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Which Way to Die: the Regulation of Acinar Cell Death in Pancreatitis by Mitochondria, Calcium, and Reactive Oxygen Species

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Acute pancreatitis is an inflammatory disorder of exocrine pancreas, which carries considerable morbidity and mortality; its pathophysiology remains elusive.[1] During the past decade, significant progress has been achieved in our understanding of the inflammatory response in pancreatitis.[1] Much less is known about the mechanisms mediating another key pathologic response of pancreatitis, namely acinar cell death. In human disease and experimental pancreatitis, acinar cells die through both apoptosis and necrosis. These 2 main types of cell death differ morphologically and biochemically.[2,3] Key molecular steps in the apoptotic pathway are the release of cytochrome c from mitochondria and activation of caspases, a specific class of cysteine proteases. Importantly, apoptosis preserves the plasma membrane integrity, whereas necrotic cell releases its constituents, damaging neighboring cells and promoting inflammation. Therefore, necrotic death is “deadlier” to the organism than apoptotic death.[2,3]

Parenchymal necrosis is a major complication of pancreatitis, and greater amounts of necrosis are associated with a worse prognosis in the disease.[1] Intriguingly, the severity of pancreatitis in animal models correlates directly with the extent of necrosis and inversely with apoptosis.[4–8] Furthermore, stimulating acinar cell apoptosis in experimental models decreased necrosis and the severity of pancreatitis, whereas inhibition of acinar cell apoptosis (eg, with caspase inhibitors[7]) potentiated necrosis and worsened pancreatitis. Thus, shifting the pattern of death responses of pancreatitis toward apoptosis and away from necrosis may have a therapeutic value.

Mitochondria play a central role in regulating cell death because mitochondrial membrane permeabilization is a universal trigger of both necrosis and apoptosis.[2,3] Permeabilization results in loss of the mitochondrial membrane potential, $\Delta\psi m$, through opening of the permeability transition pore (PTP), a nonselective channel permeating both outer and inner mitochondrial membranes.[3] Loss of $\Delta\psi m$ leads to adenosine triphosphate (ATP) depletion, inability to maintain ionic gradients across the plasma membrane, and ultimately necrosis. Mitochondrial permeabilization also triggers the apoptotic pathway through release of the

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mitochondria resident protein cytochrome c (likely, not via PTP[3]). Once in the cytosol, cytochrome c interacts with and activates caspases, leading to the downstream apoptotic events.[2,3]

Mitochondria are involved in cellular Ca

Until recently,[7,8,11–16] little was known about the properties of pancreatic mitochondria, their regulation by Ca^{2+} and ROS, and the roles of mitochondria, Ca^{2+}, and ROS in acinar cell death. The study by Booth et al in this issue of gastroenterology[17] advances further our knowledge of these regulations, especially the mechanisms involving ROS. This study applied an ex vivo model of acinar cell injury induced by bile acid (taurolithocholic acid salt [TLC-S]), which corresponds to animal (rodent) models of pancreatitis induced by administration of bile acids.[1,18] The bile acid models are clinically relevant, because gallstones are a major cause of acute pancreatitis. The previous data[7,8,11–14] were largely obtained in rodent and ex vivo models of pancreatitis induced by supramaximal CCK-8 or its analog cerulein, the most widely used and well-characterized experimental system to study acute pancreatitis[1,18] Both bile acid- and CCK/cerulein-induced pancreatitis is associated with acinar cell necrosis and apoptosis. The recent findings in these 2 dissimilar models, particularly the study by Booth et al,[17] reveal common mechanisms through which mitochondria, Ca^{2+}, and ROS regulate acinar cell death (Figure 1).

First, the results[7,8,11–13,16,17,19] provide convincing evidence that abnormal Ca^{2+} signal promotes acinar cell necrosis through mitochondrial depolarization and subsequent ATP drop. Both the supramaximal CCK/cerulein and TLC-S cause Ca^{2+}-dependent loss of $\Delta\psi m$. Experiments on isolated pancreatic mitochondria showed that Ca^{2+} directly depolarizes the mitochondria through PTP opening.[13] Moreover, it seems that pancreatic mitochondria are highly sensitive to Ca^{2+}-induced depolarization[13] (as compared, for example, with the "classical" liver mitochondria) and lose $\Delta\psi m$ even at submicromolar Ca^{2+} owing to a greater sensitivity of the pancreatic mitochondria PTP to Ca^{2+}. CCK-induced mitochondrial depolarization significantly decreases ATP level in acinar cells and leads to necrosis.[8,14] Furthermore, Booth et al[17] now show that TLC-S induced acinar cell necrosis can be reversed by patched ATP supplementation. Evidence for a key role of mitochondrial depolarization in acinar cell necrosis also comes from a study[8] that showed that the prosurvival Bcl-xL and Bcl-2 proteins (known to stabilize mitochondria[2,3]) protect acinar cells from necrosis and counteract the loss of $\Delta\psi m$ induced by the supramaximal CCK/cerulein. For example, Bcl-xL/Bcl-2 inhibitors or Bcl-xL small interfering RNA depolarized isolated pancreatic mitochondria and markedly decreased ATP level in acinar cells, resulting in necrosis.[8]

Second, the findings illuminate the role of ROS in acinar cell death. Both the supramaximal CCK-8 and TLC-S increase the mitochondrial ([ROS]m) as well as intracellular (total) ROS ([ROS]i) levels in acinar cells.[13,17] However, the data from Booth et al[17] indicate that neither [ROS]m nor [ROS]i mediate TLC-S induced acinar cells necrosis. Moreover, the data suggest that ROS provide protection from TLC-S induced necrosis, as necrosis was potentiated by the antioxidant *N*-acetylcysteine and was decreased by elevating acinar cell [ROS]i.[17] This is different from many other cell types, for example, hepatocytes, in which

ROS play the pro-necrotic role, so that antioxidants (eg, the same *N*-acetylcysteine) inhibit necrosis, whereas increasing ROS level (eg, by applying exogenous H_2O_2) facilitates necrosis.[20,21] Why pancreatic acinar cells behave so differently is not clear; one reason could be that pancreatic mitochondria are less prone to ROS-induced depolarization,[13] a pathway leading to necrosis. Indeed, exogenous H_2O_2 did not depolarize isolated pancreatic mitochondria,[13] differently from liver[22] and kidney[23] mitochondria.

Thus, in contrast with abnormal Ca^{2+} signal, ROS do not mediate, and may even protect from, necrosis in pancreatitis (at least in the ex vivo models).

At the same time, the results show that ROS, and in particular mitochondrial ROS, mediate acinar cell apoptosis in pancreatitis. Both CCK– and TLC-S–induced apoptosis was inhibited by blocking mitochondrial ROS production.[13,17] Conversely, increasing [ROS]m with rotenone,[13] or [ROS]i with the pro-oxidant menadione[15,17] stimulated acinar cell apoptosis. TLC-S induced caspase activation was inhibited by *N*-acetylcysteine and potentiated by elevating acinar cell [ROS]i.[17] A key effect whereby mitochondrial ROS promote apoptosis in acinar cells (similar to other cells[24]) is through stimulating cytochrome c release.[13] The underlying mechanism is likely oxidation by ROS of cardiolipin, an anionic phospholipid that anchors cytochrome c to the inner mitochondrial membrane. Cardiolipin oxidation facilitates cytochrome c detachment from the inner membrane and, thus, its availability for release.[24]

Finally, the findings in the bile acid and CCK models reveal complex regulation of acinar cell apoptosis by Ca^{2+}, which involves Ca^{2+} effects on cytochrome c release, $\Delta\psi m$, and ROS. Experiments on isolated pancreatic mitochondria[13] showed that Ca^{2+} has 2 opposing effects on cytochrome c release: Ca^{2+} per se stimulates cytochrome c release, whereas Ca^{2+}-induced mitochondrial depolarization inhibits cytochrome c release. In accord with the results on isolated mitochondria, increasing the cytosolic Ca^{2+} stimulated, whereas mitochondrial depolarization inhibited, cytochrome c release, caspase activation, and apoptosis in intact acinar cells.[12,13,16] One mechanism whereby mitochondrial depolarization in acinar cells inhibits cytochrome c release is by blocking mitochondrial ROS generation, which is driven by $\Delta\psi m$.[13] Similarly, the data from Booth et al[17] indicate that decreasing the cytosolic Ca^{2+} does not inhibit TLC-S–induced acinar cell apoptosis unless the mitochondrial ROS production is also blocked.

Thus, the pattern of acinar cell death in pancreatitis is regulated at the mitochondrial level by interplay between Ca^{2+}, $\Delta\psi m$, and ROS. The schematic in Figure 1 illustrates the negative feedback regulations between mitochondrial signals mediating necrotic and apoptotic pathways in acinar cells. In particular, $\Delta\psi m$ loss caused by abnormal Ca^{2+} signal not only promotes necrosis but also inhibits apoptosis by limiting cytochrome c release (*left panel*). In addition, the ensuing decrease in cellular ATP limits caspase activation. Thus, mitochondrial depolarization plays a role of fulcrum in the balance between apoptosis and necrosis. In the opposite direction, caspase activation in acinar cells not only mediates apoptosis but also inhibits necrosis (*right panel*), as shown, for example, by applying caspase inhibitors in cerulein pancreatitis.[7] One plausible mechanism[7] by which caspases inhibit necrosis is through cleavage, and hence inactivation, of the receptor-interacting

protein kinases, important mediators of necrosis.[25,26] Genetic ablation of receptor-interacting protein kinase-3 greatly decreased necrosis in cerulein pancreatitis.[26] The pro-necrotic effect of receptor-interacting protein kinases is mediated likely via mitochondrial permeabilization.[25] Caspases may also limit acinar cell necrosis by preventing accumulation of dysfunctional mitochondria with low $\Delta\psi m$.[4]

A novel aspect of these regulations, uncovered by Booth et al,[17] is that ROS may protect acinar cells from necrosis. Although the authors do not speculate on the nature of this effect, it could be explained by stimulation of cytochrome c release by ROS, leading to caspase activation (Figure 1, *right panel*).

The mutually negative regulations between necrosis and apoptosis in acinar cells are of general interest for understanding cell death responses. Specifically for pancreatitis, these negative feedbacks suggest a molecular mechanism underlying the puzzling inverse correlation between necrosis and apoptosis observed in experimental models of acute pancreatitis.[1,4–8]

Some of these regulations are due to peculiar (or even unique) properties of pancreatic mitochondria. In pancreatic mitochondria,[13] Ca^{2+} overload causes a pronounced loss of $\Delta\psi m$, but only limited amounts of cytochrome c are released, whereas ROS greatly stimulate cytochrome c release, but cause little depolarization. By contrast, in mitochondria from liver and other organs, Ca^{2+} and ROS stimulate both cytochrome c release and depolarization, resulting in parallel induction of both apoptosis and necrosis.[3] These differences could be due to “non-classical” properties of the pancreatic mitochondria PTP, that is, its high sensitivity to Ca^{2+}-induced depolarization and low sensitivity to ROS-induced depolarization.[13]

The prediction from the above analysis is that a greater extent of mitochondrial depolarization, lower levels of acinar cell ROS, or lower Bcl-xL/Bcl-2 levels facilitate pancreatic necrosis and limit apoptosis, thus worsening the disease; in other words, these parameters could serve as a prognostic factor for a more severe, necrotizing pancreatitis. Thus, approaches aimed to inhibit PTP opening, maintain a higher level of mitochondrial ROS, or up-regulate Bcl-xL/Bcl-2 could prevent or attenuate necrosis in pancreatitis.

The results from Booth et al[17] offer an explanation for the conflicting data on the effects of antioxidants in experimental pancreatitis and failure of antioxidant therapy in clinical trials. The authors emphasize the opposing roles of ROS produced by acinar and inflammatory cells in pancreatitis. Indeed, it was shown that neutrophils infiltrating the pancreas facilitate necrosis,[5,27,28] and, moreover, that ROS generated by neutrophils mediate pathologic responses of the disease.[27] Thus, rather than using broad-spectrum antioxidants, strategies to manipulate ROS in pancreatitis should be more targeted.

Another interesting question addressed by Booth et al[17] is the role of autophagy in acinar cell death. The authors conclude that autophagy does not play a significant role, based in part on the absence of the effect of 3-methyladenine, a blocker of autophagosome formation, on TLC-S induced acinar cell death. However, an alternative explanation is that TLC-S may inhibit autophagy by impairing autolysosomal function downstream of autophagosomes, as

was shown in other cells[29] (and similar to the action of CCK-8 in acinar cells[30–32]); hence, little additional effect of 3-methyladenine on cell death. Investigation of autophagy in pancreatitis has only started,[30–34] and its role in acinar cell death requires more detailed studies. In general, the role of autophagy in cell death is a subject of intense research and much debate.[35] The predominant point of view is that efficient, physiologic autophagy is prosurvival, whereas defective, inefficient autophagy promotes cell death. Relevant to the study of Booth et al,[17] 1 mechanism whereby defective autophagy stimulates cell death is through accumulation of uncoupled mitochondria overproducing ROS.[35]

As with any good story, the study of Booth et al[17] provokes further questions. There is much more to be learned about the pathways of acinar cell death depicted in Figure 1. What are the mechanisms whereby TLC-S (as well as CCK-8) stimulates mitochondrial and cellular ROS? Is there a role for non-mitochondrial ROS sources in the acinar cell? How exactly does ROS protect acinar cells from necrosis? What properties of pancreatic mitochondria (ie, their PTP) make them more sensitive to Ca^{2+}-induced and less sensitive to ROS-induced depolarization? Do similar mechanisms operate in other models of pancreatitis, and importantly, in human disease (the data of Booth et al[17] on human acinar cells suggest so)?

It should be also emphasized that Ca^{2+}, $\Delta\psi m$, and ROS are critical but not the sole regulators of cell death in pancreatitis. Other players include cathepsin B, PI 3-kinase, p53, and nuclear factor-κB.[1] How these players act in the acinar cell death drama, within and outside pathways discussed here, remains to be determined.

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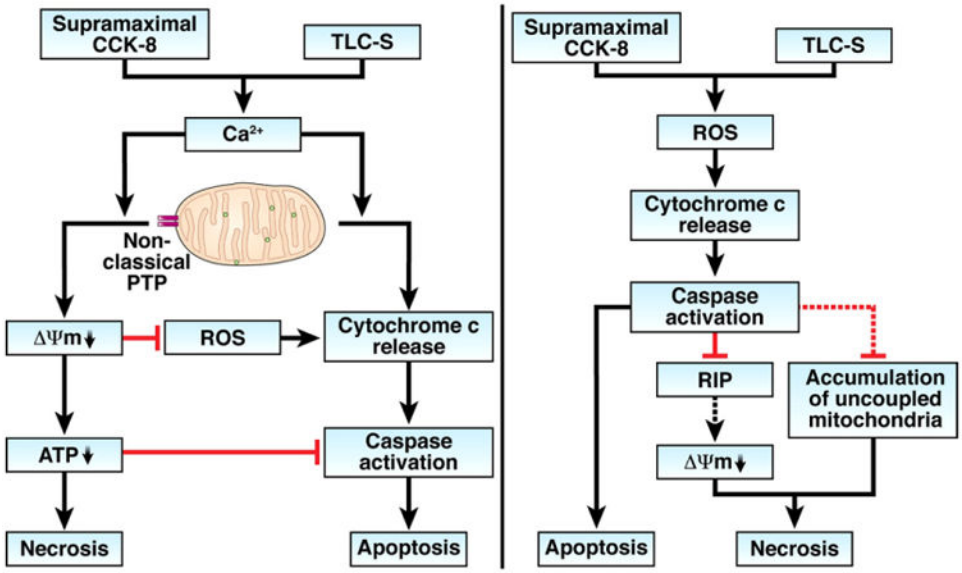

Figure 1.

Pathways mediating acinar cell death in models of pancreatitis induced by supramaximal CCK-8 or the bile acid TLC-S through Ca^{2+} (*left panel*) and ROS (*right panel*). *Dashed lines* indicate pathways that are likely but not yet experimentally proven in acinar cells.