Liver cell death: Update on apoptosis

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Hepatocyte cell death is a cardinal feature of almost every liver disease. Apoptosis is a mode of cell death characterized by specific morphological and biochemical features. Over the past decade, the importance of apoptosis has been appreciated, and it is now thought to be the main mode of cell death in liver diseases. The recognition that apoptosis can be modulated by the cell itself or by the extracellular environment has given hope that treatments can be designed to modify the evolution of disease. This article presents an overview of this important phenomenon, as well as models of hepatocyte apoptosis and goals of current research. The significance of apoptosis to the pathophysiology of liver disease is discussed.

Key Words: *Apoptosis; Hepatocyte; Liver diseases*

La mort des hépatocytes : le point sur l'apoptose

La mort des hépatocytes est une caractéristique fondamentale de presque toutes les maladies du foie. L'apoptose est un type de mort cellulaire qui se caractérise par des aspects biochimiques et morphologiques particuliers. On s'est rendu compte, au cours de la dernière décennie, de l'importance de l'apoptose et on croit maintenant qu'il s'agit du principal type de mort cellulaire dans les hépatopathies. Le fait que l'apoptose puisse être modulée par les cellules elles-mêmes ou par le milieu extracellulaire permet de croire à la mise au point de traitements conçus pour modifier l'évolution du processus morbide. Le présent article donne un aperçu du phénomène, explique les modèles d'apoptose d'hépatocytes et expose les buts de la recherche actuelle. Il sera finalement question de la signification clinique de l'apoptose dans la physiopathologie des maladies du foie.

CLINICAL SCENARIO

A 44-year-old, human immunodeficiency virus (HIV)-infected man is referred for evaluation of elevated serum liver enzymes. Ten years ago, he contracted acute hepatitis B, which has subsequently cleared completely. He is a known carrier of the hepatitis C virus (HCV). He has been treated for HIV with zidovudine, lamivudine and efavirenz for one year. Three months ago, measurement of the HIV viral load showed that viral replication was under control. However, antiviral treatment was stopped four weeks ago when aspartate transaminase and alanine transaminase levels rose from 40 to greater than 100 U/L. The patient does not drink alcohol and is not obese. Currently, the aspartate transaminase level is 396 U/L, the alanine transaminase level is 460 U/L and the alkaline phosphatase level is 119 U/L. An abdominal ultrasound reveals normal liver echogenicity without splenic enlargement. A liver biopsy shows significant inflammation consistent with a diagnosis of chronic, active hepatitis C. The activity index is 12/18 and the fibrosis score is I/IV. Inflammation is especially prominent in the liver lobules with abundant apoptotic figures.

INTRODUCTION

The term 'apoptosis' is increasingly mentioned in medical meetings, scientific articles and pathology reports. Many new drugs are being developed with the aim of modifying this process. The Nobel Prize in Medicine and Physiology was recently awarded to three researchers who unraveled the genetics of cell death. This has illustrated the importance of apoptosis as one of the basic cell processes, in the same league as DNA replication, gene expression and protein synthesis. Therefore, it is a good time to review what we know about liver cell apoptosis and how research in the field is evolving.

Overview of apoptosis

The word 'apoptosis' is derived from the Greek 'apo' (off) and 'ptosis' (falling). This neologism was chosen because of the analogy between this type of cell death and what happens to leaves when they fall from trees. The comparison is indeed very appropriate as we understand more thoroughly what happens to apoptotic cells. The word was first used in 1972 by Kerr et al (1), who described the morphological characteristics of a particular form of cell death. Kerr was a liver pathologist who had earlier reported that necrosis could sometimes be accompanied by cell shrinkage, in opposition to the dogma that an increase in cell volume was one of the hallmarks of this form of cell death (2). The publication of further key articles in the 1980s led to an explosion of enthusiasm for the study of apoptosis. In 1980, Wyllie (3) discovered that apoptosis was accompanied by a nonrandom form of DNA fragmentation that he postulated was due to a cellular endonuclease (an enzyme whose function is to cut nucleic acids). In 1984, the protein of a gene named *Bcl-2* was found to be overexpressed in a particular type of B-cell leukemia (hence the name Bcl-2) (4). This protein was also found to enhance the survival of these cells by making them resistant to apoptosis (5). These cardinal observations demonstrated that something inside cells seems to be necessary for the process of apoptosis to occur and some cell constituents have the capacity to modulate the process of apoptosis. Therefore, our understanding of how cells die changed radically: cells were not just innocent victims but

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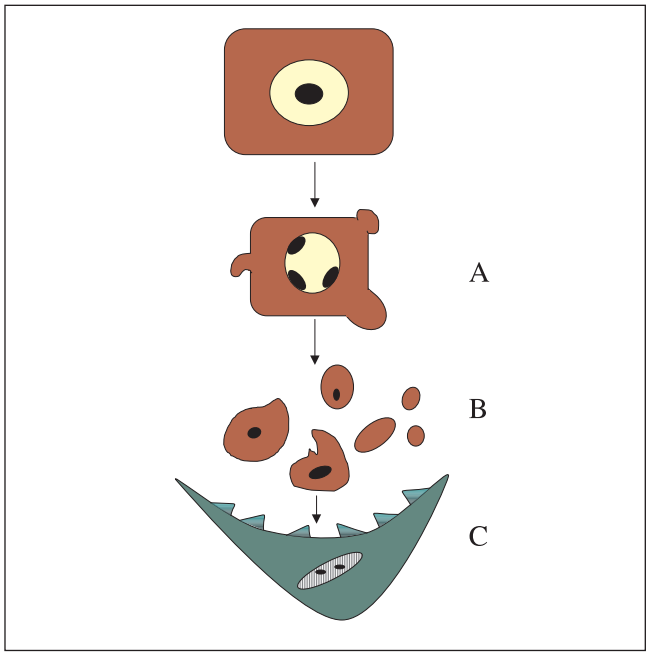

Figure 1) *Schematic depiction of the morphological characteristics of the apoptotic process. The first step is characterized by a reduction in cell volume associated with the condensation of nuclear chromatin and the formation of membrane blebs (***A***). The next step occurs when cells become fragmented and apoptotic bodies, which may or may not contain nuclear residues, can be identified (***B***). These fragments are eventually phagocytosed by macrophages or neighbouring cells (***C***)*

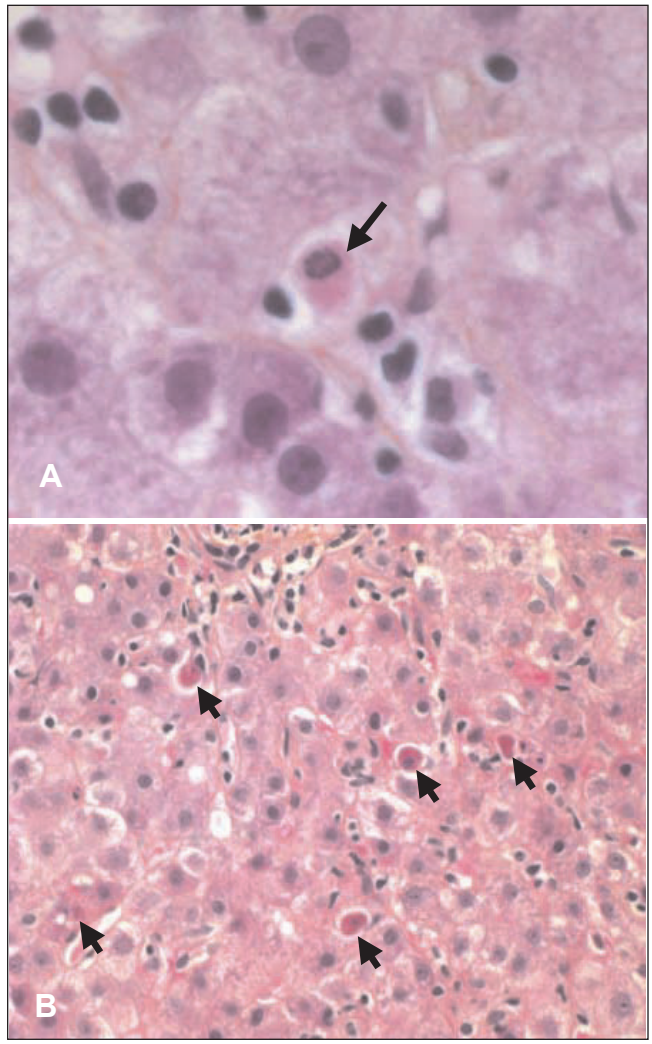

Figure 2) *Representative photomicrographs of liver tissue from a hepatitis C-infected patient. The liver section was stained with hematoxylin-phloxin-safranin.* **A** *High-power view showing the morphological characteristics of apoptosis. The arrow shows a small, acidophilic hepatocyte with condensation of the nuclear chromatin. A small lymphocyte is located adjacent to it.* **B** *Low-power view showing numerous apoptotic hepatocytes (small arrows) in the liver lobule*

could commit suicide as well. The expression 'active cell death' was then regarded as equivalent to apoptosis. That being said, cells can also die passively and involuntarily from an overwhelming injury; this mode of death is usually referred to as necrosis.

Perhaps more important, the work of the 2002 Nobel Prize winners in Medicine and Physiology clearly demonstrated that a component of the genome is required for some forms of apoptosis to occur. In the early 1960s, Dr Sydney Brenner chose a small nematode (*Caenorhabditis elegans*) to study fundamental questions regarding cell differentiation and organ development that he believed would be difficult to answer in higher animals. In the 1970s, John E Sulston discovered that a precise number of cells died at a predictable stage of the worm's development (6). In 1986, Robert Horvitz identified the first genes that were essential for these cells to die (7). Thanks to their work, the key enzymes involved in the regulation of apoptosis have now been identified not only in worms but also in all other higher animals, including humans. The exact contributions of each Nobel awardee are described at http://www.nobel.se/medicine/laureates/2002/index.html.

The 131 of 1090 cells that undergo apoptosis in the *C elegans* model provide an excellent example of 'programmed cell death'. This term has often been used as a synonym for apoptosis, but the processes are not always equivalent. For example, it is difficult to recognize that the death of hepatocytes from the immune reaction to the hepatitis B virus is a form of autodestruction imprinted in the hepatocyte genome. The exact terminology is still being debated. Generally speaking, programmed cell death refers to a type of cell death that depends on the expression of cellular genes, whereas the definition of apoptosis is based on morphological criteria. It is probable that not all programmed cell deaths are apoptotic and that not all apoptotic cell deaths are predetermined.

Characteristics of the apoptotic process

The morphological characteristics of apoptotic cells are a decrease in cell volume; condensation and fragmentation of the nuclei; and fragmentation of the cell into small bodies that remain surrounded by plasma membranes (Figure 1) (8). Not all apoptotic cells exhibit these morphological features. This fact makes it difficult to determine the number of apoptotic cells in tissue specimens. Furthermore, apoptotic bodies have a short lifespan because they are readily eliminated by macrophages and/or neighbouring cells (9). Some of the best pathological examples of apoptosis can be observed in cases of chronic hepatitis C and B (Figure 2).

Three important concepts underlie the molecular steps that occur in apoptotic cells. First, the process can be initiated by

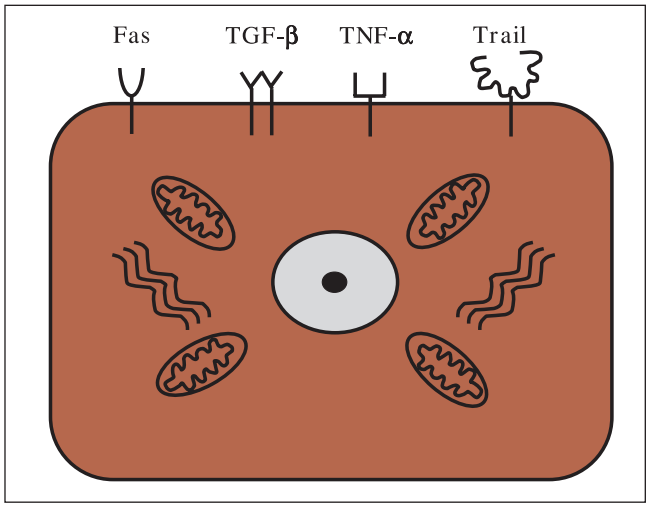

Figure 3) *Schematic view of the hepatocyte death receptors. All receptors are present at the plasma membrane. Activation of these receptors has been shown to induce hepatocyte apoptosis. TGF-β Transforming growth factor-beta; TNF-α Tumour necrosis factor-alpha*

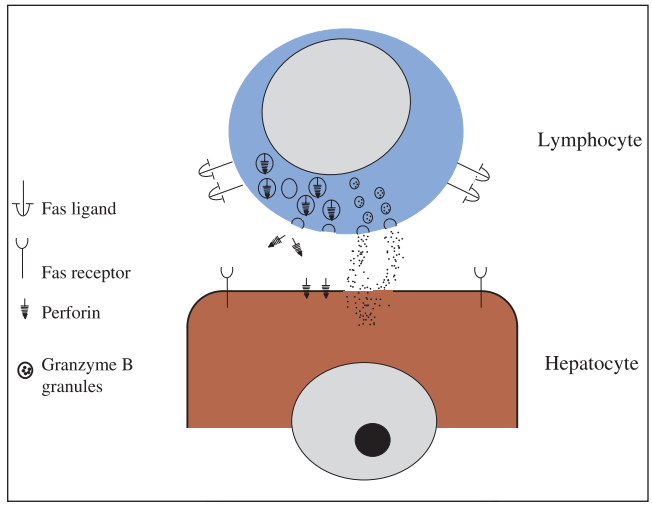

Figure 4) *Schematic representation of T cell-induced apoptosis. T cells can kill their target cells because they express Fas ligand on their surface and can elicit the Fas apoptotic pathway in Fas-expressing target cells. Alternatively, T cells can release perforins and granzyme B from their cytoplasmic granules. Perforin creates holes in the target cell membrane, which enable granzyme B to activate the apoptotic machinery inside the cell*

intracellular events or by extracellular signals (often transmitted through the activation of death receptors). Second, many of these signals are transmitted through the mitochondria, which have an important role in the regulation of apoptosis. Third, the execution of the apoptotic process depends on specific proteases that act in sequence. This is similar to the coagulation cascade, which is also driven by proteases.

Death receptors

It is somewhat surprising to learn that almost every cell possesses death receptors on its surface. Fortunately, the expression of these receptors differs among various tissues. Moreover, cells possess many antiapoptotic survival receptors that can block the transmission of the message derived from the activation of death receptors. Nevertheless, one must recognize that cell death occurs continuously throughout life. For example, there is a degree of cell turnover taking place in almost every tissue (eg, liver and gastrointestinal mucosa). This requires the organized loss of a definite number of cells, which is currently thought to occur by apoptosis (10,11). Figure 3 is a schematic representation of the hepatocyte death receptors.

T cell-induced apoptosis

A good example of an extracellular event that leads to apoptosis is the killing of target cells by activated T lymphocytes that have recognized foreign antigens expressed on the surface of an infected cell. The lymphocyte binds the target cell through specific receptors with the help of human leukocyte antigen molecules expressed by the target cell. The lymphocyte can deliver the 'kiss of death' by means of two distinct pathways (12). First, the Fas ligand, which is expressed on the surface of the T lymphocyte, can bind the Fas death receptor when it is expressed on the surface of the target cell. The Fas receptor is constitutively expressed on hepatocytes (13). This leads to the activation of the intracellular apoptotic cascade (see below). Alternatively, the lymphocyte can literally drill a hole in its target cell by using a protein that is appropriately named 'perforin'. Perforin is present in the secretory granules of activated lymphocytes. Following the action of perforin, other components of the secretory granules (called granzymes) enter the target cell and directly activate the apoptotic cascade, thereby circumventing the death receptor (Figure 4). One must recognize that in addition to these mechanisms, lymphocytes can also kill cells indirectly and nonspecifically by secreting apoptotic cytokines (eg, tumour necrosis factor alpha [TNF-α], interferon gamma).

The role of mitochondria

The importance of mitochondria in the apoptotic process was first suspected when Bcl-2 was found to be localized to the outer membrane of these organelles (14). Mitochondria possess a membrane that is rich in enzymes that play a major role in the oxygenation of cells (ie, for the genesis of ATP). The activity of these enzymes depends on the maintenance of an electrochemical gradient between the inner membrane and the mitochondrial matrix. Therefore, the intermitochondrial space needs to be isolated from the cell cytoplasm. Members of the Bcl-2 family govern the permeability of the outer membrane to ions (15). A number of other proteins are structurally similar to Bcl-2; they form the large family of Bcl-2-related proteins (16). Some of these are antiapoptotic (like Bcl-2 itself), whereas others promote apoptosis by binding Bcl-2 and blocking its activity at the outer mitochondrial membrane. When this occurs, the contents of the intermitochondrial space are released into the cytoplasm (Figure 5). At least three proteins thereby released are able to activate the downstream executioner caspases, leading to the actual events of apoptosis. This means that an apoptotic message can be transmitted through mitochondria, where it is either amplified or aborted, depending on the balance between anti- and proapoptotic factors (17). Hepatocytes, known to be rich in mitochondria, are a good example of cells that have a propensity for mitochondrial regulation of the apoptotic response. However, not all apoptotic signals are transmitted through mitochondria.

Caspases: The executioners of apoptosis

The intracellular execution of apoptosis is driven by a recently identified class of proteases. Proteases are enzymes whose func-

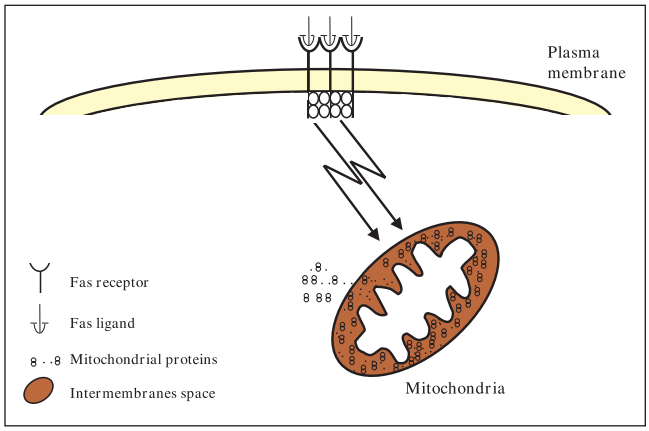

Figure 5) *Schematic drawing of the mitochondrial activation of apoptosis. Following activation of the death receptor (Fas), a signal is conveyed to mitochondria. This event causes the release of molecules normally present in the intermitochondrial space, leading to further activation of the apoptotic cascade*

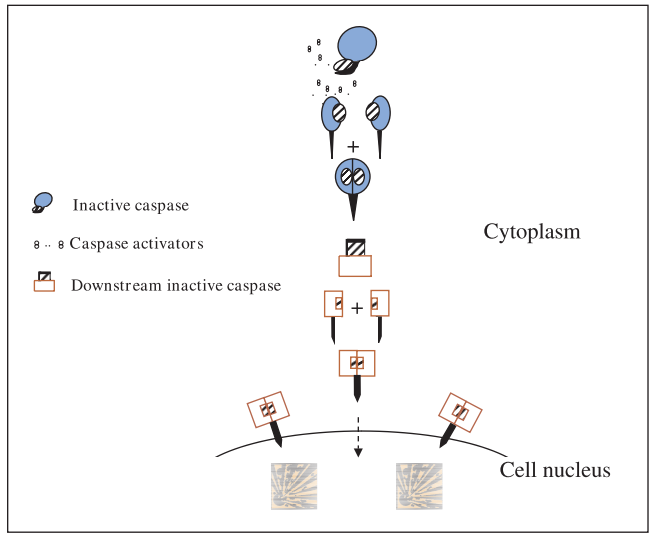

Figure 6) *The caspase cascade. An inactive caspase becomes activated after the release or the previous activation of caspase activators (in this case, the molecules released from the mitochondria). An active upstream caspase then activates an executioner caspase that targets the nuclear constituents, leading to the architectural characteristics of the apoptotic process (nuclear fragmentation and breakdown).*

tion is to cut proteins. Some proteases are nonspecific: they cut all sorts of proteins at a variety of cleavage sites. Such are the pancreatic enzymes, whose role is to render large proteins small enough to be absorbed by the intestinal mucosa. Other proteases are very specific and will cleave only proteins that have a particular shape or amino acid sequence. The latter group includes proteases involved in the apoptotic process. They have been given the name caspases because they are rich in Cysteine amino acids, they recognize a special target sequence that contains ASPartic acid and they are proteASES. Fourteen caspases have been identified so far and their roles have not all been well described (18). We know that they act in sequence, with one caspase activating another by cleavage of the inactive form called a procaspase (Figure 6). A group of caspases that are activated early in the process (upstream) are called activation caspases, whereas others that deliver the important structural hits of apoptosis are called executioner caspases (19).

Experimental models of hepatocyte apoptosis

There are a number of experimental models of hepatocyte apoptosis. Maybe the best known, and the one that has generated the widest interest, is the mouse model of massive liver apoptosis after the intraperitoneal injection of Fas antibodies (20). These antibodies bind to the Fas receptor and, instead of preventing its activation (as is the case for other antibodies such as the anti-TNF-α antibodies), they induce oligomerization of the Fas receptors and their consequent activation. When injected in vivo, this causes a rapid and massive liver injury characterized by overwhelming apoptosis. Other liver cells, such as endothelial cells, are also injured and there is some debate about the exact sequence of pathological events. Nevertheless, this model has been used to assess the capacity of agents to block hepatocyte apoptosis and has demonstrated the importance of the Fas system in this process.

TNF-α has been reported to induce hepatocyte apoptosis, but only in the presence of a transcriptional block (ie, an arrest of the synthesis of new messenger RNA) (21). Models of inflammatory liver disease, in which TNF-α levels are increased, have been assessed for the presence of apoptosis. Liver injury induced by endotoxin, lipopolysaccharide or concanavalin have all been shown to exhibit hepatocyte apoptosis (22-24).

In vitro, hepatocyte apoptosis has been reproducibly induced by exposure to chenodeoxycholate, a known hepatotoxic bile salt, in concentrations similar to those found in cholestatic disorders (25). However, as is often the case with toxic agents, there is a concentration beyond which necrosis becomes more prominent than apoptosis. This well-known observation has not yet been explained. A recent report suggests that the degree of ATP availability determines the mode of cell death (26).

Clinical importance of apoptosis in liver diseases

The relevance of studying apoptosis and of trying to change the apoptotic response in liver diseases depends on the actual occurrence of this type of cell death and its importance relative to other forms of cell death. This can be expected to vary widely depending on the etiology of the liver injury.

There is accumulating evidence that hepatocyte apoptosis is significant in alcoholic liver disease. Surgical specimens from patients with inactive alcoholic liver disease reveal increased numbers of acidophilic bodies, which are considered apoptotic (see below) (27). Apoptotic hepatocytes have been reported in liver biopsy material from patients with alcoholic hepatitis (28). There is experimental evidence that chronic ethanol feeding increases hepatocyte apoptosis in animals (29,30). Furthermore, Fas ligand upregulation has been described in the liver and the plasma in cases of alcohol-induced liver disease (31,32). Recent evidence has linked the upregulation of the Fas ligand with increased hepatocyte apoptosis (33).

Chronic viral hepatitis is probably the best example of a pathological process characterized by hepatocyte apoptosis. Liver pathologists now recognize that acidophilic bodies, which have long been identified in patients with viral hepatitis, represent apoptotic hepatocytes. The reported occurrence of apoptotic hepatocytes in hepatitis C liver biopsy samples varies depending on the method used to assess apoptosis (34-38). It is generally accepted that there is a correlation between the

intensity of histological inflammation and the degree of apoptosis. It is accepted that the immune response plays a key role in causing apoptosis during the course of viral hepatitis. It is known that activated T lymphocytes recognize viral antigens expressed on the cell surface of infected cells. As previously described, the presence of the Fas ligand on the surface of the lymphocyte can activate Fas receptors normally expressed on the hepatocyte plasma membrane. There are several reports describing an increased expression of Fas in human specimens of chronic hepatitis C and B (39-42). Lymphocytes can also use the perforin-granzyme pathway to deliver their death signal. On the other hand, the role of viruses in the induction of apoptosis is controversial. Some authors have demonstrated that HCV proteins can induce apoptosis, whereas others have postulated that these proteins have the capacity to block the apoptotic signal generated by cytokines (38,43-46). Thus far, experimental models of HCV infection or replication have not shown evidence of increased hepatocyte cell death.

Hepatocyte apoptosis has also been demonstrated in human cholestatic disorders. Bile salts can induce hepatocyte apoptosis in vitro, and the same process has been identified in an animal model of extrahepatic cholestasis induced by bile duct ligation (47). Hepatocyte and bile duct apoptosis have been reported in primary biliary cirrhosis (48-51). Finally, there is experimental evidence that part of the protective effect of ursodeoxycholic acid in these disorders is explained by its capacity to block hepatocyte apoptosis (52).

There has been some degree of controversy concerning the importance of apoptosis in drug-induced liver injury. Earlier studies reported evidence of DNA fragmentation in hepatocyte cultures exposed to toxic doses of acetaminophen (53), but most recent studies have concluded that oncotic necrosis is more important than apoptosis in this situation (54). There is some evidence that toxic mushroom poisoning (mediated by *Amanita phalloides* toxin) involves apoptosis (55). Indeed, amanitin is a transcriptional blocker (see above) and has been shown in vitro to cause all the characteristics of hepatocyte apoptosis. Besides these observations, there is experimental evidence that drugs can trigger hepatocyte apoptosis by directly activating the caspase cascade or by sensitizing hepatocytes to the lethal effects of cytokines (56). However, there have been few pathological studies assessing the amount of hepatocyte apoptosis in biopsy samples of drug-induced liver injury.

It has been suggested that much of the ischemia-reperfusion injury that occurs early after liver transplantation is due to hepatocyte apoptosis. Antiapoptotic agents that would allow better and longer preservation of the liver have been sought in animal models. However, whether apoptosis or necrosis predominates in this setting has not been resolved (57).

It has long been suggested that carcinogenesis is due to an imbalance between cell proliferation and cell death (1,58). Most studies have shown that the amount of apoptosis in human liver tumours decreases as the degree of dedifferentiation increases (59-62). A more recent hypothesis is that cancer cells can display the capacity to kill immune cells that have been deployed to eliminate them. Experimental evidence for this hypothesis has been obtained in studies using liver tumour cell lines (63). Nevertheless, formal in vivo evidence of this phenomenon is still lacking.

PERSPECTIVES

The current goals of research done in this field are to further elucidate the mechanisms governing the cellular response to apoptotic stimuli. A number of intracellular pathways are involved. The manipulation of these intracellular responses may alter the course of liver diseases. For example, liver function could be maintained in patients with cholestatic disorders with the inhibition of hepatocyte apoptosis. It is not always possible to address the underlying cause of liver disease (eg, in cases of autoimmune or chronic viral hepatitis); therefore, finding ways to make hepatocytes resistant to apoptotic signals may be useful in mitigating the liver injury.

Several drug companies have already developed vast research programs targeted at apoptosis. A number of nonspecific apoptosis inhibitors are already used in experimental models. In addition to their lack of specificity, these compounds exhibit poor pharmacodynamic properties. Caspase inhibitors are effective at the molecular level, but are unable to prevent cell death that occurs through other undefined mechanisms. Inhibitors of apoptosis might be useful if administered before a nonrecurrent, predictable event (eg, during an episode of acute hypotension) to prevent tissue injury. However, the real challenge will be to develop compounds that can be used chronically to combat specific types of cell injury, such as that seen in chronic viral hepatitis. The obvious risk of the long term use of such drugs is that abnormal cells would obtain a survival advantage. Indeed, the future is full of hope, but we should not forget that unexpected consequences typically occur when we make changes to our environment.

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