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Perspectives

PERSPECTIVES

Calcium in Pancreatitis . . . Immune Cells, Too?

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A Perspective on "Calcium Signaling in Pancreatic Immune Cells In Situ"

In this issue of the Journal, Gryshchenko et al. describe the identification and characterization of pancreatic macrophages (PMs) in mouse pancreatic lobules *in vitro*.[1] Much progress has been made in recent years in the understanding of the mechanisms of acute pancreatitis.[2] The use of experimental animal models *in vivo* has accounted for some of this progress, but most of the insight into the fundamental mechanisms underlying acute pancreatitis has come from studies of various pancreatic tissue preparations *in vitro*. Most studies of the physiology and pathophysiology of the exocrine pancreas *in vitro* have been performed using pancreatic acini. The acinus is the functional unit of the exocrine pancreas and consists mainly of digestive enzyme-secreting acinar cells and some stellate cells.[3,4] Many other cell types are also in the exocrine pancreas such as duct cells, centroacinar cells, connective tissue cells, immune cells, and others, but it is generally not known that pancreatic acini contain these cell types. Pancreatic acini prepared from rodents such as rats, guinea pigs, and mice by gentle collagenase digestion and physical shearing forces have been used to reveal the basic mechanisms of digestive enzyme stimulus-secretion coupling in normal physiology and acute inflammation in pancreatitis pathophysiology. Some insight into other aspects of exocrine pancreatic function has also been obtained using other *in vitro* preparations such as pancreatic lobules to demonstrate the role of intrinsic pancreatic nerves in scorpion toxin-induced enzyme secretion and acute pancreatitis[5]; acini do not contain nerves. Pancreatic lobules are larger than acini and can be prepared by inflating the pancreas with buffer followed either by simple dissection with scissors or by very gentle collagenase digestion. Differentiation of the pancreas and its regeneration has also been studied using tissue culture of pancreatic organoids *in vitro*.[6]

Most studies of exocrine pancreatic function are directed at understanding the role of acinar cells in normal physiology and in pathologies such as pancreatitis. This is because acinar cells secrete digestive enzymes and because the hallmarks of acute pancreatitis in people and research animals are damage to acinar cells resulting in release of digestive enzymes such as α-amylase and lipase into the blood and trypsin into the pancreatic extracellular spaces, edema, and necrosis. Isolated, single acinar cells do not survive well *in vitro*[7] and this has led to the widespread use of pancreatic acini for these studies.

Although pancreatic acini have proven invaluable for studying early acinar cell responses to injury, pancreatitis is a complicated biological process that involves both pancreatic acinar cell damage and a subsequent inflammatory response. Modeling this process *in vitro* is incomplete using only pancreatic acini.

In this article, Gryshchenko et al. identify and characterize macrophages in pancreatic lobules.[1] It is not clear whether lobules were used because acini do not contain macrophages or whether acini were not examined. The PMs were identified by immunocytochemistry and by nuclear morphology. These cells had been termed "X" cells in a previous publication.[8] In this article, intracellular Ca^{2+} signals in response to various agents are characterized in PMs and compared with the Ca^{2+} signals in neighboring acinar cells and stellate cells (Figure 1). They show that PMs express purinergic receptors, identified pharmacologically as P2Y1 and P2Y13 receptors, sensitive to adenosine triphosphate (ATP) and adenosine diphosphate (ADP) that signal intracellularly via releasing Ca^{2+} from internal stores followed by store-operated Ca^{2+} entry through Ca^{2+} release-activated Ca^{2+} (CRAC) channels. Some PMs also produced Ca^{2+} signals in response to acetylcholine and high concentrations of bradykinin.

This study reinforces the critical role tightly regulated intracellular Ca^{2+} concentrations play in maintaining acinar cell integrity and provides new insights into a role for Ca^{2+} signaling in immune cells during the evolution of pancreatitis. The

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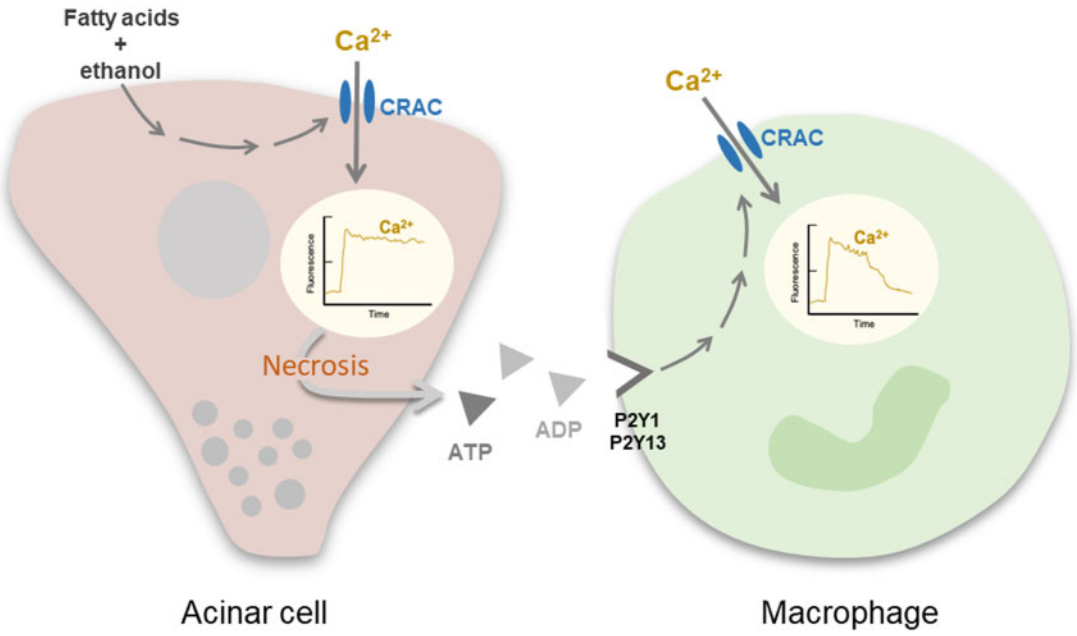

Figure 1. A Diagram Illustrating the Possibility that Acute Pancreatitis Caused by the Combined Effects of Ethanol and Fatty Acids Resulting in Acinar Cell Necrosis May Release ATP and/or ADP from Acinar Cells into the Periacinar Cell Space Occupied by PMs. Ethanol and fatty acids ultimately cause elevations in cytoplasmic Ca^{2+} concentrations in acinar cells by a series of steps culminating in the entry of extracellular Ca^{2+} into the cell via CRAC channels as indicated in the figure by several arrows and described in full elsewhere.[2] This ATP/ADP may bind to macrophage P2Y1 and/or P2Y13 receptors and stimulate Ca^{2+} entry from outside the cell via CRAC channels. The effect of elevated cytoplasmic Ca^{2+} levels in PMs is unknown.

authors demonstrate that the density of PMs is very low in normal pancreatic lobules, but that induction of acute pancreatitis by administering a combination of ethanol and fatty acids *in vivo* markedly increased the number of PMs. They speculate that the ensuing acinar cell necrosis may result in the release of ATP and/or ADP into the periacinar space leading to stimulation of PMs via purinergic receptors thus contributing to the mechanism of acute pancreatitis. Previous studies of PMs have shown that in the initial stages of acute pancreatitis most PMs are the proinflammatory M1 subtype but that later macrophages switch to the reparative M2 subtype and play an important role in parenchymal regeneration.[9] The system introduced herein offers a unique model for studying PM function *in vitro*. It will be fascinating to see in future studies of PMs how Ca^{2+} responses induced by local mediators such as ATP, ADP, acetylcholine, and bradykinin affect the PM phenotype and, in particular, the M1 and M2 transition. For example, if PMs contribute to disease severity, their activation may be blocked by use of CRAC channel inhibitors, at least one of which is currently under clinical development. Thus, limiting intracellular calcium influx in either acinar cells or immune cells in the pancreas may be useful in treating pancreatitis.

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Conflict of Interest Statement

The authors declare no conflict of interest.

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