Apoptosis versus necrosis in acute pancreatitis

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Bhatia, Madhav. Apoptosis versus necrosis in acute pancreatitis. Am J Physiol Gastrointest Liver Physiol 286: G189–G196, 2004; 10.1152/ajpgi.00304.2003.—Acute pancreatitis is a disease of variable severity in which some patients experience mild, self-limited attacks, whereas others manifest a severe, highly morbid, and frequently lethal attack. The events that regulate the severity of acute pancreatitis are, for the most part, unknown. It is generally believed that the earliest events in acute pancreatitis occur within acinar cells and result in acinar cell injury. Other processes, such as recruitment of inflammatory cells and generation of inflammatory mediators, are believed to occur subsequent to acinar cell injury, and these “downstream” events are believed to influence the severity of the disease. Several recently reported studies, however, have suggested that the acinar cell response to injury may, itself, be an important determinant of disease severity. In these studies, mild acute pancreatitis was found to be associated with extensive apoptotic acinar cell death, whereas severe acute pancreatitis was found to involve extensive acinar cell necrosis but very little acinar cell apoptosis. These observations led to the hypothesis that apoptosis could be a favorable response to acinar cells and that interventions that favor induction of apoptotic, as opposed to necrotic, acinar cell death might reduce the severity of an attack of acute pancreatitis. Indeed, in an experimental setting, the induction of pancreatic acinar cell apoptosis protects mice against acute pancreatitis. Little is known about the mechanism of apoptosis in the pancreatic acinar cell, although some early attempts have been made in that direction. Also, clinical relevance of these experimental studies remains to be investigated.

acute pancreatitis; pancreatic acinar cells

ACUTE PANCREATITIS IS A COMMON clinical condition, whose incidence has been increasing over recent years (60, 75). In the United States alone, >300,000 patients are hospitalized annually with acute pancreatitis leading to 3,200 deaths. Acute pancreatitis is a contributing factor in an additional 4,000 deaths annually. It also inflicts a heavy economic burden; the direct cost in the United States alone is more than $2,000,000,000 annually (75). In the majority of patients, the condition is mild, but ~25% of patients suffer a severe attack, and between 30 and 50% of these will die (8–10). Most cases are secondary to biliary disease or excess alcohol consumption. The events that regulate the severity of acute pancreatitis are, for the most part, unknown. The exact mechanisms by which diverse etiological factors induce an attack are still unclear, but once the disease process is initiated, common inflammatory and repair pathways are invoked. There is a local inflammatory reaction at the site of injury; if marked, this leads to a systemic inflammatory response syndrome, and it is this systemic response that is believed to be ultimately responsible for the majority of the morbidity and mortality (8–10). Several recent studies have, however, suggested that acinar cell response to injury may, itself, be an important determinant of disease severity. This review focuses on the contribution of pancreatic acinar cell death as apoptosis vs. necrosis as a determinant of the severity of acute pancreatitis and our current understanding of the mechanism of pancreatic acinar cell apoptosis.

Modes of Cell Death: Apoptosis and Necrosis

A distinction between the morphology of cells undergoing physiological cell death and pathological cell death was observed more than 50 years ago (24). However, it was not until the report by Kerr et al. (46) in 1972 that investigators adopted the term “apoptosis” for a morphologically distinct form of cell death. The perpetual flow of information in the past two decades defining the effector and regulatory pathways that result in apoptosis and necrosis (summarized in Fig. 1) has raised further interest in the role of this mode of cell death in various diseases and pathological conditions, including acute pancreatitis.

Apoptosis is a highly coordinated process and is generally believed to be mediated by active intrinsic mechanisms, although extrinsic factors can contribute (6, 16, 42, 70). Apoptosis is genetically controlled and is defined by cytoplasmic and nuclear shrinkage, chromatin margination and fragmentation, and breakdown of the cell into multiple spherical bodies that retain membrane integrity (14, 57). The factors contributing to necrosis are mostly extrinsic in nature, such as osmotic, thermal, toxic, hypoxic-ischemic, and traumatic insults (14, 57). Necrosis is characterized by progressive loss of cytoplasmic membrane integrity, rapid influx of Na\(^+\), Ca\(^{2+}\), and water, resulting in cytoplasmic swelling and nuclear pyknosis (4, 7, 94). The latter feature leads to cellular fragmentation and release...
of lysosomal and granular contents into the surrounding extracellular space, with subsequent inflammation (14, 57, 70, 77).

**Apoptosis.** Apoptosis is defined by distinct morphological and biochemical changes mediated by a family of cysteine aspartases (caspases), which are expressed as inactive zymogens and are proteolytically processed to an active state following an apoptotic stimulus. Early studies by Horvitz et al. (38) first elucidated the existence of a genetically controlled cell death program in which at least three gene products, CED-3, CED-4, and CED-9, participate to cause selective programmed cell death during *Caenorhabditis elegans* development. Subsequent studies in other organisms revealed that several cysteine proteases that share homology to CED-3 are present in mammalian cells. Fourteen such cysteine proteases have been identified so far, and they are identified as caspase-1 to caspase-14 (84, 85).

Caspases are the molecular executioners of apoptosis because they bring about most of the morphological and biochemical characteristics of apoptotic cell death. They are a family of constitutively expressed proenzymes that undergo proteolytic processing to generate its activated form (80, 84, 85). Functionally, the caspase family can be divided into two major subfamilies. Caspases-1, -4, and -5 are involved in the maturation of cytokines such as interleukin-1β and interleukin-18 and promote proinflammatory functions. The other members of the family function as part of the apoptotic pathway, and they are subdivided into initiator (caspases-2, -8, -9, and -10) and “executioner” or effector caspases (caspases-3, -6, and -7) (80, 84). The “apical” or initiator caspases are cleaved in response to apoptotic stimuli. The effector caspases are activated through cleavage by initiator caspases (26). It has recently been shown that the executioner caspases exist as inactive dimers in their proforms and reside predominantly in the cytosol. They are activated by cleavage at specific sites between what will be the large and small subunits in the mature enzymes, and it appears that this is the only way they can be activated (13, 26). Unlike the executioner caspases, the initiator caspases cannot be activated by cleavage (such cleavage occurs, but this does not cause the formation of an active site). The initiator caspases exist in their proforms as monomers, and it appears that they can only be activated by their enforced dimerization. This activation occurs when adapter molecules bind to protein-interaction domains near the NH2-terminal prodomains of the initiator caspases. Such protein-interaction domains are absent from the much shorter prodomains of the executioner caspases (13, 26).
During apoptosis, the effector caspases cleave numerous proteins located in the cell membrane, nucleus, and cytoplasm, and the significance of this proteolysis in the apoptotic process is incompletely elucidated. The activation of caspase-activated DNase to facilitate DNA degradation (62), cleavage of nuclear lamins to facilitate nuclear shrinkage and budding (27, 72), and activation of p21-activated kinase 2 to cause active blebbing in apoptotic cells (74) are a few of the important functions mediated by caspases in the apoptotic process.

Two separable pathways leading to caspase activation have been characterized (26, 42, 92) (Fig. 1). The extrinsic pathway is initiated by ligation of transmembrane death receptors (CD95, TNF receptor, and TNF-related apoptosis-inducing ligand receptor) to activate membrane-proximal (activator) caspases, which, in turn, cleave and activate effector caspases. This pathway can be regulated by c-FLICE-like inhibitory protein, which inhibits upstream activator caspases, and inhibitor of apoptosis proteins (IAPs), which affect both activator and effector caspases. The intrinsic pathway requires disruption of the mitochondrial membrane and the release of mitochondrial proteins including Smac/DIABLO (second mitochondria-derived activator of caspase/direct IAP-binding protein with low pl), HtRA2 (high temperature requirement protein A2), and cytochrome c. Cytochrome c functions with apoptotic protease activating factor (Apaf)-1 to induce activation of caspase-9, thereby initiating the apoptotic caspase cascade, whereas Smac/DIABLO and HtRA2 bind to and antagonize IAPs (26, 42, 64, 83).

The release of cytochrome c by mitochondria is almost a universal feature found in response to various intracellular stimuli, including DNA damage, glucocorticoids, oxidative injury, and growth factor deprivation, although they may not play a significant role in receptor-mediated apoptosis (3, 51). Although classically considered the powerhouse of the cell, it now understood that mitochondria are also “gatekeepers” that ultimately determine the fate of the cell. The mitochondrial decision as to whether a cell lives or dies is complex, involving protein-protein interactions, ionic changes, reactive oxygen species, and other mechanisms that require further elucidation. Once the death process is initiated, mitochondria undergo conformational changes, resulting in the release of cytochrome c, caspases, endonucleases, and other factors leading to the onset and execution of apoptosis (3, 51). The activation of caspase-9 is mediated by a macromolecular complex, the apoptosome, that is formed in response to a cellular commitment to apoptotic death. Formation of the apoptosome is initiated on release of certain mitochondrial proteins, such as cytochrome c, from the mitochondrial intermembrane space. Released cytochrome c binds to monomers of Apaf-1 in the cytosol, inducing a conformational change that enables stable assembly with (deoxy)adenosine triphosphate. Apaf-1 monomers then assemble into the heptameric apoptosome, which, in turn, binds to procaspase-9. Once recruited, procaspase-9 acquires catalytic competency, is proteolytically cleaved, and activates the effector caspases, a process that culminates in apoptotic cell death (64).

Cytochrome c-deficient embryonic stem cells have been shown to be resistant to ultraviolet light and staurosporine-induced apoptosis and partially resistant to apoptotic stimuli from serum deprivation. However, cytochrome c-deficient cells readily undergo apoptosis in response to receptor-mediated apoptotic stimuli (43, 45).

The mechanism by which mitochondria release cytochrome c during apoptosis is still controversial, and the mechanism of release may differ depending on cell type, the cellular environment, and the apoptotic trigger (71). Cytochrome c is bound to the inner mitochondrial membrane by its association with the anionic phospholipid cardiolipin. Cardiolipin is unique to mitochondria and is present predominantly, if not exclusively, in the inner mitochondrial membrane. Evidence indicates that the dissociation of cytochrome c from cardiolipin is a crucial first step for cytochrome c release into the cytosol and for the induction of apoptosis. The dissociation of cytochrome c is facilitated by the peroxidation of cardiolipin, which results in a decreased binding affinity for the hemoprotein. In addition, Ca$^{2+}$ can bind to cardiolipin in the inner mitochondrial membrane, leading to decreased lipid mobility, formation of cardiolipin-enriched domains, and protein aggregation. In turn, this rearrangement leads to an increased production of reactive oxygen species by the respiratory chain, which promotes the oxidation of membrane phospholipids and proteins and, as a result, an increase in membrane permeability (69). Recent work has shown that cytochrome c is nitrosylated on its heme iron during apoptosis (78). In vitro nitrosylation of cytochrome c increases caspase-3 activation in cell lysates (78). Also, the inhibition of intracellular cytochrome c nitrosylation is associated with a decrease in apoptosis, suggesting that cytochrome c nitrosylation is a proapoptotic modification (78). A recent paper (33) has also reported that mitochondrial aggregation is an event upstream of cytochrome c release during apoptosis and that changes in the localization of mitochondria participate in the regulation of apoptosis through cytochrome c release.

Mitochondrial membrane permeabilization, which leads to the release of proapoptotic mitochondrial proteins including cytochrome c, is regulated by the opposing actions of pro- and antiapoptotic Bcl-2 family members. The ever-growing mammalian Bcl-2 family of apoptotic regulators shares homology with the C. elegans antiapoptotic molecule CED-9 (17, 50, 69). On the basis of their structure and functional similarities, Bcl-2 family members are divided into the proapoptotic (Bax, Bak, and Bok) and antiapoptotic (Bcl-2, Bcl-XL, Bcl-w, Mcl-1, and A1) groups (43). A third class of death effector molecules sharing homology only to the Bcl-2 homology-3 (BH3) domain can activate proapoptotic Bcl-2 family members or inactivate antiapoptotic members (39, 81). The family of BH3-only proteins include Bin, Bid, Bad, Bik, BNIP3, Noxa, Puma, and Hrk (39, 81). Recent studies have shown that micromolar to submicromolar concentrations of selected BH3 peptides enable the release of cytochrome c from either mitochondria or outer mitochondrial membrane vesicles. This action, which is inhibited by Bcl-2 or Bcl-XL, is reduced when mutant peptides are used (especially those mimicking apoptosis defects observed in cells) (19, 71). In one study, BH3 peptide (0.1–60 μM) released cytochrome c from mitochondria in the presence of physiological concentrations of ions in a cell type-selective manner, whereas a BH3 peptide with a single amino acid substitution was ineffective. The release of cytochrome c by BH3 peptide correlated with the presence of endogenous Bax at the mitochondria and its integral membrane insertion. Cytochrome c release was accompanied by adenylate kinase release, was not associated with mitochondrial swelling or...
substantial loss of electrical potential across the inner membrane, and was unaffected by inhibitors of the permeability transition pore. Cytochrome c release was, however, inhibited by Bcl-2. Although energy-coupled respiration was inhibited after the release of cytochrome c, mitochondria maintained membrane potential in the presence of ATP due to the reversal of the ATP synthase (71).

Pro- and antiapoptotic members of the Bcl-2 family can homodimerize or heterodimerize, thus forming a large number of combinations within a cell. Heterodimerization between a proapoptotic member and an antiapoptotic member can nullify the functions of each (73). The outcome of a cell that received an apoptotic stimulus is thought to depend partly on the ratio of the death promoter to the death suppressor (1, 2). The precise mechanisms by which the Bcl-2 family members modulate apoptosis are still not completely elucidated, but their key functions revolve around the release of proapoptotic factors, especially cytochrome c from the mitochondrial intermembrane compartment into the cytosol (66, 82). Multidomain proapoptotic Bcl-2 proteins (e.g., Bak and Bax) can be activated directly following interaction with the BH3-only Bcl-2 protein Bid. Alternatively, binding of other BH3-only proteins (e.g., Noxa, Puma, Bad, and Bim) to antiapoptotic Bcl-2 proteins (e.g., Bcl-2 and Bcl-XL) results in activation of Bax and Bak (1, 39). Whether Bcl-2 proteins control mitochondrial membrane permeability by directly forming pores in the outer membrane and/or by regulating the opening and closing of the permeability transition pore remains the topic of much debate (59). The net effect, however, is the regulated release of proapoptotic factors from the mitochondria, induction of downstream caspases, and potential loss of mitochondrial functionality. According to a recent report (95), Bid may activate mitochondria by two mechanisms; one is related to permeability transition, and the other is related to Bak oligomerization. Bid can further affect mitochondria potentials by indirectly regulating caspase activity (95).

There is considerable cross-talk between the extrinsic and intrinsic pathways. For example, caspase-8 can proteolytically activateBid, which can then facilitate cytochrome c release (26, 42). This apparently amplifies the apoptotic signal following death receptor activation, and different cell types may be more reliant on this amplification pathway than others (21). Conversely, activators of the intrinsic pathway can sensitize the cell to extrinsic death ligands. Although the extrinsic and intrinsic signals are considered to take two distinct pathways to execute cell death, receptor-initiated cell death can involve the mitochondrial pathway through the BH3-only protein Bid. In hepatocytes, for example, activation of caspase-8 by Fas leads to cleavage of Bid to its active form t-Bid. t-Bid translocates to mitochondria and associates with Bcl-2-like proteins to disrupt mitochondrial integrity (93). It should be noted, however, that the cross-talk between the two pathways is minimal under most conditions.

A recent report (53) indicates that cytotoxic stress-induced mitochondrial permeability and release of various apoptogenic factors is mediated by caspase-2 in human fibroblasts transfected with adenoviral oncogene E1A. Small interfering RNA-mediated silencing of caspase-2 expression prevented cisplatin, etoposide, and ultraviolet light-induced apoptosis in these cells. It is argued that in this setting, mitochondria are amplifiers of caspase activity rather than initiators of caspase activation (53).

**Necrosis.** Necrosis is the prominent mode of cell death that occurs in various neurodegenerative conditions and as a consequence to ischemic injury in various organs including the brain and heart. Although progress has been made in the last decade in understanding the molecular mechanisms of apoptosis, the biochemical pathways leading to necrotic cell death remain poorly understood (58). Necrosis has long been thought to be a “passive” process occurring as a consequence of acute ATP depletion. Several ATP-dependent ion channels become ineffective, leading to ion dyshomeostasis, disruption of the actin cytoskeleton, cell swelling, membrane blebbing, and eventual collapse of the cell (15, 67, 68). Recent reports suggest that in addition to the passive mechanisms, “active” mechanisms, such as Na$^+$ overloading, Ca$^{2+}$ accumulation, and changes in mitochondrial permeability, may also participate in the necrotic process (Fig. 2) (5, 55, 56, 70).

In ischemic or hypoxic injury, energy depletion occurs by defective ATP production combined with the rapid consumption of ATP by ion pumps and through hydrolysis and leakage. The necrotic volume increase associated with necrotic cell death is initiated by an influx of Na$^+$ and release of ATP due to membrane leakage (70). The increased Na$^+$ level in the cytosol activates Na$^+$-K$^+$-ATPase, resulting in dissipation of ATP. In the beginning stages of the injury, a simultaneous efflux of K$^+$ maintains ion homeostasis. Severe depletion of

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**Fig. 2.** Sequence of biochemical events that may lead to necrotic cell death based on current information in the literature.
ATP leads to failure of the pump-leak balance mechanism, leading to an influx of Na\(^+\) and water that results in swelling and collapse of the cell. Thus the overload of Na\(^+\) concomitant with severe ATP depletion seems to be the major determinant of a necrotic outcome (15).

Cytosolic Ca\(^{2+}\) plays a role in linking ATP depletion and necrosis in some cell types (4), but several other cell types including hepatocytes and renal tubules can undergo necrotic cell death in its absence (40). The reactive oxygen species-mediated necrotic volume increase and Na\(^+\) influx are suggested to be initiated by the binding of the free radicals to ion channels including nonselective Ca\(^{2+}\) channels (5, 36, 37, 49). The increased levels of Na\(^+\) activate Na\(^+\)-K\(^+\)-ATPase and consume ATP, which, in turn, activates nonselective Ca\(^{2+}\) channels, resulting in massive cytosolic Ca\(^{2+}\) accumulation. High levels of Ca\(^{2+}\) can participate in ATP depletion by activating Ca\(^{2+}\)-ATPase and mitochondrial depolarization.

The increased levels of Ca\(^{2+}\) activate endonucleases to degrade DNA and activate cellular proteases such as calpain to degrade several structural and signaling proteins (91).

Mitochondria participate in necrotic as well as apoptotic cell death by opening the mitochondrial permeability transition pore. Indeed, despite the presumed fundamental difference between apoptosis and necrosis, growing evidence supports an essential role of the mitochondrial permeability transition (MPT) in the release of cytochrome c and initiation of apoptosis in many models, including hepatocytes exposed to TNF-\(\alpha\) and Fas ligation (35). Several second messengers and proapoptotic proteins including Bcl-2 family members can induce the permeabilization of the MPT pore (18, 52). BNIP3 is a member of the Bcl-2 family that is loosely associated with mitochondria in the normal state but gets fully integrated into the mitochondrial outer membrane after a death stimulus. BNIP3-transfected cells are found to undergo cell death independently of Apaf-1, caspase activation, cytochrome c release, and nuclear translocation of apoptosis-inducing factor. The cells exhibited morphology typical of the necrotic form of cell death with plasma membrane permeability, mitochondrial damage, extensive cytoplasmic vacuolation, and mitochondrial autophagy. It is proposed that BNIP3 can mediate necrosis-like cell death through mitochondrial permeability transition pore opening and mitochondrial dysfunction (87). The expression of BNIP3 is shown to be induced in several cell lines in response to hypoxic injury. Overexpression of the hypoxia-inducible factor-\(\alpha\) has also been shown to induce the expression of BNIP3, resulting in a necrotic form of cell death (32). Moreover, although ischemia-reperfusion is usually associated with necrotic cell death (86), more recent studies have shown that apoptosis also occurs after ischemia-reperfusion in cells from liver and other organs (22, 25). In a recent study (47), it has been shown that the balance between ATP depletion after the MPT and ATP generation by glycolysis operates a switch determining whether the fate of the cell would be necrosis or apoptosis after simulated ischemia-reperfusion-induced onset of the MPT. These findings also highlight the importance of apoptotic commitment and of common pathways, such as MPT, which initiate events that culminate in either apoptosis or necrosis, depending on other variables, such as ATP supply (47). Thus death/toxic signals, through common mechanisms, can cause both apoptosis and necrosis, depending on variables such as ATP supply.

**Apoptosis and Necrosis in Acute Pancreatitis**

Clinical as well as experimental acute pancreatitis is characterized by progressive cell death, the mechanisms of which remain poorly understood. Necrosis has classically been considered the major form of cell death in acute pancreatitis (48, 63), whereas apoptosis was suggested to mediate atrophy in the organ (88, 89). However, careful biochemical and morphological examination of experimental models of acute pancreatitis has shown that severe acute pancreatitis (e.g., that induced by pancreatic duct ligation in the opossum, by choline-deficient and ethionine-supplemented diet in the mouse, and by caerulein hyperstimulation in the mouse) is associated primarily with necrosis but little apoptosis, whereas mild acute pancreatitis (e.g., that induced by pancreatic duct ligation and by caerulein hyperstimulation in the rat) is associated primarily with apoptotic cell death and little necrosis (30, 44).

The mechanisms of acinar cell apoptosis and necrosis in acute pancreatitis remain poorly understood, although several recent studies have contributed significantly in this direction (Fig. 3). An early study in this direction using the caerulein-hyperstimulation model of acute pancreatitis showed that caerulein stimulates pancreatic production of platelet activating factor (PAF). PAF mediates both apoptosis and neutrophil chemotaxis in the pancreas. Neutrophils in turn may convert acinar cells undergoing apoptosis into necrotic cells (76). A subsequent study by the same group has shown the evidence of the death receptor (TNF-\(\alpha\) receptor) on pancreatic acinar cells. In this study, it was shown that pancreatic acinar cells produce, release, and respond (by apoptosis) to TNF-\(\alpha\) (29). In a later study (31), these authors showed a potential role of NF-\(\kappa\)B in cell death, possibly by activation of TNF-\(\alpha\) transcription. Another study (79) has shown that duct ligation-induced apoptosis of the pancreatic acinar cells is p53 dependent. A recent report (28) has shown the role of the intrinsic pathway in acinar cell apoptosis. The results show that CCK stimulates death signaling pathways in rat pancreatic acinar cells, including caspase activation, cytochrome c release, and mitochondrial depolarization, leading to apoptosis. The mitochondrial dysfunction is mediated by upstream caspase(s). CCK causes mitochondrial alterations through both permeability transition pore (PTP)-dependent (cytochrome c release) and -independent (mitochondrial depolarization) mechanisms. In addition to apoptosis, caspases also regulate other processes in the pancreatic acinar cell that play key roles in pancreatitis; in particular, caspases negatively regulate necrosis and intra-acinar cell activation of trypsin (28). Caspase-mediated protection against necrosis and trypsin activation can explain the inverse correlation between the extent of apoptosis on the one hand and necrosis and the severity of the disease on the other hand observed in experimental models of pancreatitis. Indeed, these signaling mechanisms may play an important role in acinar cell injury and death in pancreatitis.

In addition to CCK, other inducers of pancreatic acinar cell apoptosis have been used to investigate cell death in relation to acute pancreatitis. Examples of these compounds are menadione and crombene (1-cyano-2-hydroxy-3-butene). Menadione is a quinone that is metabolized by flavoprotein reductase to semiquinone, which can be oxidized back to quinone in the presence of molecular oxygen. In this redox cycle the superoxide anion radical, hydrogen peroxide, and other reactive oxygen species play a role in linking ATP depletion and necrosis. The increased levels of Ca\(^{2+}\) can participate in ATP depletion by activating Ca\(^{2+}\)-ATPase and mitochondrial depolarization. The increased levels of Ca\(^{2+}\) activate endonucleases to degrade DNA and activate cellular proteases such as calpain to degrade several structural and signaling proteins (91).
Figure 3. Factors that determine the cellular fate of pancreatic acinar cell. This figure summarizes the information currently available in the literature. More research is needed to characterize the pathways responsible for acinar cell death in the form of necrotic and apoptotic cell death.
acinar cell death in the form of necrosis vs. apoptosis from bench to bedside.

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REFERENCES

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G196


