Physiology and pathophysiology of apoptosis in epithelial cells of the liver, pancreas, and intestine

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Jones, Blake A., and Gregory J. Gores. Physiology and pathophysiology of apoptosis in epithelial cells of the liver, pancreas, and intestine. Am. J. Physiol. 273 (Gastrointest. Liver Physiol. 36): G1174–G1188, 1997.—Cell death of gastrointestinal epithelial cells occurs by a process referred to as apoptosis. In this review, we succinctly define apoptosis and summarize the role of apoptosis in the physiology and pathophysiology of epithelial cells in the liver, pancreas, and small and large intestine. The physiological mediators regulating apoptosis in gastrointestinal epithelial cells, when known, are discussed. Selected pathophysiological consequences of excessive apoptosis and inhibition of apoptosis are used to illustrate the significance of apoptosis in disease processes. These examples demonstrate that excessive apoptosis may result in epithelial cell atrophy, injury, and dysfunction, whereas inhibition of apoptosis results in hyperplasia and promotes malignant transformation. The specific cellular mechanisms responsible for dysregulation of epithelial cell apoptosis during pathophysiological disturbances are emphasized. Potential future areas of physiological research regarding apoptosis in gastrointestinal epithelia are highlighted when appropriate.

cholestasis; colon cancer; transforming growth factor-β; pancreatitis

APOPTOSIS, a morphologically and biochemically distinct form of cell death, is an important physiological process in epithelial cell biology. Cell death by apoptosis is a highly conserved evolutionary process for deleting senescent, damaged, redundant, and deleterious cells from the organism. In addition, rates of apoptosis are paired with rates of mitosis so that epithelial cell numbers remain constant and tissue homeostasis is maintained (54). Given the widespread and critical role of apoptosis in physiology, it is not surprising that dysregulation of apoptosis occurs frequently during pathophysiological disturbances. Indeed, several key concepts have recently emerged with respect to the dysregulation of apoptosis in pathophysiological processes, making a review focused on gastrointestinal epithelial cells timely and topical. First, tissue hyperplasia and atrophy can result from inhibition or potentiation of apoptosis, respectively. Second, pathophysiological processes can trigger the cellular apoptotic machinery leading to rapid and extensive cell death and tissue dysfunction. Finally, failure of apoptosis to delete genetically altered cells appears to contribute to malignant transformation. The therapeutic corollaries of these concepts are that 1) inhibition of apoptosis may prevent tissue injury and/or promote tissue regeneration and restitution, 2) induction of apoptosis of dysplastic and transformed cells may be useful in preventing and treating malignant diseases, and 3) conversion of necrotic inflammatory injury to an apoptotic noninflammatory process may ameliorate disease processes (see below). Indeed, enhanced and/or deregulated apoptosis has already been implicated in several diseases (Table 1). Although several reviews on apoptosis are available (6, 177, 178), especially regarding the intracellular mechanisms regulating apoptosis (20, 36, 88, 108, 139), this review provides an update on the physiology and pathophysiology of apoptosis as it relates to gastrointestinal epithelial cells. Because mechanisms of apoptosis are best studied when they are exaggerated or disturbed during pathological events, we frequently use pathophysiological paradigms to illustrate the mechanisms regulating apoptosis of gastrointestinal epithelia. We first review, succinctly, key general concepts on the cell physiology of apoptosis, followed by a more in-depth review of apoptosis in the liver, pancreas, and small and large intestine. The lack of information on apoptosis in the esophagus and stomach precludes a review of apoptosis in these tissues. We also highlight those areas of apoptosis that deserve further investigative attention by the physiologist.

CELL PHYSIOLOGY OF APOPTOSIS

Apoptosis is characterized by stereotypical morphological features including cell shrinkage, the disappear-
ance of microvilli, the formation of cell surface blebs containing organelles, nuclear chromatin condensation and margination, and nuclear fragmentation (Fig. 1). Ultimately, the cell separates into intact, discrete, membrane-bound bodies, referred to as apoptotic bodies. These morphological changes of apoptosis are currently the “gold standard” for identifying apoptosis. Apoptotic bodies are phagocytosed in vivo by neighboring epithelial cells and professional phagocytic cells (mononuclear cells). Indeed, phagocytosis of apoptotic bodies has been observed in both hepatocytes and intestinal epithelial cells (111A, 126). Apoptosis is difficult to detect in tissues because the changes of apoptosis occur rapidly (over 2-4 h) and the apoptotic bodies are rapidly phagocytosed and removed from the tissue. Indeed, identifying apoptotic cells in tissues has been likened to counting meteors in the night sky (23).

The intact plasma membrane of the apoptotic body and

Table 1. Gastrointestinal disease in which apoptosis has been implicated

<table>
<thead>
<tr>
<th>Colon</th>
<th>Pancreas</th>
<th>Liver</th>
<th>Small Bowel</th>
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<tr>
<td>?Inflammatory bowel disease</td>
<td>Acute pancreatitis</td>
<td>Cholangiopathies*</td>
<td>?Malabsorption syndromes</td>
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<td>Colon cancer</td>
<td>Chronic pancreatitis</td>
<td>Hepatocellular carcinoma</td>
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<td>Radiation proctitis</td>
<td>Pancreatic cancer</td>
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*Cholangiopathies refer to diseases with loss of cholangiocytes, including primary sclerosing cholangitis, primary biliary cirrhosis, allograft rejection, and acquired immunodeficiency syndrome-associated cholangitis.

![Fig. 1. Morphological features and identification of apoptosis. A: schematic representation of the morphological features of apoptosis. Normal cell at far left exhibits a uniformly distributed chromatin in the nucleus, and phosphatidylserine (lollipops) is restricted to the inner leaflet of the plasmalemma. Early morphological features of apoptosis include blebbing of the plasma membrane and condensation and margination of nuclear chromatin (second cell). Externalization of phosphatidylserine occurs at this early stage. Subsequently the nucleus becomes fragmented (third cell), and the cell separates into membrane-bound apoptotic bodies containing intact organelles (fourth cell). Apoptotic bodies are phagocytosed by neighboring cells. B: representative approaches for the identification of apoptotic cells are demonstrated. Left: fluorescein-conjugated annexin V binds to phosphatidylserine on the external membrane leaflet of the plasmalemma of 2 early apoptotic hepatocytes. Middle: fluorescence photomicrograph showing nuclear fragmentation identified using the fluorescent DNA binding dye 4',6-diamidino-2-phenylindole. Right: DNA fragmentation of apoptotic cells assessed by DNA agarose gel electrophoresis. The “ladder” pattern of DNA cleavage results from internucleosomal cleavage.]
its rapid phagocytosis are thought to limit release of intracellular constituents into the extracellular space. Because release of intracellular constituents into the extracellular space is limited, the inflammatory response to the dead cell is postulated to be nonexistent. However, apoptosis may not be as “silent” as presumed.

For example, hepatocyte apoptosis is associated with the appearance of hepatocyte intracellular enzymes in the circulation (102, 104, 146). Furthermore, the mediators of apoptosis, such as transforming growth factor-β1 (TGF-β1), may have other consequences (e.g., tissue fibrogenesis). Current dogma suggests that isolated cell apoptosis also occurs without a disruption of the epithelial cell permeability barrier. However, few functional data exist to support this morphological observation, and enhanced rates of apoptosis may potentially alter the transmembrane resistance of epithelia, leading to alterations in absorption and secretion.

The biochemical features of apoptosis identified to date include changes in the plasma membrane phospholipid orientation, alterations of intracellular ion homeostasis, activation of proteases and endonucleases with cleavage of proteins and DNA, respectively, intracellular generation of ceramide via sphingomyelinase, and activation of transglutaminase (21, 36, 108, 140). The precise roles of each of these events and their relationship to each other is a topic of current investigation. Phosphatidylinositol is located predominantly on the inner or cytoplasmic face of the plasma membrane in healthy cells. However, early in apoptosis, phosphatidylinositol is translocated to the outer leaflet of the plasma membrane, presumably for phagocytic recognition (32). The externalization of phosphatidylinositol can be readily detected using fluorescently labeled annexin V, which has a strong affinity for phosphatidylinositol. Assays employing fluorescently labeled annexin V are frequently used to identify apoptotic cells experimentally (Fig. 1) (73). Increases in cytosolic free calcium and magnesium and decreases in cytosolic pH and potassium have been implicated as mechanisms contributing to apoptosis (10, 42, 89, 106, 127). Despite the widespread recognition of cell volume changes and disturbances of ion homeostasis in apoptosis, this facet of apoptosis has received little attention by transport physiologists and is a neglected but potentially fruitful area of investigation. A variety of proteases have been implicated in apoptosis, including members of the caspase family (previously known as the interleukin-1β-converting enzyme family of proteases), calpains, cathepsins, and the proteasome (108). In particular, caspases (cysteine proteases recognizing aspartate in the P1 position of the substrate) have been strongly implicated in apoptosis. Caspase protease cascades analogous to the coagulation protease cascade have been suggested as a mechanism leading to the structural changes of apoptosis. Endonuclease activation with DNA cleavage follows protease activation in apoptosis. DNA is initially cleaved into fragments of 300,000 and/or 50,000 base pairs. This type of DNA cleavage appears to be universal in apoptosis and can be detected by pulse-field gel electrophoresis or field inversion gel electrophoresis of DNA. The large-order DNA cleavage is often, but not always, followed by internucleosomal DNA cleavage into fragments of 180–200 base pairs (the so-called “ladder” pattern of DNA cleavage) (Fig. 1). Different endonucleases are thought to mediate the two types of DNA cleavage. Detection of DNA cleavage in extracted DNA by gel electrophoresis techniques or in situ using cytochemical and histochemical techniques is frequently employed to confirm and identify apoptosis (39, 102). Activation of either neutral or acidic sphingomyelinase occurs in many models of apoptosis, leading to the generation of ceramide from sphingomyelin; ceramide activates a proapoptotic cell signaling cascade (56). Cross-linking of proteins by transglutaminase, which catalyzes the formation of ε-(γ-glutamyl)lysine peptide bonds between appropriate substrates, keeps the apoptotic bodies intact during the fragmentation of the cell (34).

The intracellular signaling pathways for apoptosis have not yet been completely delineated. However, the Fas receptor/Fas ligand pathway of apoptosis has been elucidated more fully and remains the best characterized model of apoptosis (Fig. 2). In this model of apoptosis, binding of the Fas ligand to the Fas receptor results in trimerization of the receptor (98). The trimerized receptor then recruits the binding protein FADD/MORT1 to its death domain. The binding of FADD to the death domain results in the recruitment of caspase 8 to the resulting death-inducing signaling complex (77). Via as yet unknown mechanisms, the interaction
of caspase 8 with the death-inducing signaling complex leads to caspase 8 activation. After caspase 8 activation mitochondrial dysfunction occurs, leading to the release of cytochrome c and perhaps also apoptosis-inducing factor (AIF) into the cytosol (77). Either cytochrome c or AIF can potentiate caspase 3 activation, a key protease causing the structural changes of apoptosis.

The cellular threshold for apoptosis is also highly regulated, especially by members of the Bcl-2 family of proteins. Multiple mammalian members of this family have been reported to date, including Bcl-2, Bax, Bcl-x, Bcl-w, Bak, Bad, A1, NR-13, and Mcl-1 (37). These proteins (except for Bad) are integral membrane proteins localized predominantly to the nuclear membrane, endoplasmic reticulum, and outer mitochondrial membranes. Members of this family can be antiapoptotic [Bcl-2, Bcl-xL (long), Bcl-w, A1, NR-13] and proapoptotic [Bcl-xS (short), Bax, Bad]; however, the pro- or antiapoptotic function of these proteins may also depend on the cell type, the apoptotic stimulus, the cellular context (e.g., cell cycle dependence of the process), and the cellular environment (e.g., presence or absence of growth factors). The mechanism by which these proteins modulate apoptosis is unclear, but these proteins appear to regulate each other by forming homo- and heterodimers. The crystal structure of Bcl-x has been reported; this protein appears to have a channel configuration similar to diphtheria toxin, and anion-transporting activity has been observed (96). Thus these proteins may modulate apoptosis by altering the electrochemical responses of cells to pathophysiological processes.

Rates of epithelial cell apoptosis, as with other cells, can be controlled by the presence of growth factors. Because growth factors often inhibit apoptosis by paracrine mechanisms, Raff (115) has suggested that apoptosis is a socially regulated process in that cells need each other to survive. This concept has four important conceptual ramifications. First, the default response of a cell may be to die by apoptosis unless it is kept alive by cell survival signals originating from other cells. The social control of apoptosis may be important in maintaining tissue homeostasis with regard to cell number. Second, the dependence on neighboring cells for cell survival is a strong stimulus to prevent cell metastases. Third, therapeutic administration of growth factors may block apoptosis in disease processes. Finally, inhibition of apoptotic programs may be required for cell growth.

LIVER

Physiology of apoptosis in the liver. There are two epithelial cell types in the liver, hepatocytes and cholangiocytes (bile duct epithelial cells). Because much of what we know about apoptosis in the liver is based on studying hepatocytes, in this review we primarily discuss hepatocyte apoptosis. Where information is available, we also discuss what is known regarding the physiology of cholangiocyte apoptosis. Characterization of apoptosis rates in epithelial tissues such as the liver with low rates of cell turnover is problematic due to the transient nature of recognizable apoptotic events. Indeed, even in a tissue undergoing 50% involution in 3 days by steady-state apoptosis, at any given time point only 9% of the cells would be identified as apoptotic (6). In the liver, it is estimated that only 2–4 cells per 10,000 will be detected as apoptotic given the low cell turnover of both hepatocytes and cholangiocytes under physiological conditions (126). Despite the low endogenous rates of apoptosis in the liver, the importance of apoptosis in regulating liver volume is underscored by two observations. First, the nongenotoxic, peroxisome-proliferating drugs lead to increases in liver cell volume by inhibiting apoptosis (7) and the Fas knockout mouse has substantial liver cell hyperplasia (3). Second, segmental liver atrophy occurring during portal vein ligation results from enhanced hepatocyte apoptosis (70). We believe a better understanding of epithelial cell apoptosis in tissues with low turnover rates will require new and different methods for identifying apoptotic cells.

Physiological mediators of apoptosis: growth factors, cytokines, and Fas receptor/Fas ligand. Although primary cultures of hepatocytes do not appear to require growth factors for survival, this observation is confounded by the extremely rapid dedifferentiation of hepatocytes in culture, which precludes an assessment of their dependence on growth factors for survival. Hepatocyte growth factor (HGF) is a potent mitogen for hepatocytes in primary culture and appears to provide a key physiological growth stimulus after partial hepatectomy (91). After chronic treatment of hepatocytes in vitro with HGF, acute withdrawal of the growth factor induces hepatocyte apoptosis. These observations suggest that in vivo, where growth factors are continually present, hepatocytes may be dependent on growth factors for their survival (24). HGF is able to prevent apoptosis induced by treatment of murine hepatocytes with interferon-γ (95). The HGF receptor associates with the antiapoptotic protein BAG-1, providing a mechanism for inhibition of apoptosis by HGF (6b). Indeed, the HGF receptor when expressed as a constitutively active form blocks apoptosis and permits hepatocyte immortalization (5). Likewise, epidermal growth factor, also a hepatic mitogen, inhibits hepatocyte apoptosis induced by TGF-β (31). Growth factors may potentially inhibit apoptosis by enhancing expression of the antiapoptotic members of the Bcl-2 family of proteins. For example, liver regeneration after partial hepatectomy is associated with increases in the mRNA transcript for Bcl-xL, suggesting that inhibition of the apoptotic machinery by this protein promotes liver regeneration (76). Interleukin-6 (IL-6), also a potent growth factor in the regenerating liver, upregulates Bcl-xL expression in myeloma cells, preventing apoptosis (129). Liver failure from extensive cell death, presumably by apoptosis, occurs after a partial hepatectomy in IL-6-deficient mice (25).

The proapoptotic response of hepatocytes to the injurious growth factor TGF-β1 and the toxic cytokine tumor necrosis factor-α (TNF-α) has been more extensively studied. Physiological regression of the liver to
Apoptotic cells secreting transforming growth factor-
1 (TGF-1) in liver by activin, a member of the TGF-
family (128). Although TGF-1 expression in the liver
is abnormal in a variety of disease states (45), the role
of TGF-1 in hepatocyte apoptosis in disease processes
requires further documentation. Escape from TGF-
1-induced apoptosis may contribute to hepatocarci-
genesis. Indeed, the development of resistance to TGF-
1, with a consequent loss of growth inhibition and apo-
tonosis (46). Given the importance of both apoptosis and
fibrogenesis in liver diseases, the relationship between
the two is an important physiological puzzle requiring
further delineation.

TNF-α, a cytokine primarily produced by macro-
phages, cholangiocytes, and Kupffer cells, is capable of
producing a wide range of effects in vivo, including
hepatotoxicity (35). It is now appreciated that TNF-α
causes liver injury by inducing hepatocyte apoptosis
(82, 104, 109). TNF-α may mediate hepatocyte apo-
ptosis occurring during Kupffer cell activation by lipopoly-
 saccharide (LPS) and ischemia-reperfusion injury, the
cytokine syndromes associated with septic shock, and
ethanol-mediated liver injury (141). Indeed, TNF-α
peripheral serum concentrations are increased in alco-
holic hepatitis and may contribute to the hepatocyte
injury observed in this syndrome (13). Induction of
apoptosis of mouse hepatocytes by TNF-α requires
transcriptional arrest but functional translation, impli-
cating protein synthesis as a necessary component of
the pathway (81, 82). Translation of proapoptotic pro-
teins from preformed RNA or the activation of an
immediate-early gene response (with preformed tran-
scriptional machinery) may thus be inferred to be
apoptotic mechanisms mediating the signaling cascade
distal to TNF-α receptor ligation. In both TNF-α and
LPS models of hepatocyte apoptosis the production of
nitric oxide (NO) by increased expression of inducible
nitric oxide synthase (iNOS) has been suggested as a
cytoprotective mechanism; prevention of iNOS upregu-
lation was thus suggested to be a candidate mechanism
by which transcriptional inhibition sensitizes hepatocytes
to undergo apoptosis (82). However, subsequent
investigations have demonstrated that NO generated
by increased iNOS expression may itself be a toxic
mediator enhancing hepatocyte death (78). Further
work is required to clarify the potential cytoprotective
and injurious actions of iNOS and NO in hepatocyte
apoptosis.

Fas receptor/Fas ligand interactions are also impor-
tant inducers of apoptosis in hepatocytes (38). The Fas
receptor is a member of the nerve growth factor recep-
tor family. Binding of the receptor by Fas ligand results
in apoptosis of the cell expressing the Fas receptor.
Unlike many ligands, Fas ligand is predominantly cell
bound and it is expressed in high numbers of cytotoxic
T lymphocytes (68). Hepatocytes constitutively express
Fas receptor and may upregulate expression of this
receptor in a variety of liver diseases, including viral
hepatitis and alcohol-induced liver disease (38, 60, 92).
For example, immunohistochemical studies demon-
strate Fas receptor expressed on hepatocytes attached
to infiltrating lymphocytes near the regions of "piecemeal
necrosis" in hepatitis C-positive patients, suggest-
ing that hepatocyte apoptosis occurs via a T cell-
mediated Fas pathway in this viral liver disease (60). In
a model of fulminant hepatic failure, intraperitoneal
injection of agonistic anti-Fas antibody leads to mas-
sive hepatocyte apoptosis and liver failure (104). In
pathophysiological processes, hepatocytes may also
express Fas ligand, raising the possibility that a Fas

Fig. 3. Mechanisms interrelating hepatocyte apoptosis and fibrogen-
esis. Apoptotic cells secreting transforming growth factor-
1 (TGF-1) may be the driving force for hepatic fibrogenesis. Stellate cells
exposed to TGF-1 originating from apoptotic hepatocytes become
activated (myofibroblasts) and contribute to liver fibrogenesis by
proliferating and increasing collagen deposition. Alternatively, patho-
physiological events may lead to direct activation of stellate cells.
Activated stellate cells (myofibroblasts) secrete TGF-1, inducing
apoptosis of neighboring hepatocytes.
ligand-positive hepatocyte may induce apoptosis in a Fas receptor-positive neighbor, an example of fratricide (38). Because Fas ligand is constitutively expressed on cytotoxic lymphocytes (CTL), cell-mediated immunity, a common process contributing to hepatocyte apoptosis in autoimmune and viral hepatitis, likely occurs via Fas-dependent pathways (68). CTL-induced apoptosis of target cholangiocytes occurring in allograft rejection, graft vs. host disease, and primary biliary cirrhosis may also occur via Fas-mediated pathways (11, 75). Table 2 provides a summary of the physiological mediators and inhibitors of apoptosis.

Cholestasis as a pathophysiological model of liver cell apoptosis. Cholestasis is a common but complex pathophysiological process in human liver disease leading to impaired bile formation, which affects both hepatocytes and cholangiocytes. As a pathophysiological process, it provides a useful model to study apoptosis in both cell types. The prominence of hepatocyte-derived acidophilic (apoptotic) bodies and cell dropout rather than extensive necrosis in cholestatic liver biopsy specimens is testimony to the role of hepatocyte apoptosis in cholestasis (107). Retention and accumulation of toxic bile salts during cholestasis is thought to trigger hepatocyte apoptosis. Indeed, exposure of hepatocytes in primary culture to toxic, hydrophobic bile salts has been demonstrated to directly cause apoptosis of hepatocytes (106). The mechanism of apoptosis by toxic bile salts has been partially clarified in recent years (Fig. 4). The induction of apoptosis by toxic bile salts appears to proceed through activation of protein kinase C (PKC) (67). Activation of PKC appears to cause magnesium influx into the cell, activating magnesium-dependent endonucleases that cleave DNA (66, 106). Through a process that is not yet clear, PKC activity is also associated with activation of cathepsin B, which appears to function as a key effector protease in this model of apoptosis (67, 118).

Mechanical obstruction of the bile duct (i.e., obstructive cholestasis) induces hyperplasia of cholangiocytes, presumably due to a growth stimulus. Release of the mechanical obstruction with loss of the growth stimu-

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**Table 2. Physiological mediators and inhibitors of apoptosis**

<table>
<thead>
<tr>
<th>Mediators</th>
<th>Targets</th>
<th>Effects</th>
<th>Ref. Nos.</th>
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<tr>
<td>Butyrate</td>
<td>Dietary fiber</td>
<td>Apoptosis of tumor cells</td>
<td>50</td>
</tr>
<tr>
<td></td>
<td>CTL</td>
<td>Cholangiocytes</td>
<td>Apoptosis of cells bearing FasR</td>
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<tr>
<td>Fas ligand</td>
<td>sFasL</td>
<td>Cholangiocytes</td>
<td>Apoptosis</td>
</tr>
<tr>
<td>NO</td>
<td>Endothelial cells</td>
<td>Apoptosis of epithelial cells</td>
<td>78</td>
</tr>
<tr>
<td>TGF-β</td>
<td>Endothelial cells</td>
<td>Apoptosis of epithelial cells</td>
<td>5, 9, 97, 102, 103, 124</td>
</tr>
<tr>
<td>TNF-α</td>
<td>Cholangiocytes, Kupffer cells, Stellate cells</td>
<td>Apoptosis</td>
<td>81, 83, 109, 141</td>
</tr>
<tr>
<td>EGF</td>
<td>Salivary glands</td>
<td>Prevents TGF-β-induced apoptosis</td>
<td>31</td>
</tr>
<tr>
<td>Eicosanoids</td>
<td>Endogenous epithelial cell production</td>
<td>Inhibition of butyrate-induced apoptosis</td>
<td>29, 30, 114, 138</td>
</tr>
<tr>
<td>HGF</td>
<td>Liver</td>
<td>Prevention of apoptosis by IFN-γ</td>
<td>95</td>
</tr>
<tr>
<td>TGF-α</td>
<td>Endogenous epithelial cell production</td>
<td>Prevents retinoid-induced apoptosis</td>
<td>33, 99</td>
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NO, nitric oxide; TGF, transforming growth factor; TNF-α, tumor necrosis factor-α; EGF, epidermal growth factor; HGF, hepatocyte growth factor; IFN-γ, interferon-γ; CTL, cytotoxic T lymphocyte; sFasL, soluble Fas ligand; FasR, Fas receptor.
lars results in regression of cholangiocyte numbers by apoptosis (12). Interestingly, the apoptotic cholangiocytes are shed into the biliary lumen, where they appear in bile (12). Cholangiocyte apoptosis in this model likely represents the classic paradigm of epithelial cell apoptosis on withdrawal of a growth factor.

In contrast to this model of extrahepatic restriction to bile flow, loss of cholangiocytes and consequent ductopenia characterize the cholestasis observed in the majority of chronic cholestatic liver diseases, such as primary biliary cirrhosis, primary sclerosing cholangitis, and biliary atresia. This loss of biliary epithelium appears to be mediated by apoptosis induced by CTL in these presumed autoimmune syndromes (11). Indeed, cholangiocytes are known to express Fas receptor, supporting this hypothesis (38).

Dysregulation of apoptosis and hepatobiliary malignancy. Development of a malignant clone may be conceptualized as proceeding in a stepwise manner. Among the requirements for successful establishment as a malignancy may be the sequential accumulation of mutations necessary to block apoptosis. For example, dysregulation of apoptosis may be necessary to promote growth, prevent elimination by CTL, and allow survival despite detachment from the substratum during metastases (8). These tenets do not mean that neoplastic cells do not undergo apoptosis, because apoptosis is common in neoplastic tissues (19). However, the mechanisms of apoptosis appear to be altered during cell dedifferentiation and malignant transformation.

p53 mutations are common in hepatocellular carcinoma. The p53 gene product acts as a genetic sentinel, acting to initiate the apoptotic process if excessive DNA damage occurs (79). A defective copy of p53 behaves as a dominant negative, resulting in a cell that is resistant to undergoing many forms of apoptosis (84). In regions of the world where both chronic hepatitis B virus infection and dietary aflatoxin B1 exposure are widely prevalent, hepatocellular carcinoma is frequently accompanied by mutation of p53 (57). Moreover, the protein product of the hepatitis X gene binds to p53 and abrogates p53-mediated apoptosis (144).

A novel mechanism to escape immune recognition by neoplastic cells is loss of Fas receptor expression and the development of Fas ligand expression by the cancer (53, 93). Expression of the Fas ligand results in apoptosis of Fas-receptor-expressing CTL as they attempt to attack the neoplastic cell; loss of Fas receptor by the neoplastic cell ensures its survival despite recognition by the CTL. Indeed, hepatocellular cancers do not express Fas receptor and frequently express Fas ligand, apparently to escape immune surveillance (134). Similar observations have been made in colon cancers (93).

**PANCREAS**

Physiology of apoptosis in the pancreas. The pancreas contains two epithelial cell types, acinar cells and pancreatic ductal cells. Analogous to the epithelial cells in the liver, the turnover of both acinar and ductal cells in the pancreas is limited. Under basal conditions, tritiated thymidine uptake by cells in the pancreas is only 0.1–0.2% (40). Assuming basal cell proliferation is matched to apoptosis, rates of apoptosis in the normal pancreas should be equally as low. Unfortunately there is a lack of information regarding the physiological mediators of pancreatic cell apoptosis in either health or disease. Most of what we know about apoptosis in the pancreas is derived from studies of pathophysiological models of pancreatitis.

Pathophysiology of pancreatitis and apoptosis. Pancreatitis results from acinar cell injury and can be characterized as either acute or chronic. Acute pancreatitis is associated with extracellular release of digestive enzymes, which further propagate the inflammatory injury. In various animal models of acute pancreatitis, including pancreatic duct ligation, infusing supramaximal stimulating concentrations of caerulein, and feeding a choline-deficient, ethionine-supplemented diet, the severity of pancreatitis is directly related to the magnitude of acinar cell necrosis and inversely related to the magnitude of acinar cell apoptosis (69). The extent of the inflammation and necrosis appears to be dependent on the recruitment of neutrophils to the pancreas (48). Neutrophils may convert the process of acinar cell death from apoptosis to necrosis (122). Thus the pathophysiological form of cell death during pancreatic injury may be apoptosis, with neutrophils acting as an exogenous necrotic trigger. These data have led to the concept that potentiation of acinar cell death by apoptosis instead of necrosis may ameliorate disease severity during pancreatitis (Fig. 5) (48, 69). Indeed, feeding mice a raw soy diet to stimulate pancreatic growth followed by a switch to a normal chow diet to induce involutional acinar cell apoptosis protects against caerulein-induced apoptosis (121). Thus purposefully inducing acinar cell apoptosis may reduce the severity of pancreatitis in humans. The concept of pharmacological induction of pancreatic apoptosis during the early stages of acute pancreatitis provides a new therapeutic strategy for the treatment of this disease. To our knowledge, this is one of the few examples in which intentional induction of apoptosis in a benign disease would be of potential therapeutic benefit. Potential pharmacological approaches would include prevention of neutrophilic infiltration of the pancreas (122).

In contrast to acute pancreatitis, chronic pancreatitis is characterized by acinar cell atrophy and replacement of the gland architecture with fibrotic tissue. Acinar cell atrophy likely occurs through an apoptotic process (142). Indeed, chronic obstruction of the pancreatic duct, feeding a copper-deficient diet, and ethionine administration have also been shown to lead to apoptosis of pancreatic acinar cells in the rat, with resultant pancreatic atrophy (72, 142, 143). Acinar cell atrophy by apoptosis may also contribute to the transdifferentiation of ductal epithelial cells to hepatocytes in the copper-deficient pancreas by altering the cellular communications in their microenvironment (117). The acinar cells of the splenic lobe of mouse pancreas also undergo apoptosis after selective ductal ligation as a model of chronic pancreatitis, suggesting that this is
the predominant form of cell death leading to atrophy of
the exocrine pancreas in this condition (145). Apoptosis
of acinar cells has also been identified in rodents fed
ethanol plus a low-protein diet, in a model perhaps
more germane to human chronic pancreatitis. TGF-β is
capable of inducing fibrogenesis in pancreatic tissue,
and conceivably replacement of acinar tissue by fibrous
scarring is in part mediated by the apoptotic clearance
of pancreatic acinar cells (124). The role of TGF-β in
inducing acinar cell apoptosis as well as fibrous scar-
ing in vivo remains unknown. However, overexpression
of the TGF-β-regulated zinc finger encoding gene,
TIEG, induces apoptosis in pancreatic epithelial cells
(135a). Thus it is highly likely that TGF-β can induce
pancreatic cell apoptosis.

Dysregulation of apoptosis and pancreatic malignancy.
Pancreatic adenocarcinoma is an extremely
aggressive cancer with an accordingly poor prognosis.
Current concepts suggest pancreatic cancers arise pri-
marily from the ductal epithelial cells in the pancreas.
To date, studies investigating dysregulation of apopto-
sis as a mechanism of pancreatic ductal cell carcinogen-
esis have focused on p53, the adenomatous polyposis
coli (APC) gene, and members of the Bcl-2 family of
proteins. p53 null mice heterozygous for the APC gene
lose their remaining copy of wild-type APC through a
somatic mutation. A high proportion of pancreatic cells
in this model exhibit a range of pancreatic abnormalities,
including dysplasia and preneoplastic foci (61%) and
adenocarcinoma (22%) (22). In humans, the p53
gene is mutated in the majority of pancreatic adenocar-
cinomas and may contribute to the clinical aggressive-
ness of these tumors (27). The antiapoptotic oncopro-
tein Bcl-xL is also strongly expressed in human
pancreatic adenocarcinomas (64). The enhanced expres-
sion of Bcl-xL suggests that an altered threshold for the
induction of apoptosis (in addition to the mutation of
p53 observed in most pancreatic cancers) has permitted
clonal expansion of the cancer (85, 132). Finally, it is
interesting to note that the tumor suppressor gene first
identified in pancreatic cancer, DPC4, is a downstream
signaling factor for TGF-β and is now referred to as
smad4 (88a). This observation suggests that inhibition
of TGF-β-induced pancreatic apoptosis may be impor-
tant in pancreatic carcinogenesis. Although these obser-
vations suggest that dysregulation of apoptosis may
occur in pancreatic carcinogenesis, this concept has not
been adequately tested and remains a hypothesis.

SMALL AND LARGE INTESTINE

Physiology of apoptosis in the small and large intesti-
tine. Both the small and large intestine have rapid cell
turnover rates (3–6 days), suggesting that rates of
apoptosis are equally high to provide a counterbalance
to the increased cell division (110, 130). Apoptosis of
cells at villus tips in the small intestine and luminal
surface of the colon is an attractive hypothesis to help
explain the high rates of epithelial cell turnover in
these organs. However, documentation of enhanced
rates of apoptosis in the villus tips and luminal surface
of the small and large intestine has proved surprisingly
controversial (39, 54, 90). Cells at the villus tip have
been noted to be positive for DNA strand breaks with
the use of the terminal dideoxynucleotide transferase (TdT) labeling technique, suggesting the cells are apoptotic (39, 54). In contrast, other investigators using the same technique did not identify labeling of cells on the villus tips (90). The controversy resulting from these disparate studies centers on the technique of TdT labeling of 3’-OH ends of DNA as a marker for apoptosis. This technique relies on digestion of tissue with proteinase K before the enzymatic labeling step. It is now apparent that the apparent of proteinase K digestion and the use of diethyl pyrocarbonate-treated water influence which and how many cells are labeled using this technique (133). TdT labeling should be combined with complementary morphological techniques before it can be considered a marker for apoptosis. Furthermore, the TdT-based technique is not specific for apoptosis and can also be observed in cell death by necrosis (44). In the small intestine, TdT will label approximately four times as many cells as would be detected by morphology in the villus tip with the use of electron microscopy (130). Nonetheless, apoptosis occurs rapidly in vivo and may therefore appear underrepresented in histological sections, and kinetic analysis suggests that apoptosis rather than shedding from the luminal surface accounts for a large proportion of the cells lost (54). Although further studies will be necessary to prove or disprove the hypothesis that apoptosis is responsible for shedding of cells from the villus tips in the small intestine or luminal surface of the colon, the observation that Bcl-2 is expressed in the proliferation compartment of colonic crypts, whereas Bax is expressed near the lumen, supports this hypothesis in the colon (61, 74, 111a).

Stem cells in the large and small intestine also appear to undergo apoptosis (112). The proportion of stem cells in the small intestinal crypt that undergo apoptosis under physiological conditions has been estimated to be 10% (113). The spontaneous apoptosis of stem cells not exposed to exogenous insults may repress detection and deletion of defective cells (i.e., random genetic defects), as well as control of cell number per se (111). p53 null mice exhibit normal levels of spontaneous apoptosis in the intestinal crypts (28). This suggests either that p53 is not involved in the detection of genetic flaws in recent progeny of stem cells or that the prime function of induction of apoptosis is regulation of cell number. The low incidence of small intestinal tumors despite rapid cellular division in contrast to the relatively high rate of neoplasia in the colon implies more effective eradication of malignant precursor lesions via apoptosis in the small vs. large intestine. The absence of Bcl-2 expression and the presence of the proapoptotic Bax protein in the small intestinal crypt would favor a proapoptotic threshold helping to facilitate apoptosis of genetically altered stem cells (74, 90). In contrast, stem cells in the colon do express the antiapoptotic Bcl-2 protein favoring cell survival despite genetic damage. These observations provide insight into the mechanisms contributing to the high rates of colon cancer in the large intestine compared with the low rates of cancer in the small intestine (80, 87, 119).

Expression of Fas ligand has been described as a feature of “immune privileged” sites. Cells expressing Fas ligand can produce apoptosis of Fas receptor-expressing immune effector cells, thereby conferring an immune privilege (47). The Paneth cells of the small intestine express Fas ligand at a high level under normal circumstances (94). The reason for this unique status of Paneth cells is currently unclear. Normal colonic epithelial cells also constitutively express Fas receptor and undergo apoptosis on Fas ligation. Based on these observations it has been proposed that the Fas system contributes to epithelial cell injury in ulcerative colitis and graft vs. host disease (120a, 134a).

Physiological mediators of apoptosis: growth factors and dietary factors. As we have discussed, TGF-β directly induces apoptosis in most epithelial cells, including colonic epithelial-derived cell lines (6c). Immunohistochemical studies have shown that, in the small bowel, TGF-β1 is localized primarily in the villus tip (6a, 6c). In colonic tissue, TGF-β1 is expressed predominantly in nonreplicating cells at the top of the colonic crypts (6a). Positive expression of TGF-β and the absence of Bcl-2 expression by the cells at the top of the crypts would appear to prime these cells for apoptosis (54).

Sodium butyrate, a fermentation product of dietary fiber in the colon, has been shown to induce apoptosis in human colonic cancer cell lines independent of p53 (51). Butyrate-induced apoptosis of colonic epithelial cells is associated with increased expression of p21\(^{WAF-1/cip1}\) (a tumor suppressor protein that inhibits various cyclin-dependent kinases). p21\(^{WAF-1/cip1}\) expression induces apoptosis and cell cycle arrest in a variety of cells and also likely mediates butyrate-induced apoptosis of colonic epithelial cells (41, 65). Of note is the apparent structural specificity for a four-carbon acyl chain for butyrate-induced apoptosis; acetate, proprionate, and isobutyrate (which is branched) appear to be much less effective (50, 59). In contrast, acute butyrate deprivation induces apoptosis in tissue sheets from guinea pig proximal colon by inducing Bax, a model more relevant to in vivo conditions than the use of cell lines (58). The differences between the survival effects of butyrate in vivo and its apoptotic effects on cell lines remain unclear but have been comprehensively discussed in a recent editorial (52). Nonetheless, it appears that dietary products that are metabolized by luminal bacteria can regulate apoptosis of colonic epithelia.

Dysregulation of apoptosis and colorectal cancer. Although there are scant data on apoptosis as a mechanism of tissue injury in the small and large intestine, dysregulation of apoptosis as a mechanism contributing to colon carcinogenesis has received considerable attention (Fig. 6). Studies in the colon on dysregulation of apoptosis as a mechanism of carcinogenesis are providing fundamental, pioneering observations highly relevant to the broad field of carcinogenesis in general. Therefore, instead of the the pathophysiological consequences of excessive apoptosis in the intestine, this
portion of the review focuses on dysregulation of apoptosis as a cellular mechanism of colon carcinogenesis. Bcl-2, an antiapoptotic oncogene product first implicated in the pathogenesis of follicular lymphoma, has been implicated in the genesis of the adenoma/carcinoma sequence of events in colon carcinogenesis. In normal colon, Bcl-2 protein is expressed only in the base and the lower third of the epithelial column (26). However, increased expression of Bcl-2 was found in most dysplastic, adenomatous, and early adenocarcinomatous lesions (16, 90). Although Bcl-2 may not be expressed in advanced, anaplastic cancers, it is expressed in virtually 100% of adenomas, suggesting that Bcl-2 contributes to the early stages of neoplastic transformation by blocking apoptosis during the transformation process of genetically altered cells (15, 16). These observations suggest that impaired induction of apoptosis due to aberrant Bcl-2 expression facilitates the development of colonic neoplasia.

One of the most convincing examples of dysregulation of apoptosis as a mechanism of carcinogenesis is in the hereditary nonpolyposis colorectal cancer (HNPCC) syndromes. Individuals with HNPCC syndrome have defects in the base-base mismatch repair pathway of DNA repair, leading to frameshift mutations (1, 147). Recently, frameshift mutations in the bax gene have also been identified in colon cancers from patients with this syndrome (116). Loss of Bax function by this mutation would alter the apoptotic threshold. How failure of TGF-β- or Bax-mediated apoptosis of colonic epithelium leads to colon carcinogenesis remains unclear; however, failure of apoptosis likely helps to prolong cell survival during the multiple mutations involved in the multistep stages of colon carcinogenesis.

Alterations in eicosanoid production within colonic epithelium may also promote colon carcinogenesis by blocking apoptosis. Most colon cancers overexpress prostaglandin endoperoxide synthase 2, commonly re-
ferred to as cyclooxygenase-2 (COX-2) (123). Intestinal epithelial cells overexpressing COX-2 increase expression of Bcl-2, decrease expression of retinoblastoma kinase, cyclin D1, and TGF-β receptors, and enhance cellular adherence to matrix proteins such as E-cadherin (29, 30, 138). These phenotypic alterations lead to a cellular phenotype resistant to butyrate-induced apoptosis (138). The resistance to apoptosis can be reversed by nonsteroidal anti-inflammatory drugs (NSAIDs), which inhibit the enzymatic activity of COX-2, suggesting that enhanced eicosanoid production leads to the altered phenotype. Compared with histologically normal mucosa, tissue from human colon cancers showed a 2.3-fold increase of prostaglandin E2 and a 3.5-fold decrease of prostaglandin I2 levels, with unaltered levels of thromboxanes and leukotrienes (representing products of the 5-lipoxygenase pathway of arachidonic acid metabolism) (114). However, leukotriene B4 and 12-(R)-hydroxyecosapentaenoic acid have also been shown to increase the rate of proliferation of colon cancer cells in vitro, suggesting that at least some metabolites from the lipoxygenase and cytochrome P-450 pathways may also be responsible for increased proliferation in colon cancer (14). These exciting studies provide insight into the resolution of colonic adenomas, which have been reported in patients taking NSAIDs. The use of specific COX-2 inhibitors currently under development may prove efficacious in the chemoprevention of human colonic neoplasia. Nonetheless, it should be noted that induction of apoptosis by NSAIDs may be independent of blocking eicosanoid pathways. For example, induction of apoptosis cannot be prevented by the addition of exogenous prostaglandins, and these compounds induce apoptosis in HCT-116 cells, which lack both COX-1 and COX-2 transcripts (55). The NSAID sulindac and its metabolite sulindac sulfide reduce the level and activity of multiple cyclin-dependent kinases and induce expression of p21WAF-1/cip1. Multiple cellular mechanisms may lead to apoptosis by these pharmacological agents (41, 131).

Finally, alterations in the APC gene, which lead to colon carcinogenesis, may also regulate apoptosis. The protein product of this gene is expressed in the nonproliferating, differentiated cell in the upper half of the crypt. Patients with germline mutations of this gene produce a truncated protein that presumably has loss of function (17, 71, 101). APC has been reported to associate with α- and β-catenin, proteins that associate with E-cadherin, suggesting that APC is involved in cell adhesion (120, 135). Recent data suggest APC may be involved in the apoptotic pathway, and loss of function could inhibit apoptosis, promoting an expansion of the proliferative zone in the colon crypt, or could conceivably facilitate metastasis (17). Expansion of the proliferating zone and prolonged cell survival may provide the cellular substrate for the enhanced mutagenesis and the early development of colon cancers observed in affected individuals.

**SUMMARY**

Recent knowledge regarding cell death by apoptosis has markedly altered our concepts of the physiology and pathophysiology of gastrointestinal epithelial cells. We now realize that enhanced apoptosis can lead to tissue injury during vascular, inflammatory, infectious, metabolic, and drug-induced disease processes. Dysregulation or inhibition of apoptosis appears to be important in cell proliferation, tissue hyperplasia, and malignant transformation of gastrointestinal epithelia. Nonetheless, we believe much remains to be learned regarding the role of apoptosis in gastrointestinal diseases. Two fundamental questions remain: 1) What are the initiators and regulators of apoptosis in gastrointestinal epithelia? and 2) What are the intracellular pathways culminating in apoptosis in gastrointestinal epithelial cells? Although the role of Fas receptor and Fas ligand pathways of apoptosis (a pathway fully elucidated in lymphocytes) needs to be further explored in gastrointestinal diseases, many of the initiators and regulators of apoptosis in the gastrointestinal tract will not be shared by lymphocytes (i.e., private pathways of apoptosis). In particular, characterization of the expression of the Bcl-2 family members of proteins and their role in promoting or preventing apoptosis as well as their regulation by cytokines, dietary factors, neuropeptide, and neurotransmitter needs further delineation. In contrast, the intracellular pathways of apoptosis (e.g., caspase activation, mitochondrial dysfunction) are likely to be shared between lymphocytes and gastrointestinal epithelial cells. Investigators interested in intracellular mechanisms of apoptosis in epithelial cells will need to compare and contrast differences and similarities between the intracellular mechanisms known in lymphocytes and those of epithelial cells. As new knowledge becomes available, the purposeful, therapeutic regulation of apoptosis should prove useful in disease processes.

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