Apoptosis: A Friend Or Foe?
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Citation

Abstract
Over the past several years, a growing number of researchers have become fascinated with apoptosis, a form of programmed cell death, and it is now widely accepted that this physiological cell death is a fundamental feature of life. The reason why apoptosis research has experienced such a rapid development relates to its biological relevance. Apoptosis is a physiological way for nucleated cells to die. Apoptosis takes care of unwanted, injured, or virus-infected cells. Auto reactive T and B cells, millions of which are produced by the immune system every day, are also eliminated by apoptosis. Recently, dysregulation ("too much or too little") of apoptosis has emerged as a new concept to explain important features in the development of several as yet poorly understood diseases. Unregulated excessive apoptosis may be the cause of various degenerative and autoimmune diseases that are characterized by an excessive loss of normal or protective cells, such as in multiple sclerosis, type-I diabetes mellitus, Hashimoto thyroiditis, Sjögren syndrome, and certain cancers such as melanoma. Conversely, an inappropriately low rate of apoptosis may promote survival and accumulation of abnormal cells that can give rise to tumor formation and prolonged autoimmune stimulation such as in cancers and Graves disease. With the dawning of the 'age of apoptosis', substantial progress has been made in understanding the molecular cell biological mechanisms that underlie the initiation, execution and regulation of this cell death program. It seems likely that pro- and anti-apoptotic factors determine either susceptibility or resistance to apoptosis, and, consequently, play a crucial role in the evolution, propagation, and chronicity of degenerative, cancerous, and autoimmune conditions. Thus, precise identification of the distinct errors in the complex apoptotic machinery holds great promise for elucidating the pathogenesis of various important diseases and for devising more specific and effective treatments.

INTRODUCTION
Control of cell proliferation, differentiation, activation and cell removal is crucial for the development and existence of multicellular organisms. Each cell cycle progression, with sequences of DNA replication, mitosis, and cell division, is a tightly controlled and complicated process that, when deregulated, may become dangerous not only to a single cell, but also to the whole organism. Regulation and the proper control of the cell cycle and of programmed cell death are therefore essential for mammalian development and the homeostasis of the immune system. The molecular networks that regulate these processes are critical targets for drug development, gene therapy, and metabolic engineering. In addition to the primary, intracellular apoptotic suicide machinery, components of the immune system can detect and remove cells and tissue fragments that no longer serve their defined functions.

There are two major mechanisms of cell death-necrosis and apoptosis. Cells that are damaged by external injury undergo necrosis, while cells that are induced to commit programmed suicide because of internal or external stimuli undergo apoptosis. Apoptosis, derived from the Greek word for a natural process of leaves falling from trees. Apoptosis, or programmed cell death, is a major control mechanism by which cells die if DNA damage is not repaired. Apoptosis is also important in controlling cell number and proliferation as part of normal development.

The apoptosis process can be divided into at least three functionally distinct phases: initiation, effector and degradation. During the heterogeneous initiation phase, cells receive death-inducing signals: lack of obligatory survival factors, shortage of metabolite supply, ligation of death-signal transmitting receptors, subnecrotic damage by toxins, heat or irradiation. During the effector phase, these signals are translated into metabolic reactions and the decision to die is taken. The ultimate fate of the cell is subjected to regulatory events.

Biochemically, apoptotic cells are characterized by reduction in the mitochondrial transmembrane potential (MTP), intracellular acidification, production of reactive oxygen
species, externalization of phosphatidylserine residues in membrane bilayers, selective proteolysis of a subset of cellular proteins and degradation of DNA into internucleosomal fragments. Apoptosis or programmed cell death is, unlike necrosis, a highly regulated and energy requiring process. Apoptosis is characterized by shrinkage of the cell and the nucleus. The nuclear chromatin is condensed into sharply delineated masses, and eventually breaks up. The cell then detaches from the surrounding tissue. At this stage, extensions bud out from its membrane, which eventually seals off to form membrane enclosed vesicles, called apoptotic bodies, containing condensed cellular organelles and nuclear fragments. These apoptotic bodies are either rapidly phagocytosed by neighboring cells or undergo degradation, which resembles necrosis in a process called secondary necrosis. However, apoptosis is generally considered not to trigger an inflammatory response (1). While necrosis is characterized by the rapid loss of cellular homeostasis, rapid swelling as a result of the accumulation of water and electrolytes, early plasma membrane rupture, and the disruption of cellular organelles. As a result of the membrane rupture and subsequent leakage of a broad array of cellular material, necrosis induces an inflammatory response (2,3).

This concept of cell suicide has gained increasing interest in cytology and pathology. In terms of tissue kinetics, apoptosis may be considered a mechanism that counterbalances the effect of cell proliferation by mitotic division. In fact, deregulated apoptosis has been implicated as a fundamental pathogenetic mechanism in a variety of human diseases. Excessive apoptotic cell death may cause organ atrophy and organ failure, as suggested for neurodegenerative diseases and viral hepatitis. On the other hand, inefficient elimination of malignant, auto reactive, infected, or redundant cells may lead to the development of neoplasia, autoimmunity, viral persistence, and congenital malformations.

**APOTOPSIS PATHWAY**

Apoptosis occurs through two main pathways. The first, referred to as the extrinsic or cytoplasmic pathway, is triggered through the Fas death receptor, a member of the tumor necrosis factor (TNF) receptor superfamily (4). The second pathway is the intrinsic or mitochondrial pathway that when stimulated leads to the release of cytochrome-c from the mitochondria and activation of the death signal (5). Both pathways converge to a final common pathway involving the activation of a cascade of proteases called caspases (cysteine-requiring aspartate-directed proteases) that cleave regulatory and structural molecules, culminating in the death of the cell.

**EXTRINSIC PATHWAY**

This pathway comprises several protein members including the death receptors, the membrane-bound Fas ligand, the Fas complexes, the Fas-associated death domain, and caspases 8 and 10, which ultimately activate the rest of the downstream caspases leading to apoptosis. Binding of Fas ligand with death receptors (DRs) activates extrinsic pathway. Fas is a member of the tumor necrosis factor receptor superfamily and is also called Apo-1 or CD95. Other TNF receptors include TNF R1, DR3 (Apo 2), DR4 (tumor necrosis factor-related apoptosis-inducing ligand receptor 1 [TRAIL R1]), DR5 (TRAIL R2), and DR6 (5). Fas signaling plays an important role in immune surveillance of transformed or virus-infected cells and in the removal of self-reactive lymphocytes. Therefore, defects in this pathway have been implicated in many malignancies and autoimmune diseases (5,6). The Fas death-inducing signaling complex contains the adaptor protein Fas-associated death domain protein and caspases 8 and 10 and leads to activation of caspase 8, which in turn can activate the rest of the downstream caspases.

**INTRINSIC PATHWAY**

One of the most important regulators of this pathway is the Bcl-2 family. The bcl-2 gene was originally identified at the chromosomal breakpoint of the translocation of chromosome 18 to 14 in follicular non-Hodgkin lymphoma (NHL) (7). The Bcl-2 family includes proapoptotic members such as Bax, Bak, Bad, Bcl-Xs, Bid, Bik, Bim, and Hrk, and antia apoptotic members such Bcl-2, Bcl-XL, Bcl-W, Bfl-1, and Mcl-1 (8). Antiapoptotic Bcl-2 members act as repressors of apoptosis by blocking the release of cytochrome-c, whereas proapoptotic members act as promoters. These effects are more dependent on the balance between Bcl-2 and Bax than on Bcl-2 quantity alone (8).

The Bcl-2 is overexpressed in many malignancies. Increased expression of Bcl-2 causes resistance to chemotherapeutic drugs and radiation therapy, while decreasing Bcl-2 expression may promote apoptotic responses to anticancer drugs. In addition, overexpression of Bcl-2 may result in accumulation of cells in the G0 phase of cell cycle division and contribute to chemoresistance (9).

In response to apoptotic stimuli, the outer mitochondrial membrane becomes permeable, leading to the release of
Cytochrome-c, once released in the cytosol, interacts with Apaf-1 (Apoptotic protease-activating factor-1), leading to the activation of caspase-9 proenzymes. Active caspase-9 then activates caspase-3, which subsequently activates the rest of the caspase cascade and leads to apoptosis. Activated caspases lead to the cleavage of nuclear lamin and breakdown of the nucleus through caspase-3.

**Caspases (Final Pathway)**

The final pathway that leads to execution of the death signal is the activation of a series of proteases termed caspases. Caspases are an endogenous family of intracellular cysteine proteases that participate in critical steps of the apoptotic cascade in numerous pathologic processes. Not all caspases are involved in apoptosis. The caspases that have been well described are caspases-3, -6, -7, -8, and -9. The intrinsic and extrinsic apoptotic pathways converge to caspase-3, which cleaves the inhibitor of the caspase-activated deoxyribonuclease, and it becomes active leading to nuclear apoptosis. The upstream caspases that converge to caspase-3 are caspases-9 and -8 in the intrinsic and extrinsic pathways, respectively. The downstream caspases induce cleavage of protein kinases, cytoskeletal proteins, DNA repair proteins, and finally, destruction of “housekeeping” cellular functions. Caspases also affect cytoskeletal structure, cell cycle regulation, and signaling pathways, ultimately leading to the morphologic manifestations of apoptosis, such as DNA condensation and fragmentation, and membrane blebbing.

**Regulators of the Apoptosis Pathway**

**P53**

p53 functions as a transcription factor regulating downstream genes important in cell cycle arrest, DNA repair, and apoptosis. The critical role that p53 plays is evident by the large number of tumors that bear a mutation in this gene. Loss of p53 in many cancers leads to genomic instability, impaired cell cycle regulation, and inhibition of apoptosis. After DNA damage, p53 holds the cell at a checkpoint until the damage is repaired. If the damage is irreversible, apoptosis is triggered.

**NFκB**

NFκB is a nuclear transcription factor that regulates expression of a large number of genes involved in the regulation of apoptosis, viral replication, tumorigenesis, inflammation, and many autoimmune diseases. NFκB is activated by a variety of stimuli that include growth factors, cytokines, lymphokines, radiation, pharmacologic agents, and stress. In its inactive form, NFκB is sequestered in the cytoplasm, bound inhibitor proteins of the IκB (inhibitor of NFκB) family. The various stimuli that activate NFκB cause phosphorylation of IκB, which is followed by its degradation. This results in exposure of the nuclear localization signals on NFκB subunits and the subsequent translocation of the molecule to the nucleus. In the nucleus, NFκB binds with the consensus sequence of various genes and thus activates their transcription. NFκB has been shown to have both anti- and proapoptotic functions that may be determined by the nature of the death stimulus rather than by the origin of the tissue. Under physiologic conditions, the activation of NFκB induces resistance to apoptotic stimuli through the activation of many complex proteins including TNF receptor-associated factor, IAP (Inhibitor of Apoptotic Protein), and X-linked IAP. However, in response to certain stimuli, NFκB activation may lead to induction of apoptosis.

**Figure 1**

<table>
<thead>
<tr>
<th>Too much</th>
<th>Too little</th>
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<tbody>
<tr>
<td>AIDS</td>
<td>Canavan-Smith syndrome (CSS, autosomal recessive syndrome)</td>
</tr>
<tr>
<td>Cancers (e.g. melanoma, hepatoma colon cancer)</td>
<td>Lymphoma</td>
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<tr>
<td>Liver failure</td>
<td>Leukemia</td>
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<tr>
<td>Wilson disease</td>
<td>Solid tumors</td>
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<tr>
<td>Myelodysplastic syndromes, Multiple myeloma, Neurodegenerative diseases, Ulcerative colitis, Diabetes mellitus, Acute anemia, Chronic neutropenia</td>
<td>Autoimmune diseases (e.g. inflammatory bowel syndrome, lupus erythematosus, rheumatoid arthritis, Hashimoto thyroiditis, Graves disease)</td>
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Listed are diseases in which dysregulation of apoptosis has been shown.
APOPTOSIS INDUCERS

Most chemotherapeutic agents presently used for cancer treatment were developed by screening in a growth inhibition assay for chemical substances, which inhibit the growth of neoplastic cells. Inherent to the growth inhibition assay, agents active in this screen fall into two general classes, cytostatic and cytotoxic, the latter falling into two modes of action, necrotic and apoptotic. Subsequently, it was determined that the resultant clinically useful cytotoxic agents primarily act by inducing apoptosis in cancer cells \((16,17,18)\). The principal pro-apoptotic chemotherapeutic agents used for both childhood and adult cancers target tubulin (taxanes like Taxol, Taxotere, and Vinca alkaloids consisting of vincristine, vinblastine). The limitations for their widespread use are the emergence of drug-resistant tumor cells as well as dose-limiting levels of neurologic and bone marrow toxicity. A subclass of tubulin inhibitors was shown to preferentially target tumor endothelial cells while sparing the normal vasculature. These compounds, called vascular targeting agents, act by disrupting the tumor vasculature by targeting endothelial cells \((19,20)\). Two such agents, combretastatin A-4 phosphate prodrug (CA-4P) and ZD6126, are undergoing clinical trials \((21,22)\). Novel and synthetic compounds that induce apoptosis in cancer cells targeting the clinically validated tubulin/microtubule system as well as lacking neurotoxicity and retaining activity in multidrug-resistant tumors remain compelling for drug discovery in oncology \((21,22,23)\). It has been reported that MX-116407 possesses anti-tumor activity by disrupting tubulin. It has also been reported that this compound shows a time dependent block in the G2-M phase followed by a decrease in mitotic cells and an increase in apoptotic cells, suggesting a mitotic catastrophe as the cause for apoptosis.

APOPTOSIS INHIBITORS

The inhibitor of apoptosis (IAP) family of proteins prevent cell death by binding to and inhibiting active caspases and are negatively regulated by IAP-binding proteins, such as the mammalian protein DIABLO/Smac. The inhibitor of apoptosis proteins (IAPs) were originally identified in baculoviruses, where they provide a mechanism for enhancing viral propagation through inhibition of defensive apoptosis by host insect cells \((26,27)\). The cIAP-1 and cIAP-2 proteins were initially identified in a complex with TNF receptor 2, an indirect association resulting from direct interaction with TNF-receptor-associated factors (TRAFs) 1 and 2 involving the BIR and TRAF domains of the respective proteins \((28)\). The expression of cIAP-1 and cIAP-2 is increased following activation of the NF-\(\kappa\)B transcription factor by the TNF receptor, and these IAPs may have a role in protecting cells from TNF-induced apoptosis by reducing the amount of caspase 8 activation \((29)\).

APOPTOSIS IN LIVER DISEASES

The liver is exposed to many potentially harmful agents that include pathogens, toxins, tumor cells and dietary antigens. Amongst the hepatitis viruses, only hepatitis B virus (HBV)
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and hepatitis C virus (HCV) cause chronic hepatitis, this can progress to cirrhosis and hepatocellular carcinoma. Under normal conditions, they do not damage the liver cells due to the protective mechanisms and the large repair capacity of hepatocytes. Both cell proliferation and apoptosis are required for proper development of the biliary tree and parenchyma of the liver (s6). During acute and chronic liver diseases, hepatocytes are exposed to increased levels of cytokines like tumor necrosis factor-alpha (TNF), interleukin-1-alpha (IL-1α) and interferon-gamma (IFN-γ), oxidative stress and bile acids (s1). Although hepatocytes have an abundant capacity to defend themselves against these agents, excessive exposure will lead to cell death. Hepatic cell death occurs in both acute and chronic liver diseases. Therefore deep insight into the cellular mechanisms leading to cell death is of utmost importance in understanding the pathophysiology of liver diseases.

Apoptotic cell death of hepatocytes emerges as a fundamental component of virtually all acute and chronic liver diseases. The liver tissue repair, inflammation, regeneration, and fibrosis may all be triggered by apoptosis (s1,s2,s3,s4). Hepatocytes apoptosis can be induced through the death receptor-dependent pathway (extrinsic pathway) or the mitochondrial dependent pathway (intrinsic pathway). The death receptor dependent pathway is initiated in the liver by death ligands like TNF, Fas ligand (FasL, CD95L), and tumor necrosis factor-related apoptosis-inducing ligand (TRAIL), following their binding to their relevant death receptors. Among them, TNF and Fas ligand are being considered as the major players and thus they have been extensively studied.

TNF is a pleiotropic proinflammatory cytokine produced largely by activated macrophages and in smaller amounts by several other types of cells. It exerts a variety of effects that are mediated by TNF-receptor 1 and 2 (TNFR-I and TNFR-II). The apoptotic effects are only mediated by TNFR-I, whereas TNFR-II may serve to potentiate the effects of TNFR-I in promoting cell death or promoting inflammation (s5).

TNF initiates apoptosis in hepatocytes by activating different pathways, whose subsequent activation leads to liver injury. As discussed above, the main apoptotic effects of TNF are mediated by TNFR-I. Following the TNFR-I ligation, the TRADD adaptor molecule is recruited by the death domain to form the first protein complex (Complex I), which also includes TRAF2 (s5). This complex then dissociates from TNFR-I and forms a different complex in the cytosol (called Complex II). Complex II includes FADD, c-FLIP, cIAP1/2, TRAF2 and caspase-8 (s5). The anti-apoptotic Bcl-2 family proteins, such as Bcl-2 and Bcl-XL, inhibit the mitochondrial death pathway, whereas pro-apoptotic Bcl-2 family proteins, such as Bid, Bax and Bak, promote it (s6). Caspase-8 can cleave Bid. Mitochondrial permeability transition (MPT), an important regulatory mechanism for cytochrome c release, is also induced by TNF in hepatocytes with a strong dependence on Bid (s7,s8). The MPT occurs due to the opening of MPT pores, which are highly conductive to solutes with a molecular weight up to approximately 1.5 kDa (s8). As a consequence, mitochondria depolarize and the MPT can contribute to the release of apoptogenic proteins from the intermembrane space. Furthermore, activation of MPT can also lead to the generation of reactive oxygen species (s9), which may in turn further enhance the MPT (s8).

Suppression of MPT with cyclosporin A alone or in conjunction with its enhancer, trifluoperazine or aristolochic acid could lead to reduction of TNF induced cytochrome c release, caspase activation and hepatocytes apoptosis (s9). These findings on the TNF-induced mitochondria apoptotic pathway may provide a viable strategy for the treatment of liver diseases.

Fas is activated through oligomerization upon binding of FasL or the agonistic anti-Fas antibody. This causes formation of the death-inducing signaling complex (DISC), and the activation of downstream death signal pathway, the caspase cascade becomes activated (s10). Fas is ubiquitously expressed in various organs, including the thymus, liver, heart and kidney (s1).

The strict regulation of apoptotic cell death and survival pathways allows the development of therapeutic intervention strategies. In acute liver injury, inhibition of apoptosis of hepatocytes may be beneficial. Targets for anti-apoptotic interventions include caspases, through endogenous or exogenous caspase inhibitors, and preservation of mitochondrial integrity via antiapoptotic Bcl-2 family members. Anti-apoptotic Bcl-2 family members like Bcl-2, Bcl-XL or A1/Bfl -1 prevent the activation of the mitochondrial/apoptosome death pathway, which is activated in hepatocytes by many noxious stimuli. Among the members of the Bcl-2 family, A1/Bfl -1, an NF-kB-regulated gene, appears to be important for hepatocyte survival. It blocks hepatocyte cell death by inhibiting the mitochondrial/apoptosome death pathway and thus the activation of the caspase cascade (s10).
So, in summary liver is one of the largest organs in the body. Among other functions it serves as an interface that processes absorbed nutrients into chemicals that are nontoxic for the organism and can safely be utilized by other tissues and organs. It also plays an important role as a neutralizer of exo- and endotoxins. Thus, the organism cannot function without an intact liver for a prolonged time. Therefore, beside liver transplantation, several pharmacological approaches are under development that either target pathologic processes like apoptosis in the liver.

**APOPTOSIS AND MYOCARDIAL INFARCTION**

The common view on how cardiomyocytes die during or after myocardial infarction has altered in recent years. For a long time necrosis was regarded as the sole cause of cell death in myocardial infarction. Now, recent studies indicate that apoptosis also plays a role in the process of tissue damage subsequent to myocardial infarction. Although both necrosis and apoptosis result in the death of the cell, they differ in several morphological and cellular regulatory features. The fact that apoptosis plays a role in the tissue damage seen after myocardial infarction has pathological and therapeutic implications. Because apoptosis is a highly regulated process, a better understanding of the circumstances that specifically trigger apoptosis during and after myocardial infarction, and a better understanding of the cellular mechanisms that control apoptosis, could lead to therapeutic strategies to limit the amount of tissue damage in patients with myocardial infarction.

It has been found that upon permanent occlusion of a coronary vessel in rats, apoptosis occurred in the ischemic region, the area immediately bordering the ischemic region and in the remote from ischemia region. Therefore, it has been suggested that apoptosis is the major determinant of infarct size. Necrosis occurred less often and was seen only in the ischemic region. In a similar experiment in rats, it has been shown that apoptosis occurs after constant ischemia. However, they found that apoptosis appeared solely in the ischemic myocardium, not in the bordering or remote from ischemia regions. Although reperfusion after 45 minutes of ischemia appeared to attenuate apoptosis in the infarcted area, it enhanced apoptosis in the bordering zones and the remote from ischemic regions.

C5a receptor activation activates Ras. Furthermore, C5b-9 can induce significant changes in intracellular calcium fluxes and production of oxygen-derived free radicals. Because the mechanisms of apoptosis induction during MI/R may also involve several of these complement signaling events, the terminal complement components (ie, C5-C9) are well suited to provide a molecular switch for MI/R-induced apoptosis. It has been demonstrated that inhibition of rat C5 in vivo dramatically attenuated MI/R-induced apoptosis. These data suggest that the terminal complement components play an important role not only in MI/R-induced necrosis but in apoptosis as well. Furthermore, these data suggest that anti-C5 therapy in humans may attenuate complement-dependent apoptosis. Future studies on the mechanism of complement-induced apoptosis are warranted.

Increased oxidative stress was found to coexist with apoptosis in the remote non-infarcted rat myocardium after myocardial infarction. Long term treatment with the antioxidants probucol and pyrrolidine dithiocarbamate, starting three days after MI in an in vivo rat ischaemia/reperfusion model, attenuates oxidative stress, myocyte apoptosis, caspase 3 activity, and the expression of p53, Bax, and caspase 3 protein in the remote non-infarcted myocardium. These findings indicate a causal relation between oxidative stress and apoptosis in the remote non-infarcted rat myocardium after MI.

Ischaemic preconditioning is a phenomenon whereby a series of brief periods of alternating ischaemia and reperfusion increase myocardial tolerance to the subsequent prolonged ischaemia. It has been shown that ischaemic preconditioning in rats caused a significant reduction in the amount of apoptosis and infarct size. However, the exact mechanisms by which ischaemic preconditioning causes this cardioprotective effect are still unclear. A correlative effect of reduced myocardial apoptosis, reduced Bax expression, increased bcl-2 expression, and reduced neutrophil accumulation in ischaemic preconditioning experiments in rats and dogs has been suggested. Ischaemic preconditioning also seems to attenuate the ischaemia/reperfusion induced activity of caspase 1 and caspase 3 in rats.

Insulin-like growth factor I (IGF-I) appears to be cardioprotective in a rat in vivo ischaemia/reperfusion model. When administered one hour before ischaemia, IGF-I attenuates polymorphonuclear neutrophil accumulation in the ischaemic area. Furthermore, IGF-I significantly attenuates the incidence of myocyte apoptosis after myocardial ischaemia and reperfusion. In addition, in transgenic mice overexpressing human IGF-I, it was found...
that both apoptosis and necrosis were attenuated in the viable myocardium after infarction ($a_0$).

Interestingly, IGF-I also promotes the release of physiological amounts of nitric oxide (NO) ($a_0$). In an in vivo mouse model it has been shown that the inhibition of endogenous NO synthesis increases apoptosis during ischaemia ($a_1$). It seems that endogenous NO suppresses apoptosis by interfering with the caspase cascade, because the inhibition of endogenous NO correlated with increased caspase activity, whereas bcl-2 and Bax protein values were unchanged ($a_3$).

In conclusion, numerous studies indicate that apoptosis can cause cardiomyocyte cell death after myocardial infarction, although the exact mechanism of apoptosis within the heart is not known and the interpretation of studies is somewhat difficult because of the different methods used to determine apoptosis.

**APOPTOSIS AND LUNGS**

Evidence that apoptosis plays an important role in the pathophysiology of lung diseases has been accumulated. Apoptosis may play important roles in lung diseases in two different ways. First, failure to clear unwanted cells by apoptosis will prolong the inflammation because of the release of their toxic contents, and also delay repair processes. Apoptotic cells should be quickly recognized and ingested by phagocytes before releasing their toxic contents, unlike accidental cell death or necrosis. Second, excessive apoptosis may cause diseases. The epithelial barrier represents a critical line of defense against the environment, so airway epithelial cells are likely designed to be refractory to a number of potentially apoptotic stimuli, including potent death-receptor activators such as tumor necrosis factor-$\alpha$ and Fas ligand. The relative resistance of airway epithelial cells to apoptosis is likely helpful in maintaining the integrity of the epithelial barrier during an inflammatory response, when immune cells, which express or secrete these death-receptor ligands, are trafficking through the lung.

As well as death receptors/ligands, death signals such as reactive oxygen species, nitrogen species, proinflammatory cytokines, chemokines and other signaling molecules of apoptosis are involved in the pathophysiology of lung diseases. The survival and recovery of epithelial and endothelial cells and the resolution of inflammatory cells appear to be the key in the prognosis of patients. Therefore, further understanding of molecular mechanisms of apoptosis and its regulation by novel drugs may lead to the development of effective strategies against lung diseases. It has been reported that the expression of Bax and Bcl-2 protein are upregulated in alveolar epithelial cells and the number of epithelial cell apoptosis is associated with the prognosis of patients with diffuse alveolar damage ($a_{10}$).

Repair after an acute lung injury requires the elimination of proliferating mesenchymal and inflammatory cells from the alveolar airspace or alveolar wall ($a_5$). Neutrophils play an important role in endothelial and epithelial damage in lung injury. Clearance of apoptotic neutrophils by phagocytes has an important role in the resolution of inflammation and lung injury ($a_5$). Phagocytosis of apoptotic neutrophils by macrophages suppresses the production of proinflammatory cytokines, such as interleukin (IL)-1, IL-8, Granulocyte macrophage-colony stimulating factor (GMCSF), and tumor necrosis factor (TNF-$\alpha$) by macrophages, and induces the production of transforming growth factor-$\beta$ (TGF-$\beta$) and hepatocyte growth factor (HGF) to regenerate damaged tissues ($a_5$). Accordingly, the insufficiency of this system including the appropriate rate of neutrophil apoptosis, the clearance of apoptotic neutrophils by phagocytosis, and the cytokine release by phagocytes may lead to the prolongation of inflammation and the impairment of repair. It has been suggested that IL-6 attenuates hyperoxic lung injury and this protection is associated with a marked diminution in hyperoxic cell death probably through the induction of bcl-2 and tissue inhibitor of metalloproteinase (TIMP)-1 ($a_5$).

Reactive oxygen species (ROS) induce apoptosis in lung endothelial and epithelial cells. Apoptosis plays a central role in DNA damage during the pathogenesis of hyperoxic lung injury ($a_5$). Hydrogen peroxide induces Fas upregulation by promoting cytoplasmic transport of Fas to the cell surface in human airway epithelial cells. Exaggerated apoptosis through Fas mediated signaling may accelerate hyperoxia-induced acute lung injury in Legionella pneumonia ($a_5$). Hyperoxia, by virtue of activating NADPH oxidase, generates ROS, which mediates cell death of lung epithelium via ERK1/2 MAPK activation in lung epithelial cells ($a_5$). In response to this theory, strategies to reduce oxidation may be beneficial in reducing lung epithelial cell damage.

Promotion of inflammatory cell apoptosis and protection of parenchymal cells from cell death may be an effective therapeutic strategy against inflammatory lung diseases. Once parenchymal cells are damaged, accelerating the repair and regeneration in damaged tissues could also be an effective treatment. However, when parenchymal cells are...
severely damaged, rescue of these cells may lead to carcinogenesis. To avoid this problem, inhibiting apoptosis at early stage may be an effective strategy. The accumulating evidence concerning the apoptosis-signaling molecules may lead to novel treatment.

**APOPTOSIS AND CANCER**

The ultimate goal of cytotoxic therapies is to induce death of tumour cells. Drug resistance is the principal factor limiting the efficacy of chemotherapy used in the treatment of cancer. A frustrating property of such acquired resistance is that the tumours not only become resistant to the drugs specifically used in the treatment regime, but can also become cross-resistant to other drugs with different mechanisms of action (75). Drug resistance, whether intrinsic or acquired, is believed to cause treatment failure in more than 90% of patients with metastatic cancer. Cancer-associated defects in apoptosis are vital to the development of resistance to chemotherapy and radiotherapy (76). Essentially all cytotoxic anticancer drugs that are currently in clinical use — for example, microtubule-binding drugs, DNA-damaging agents and nucleosides — induce apoptosis of malignant cells (including cisplatin, 5-flourouracil, doxorubicin, docetaxel, fludarabine, cyclophosphamide, dacarbazine and dexamethasone (77). Although these conventional drugs are important weapons in the treatment of cancer, new classes of targeted therapeutics are emerging that are based on strategies that rely on a deeper understanding of the molecular mechanisms that underlie the phenomenon of apoptosis. In view of this, strategies aimed at inhibiting the expression or function of anti-apoptotic proteins have gained considerable attention (78,79).

The understanding of apoptosis has provided the basis for novel targeted therapies that can induce death in cancer cells or sensitize them to establish cytotoxic agents and radiation therapy. These novel agents include those targeting the extrinsic pathway such as tumor necrosis factor-related apoptosis-inducing ligand receptor 1, and those targeting the intrinsic Bcl-2 family pathway such as antisense bcl-2 oligonucleotides. Many pathways and proteins control the apoptosis machinery. Examples include p53, the nuclear factor kappa B, the phosphatidylinositol 3-kinase (PI3K)/Akt leading to defects in apoptosis (80).

Death receptors have been pursued as potential targets for cancer therapy for many years. TNF, Fas, and Fas L have extensive in vitro antitumor activity and have been utilized as potential therapeutic targets in vivo. Unfortunately, they were also found to activate nonspecific TNF receptors resulting in extensive ischemic and hemorrhagic lesions in several tissues leading to septic shock and fulminant hepatic failure in animal models (81,82).

TRAIL, or APO2L, has been introduced as an extrinsic pathway inducer that does not have the toxicities of Fas and TNF. TRAIL induces apoptosis in a variety of tumor cell types and suppresses the growth of colon and breast xenografts. Synergistic antitumor effects are also seen when combined with chemotherapy or radiation (83,84). TRAIL was found to induce apoptosis in human hepatocytes (85) and in human brain cells in vitro; however, it did not lead to apoptosis in the brain of animals in preclinical studies (86). Preclinical testing of TRAIL in combination with conventional chemotherapeutic agents such as doxorubicin has demonstrated significant inhibition of tumor growth in a prostate cancer in vivo model (87).

Lonidamine is a derivative of indazole-3-carboxylic acid that acts on the mitochondria to induce apoptosis through the disruption of the intrinsic transmembrane potential. It has a potent antiproliferative effect on neoplastic cells by inhibiting oxygen consumption and interfering with the energy metabolism of neoplastic cells (88). This drug has been shown to potentiate the cytotoxic effects of chemotherapeutic agents, especially anthracyclines in human breast cancer cell lines. It also potentiates radiotherapy. The most frequent toxicities are gastrointestinal and hematologic side effects. A Phase II trial of lonidamide in combination with epirubicin and cisplatin as a first line therapy for metastatic breast cancer has shown an overall response rate of 73% with 13% complete response (89). Bortezomib is a boronic acid inhibitor that selectively and potently inhibits chymotryptic threonine protease activity, the rate-limiting
proteolytic step in the proteosome. In vitro and mouse xenograft studies of bortezomib have shown antitumor activity in a broad range of tumor types including myeloma, CLL, prostate cancer, pancreatic cancer, and colon cancer (\textit{gi50}Ki).

Increased understanding of the molecular genetic defects and the regulation of the complex signaling pathways in tumors, especially the regulation of apoptosis, can result in rationally designed anticancer strategies. Our knowledge of the intrinsic and extrinsic apoptotic pathways and the other signaling modulators such as the p53, proteosome/ubiquitin system, NF\textsubscript{K}B, and the PI3K/Akt pathways have led to the discovery of many novel agents that are showing effectiveness whether as single agents or in combination with conventional cytotoxic therapy or radiation. Further understanding of the different signaling pathways that control apoptosis in the different tumor types will help with the discovery of novel targeted agents and the design of clinical trials that are based on the molecular defects their numbers so as to provide appropriate innervation of their targets. It has since become a common theme that aberrant neuronal apoptosis may also occur as a feature of disease processes of the adult brain. Consequently, it has been suggested that blocking apoptosis may be a treatment specific to the targeted tumor.

**APOPTOSIS AND NEURODEGENERATIVE DISEASES**

In recent years, the investigation of erroneous regulation of apoptotic mechanisms during acute and chronic injury of neuronal cells has gained increasing attention. Besides acute neuronal trauma and ischemia, chronic neurodegenerative diseases like Alzheimer’s, Huntington’s, Parkinson’s and Lou-Gehrig’s disease (amyotrophic lateral sclerosis) are of particular interest. It is generally accepted that neurons are generated in excess so that they may compete for contacts with their cellular partners and thus adjust strategy for several acute or chronic neurodegenerative diseases of the adult brain. In several neurodegenerative diseases, including Alzheimer’s disease, and in appropriate transgenic animal models of these diseases, the loss of synapses may precede neuronal loss.

Acute and chronic neurodegenerative diseases are illnesses associated with high morbidity and mortality, and few or no effective options are available for their treatment. A characteristic of many neurodegenerative diseases — which include stroke, brain trauma, spinal cord injury, amyotrophic lateral sclerosis (ALS), Huntington’s disease, Alzheimer’s disease, and Parkinson’s disease — is neuronal-cell death (\textit{gi50}). Given that central nervous system tissue has very limited, if any, regenerative capacity, it is of utmost importance to limit the damage caused by neuronal death (\textit{gi50}). During the past decade, considerable progress has been made in understanding the process of cell death (\textit{gi50}).

It has been reported that the biochemical and molecular cascade of apoptosis does not only exist in neuronal cell bodies but that it can also be engaged in synaptic terminals and neurites. These results suggest a role for apoptotic signaling in synaptic plasticity. Actin and spectrin, modulators of synaptic plasticity, are substrates of activated caspases. Further, the activation of caspases leads to proteolysis of AMPA receptor subunits and may thereby prevent excitotoxic necrosis and steer the cells to apoptosis. Here, the apoptotic signaling pathway including the activation of caspases may not represent a suicide mechanism but suggests a physiological function, which is important for learning and memory as well as for regeneration after tissue injury. These results suggest that the expression of procaspases in differentiated neurons is not exclusively a potential guillotine leading to suicide but may be required as a physiological and important response to different stimuli. If, however, these apoptotic signals propagate to the nucleus, they may induce apoptotic cell death (\textit{gi50}).

Dementia of the Alzheimer type affects about 10% of the population over 65 years of age and up to 50% over 85 years of age. Degeneration of neurons in the basal forebrain, hippocampus and cortex is a hallmark of Alzheimer’s disease. Besides decreased synaptic density and the loss of neurons, the brains of Alzheimer patients show characteristic histological changes at the neuronal level, i.e. the formation of so-called senile plaques, consisting of aggregates of the \(\beta\)-amyloid protein, and tangles, which occur through the accumulation of hyperphosphorylated tau, a protein associated with microtubuli. Although the number of neurons showing these characteristics is too small to explain the dysfunction and death of so many neurons in Alzheimer brains, changes in \(\beta\)-amyloid metabolism are believed to play a predominant role in the observed pathology. \(\beta\)-Amyloid is generated by cleavage of amyloid precursor protein, which is mutated in a hereditary form of Alzheimer’s disease. Exposure to \(\beta\) amyloid induces apoptosis in neurons. Cell death is preceded by the activation of caspases and altered expression levels of Bcl-2 family proteins.
proteins (proteases). In other forms of familial Alzheimer’s disease, mutations in the presenilin gene 1 and 2 were identified. These mutations also alter the normal proteolytic cleavage of amyloid precursor protein, sensitizing neuronal cells to apoptotic stimuli in vitro. As is the case with other neurodegenerative illnesses, the presence of DNA fragmentation, caspase activation, and the expression of other apoptosis-related genes has been described. Although apoptosis may not be the primary cause of neuronal degeneration in Alzheimer’s disease, programmed cell death may contribute to the continued progression of disease pathology. Thus, interfering with apoptosis is still of interest as a potential therapeutic strategy in light of the fact that current treatments for Alzheimer’s disease are mainly symptomatic and rather inefficient. One anti-apoptotic drug that has been approved for clinical use is Akatinol® (memantine). Originally released for the treatment of Parkinson’s disease and dementia more than 10 years ago, this drug has experienced a renaissance after being tested successfully in an US phase III trial for the treatment of severe Alzheimer’s disease. Memantine is classified as a non-competitive inhibitor of the NMDA-type glutamate receptor that has been shown to interfere with glutamate-induced apoptosis and processes of learning and memory. Currently, derivatives are being developed in order to optimize neuroprotective benefits and minimize adverse effects.

**SUMMARY**

Recent advances in the understanding of the molecular mechanisms of apoptosis have allowed researchers to begin targeting undesired apoptosis, in an attempt to moderate its occurrence. Unscheduled apoptosis appears to transpire in numerous diseases, both acute (e.g., stroke, liver degeneration) and chronic (e.g., cancer, osteoarthritis, neurodegeneration). There is a clear unmet medical need for better therapies for such diseases. The most active area of research involves a novel family of cysteine proteases which have been termed the caspases, TNF-α, Fas-L, NO. In a number of isolated cell systems, all of these have been shown to be involved in molecular pathways leading to apoptosis in response to apoptotic stimuli and evidence is mounting for pivotal roles for members of this novel protease family in degenerative diseases. Inhibition or induction of all of these is predicted to be beneficial for degenerative diseases and for uncontrolled proliferation of tissue. This review summarizes the current status and some of the issues of targeting apoptosis as a disease-modifying therapy.

**References**

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