (SPINK1) (2). Inadvertently, activated enzymes, particularly trypsin, are cleaved by cytosolic enzyme Y and mesotrypsin (3). Because optimal pH levels and Ca²⁺ concentrations are required to activate the enzymes, a controlled pH range within the ZG is maintained by a ZG membrane-bound proton pump and a vacuolar ATPase (4), and low cytosolic Ca²⁺ concentration is maintained by Ca²⁺ sequestration into intracellular stores within a smooth endoplasmic reticulum compartment by a Ca²⁺ ATPase (5).

The current dogma for the pathogenesis of acute pancreatitis is the premature activation of trypsinogen within the pancreatic cell (6,7). The major established experimental model of mild acute pancreatitis is the hyperstimulation (cholecystokinin [CCK] or its analogue cerulein/carbachol)

De récents aperçus des mécanismes cellulaires de la pancréatite aiguë

En cas de pancréatite aiguë, l'initiation d'événements cellulaires causant des lésions cellulaires acineuses inclut la colocalisation de proenzymes avec des hydrolases lysosomiales entraînant une activation enzymatique prématurée et une exocytose pathologique des proenzymes dans l'espace interstitiel. Ces phénomènes sont suivis de processus qui accentuent la lésion cellulaire, déclenchant des médiateurs inflammatoires aigus, intensifiant le stress oxydatif, compromettant la microcirculation et activant les rétroactions neurogènes. Ces événements localisés évoluent ensuite vers une réaction inflammatoire systémique provoquant un syndrome de dysfonctionnement multiviscéral entraînant un taux de morbidité et de mortalité élevé. La présente analyse expose certains des aperçus les plus récents sur chacun de ces processus cellulaires qu'on pense causer ou propager le processus de pancréatite aiguë, et également le rôle de l'alcool et de la génétique.

model (6,7), which leads to missorting and co-localization of zymogens with lysosomal cathepsin B within large cytoplasmic vacuoles, where trypsinogen is cleaved and activated (6,7). Within this compartment, zymogen activation is favoured by an acidic pH that is effected by a hyperstimulation-induced translocation of cytosolic vacuolar ATPase (4). Hyperstimulation also causes a high and persistent rise in cytosolic Ca²⁺ concentration, which facilitates enzyme activation and large vacuole formation (8,9). Pharmacological blockade of cathepsin B decreases necrosis in cerulein-induced pancreatitis (10). However, genetic deletion of cathepsin B in mice only partially prevented trypsinogen activation and did not completely abrogate cerulein-induced pancreatitis (11), indicating that additional factors must be involved in the pathogenesis of acute pancreatitis.

BASOLATERAL EXOCYTOSIS INTO THE INTERSTITIAL SPACE

Interstitial pancreatitis is a mild stage of clinical pancreatitis, which is due to the misdirection of pancreatic enzymes into the interstitial space (12). It was postulated that the normal release of enzymes into the ductal lumen from exocytosis of ZGs located at the apical pole becomes misdirected to the basolateral plasma membrane (PM), which then undergoes basolateral exocytosis (13). In fact, basolateral exocytosis in pancreatic acinar cells was demonstrated 20 years ago by

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ultrastructural studies in not only hyperstimulation rodent models (13), but also in human pancreatitis (14). However, it was only recently that we were able to show basolateral exocytosis by real-time imaging of single ZG exocytosis in supramaximal CCK-stimulated rat pancreatic acinar cells (15). We have further elucidated the molecules mediating basolateral exocytosis (15,16). It is well established that the fundamental mechanism controlling the fusion of membranes in all cell types revolves around the soluble N-ethylmaleimide-sensitive factor attachment protein (SNAP) receptor (SNARE) hypothesis, which proposed that cytosolic N-ethylmaleimide-sensitive factors and soluble SNAPs bind SNAREs on donor vesicles (v-SNAREs) and target membranes (t-SNAREs) to form a series of multimolecular complexes (17). The union between v- and t-SNAREs ultimately mediates the fusion of the two membranes (17,18). Specificity of membrane fusion events is due to the compartmental specificity of distinct sets of v-SNARE (vesicle-associated membrane proteins [VAMPs]) and t-SNARE (syntaxin and SNAP-25) proteins (19), and accessory proteins (particularly Munc18), which regulate the SNARE complex assembly (20). In the pancreatic acinar cell, we have identified the key SNARE proteins on the basolateral membrane (Syntaxin 4 and SNAP-23) and ZGs (VAMP), as well as the regulatory protein Munc18c (15,16,21-23). Munc18c binds Syntaxin 4 to prevent its assembly with the other SNARE proteins (24,25), but with supramaximal CCK stimulation, Munc18c dissociates from the basolateral PM, relieving Syntaxin 4 to bind to SNAP-23 and VAMP (most likely VAMP-8) (26) to effect basolateral exocytosis (15,16). In fact, we observed Munc18c displacement from the basolateral PM not only in the rat model of hyperstimulation-induced pancreatitis (15), but also in human alcoholic chronic pancreatitis tissues (27).

Although the SNARE proteins bring the cognate membrane compartments in close proximity, Ca^{2+} is nonetheless the final fusogenic agent. Ca^{2+} can be released from different locations of the acinar cell. Physiological CCK stimulation acts on inositol 1,4,5-triphosphate receptors of Ca^{2+} stores that overlap the ZGs in the apical pole to effect normal apical exocytosis (28,29). In contrast, hyperstimulation acts on ryanodine receptors (RyR), which release a distinct Ca^{2+} store located at the basolateral pole (30). This site of Ca^{2+} release is strategically located to effect basolateral exocytosis. Remarkably, depletion of RyR-sensitive Ca^{2+} stores or RyR blockade, *in vitro* and *in vivo*, reduced hyperstimulation-induced intracellular zymogen activation, but did not affect enzyme secretion (30). This intriguingly suggests that zymogens could already be activated within the ZGs before their release, by basolateral exocytosis into the interstitial space, which would initiate an inflammatory response.

ROLE OF INFLAMMATORY MEDIATORS

Acini undergoing injury release zymogens, particularly trypsin, that induce macrophages to synthesize and release proinflammatory cytokines, such as tumour necrosis factor- α (TNF- α) and interleukin-1 (IL-1) beta, which are capable of inducing neutrophil recruitment and activation within the pancreatic tissue (31,32). Acinar cells themselves can synthesize cytokines, which amplify the local inflammatory response (33). TNF- α has direct actions on acini that

mimic CCK hyperstimulation, including nuclear factor- κ B (NF- κ B) activation and disruption of the actin cytoskeleton; but unlike CCK, TNF- α can also induce apoptosis (34). NF- κ B is a transcription factor that promotes the expression of proinflammatory cytokines (35). Supramaximal CCK stimulation (hyperstimulation), *in vitro* or *in vivo*, causes NF- κ B activation within acinar cells during the early phase of pancreatitis (36) by inducing I κ B, the enzyme degrading NF- κ B proteolysis. Satoh et al (36) have revealed that CCK-induced I κ B degradation is partly mediated by novel protein kinase C isoforms delta and epsilon, which are activated by diacylglycerol, generated by phosphatidylinositol- and phosphatidylcholine-specific phospholipase C.

Zymogens and cytokines released by the inflamed pancreas into the ascitic fluid are absorbed into the circulation, leading to the systemic inflammatory response syndrome (31,32,37). These molecules induce leukocyte recruitment, which in turn exacerbates the synthesis and release of proinflammatory mediators in distant targeted organs (ie, lung), with consequent multiorgan dysfunction syndrome (38). In fact, disease severity can be correlated to the level of these circulating cytokines (39). Consistent with the postulate that cytokines and NF- κ B contribute to both local and systemic inflammatory response (40), pharmacological inhibition of NF- κ B (40-42), leukocyte depletion (43) or genetic deletion of cytokine IL-1 β (44), receptors to IL-1 type I or TNF- α type I (45) or intercellular adhesion molecule-1 (46) have uniformly decreased the severity of pancreatitis, including the associated lung injury (43).

Compromised pancreatic blood circulation also contributes to the pathogenesis of acute pancreatitis (47). A major factor modulating the pancreatic microcirculation is nitric oxide metabolism (48), which when altered in gene knockouts of nitric oxide synthase (49) or IL-18 (50), profoundly influences cerulein-induced pancreatitis (50).

NEUROGENIC INFLAMMATION

The perivaterian duodenum, the region where the pancreas meets the duodenum, has a very rich autonomic innervation (51). Any obstruction and/or irritative noxa in this region (ampulla of Vater) caused by gallstones, biliary sludge or endoscopic manoeuvres, stimulates primary sensory neurons which trigger autonomous arc reflexes (AARs), that initiate an acute, neurogenic inflammatory response in the pancreatic tissue (52). The AARs integrate the perivaterian duodenum autonomic nerve fibres with those from the celiac ganglion and bulbar-hypothalamic nuclei (52,53). These sensory neurons in the pancreas contain unmyelinated, capsaicin-sensitive (type C) nerve fibres that release sensory peptides such as substance P, neurokinin A and vasoactive intestinal peptides (52). These peptides act on mast cells, causing release of histamine and proinflammatory mediators, which are responsible for vasodilation and increased vascular permeability. This leads to edema and recruitment of neutrophils (52,54,55). Stimulation of the AARs also induces a local, sympathetic overstimulation that acts on the pancreatic microcirculation, causing vasoconstriction and consequent ischemia-reperfusion injury (56). These AAR-mediated events are observed using biliopancreatic duct outlet exclusion-closed duodenal loops model (57). Using this model, in one study (57) topical lidocaine was

able to interrupt the AARs, which remarkably ameliorated pancreatitis.

FACTORS INFLUENCING RECOVERY AND REGENERATION AFTER PANCREATITIS

A number of proteins within acinar cells act to protect the exocrine pancreas from injury or to facilitate its recovery. These proteins include heat shock proteins (Hsps), pancreatitis-associated proteins (PAPs) (58) and cytoskeletal actin (59). Hsp27, Hsp60, Hsp70 and Hsp90, present in the exocrine pancreas (60,61), are stress-induced proteins inducible by hyperthermia (62) and water immersion (63). Both hyperthermia and water immersion protect against cerulein hyperstimulation and arginine-induced pancreatitis via their actions on Hsp27 and Hsp70 (62,64). Hsp27, when phosphorylated, is able to inhibit trypsin activity as well as prevent degradation and disruption of the actin cytoskeleton, thereby hastening acini recovery after injury (65). In fact, transgenic mice overexpressing human Hsp27 or a constitutively phosphorylated mutant were resistant to cerulein-induced pancreatitis, which correlated with reduced trypsin activation and intact actin cytoskeleton (65). Hsp70 activation inhibits NF- κ B activation in pancreatitis (66). PAP-1, whose levels are increased during pancreatitis (67), exhibits antiapoptotic and anti-inflammatory actions, primarily by reducing TNF- α -induced apoptosis in acinar cells (68) and inhibiting TNF- α -induced NF- κ B activation in macrophages (69). PAP-1 also reduces leukocyte-induced lung injury during acute pancreatitis (70).

The following proteins are also elevated very early in the acinar cells during pancreatitis and can influence pancreatic regeneration. p8 is a nuclear phosphoprotein (71), which may act as a transcription cofactor involved in cell proliferation (72,73). Interferon-inducible protein 15 is induced by interferons, alpha and delta, and inhibits cell proliferation (74). Vacuole membrane protein 1 is a proapoptotic factor that is associated with large cytoplasmic vacuoles (75,76), one of the earliest morphological evidences of mild acute pancreatitis (6,7). Mitochondrial dysfunction occurring during acinar injury results in ATP depletion and activation of poly (ADP-ribose) polymerase, which interferes with caspase-9 activation (77). This proapoptotic action of poly (ADP-ribose) polymerase is activated in cerulein-induced pancreatitis, and its pharmacological blockade inhibits pancreatic necrosis (77). The local inflammatory response can activate neural release of proteinase-activated receptor 2, which has protective effects through actions on exocrine and duct regeneration and repair (77,78). However, proteinase-activated receptor 2 can also induce vasoconstriction of the microcirculation, followed by vasodilation due to its ability to release nitric oxide from endothelial cells, which collectively contributes to pancreatic ischemia-reperfusion injury (78).

GENE MUTATIONS AND HEREDITARY PANCREATITIS

Recurrent episodes of acute pancreatitis, particularly in the young, indicate a genetic origin (79). The main gene mutations associated with pancreatitis are in the following proteins: cationic trypsinogen (*PRSS 1*), the pancreatic secretory trypsin inhibitor or *SPINK1*, the cystic fibrosis

transmembrane conductance regulator (*CFTR*), alpha-1-antitrypsin, alcohol metabolizing enzymes and the human leukocyte antigen locus (80). Recurrent acute pancreatitis can also result from familial disorders of lipid metabolism (familial hypertriglyceridemia, familial hypercholesterolemia, congenital deficits in lipase lipoprotein or apolipoprotein C-II), calcium metabolism (hyperparathyroidism, familial hypercalcemia or hypocalciuria) and other disorders (homocystinuria or acute intermittent porphyria) (81).

PRSS 1 mutations cause excessive activation of cationic trypsinogen to occur within the acinar cell and consequent autodigestion of the pancreas (82). These cases of autosomal dominant hereditary pancreatitis are mostly linked to two missense mutations in the *PRSS 1* gene on chromosome 7 (7q35), in exon 2 (*N291*) and exon 3 (*R122H*), with 80% penetrance (78,81). These mutations also increase the risk of developing pancreatic adenocarcinoma by 50 times, particularly if the allele is of paternal origin and/or combined with smoking (83).

SPINK1 blocks the active site of trypsinogen (2). Most cases are associated with the *N34S* mutation in exon 3 of the *SPINK1* gene located in chromosome 5q (2). Although 6% to 40% of patients diagnosed with idiopathic pancreatitis have a *N34S* mutation (2,80), these mutations per se are not sufficient to induce pancreatitis, and they would require other environmental (ie, high alcohol intake) or genetic (ie, *CFTR* gene mutations) triggering factors (80,84).

Cystic fibrosis is the most frequent autosomal recessive disease in the Caucasian population (one in every 2500 people) with a very high frequency of heterozygous carriers (one in 25 people) (79). The major mutation in the *CFTR* gene is situated in the long arm of chromosome 7 in 7q31 (83). The *CFTR* protein is a chloride channel on pancreatic ductal cells (85,86), which when defective, reduces pancreatic ductal fluid flow, resulting in mucoviscidosis and ductal obstruction, and leading to pancreatic insufficiency and episodes of pancreatitis (86,87).

ALCOHOL-INDUCED PANCREATITIS

Alcohol is the most common cause of pancreatitis, but the precise mechanism of alcohol-induced pancreatic injury remains elusive (88). In fact, animal models with administration of alcohol acutely or chronically did not lead to pancreatitis (89). Instead, after alcohol feeding, pancreatitis can be induced by submaximal CCK, fat and viral infections (90-92). This suggests that alcohol either serves as a sensitizing factor or activates undefined 'susceptibility' factors, which predispose the exocrine pancreas to injury by triggering factors. As a sensitizing factor, animals that were put on an alcohol diet exhibited increased expression of cytokines and NF- κ B activation upon low-dose CCK-8 stimulation (93). As a susceptibility mechanism, our recent report on pancreatic tissues obtained from a patient with mild alcoholic pancreatitis showed that exocytotic proteins in the acinar basolateral PM are perturbed; that we propose predisposed to pathological basolateral exocytosis that perpetuates the inflammatory process (27).

Nonetheless, alcohol has been shown to have direct effects on the exocrine pancreas. Alcohol metabolism in the acinar cells is similar to hepatocytes, including oxidative pathways generating acetaldehyde and nonoxidative pathways

generating fatty acid ethyl esters (94,95), both of which are toxic to acini. Reactive oxygen species are also generated, which alter actin filament polymerization to induce cytoskeletal disruption (96). Alcohol also causes hypoxia (97), as well as mitochondrial dysfunction and injury (98), which aggravates the ischemic-hypoxic injury caused by the already compromised microcirculation. Mitochondrial dysfunction perturbs Ca^{2+} release processes (99), which activate calpains, a cytosolic cysteine protease that targets the actin cytoskeleton (100), along with Ca^{2+} -induced detrimental actions on zymogen activation and pathological exocytosis. Alcohol increases lysosome (101) and ZG fragility

(102), increases trypsinogen synthesis (103) and promotes zymogen activation (102). Alcohol can also activate 'neurogenic' mechanisms, specifically by increasing the sympathetic tone, which may result in chronic alteration in vagal-vagal tone, inducing cholinergic hyperstimulation of the pancreas (52), and spasms and dysfunction of the sphincter of Oddi (52).

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