CELL DEATH

Cell death is a process accompanying many physiological and pathological situations in organisms. The first cell death pattern that was identified was cell necrosis, described by Virchow in 1871. It was subsequently seen that cell death was an integral part of normal cell and tissue differentiation mechanisms in superior organisms. In this respect early embryological studies revealed that cell death processes were required to model organisms in their final configuration. Morphogenesis systematically entails the removal and generation of new cell and tissue structures. A similar phenomenon is encountered during metamorphosis in invertebrates and inferior vertebrates, where massive tissue involution and cell clearance are coordinately developed physiological processes. This cell death process, designated apoptosis, was characterized by Kerr in 1965. Cell apoptosis and necrosis can be differentiated by a number of both morphological and biochemical parameters. Apoptosis is a controlled removal of the involved cell with no relevant changes in cell metabolism. This process is characterized by the sequential activation of a number of proteases known as caspases, which affect cysteine-aspartate bonds in the substrate. Caspase activation entails DNA fragmentation and cell architecture changes, associated with morphological changes such as nuclear DNA condensation, decreased cell volume, and the generation of apoptotic bodies with no intracellular contents release. Necrosis results from an extreme disruption of cell balance dramatically affecting cell metabolism with a drastic decrease in cell energy contents in the form of adenosine triphosphate (ATP), ion contents changes, increased mitochondrial and cell volume, and intracellular protease activation. This process ultimately leads to a disruption of cell membranes, and release of cell contents, which promotes a secondary inflammatory response. In the liver cell apoptosis usually has a focal distribution, whereas necrosis shows a regional distribution. Despite a clear-cut differentiation between apoptosis and necrosis, both types of cell death usually coexist in the liver, because one stimulus may induce apoptosis or necrosis depending on cell type involved, exposure extent, cell metabolic status, and the integrity of the machinery involved in cell death. In this sense a number of authors have suggested that apoptosis and necrosis are no separate processes but the opposing ends in only one cell mechanism designated necrapoptosis (1). Mitochondria-generated ATP contents are a key factor in the regulation of apoptosis or necrosis induction during the process of cell death. In this respect a lesion involving a few mitochondria may be solved by autophagia of altered organelles. If more mitochondria are involved, and an adequate amount of proapoptotic factors is released while intracellular ATP levels remain, the cell undergoes apoptosis. If the cell undergoes a severe lesion, the dramatic reduction of ATP contents will not allow for many ener-
Energy-consuming processes such as cell osmosis and membrane permeability control, which will induce cell death by necrosis. It should be noted that besides cell morphology in vivo no parameters are specific for cell apoptosis. Thus several quantitative and non-quantitative parameters are needed to establish the type of cell death implied by a specific pathophysiological condition.

CELL DEATH MECHANISMS

Caspases are a protease family present in the cell in an inactive form,zymogens that are sequentially activated by other active caspases. Caspase activation may occur via two processes—so-called intrinsic and extrinsic—that depend on the cell type and pathophysiological status. In the first mechanism intracellularly generated signals (e.g., Bax, Bak, Bid, Puma, VpR, p53, JNK, Ca²⁺, reactive oxygen species or ROS, ceramide, ganglioside GD3, etc.) act on the mitochondrion and induce cell death signaling. In the extrinsic pathway cell death induction is initiated by the binding of a number of ligands (TNF-α Fas, TRAIL, etc.) to their corresponding receptors, which induce the coming together of various adapter proteins and so-called initiator caspase zymogens (procaspase 8 or 10) at the receptor’s intracellular domain to make up the so-called death-inducing signaling complex (DISC), which in turn transmits the signal for cell death either directly or in most situations through mitochondria-released proapoptotic factors inducing executional caspase (caspase 3, 6 and 7) activation (Fig. 1). In both cases mitochondrial membrane permeabilization (MMP) is a crucial event occurring both in apoptosis and necrosis. Two primary mechanisms for MMP induction have been described depending on cell type, stimulus, intensity, and duration. In the first one factors such as Bid or Bax, following translocation and oligomerization in the outer mitochondrial membrane, set up pores that allow the passage of cytochrome c or Smac/DIABLO from the inter-membrane space to the cell’s cytoplasm without damaging the inner mitochondrial membrane. The formation of such channels is facilitated by the incorporation of lipid molecules (ceramide, sphingosine, and ganglioside GD3) that induce the generation of greater pores in response to proapoptotic stimuli. On the other hand, the induction of mitochondrial permeability transition (MPT) represents an alternative model to the formation of specific channels in the outer mitochondrial membrane (2). The MPT process includes a multi-protein complex by the name of voltage-dependent anion-selective channel (VDAC), located at contact sites between the mitochondrial internal and external membranes; cytochrome c, on the outer aspect of the mitochondrial internal membrane; and internal mitochondrial membrane potential (Fig. 1). VDAC consists of a porin (pore-forming protein), ATP/ADP translocator protein (ANT), hexokinase (transforms glucose into glucose-6-phosphate, and initiates glycolysis), creatinine kinase, various anti-apoptotic members in the Bcl-2 family (Bcl-2 y Bcl-Xₐ), and cyclophilin D (Fig. 1). During MPT induction the VDAC complex is modified, and mitochondrial potential collapses because of mitochondrial electron chain depolarization, oxidative phosphorylation uncoupling, ROS generation, and ionic gradient dissipation as entailed by a transient permeability pore (TPP), which will permit the passage of molecules up to 1.5 kDa in weight. However, given the greater size of inter-membrane proapoptotic factors (cytochrome c, AIF, pro-caspase 9, etc.), the presence of big pores facilitated by Bid-Bak or VDAC-Bax interactions –leads to external membrane disruption as a result of increased mitochondrial matrix volume and inter-membrane space proapoptotic factor release– has been suggested. The release of proapoptotic factors in the mitochondrial electron chain, including cytochrome c,
via a NMP or MPT has two significant effects, namely an interruption of electron transfer in complex III, with a consequent generation of ROS (3), and the initiation of cell apoptosis signaling. Inducing oxidative stress is essential for the progression of mitochondrial dysfunction. Oxidation of cardiolipin, an anion phospholipid present exclusively in some mitochondrial electron transport complexes, favors the induction and progression of mitochondrial dysfunction. The formation of wide pores in the mitochondrial outer membrane or MPT induction are likely a part of a common mitochondrial disturbance in the process of cell death. In this respect GD3 (4) and MPT (5) have been seen to play a role in hepatocyte death induction by TNF-α before cytochrome c release, caspase-3 activation, DNA fragmentation, and apoptosis-related morphology changes. Similarly, the ability of Bid to interact with pore-generating components on the external membrane (Bak, Bax), or MPT components (Bcl2), suggests an integrated role of both types of process in cell death induction. The classic apoptosis signal as induced by PMNs—or more specifically by MPT—entails a release of cytochrome, pro-caspase 9, and ATP from the inter-membrane space; these factors, together with factor apaf-1, make up the apoptosome (Fig. 2). Apoptosome formation induces caspase-9 activation, which in turn, through controlled procaspase-3 proteolysis, activates the latter factor, which acts on specific targets to determine the apoptotic phenotype. The induction of cell necrosis results from high MMP, which contributes to metabolic dysfunction, sudden ATP loss, and inability for apoptosome assembly and cell apoptosis progression. Steps following caspase-3 activation comprise caspase-activated ADNase (CAD) activation by ICAD degradation and inactivation, which results in DNA fragmentation.

While apoptosome formation represents the classical DNA fragmentation pathway, mitochondria release other inter-membrane proteins that promote cell death, including apoptosis induction factor (AIF) and endonuclease G, which induce DNA disruption in the absence of caspases, as well as Smac/DIABLO and Omi/HtrA2, which facilitate cell death through the inactivation of so-called inhibitor of apoptosis proteins (IAPs) (Fig. 2).

**CELL DEATH IN LIVER DISEASE**

Experimental hepatocellular injury models both in vivo and in vitro are useful to understand cell death processes and identify new therapeutic targets in human liver conditions. However, the relevance of results in experimental animals must be acknowledged in the various clinical settings of liver disease. The following sections describe cell death mechanisms in a number of relevant liver dysfunction processes such as ischemia-reperfusion injury, alcoholic and non-alcoholic steatohepatitis, cholestatic diseases, viral hepatitis viral, and liver cell carcinoma.

**Ischemia-reperfusion injury**

Liver injury from anoxia or hypoxia results from absolute or relative oxygen deficiency. Anoxia occurs during hepatic artery thrombosis in orthotopic liver transplant, or during portal flow interruption in liver resection (Pringle’s maneuver). Liver hypoxia occurs when blood flow decreases, as is the case in veno-occlusive disease, congestive centrolobular necrosis, alcohol-related liver injury, hypotension, and hemorrhagic shock. Similarly, reoxygenation or reperfusion injury results from oxygenated blood supply to liver tissue after anoxia or hypoxia. The extent of tissue injury after reperfusion depends on the previous presence of anoxia or hypoxia, as well as on cell type and ischemia temperature.

Initial changes during cell anoxia involve the mitochondrion. A lack of oxygen, the final electron acceptor in the mitochondrial electron transport chain, increases the organelle’s reduced status because of an increased NADH:NAD⁺ ratio and lower ATP generation from ADP. In hepatocytes with relevant glycogen levels for anaerobic glycolysis, nutritional status is an important factor for anoxic cell injury. During cell anoxia, regardless of ATP oxidative generation, intracellular mechanisms exist that lead to ATP synthase inhibition and ionic gradient support, which preserve mitochondrial potential and integrity. This adaptive cell response to anoxia seems to result from mitochondrial calcium release in liver cell cultures (6). If anoxia reverts before changes in mitochondrial potential, hepatocytes recover their full mitochondrial function and cell death is prevented. If anoxia lasts too much, MMP and mitochondrial dysfunction are induced, which

![Fig. 2. Scheme illustrating classic apoptosis induction signaling from cell death receptors. Death receptors consist of a cysteine-rich extracellular domain, a trans-membrane domain, and a cell death cytoplasmic domain (DD), which is bound to either a Fas-associated death domain (FADD) or TNF receptor-associated death domain (TRADD).](image-url)
lead to cell death. In this sense MMP changes play a more relevant role than ATP oxidative generation depletion in anoxic liver cell injury. The susceptibility of the various liver cells to anoxic injury is unrelated to the extent of ATP intracellular depletion, but is related to the extent of intracellular protease activity. Hepatocytes are cells with the highest intracellular protease activity and susceptibility in the process of cell anoxia. While anoxic liver injury may occur in a number of ischemic syndromes, hypoxia-associated conditions are more commonly seen in daily practice. Low oxygen tension induces decreased activity in some mitochondrial complexes, and diminishes ATP synthesis, associated with mitochondrial electron chain uncoupling and ROS generation. In this respect zone-II hepatocytes under hypoxia lose their viability faster than zone-I or -III cells (7).

Reperfusion injury results from the reintroduction of physiological oxygen concentrations in cells previously exposed to non-fatal hypoxia or anoxia conditions. Under such conditions huge amounts of ROS are generated in the mitochondria of hepatocytes, Kupffer cells, endothelial cells, and bile duct epithelial cells. In hepatocytes, oxygen reintroduction in the presence of reduced mitochondrial electron complex activity results in increased complex I and III autooxidation and ROS production. Hepatocytes are less susceptible to oxidative stress after reoxygenation when compared to endothelial or duct epithelial cells due to a favorable intracellular balance between oxidative stress and antioxidant contents. Endothelial cell injury is critical for survival of livers undergoing cold ischemia during transplantation, since vessel disruption reduces blood flow and ultimately exacerbates liver cell necrosis and graft failure. Similarly, Kupffer and stellate cell activation during reperfusion generates ROS, proinflammatory cytokines, and other chemotactic factors contributing to post-ischemic damage, systemic inflammatory response, and multiple organ failure associated with ischemic injury of the liver.

ALCOHOLIC AND NON-ALCOHOLIC STEATOHEPATITIS

Alcoholic steatohepatitis (ASH) and non-alcoholic steatohepatitis (NASH) exhibit similar histological characteristics, even if their primary etiology is different. In both conditions steatosis, inflammation, hepatocellular death with marked susceptibility to oxidative stress, and fibrosis that may progress to cirrhosis and liver cell carcinoma may be seen. Lipid deposition in the cytoplasm of liver cells is the initial stage of ASH and NASH development. The regulation of genes associated with fatty acid and cholesterol synthesis is controlled by a family of transcription factors by the name of steroid-regulating element binding proteins (SREBPs). A number of reports demonstrate that alcohol increases SREBP expression, as well as the expression of genes involved in lipid metabolism, in liver cell cultures and in the liver of rats fed on a alcohol-rich diet (8). Similarly, the induction of experimental diabetes with insulin resistance and obesity is associated with increased SREBP and fatty-acid expression in the liver (9). Lipid deposition in ASH and NASH hepatocytes fosters oxidative stress, inflammation, cell death, and fibrosis, which represents the second stage in liver disease progression. Mediators involved in ASH and NASH development are mostly coincidental. In this regard, TNF-α is a cytokine related to ASH and NASH progression. Alcohol use induces a markedly decreased transportation of GSH to mitochondria, which increases liver cell susceptibility to oxidative stress and TNF-related cell death in hepatocytes (10). Similarly, circulating TNF-α levels in patients with NASH are associated with greater mitochondrial dysfunction and oxidative stress in hepatocytes (11). Nutritional and genetic liver steatosis models characterized by increased triglycerides, fatty acids, and cholesterol recently revealed that cholesterol deposition in mitochondria reduces GSH transportation to said mitochondria in hepatocytes via a mechanism similar to that of ASH, which determines a higher susceptibility of these hepatocytes to TNF-α related cell death. Mitochondrial dysfunction and oxidative stress are associated with increased cell death parameters in liver cells. Oxidative stress is a determinant factor in the progression of ASH and NASH to liver fibrogenesis.

CHOLESTATIC LIVER DISEASE

Decreased bile salt secretion by hepatocytes is common in drug-induced cholestasis, primary biliary cirrhosis, primary sclerosing cholangitis, and bile obstruction. While the initial bile duct lesion is of immune, toxic, or genetic origin in many hepatobiliary conditions, liver cell injury is exacerbated by a direct cytotoxic action of hydrophobic bile salts on hepatocytes. Such cytotoxicity is histologically characterized by apoptosis and necrosis cell markers in the cholestatic liver. Cell death from hydrophobic bile salts is associated with mitochondrial function changes, and is apoptotic or necrotic as a function of cytotoxic levels. Despite bile salt detergent effects, levels required for proapoptotic activity are below the critical concentration needed to alter mitochondrial micellar structure. Proapoptotic signaling starts when hydrophobic bile salts induce Fas or TRAIL receptor aggregation, followed by caspase-8 activation, Bid/Bax translocation into mitochondria, and proapoptotic factor release in hepatocytes (12). Similarly, bile salts inhibit mitochondrial complex activity and ATP formation, which induces increased intracellular Ca²⁺ levels and the activation of various proapoptotic proteases in hepatocytes (12). MMP induction by hydrophobic bile salts is prevented by cyclosporin A, which suggests that bile salt toxicity is mediated by specific channel-opening mecha-
nisms in the mitochondrial inner membrane. Bile salts increase intracellular oxidative stress with MMP signaling amplification, which may induce necrotic death in hepatocytes.

**VIRAL HEPATITIS**

Viral hepatitis by hepatitis B virus (HBV) or hepatitis C virus (HCV) represents a serious public health concern because of the high numbers of infected patients who will progress to cirrhosis and hepatocellular carcinoma (HCC). Persistent infection entails constant tissue destruction and regeneration, which leads to increased risk for HCC development. Liver injury by HBV and HCV is primarily mediated by the host’s immune response to infected hepatocytes expressing viral proteins in their membranes in association with class I histocompatibility antigens. Cytotoxic T cells recognize infected cells, and then induce their apoptosis. Apoptotic bodies—previously designated acidophilic or Councilman’s bodies—are found in liver tissue from patients with viral hepatitis. Several studies have shown that cytotoxic T cells express Fas ligands that play a role in infected liver cell apoptosis via the Fas receptor. Fas expression has been reported in hepatocytes, and Fas ligand expression in inflammatory cell infiltrates, in the liver of HCV- and HBV-infected patients (13, 14), which correlates to liver lesion severity. Fas expression in infected hepatocytes may be induced by specific viral proteins or inflammatory cytokines such as interleukin-1, which is released during inflammatory response. Other cell death mechanisms mediated by cytotoxic T cells involve the cell pore generation system via perforin/granzyme secretion in the microenvironment of virus-infected hepatocytes. However, hepatocytes have been seen to be resistant to cytolysis by granzyme B, which suggests that cytotoxic T cells predominantly induce apoptosis via Fas in virus-infected hepatocytes. TNF-α also seems to play a highly relevant role in HCV- and HBV-infected liver cell apoptosis. In this regard an increased expression of TNF-R1 receptors has been seen in infected livers, together with increased TNF-α production in mononuclear cells from HCV- and HBV-infected patients, and increased susceptibility to viral protein-induced, TNF-α-induced apoptosis in hepatocytes (15).

Despite cell death activation by cytotoxic T cells via Fas, or TNF-α receptor activation, viral antigen-induced antiapoptotic mechanisms that may entail tolerance to infection. In this respect HBV or HCV proteins may stimulate a number of intracellular antiapoptotic pathways related to NF-κB or JNK activation, either Fas-(16, 17) or TNF-α-induced (18), in hepatocytes. The induction of cell death resistant mechanisms in infected hepatocytes is highly relevant for infection persistence, chronic lesion establishment, and HCC development.

**HEPATOCELLULAR CARCINOMA**

HCC is a primary tumor of the liver with a high incidence among the population. HCC pathogenesis is multifactorial, with a high association with chronic viral hepatitis, alcohol use, liver toxin exposure, and genetic changes such as hemochromatosis or α-antitrypsin deficiency. Cell death induction is a response leading to cell clearance so that changes are not spread to neighboring cells. In contrast, HCC progression results from changes in proapoptotic mechanisms, which allows cell proliferation and a propagation of malignant changes. Specifically, tumor cells usually have altered tumor suppressor genes, DNA repair genes, cell cycle regulation genes, and cell apoptosis genes.

Most common disturbances in HCC regarding cell death include p53 gene mutations. Protein p53 is the product of gene related to tumor suppression that becomes active as a result of DNA damage. Following the identification of a genetic change protein p53 brings the cell cycle to a halt to permit involved gene repairs. When multiple gene changes requiring cell elimination are identified, p53 induces apoptosis by increasing the expression of pro-apoptotic proteins such as Noxa, Puma, Bid, or Bax, by inducing the expression of cell death-related receptors such as Fas or TRAIL. Thus, an altered p53 allows tumor cell survival and HCC progression (19). Another change that is commonly detected in HCC is decreased Fas expression in tumor cells, which allows their escaping cytolysis by cytotoxic T cells or natural killer cells. Similarly, a loss of Fas receptor expression in HCC is usually associated with exceptional Fas ligand expression in the liver cell, the latter being usually restricted to inflammatory cells, which allows the induction of immune system cell death, and the setup of an immunologically privileged area. Decreased Fas receptor expression in HCC is negatively correlated to with differentiation extent and patient survival (20). Other HCCs have increased expression of Bcl-2 proteins with antiapoptotic activity, which confer resistance apoptosis induction intracellular mechanisms via the mitochondria. Inhibiting cell death processes by altering DNA detection and repair systems, using cell death inducers, modifying the cell cycle, or increasing antiapoptotic factors will facilitate hepatocellular injury persistence, and progression to HCC.

**REFERENCES**

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