New Insights Into the Mechanisms of Pancreatitis

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A number of discrete pathologic events contribute to the initiation and perpetuation of acute pancreatitis.[1] These processes are sequential and often interrelated. An emerging theme is that genetic or environmental factors, such as ethanol, may sensitize the gland to injury.[1] For example, mice exposed to the coxsackie A virus are dramatically sensitized to the development of ethanol-induced pancreatitis.[2] Further, chronic ethanol feeding alone does not cause pancreatitis in rodents, but it does cause animals to develop acute pancreatitis when they receive physiologic concentrations of cholecystokinin (CCK) or cholinergic agonists such as carbachol.[1] Remarkably, such noxious stimuli was recently shown to block normal secretion at the apical pole of the pancreatic acinar cell, redirecting the release of digestive enzymes across basolateral membrane into the interstitial space. Insults that target the pancreatic acinar cell often seem to initiate disease.

Early Features of Acute Pancreatitis

The acinar cell exhibits 3 phenotypic responses in the early phases of acute pancreatitis. These include changes in secretion, the intracellular activation of proteases, and the generation of inflammatory mediators. Although each response is likely required to develop pancreatitis, this review focuses on secretion and protease activation. Digestive enzymes and enzyme precursors known as zymogens are stored in zymogen granules until the cell is directed to secrete their content into the pancreatic duct when we eat a meal. Under physiologic conditions, the secretion is confined to the pancreatic duct by 2 restraints: (1) Secretion is limited to the apical membrane that forms the proximal duct lumen and (2) apically distributed junctional barriers prevent the movement of duct contents into the intracellular space. However, this physiologic pattern of secretion can be changed dramatically early in the course of acute pancreatitis and may be linked to the pathogenesis of the disease (Figure 1*A*).

An early feature of acute pancreatitis in humans and experimental models is a dramatic decrease in secretion from the pancreatic duct into the duodenum. The reduction in secretion has been attributed to 3 pathologic responses (Figure 1*A*): (1) decreased apical secretion from the acinar cell, (2) disruption of the paracellular barrier in the pancreatic duct that allows its contents to leak into the paracellular space, and (3) redirection of secretion from zymogen granules from the apical pole to the basolateral regions of the acinar cell.[3] How this reduction in secretion might relate to the pathogenesis of acute pancreatitis has not been explored fully, but 2 mechanisms have been suggested. First, the retention of activated enzymes, particularly proteases, in the acinar cell might be required to cause disease. Thus, if the barriers to acinar cell secretion can be overcome, the damaging effects of activated enzymes might be averted. The observation that cyclic adenosine monophosphate agonists enhance secretion from the acinar cell under pancreatitis conditions and that these cells are protected from injury is consistent with this hypothesis.[4] In this context, the beneficial effects of secretin in pancreatitis shown in preliminary studies might be due to its stimulation of secretion of intracellular activated enzymes from the acinar cell. Second, the redirection of pancreatic enzyme secretion from the apical to the basolateral domain of the acinar cell may deliver enzymes and enzymes precursors to the interstitial space.[3] Neither mechanism has been fully explored.

Regulated Apical and Pathologic Basolateral Exocytosis in the Pancreatic Acinar Cell

Exocytosis represents the fusion of membrane bound vesicles with the plasma membrane. Exocytosis serves 2 major delivery functions, namely, secretion of luminal cargo into the extracellular space and transfer of vesicle membrane proteins to the plasma membrane. The pancreatic acinar cell is a polarized secretory cell with distinct apical and basolateral plasma membrane domains (Figure 1*A*). Almost all of the zymogen granules are localized to the acinar cell's apical pole; this positioning is essential for the rapid delivery of their cargo of digestive enzymes and zymogens to a very limited apical plasma membrane surface area that is only 5%–10% of the total surface area of the cell. To increase the efficiency of delivering this cargo to the pancreatic duct and duodenum during a meal, the acinar cell has a membrane fusion machinery that enables both the direct fusion of

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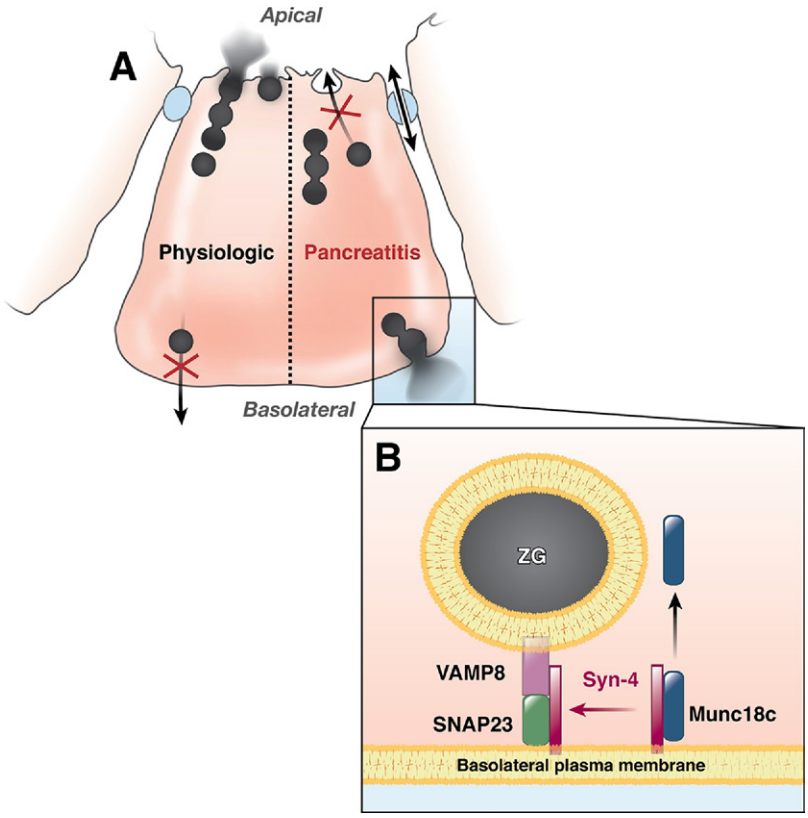

Figure 1. Physiologic regulated apical exocytosis and pathologic basolateral exocytosis in pancreatitis. (*A*, *left*) Normally, physiologic agonist stimulation causes zymogen granules (ZG) to undergo exocytotic fusion with the apical plasma membrane (PM) to release its zymogen cargo into the ductal lumen. ZGs deep in the apical pole would fuse with these apical PM-fused ZGs (compound or sequential exocytosis). ZGs would not fuse with the basolateral PM. (*Right*) Noxious stimuli (supramaximal CCK; alcohol plus postprandial CCK or carbachol) causes blockade of apical exocytosis, but ZG–ZG fusion continues. ZG granules distribute away from the apical pole and exocytosis is redirected to basolateral PM to release zymogens into the paracellular space. Intercellular junctions are disrupted allowing contents to leak into the paracellular space. (*B*) Molecular mechanism of basolateral exocytosis. Munc18c binds basolateral PM-bound Syntaxin 4 (Syn-4), which blocks Syn-4 binding to SNAP23 and ZG-bound VAMP8. Exposure to the noxious stimuli results in displacement of Munc18c (into the cytosol) from Syn-4, enabling the formation of Syn-4/SNAP23/VAMP8 fusion complex, which affects basolateral exocytosis.

the zymogen granules with the apical plasma membrane and for cargo to be released from granules that do not reside in close proximity to the apical membrane. The latter is accomplished through granule-to-granule fusion (also called sequential exocytosis and compound exocytosis) that joins the deepest layer of granules with those that are most apically distributed. Remarkably, over half of the zymogen granules become connected through fusion pores within 1–2 minutes of stimulation, and these fusion pores remain open for long periods of time to allow metered emptying of cargo at the apical membrane.[3,5] These exocytotic events were shown very elegantly with 2-photon confocal microscopy that tracked fluorescent polar tracers penetrating into sequentially fused zymogen granules.[5]

Studies reported 2 decades ago showed that this orderly secretion of zymogen at the apical pole can be disrupted when acinar cells are exposed to supraphysiologic (CCK or carbachol) stimulation, which is known to block apical exocytosis, that is, granule fusion with the apical plasma membrane.[6] Using electron microscopy, the original workers showed 2 additional features.[6] First, although there was an apical secretion blockade, granule–granule fusion still occurred. Second, exocytosis became misdirected to the lateral plasma membrane, where some of the acinar cell content was discharged into the interstitial space where they could affect the pathogenesis of pancreatitis. Recent insights into the mechanisms of exocytosis in health and disease are shown (Figure 1*B*) and discussed below. The importance of basolateral exocytosis to the pathogenesis of acute pancreatitis has not been fully elucidated, but it may have a role in the trafficking of active enzymes or provide zymogens that could become active during the course of acute pancreatitis.

New Insights Into Zymogen Activation as Underlying Basis of Pancreatitis

Although multiple mechanisms of pancreatic zymogen activation have been proposed, the intracellular sites remain unclear (Figure 2). The trypsinogen activation peptide is a surrogate for trypsinogen activation. Morphologic studies have shown that it initially appears in very small vesicles then larger vacuoles during the first hour of pancreatitis. These structures are associated with markers of lysosomes and/or endosomes.[7] Studies done in transgenic mice suggest that autophagic compartments have a role in intracellular activation.[8] A role for endocytic compartments in zymogen activation has also been described.[9] One explanation for differences among these studies may be that zymogens could be activated in distinct compartments and these responses might occur in a time-dependent manner. Supporting this conclusion is a report that inhibiting and/or removing inflammatory