REVIEW ARTICLE

MECHANISMS OF DISEASE

Cell Death

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All multicellular organisms require apoptosis, the controlled death of cells. Without apoptosis, 2 tons of bone marrow and lymph nodes and a 16-km intestine would probably accumulate in a human by the age of 80.[1] Investigations into apoptosis have revealed complex interconnections between various cell-death programs, and these networks could affect the treatment of a wide range of diseases.[2-10]

Classification of Cell Death

The most widely used classification of mammalian cell death recognizes two types: apoptosis and necrosis.[3,4,11] Autophagy, which has been proposed as a third mode of cell death, is a process in which cells generate energy and metabolites by digesting their own organelles and macromolecules.[12-15] Autophagy allows a starving cell, or a cell that is deprived of growth factors, to survive.[12-15] However, cells that do not receive nutrients for extended periods ultimately digest all available substrates and die (autophagy-associated cell death). Distinctions between apoptosis, necrosis, and autophagy entail differences in the mode of death and morphologic, biochemical, and molecular attributes (Fig. 1).[3,4,11]

Programmed cell death is an important concept. Cell death is "programmed" if it is genetically controlled. Apoptosis and autophagy-associated cell death are the two fundamental types of programmed cell death.[3,12] The recognition that cell death can occur by genetically controlled processes has enabled advances in unraveling the mechanisms of many diseases, and this new knowledge has facilitated the development of pharmacologic agents that initiate or inhibit programmed cell death.[6-8,16] Moreover, there is now evidence that necrosis, traditionally considered an accidental form of cell death, can in certain instances be initiated or modulated by programmed control mechanisms.[17-21]

Apoptosis

Definition

Apoptosis is derived from an ancient Greek word that suggests "leaves falling from a tree."[22-24] In contrast to the swelling of the cell and its organelles that defines necrosis, the principal morphologic feature of apoptosis is shrinkage of the cell and its nucleus (Fig. 2 and 3, and Fig. 1 through 4 in the Supplementary Appendix, available with the full text of this article at NEJM.org). The distinction between necrosis and apoptosis is due in part to differences in how the plasma membrane participates in these processes. In necrosis, early loss of integrity of the plasma membrane allows an influx of extracellular ions and fluid, with resultant swelling of the cell and its organelles.[17-20,25,26] In apoptosis, plasma-membrane integrity

persists until late in the process. A key feature of apoptosis is cleavage of cytoskeletal proteins by aspartate-specific proteases, which thereby collapses subcellular components.[2,5,8,23] Other characteristic features are chromatin condensation, nuclear fragmentation, and the formation of plasma-membrane blebs.

DEATH-RECEPTOR PATHWAY

Caspase activation commits cells to one of two distinct but convergent pathways: the death receptor and mitochondrial pathways (Fig. 4). The death-receptor pathway is activated when members of the tumor necrosis factor (TNF) superfamily bind to cell-surface "death receptors," members of the TNF-receptor family.[27-31] Ligation of these receptors initiates the formation of the multiprotein death-inducing signaling complex.[5,32,33] Aggregation of this complex causes conformational changes in its components that trigger the catalytic activity of caspase 8, a central mediator of apoptosis.

MITOCHONDRIAL PATHWAY

Interplay between proapoptotic and antiapoptotic members of the BCL2 family controls the mitochondrial apoptotic pathway (Table 1 in the Supplementary Appendix). Caspase 9 regulates this pathway, which comes into play after intracellular sensors indicate overwhelming cell damage.[5,23,34] Initiators of the pathway include increased intracellular reactive oxygen species, DNA damage, the unfolded protein response, and the deprivation of growth factors. These initiators ultimately lead to increased mitochondrial permeability, thereby promoting the release of proapoptotic proteins (e.g., cytochrome *c*) from the intermitochondrial membrane space into the cytosol (Fig. 4).[35-38] Another of these proteins, diablo homologue (SMAC/DIABLO), antagonizes cytosolic inhibitors of proapoptosis proteins, thus allowing the activation of caspases and hence progression to apoptosis. Activated caspase 8 (death-receptor pathway) and caspase 9 (mitochondrial pathway) in turn mobilize caspases 3, 6, and 7, proteases that herald demolition of the cell by cleaving numerous proteins and activating DNases.[23,38]

Factors that determine which death pathways are activated include the stage of the cell cycle, the type and magnitude of the apoptotic stimulus, and, for immune cells, the stage of cellular activation.[23,31,34] In sepsis, blocking of either pathway causes a moderate decrease in cell death, whereas blocking of both pathways protects a larger number of cells. Multiple pathologic stimuli triggering different apoptotic pathways may thus occur concomitantly.[30]

BCL2 FAMILY

The balance between proapoptotic and antiapoptotic BCL2 protein family members controls the mitochondrial apoptotic pathway (Table 1 in the Supplementary Appendix).[2,23,24] *BCL2*, which was originally identified as the gene that is deregulated by the t(14;18) chromosomal translocation in follicular B-cell lymphomas, inhibits apoptosis.[5] It occurs in cell populations that routinely turn over by means of apoptosis, such as hematopoietic lineages, intestinal epithelial cells, and glandular epithelium, in which hormones regulate hyperplasia or involution.[39] Membership in the BCL2 family requires at least one conserved BCL2 homology domain in a protein. This domain allows the protein to regulate apoptosis by joining other proteins through intermolecular forces.

The prosurvival members of the family — BCL2, BCL-XL, BCLW, MCL1, A1, and BOO/DIVA — have as many as four BCL2 homology regions. These six proteins are essential for cell survival and function, specifically in certain cells and on certain stimuli (Table 1 in the Supplementary Appendix).[40-43] The proapoptotic BCL2 family proteins differ not only in function but also in the number of BCL2 homology domains. BAX and BAK, which have three BCL2 homology domains, are critical for increasing permeability of mitochondrial membranes and the release of cytochrome *c*, which activates caspase 9. Other proapoptotic proteins have only the BCL2 homology 3 (BH3) domain.[44,45] These "BH3-only" proteins bind to and inhibit antiapoptotic BCL2 family members, thereby liberating the proapoptotic BAX and BAK proteins that cause loss of mitochondrial membrane permeability and subsequent cell death.[44-46]

A balance between proapoptotic BH3-only proteins and antiapoptotic BCL2 family members determines the life or death of a cell.[34,47] BH3-only proteins differ in their ability to trigger apoptosis.[47,48] Three of them (BIM, PUMA, and BID) bind with high affinity to all prosurvival BCL2 family members. Moreover, different apop-

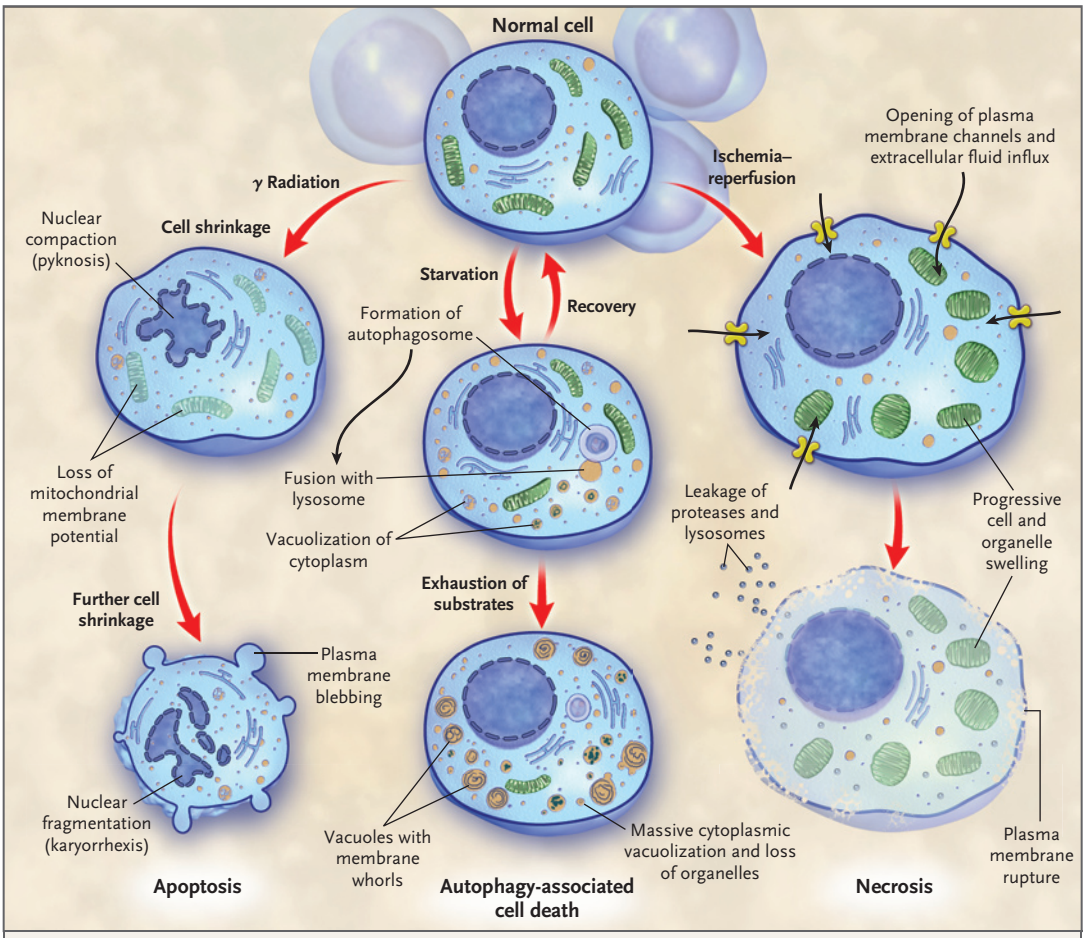

Figure 1. Three Pathways of Cell Death.

Among the three major pathways of cell death — apoptosis, autophagy, and necrosis — a particular mode of cell death may predominate, depending on the injury and the type of cell. Cross-talk among the different types of cell-death pathways exists at multiple levels and is not shown.

totic stimuli preferentially activate certain BH3-only proteins: BIM is essential for apoptosis induced by deprivation of growth factors, whereas PUMA is critical for apoptosis induced by DNA damage.[49-51] The concordant loss of BIM and PUMA is more protective against apoptotic stimuli than the loss of either alone, an indication of a functional overlap of these initiators of apoptosis.[52]

CLINICAL IMPLICATIONS OF APOPTOSIS

CANCER

More than 50% of neoplasms have defects in the apoptotic machinery. Among the best characterized of these abnormalities are the increased expression of prosurvival BCL2 family proteins and mutations in the tumor-suppressor gene *TP53*, which encodes tumor protein p53.[53-55] This gene, called the "guardian of the genome," initiates apoptosis in response to DNA damage induced by radiation, chemical agents, oxidative stress, and other agents by transcriptional induction of many proapoptotic proteins, including PUMA, NOXA, and BAX. Inherited defects in *TP53* (e.g., the Li–Fraumeni syndrome) result in numerous neoplasms, including gliomas and sarcomas.[55]

Most chemotherapeutic agents induce apoptosis in tumor cells (Table 1). The tyrosine kinase inhibitor imatinib (Gleevec) kills chronic myeloid leukemia cells by up-regulating the proapoptotic BCL2 family members BIM and BAD.[56] ABT-737, a small-molecule mimic of the BH3-only proteins that bind to antiapoptotic BCL2 and BCL-XL, kills certain tumor cells on its own and greatly en-

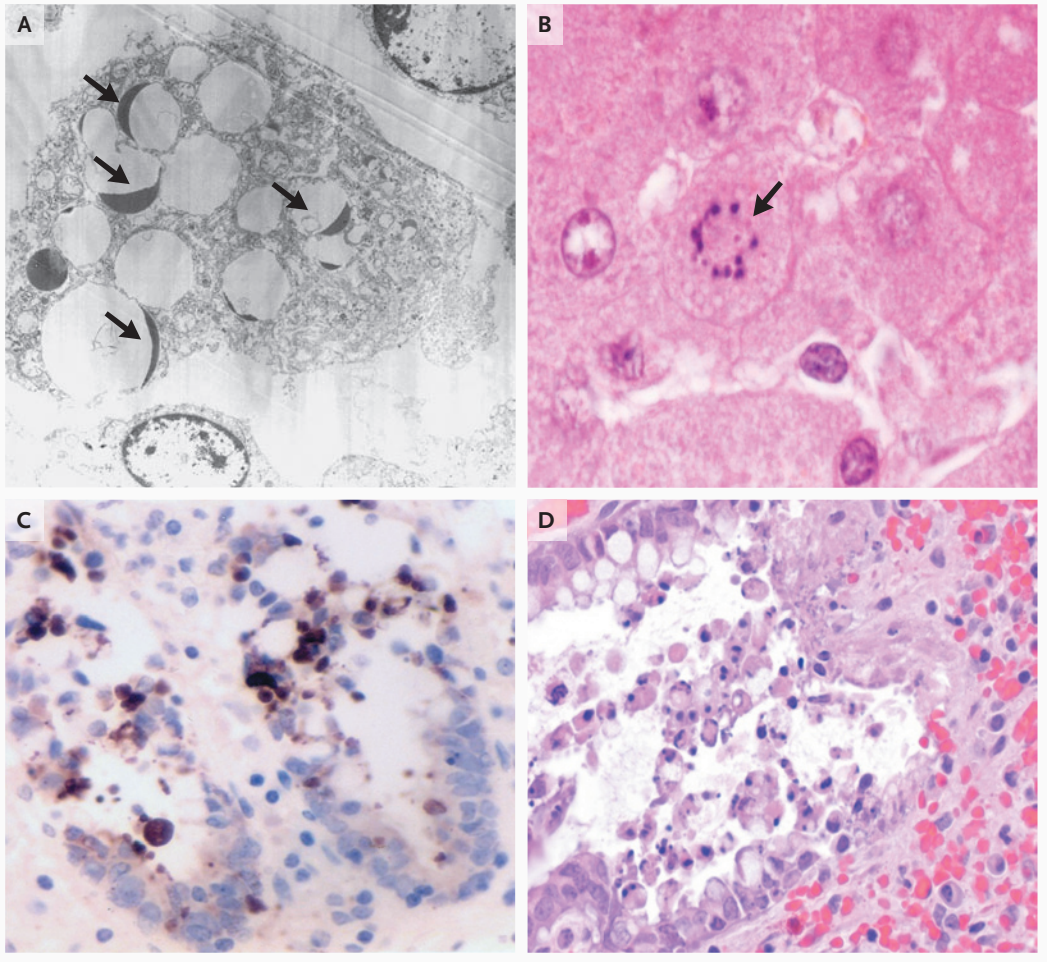

Figure 2. Apoptotic Cells in Thymus, Liver, and Intestine.

Panel A is an electron-microscopical image of a phagocytic cell that has engulfed multiple apoptotic thymocytes. The compacted thymocyte nuclei have a classic crescent-shaped appearance, owing to layering of chromatin along the nuclear membrane (arrows). Normal-appearing nuclei are present at the top and bottom of the field of view (uranyl acetate–lead citrate). The thymic tissue section was obtained from a 26-year-old woman who died after a motor vehicle accident and whose condition was complicated by the acute respiratory distress syndrome and sepsis. Panel B shows a single apoptotic hepatocyte (arrow) containing multiple compacted nuclear fragments indicative of apoptosis (hematoxylin and eosin). The sample was obtained from an 81-year-old man who had been injured in a motor vehicle accident and whose condition was complicated by ventilator-associated pneumonia. Panel C shows two adjacent crypts in colonic mucosa that had immunohistochemical staining for cytokeratin 18 cleavage fragments (brown). Cytokeratin 18 is cleaved by active caspases in both intrinsic and extrinsic apoptotic pathways. Detached cells in crypt lumens and epithelial cells that are still integrated into the crypt lining are positive; these cells also have classic apoptotic nuclear morphology (cytokeratin 18 immunostaining [clone M30] and diaminobenzidine with hematoxylin counterstaining). The tissue sample was obtained from a 24-year-old man who had aortic dissection and bowel ischemia after a motor vehicle accident. Panel D shows colonic intestinal epithelial cells with characteristic apoptotic features of nuclear compaction and fragmentation; the epithelial cells have been sloughed into the bowel lumen (hematoxylin and eosin). The sample was obtained from a 23-year-old patient with ischemic injury to the bowel after intestinal surgery.

hances the efficacy of other anticancer drugs.[10,57] An orally active analogue of ABT-737 (ABT-263) has entered clinical trials in hematologic cancers and as adjuvant therapy in solid-organ tumors.[57] Other proapoptotic chemotherapeutic agents that are in clinical trials target survivin and X-linked

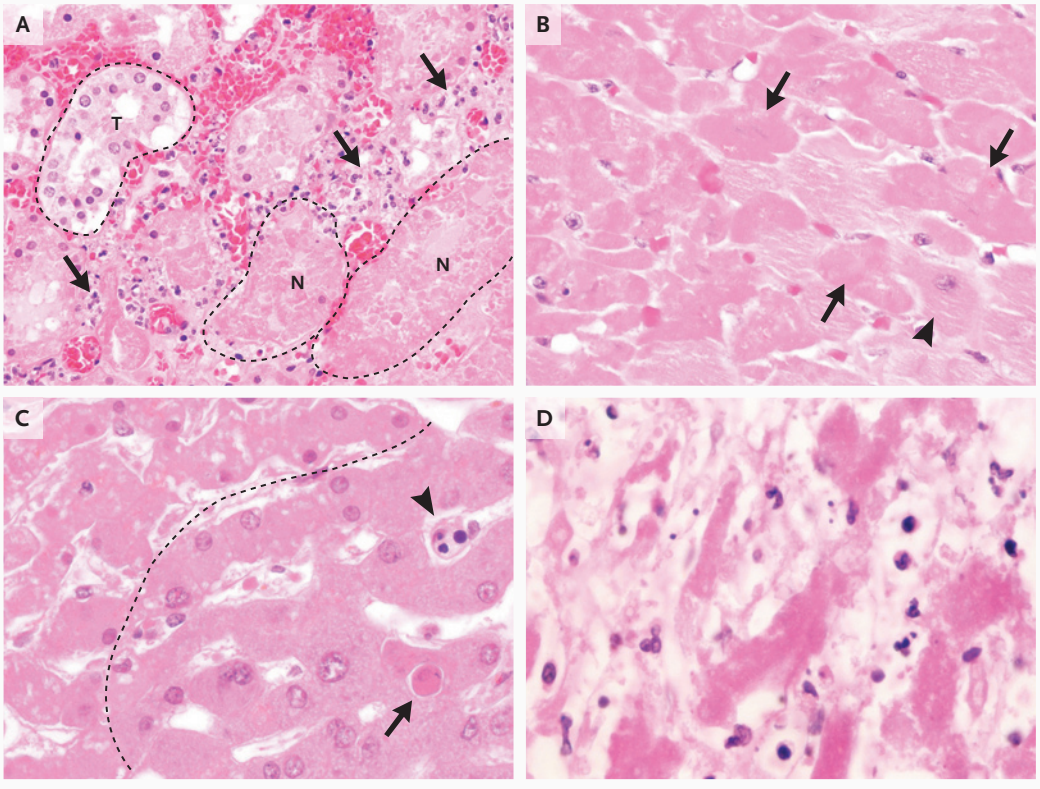

Figure 3. Apoptotic and Necrotic Cell Death.

Panel A shows the transition from viable cells to ischemic parenchyma in renal cortical necrosis. The necrotic cells are characterized by hypereosinophilia, a loss of distinct nuclear detail, and cytoplasmic vacuolization. The relatively intact tubule (T) is seen next to representative necrotic tubules (N). In the interface between viable and necrotic tubules (arrows), apoptotic cells (presumably neutrophils and mononuclear inflammatory cells) are abundant (hematoxylin and eosin). The renal sample was obtained from a 42-year-old man who had undergone renal arterial embolization for the treatment of renal-cell carcinoma. Panel B shows characteristic features of cardiomyocyte necrosis. As compared with viable cardiomyocytes with pale cytoplasm and distinct nuclear features (arrowhead), necrotic cells are hypereosinophilic with uneven cytoplasmic vacuolization and either loss of nuclear detail or nuclear absence (arrows). This morphology typifies an early manifestation of so-called coagulative necrosis (hematoxylin and eosin). Panel C shows hepatocytes with changes of early necrosis, including vacuolization of hypereosinophilic cytoplasm and loss of nuclear detail (above and to the left of the dashed line). A sinusoidal inflammatory cell (arrowhead) and a hepatocyte with compacted and fragmented nuclei indicative of apoptosis (arrow) are both visible (hematoxylin and eosin). The sample was obtained from a 53-year-old man who had pneumonia and bacteremia caused by *Streptococcus pneumoniae*. Panel D shows hepatic structures that are similar to those in Panel C, with features that are typical of more advanced necrosis, including remnants of hepatocytes (eosinophilic cords) that lack obvious cell borders or recognizable nuclei. Cells and cell fragments that are admixed with hepatocytes are products of apoptosis; they probably represent apoptotic lymphocytes or neutrophils in sinusoids (hematoxylin and eosin). The sample was obtained from an 81-year-old man with ventilator-associated pneumonia.

inhibitor of apoptosis (XIAP), which are endogenous inhibitors of proapoptotic caspases.[58]

THE IMMUNE SYSTEM

Abnormalities in apoptosis can increase susceptibility to autoimmune diseases.[59] During development, clones of B and T cells that express autoreactive antigen receptors are deleted from the immune repertoire. The deletion relies on the proapoptotic BH3-only protein BIM.[60] Moreover, killing of mature, antigen-activated B and T cells during shutdown of immune responses is mediated by both BIM and the death receptor FAS.[30] The autoimmune lymphoproliferative syndrome,

which is typified by massive lymphadenopathy, hypersplenism, and autoimmune cytopenias, develops in patients with a defect in the FAS ligand or receptor.[59] Apoptosis of intestinal epithelial cells and basal keratinocytes in graft-versus-host disease is a functionally related phenomenon. In type 1 diabetes, the loss of beta cells from the pancreatic islets is probably mediated by the FAS death receptor; CD8 T cells expressing FAS ligand interact with FAS receptors on the insulin-secreting cells to induce death.[61]

NEUROLOGIC DISEASES

There is growing evidence that neuronal apoptosis plays a key role in neonatal brain disorders.[62] Developing neurons are particularly susceptible to apoptosis in response to noxious stimuli during the period of synaptogenesis.[63] In neonatal hypoxic brain injury, the cell-death phenotype changes over time from early necrosis to apoptosis, an evolution that has been termed the "necrosis–apoptosis" continuum. There is evidence that apoptosis is a more important mechanism of neonatal brain injury than necrosis.[62] The fetal alcohol syndrome is due to apoptotic neurodegeneration that results from ethanol-induced blockade of the *N*-methyl-D-aspartate (NMDA) receptor and activation of the γ-aminobutyric acid (GABA) receptor.[64] General anesthetics also modulate NMDA and GABA receptors, and studies in animals showing that general anesthetics induce extensive neuronal apoptosis in neonates have raised considerable concern that the use of general anesthetics in neonates might cause long-term cognitive defects.[65]

HEPATITIS

Hepatocytes are particularly prone to apoptosis in response to various types of stress, including infections.[29] A trial of a potent caspase inhibitor (IDN-6556) in patients with chronic hepatitis C showed that the drug caused a highly significant lowering of serum alanine aminotransferase and aspartate aminotransferase levels in patients with chronic hepatitis C.[66] IDN-6556 is also being evaluated for its ability to reduce ischemia–reperfusion injury after liver transplantation (Table 1).

CARDIOVASCULAR DISEASES

Necrosis predominates in ischemic injury, but often there are apoptotic cells in the hypoxic penumbra in myocardial infarction and stroke and in globally hypoxic zones after reperfusion injury. When there is hypoxia-induced premature activation of the apoptotic program, the inhibition of apoptosis (e.g., by caspase inhibitors) might prevent cell loss. Cyclosporine, which inhibits

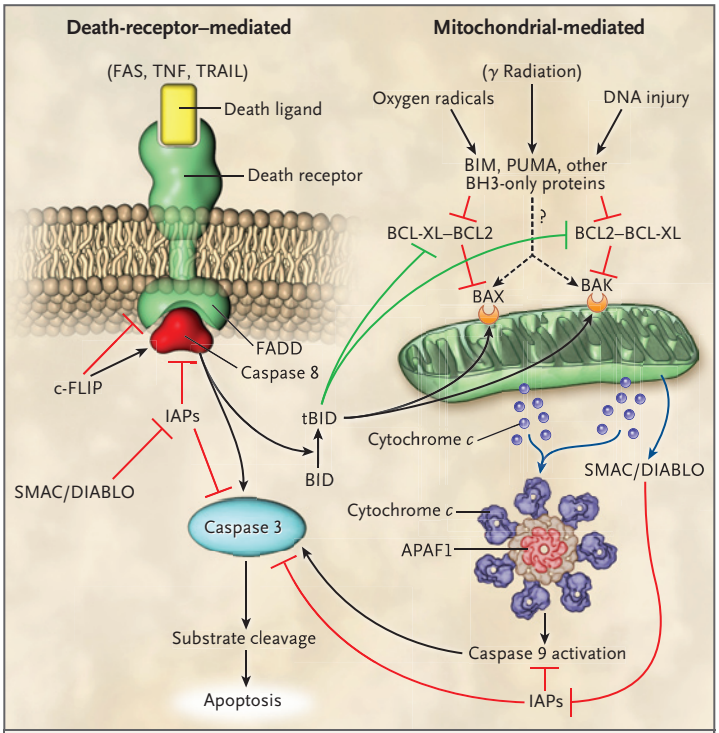

Figure 4. Pathways of Cellular Apoptosis.

There are two major pathways of apoptosis: the death-receptor pathway, which is mediated by activation of death receptors, and the BCL2-regulated mitochondrial pathway, which is mediated by noxious stimuli that ultimately lead to mitochondrial injury. Ligation of death receptors recruits the adaptor protein FAS-associated death domain (FADD). FADD in turn recruits caspase 8, which ultimately activates caspase 3, the key "executioner" caspase. Cellular FLICE-inhibitory protein (c-FLIP) can either inhibit or potentiate binding of FADD and caspase 8, depending on its concentration. In the intrinsic pathway, proapoptotic BH3 proteins are activated by noxious stimuli, which interact with and inhibit antiapoptotic BCL2 or BCL-XL. Thus, BAX and BAK are free to induce mitochondrial permeabilization with release of cytochrome *c*, which ultimately results in the activation of caspase 9 through the apoptosome. Caspase 9 then activates caspase 3. SMAC/DIABLO is also released after mitochondrial permeabilization and acts to block the action of inhibitors of apoptosis protein (IAPs), which inhibit caspase activation. There is potential cross-talk between the two pathways, which is mediated by the truncated form of BID (tBID) that is produced by caspase 8–mediated BID cleavage; tBid acts to inhibit the BCL2–BCL-XL pathway and to activate BAX and BAK. There is debate (indicated by the question mark) as to whether proapoptotic BH3 molecules (e.g., BIM and PUMA) act directly on BAX and BAK to induce mitochondrial permeability or whether they act only on BCL2–BCL-XL. APAF1 denotes apoptotic protease-activating factor 1, BH3 BCL homologue, TNF tumor necrosis factor, and TRAIL TNF-related apoptosis-inducing ligand.

Table 1. Pharmacologic Modulators of Cell Death in Clinical Trials.*

Disease	Compound	Mechanism of Action
Cancer		
Leukemia, multiple myeloma, non-Hodgkin's lymphoma, lung, solid-organ	ABT-263, gossypol, GX15-070 (obatoclax)	Induces apoptosis by inhibiting antiapoptotic BCL2 family members
Colorectal, non–small-cell lung, non-Hodgkin's lymphoma	Recombinant human Apo2L/TRAIL (dulanermin) and anti-TRAIL R1-mAb	Induces apoptosis by activation of TRAIL death receptors
Breast, pancreatic, ovarian, solid-organ, glioma	PARP inhibitors	Prevents repair of DNA strand breaks leading to apoptosis
Multiple myeloma, breast, prostate	Hydroxychloroquine	Inhibits autophagy
Ovarian, small-cell lung, cervical	Topotecan (Hycantin)	Induces apoptosis by inhibiting topoisomerase I, an enzyme essential for DNA replication
Breast, renal, rectal, large-B-cell lymphoma	Temsirolimus and sirolimus	Inhibits mTOR, resulting in autophagy; other actions
Non–small-cell lung, pancreas, breast	XIAP antisense (AEG35156)	Induces apoptosis by knockdown of endogenous caspase inhibitors
Acute myeloid leukemia	Survivin antagonist	Induces apoptosis by inhibiting survivin, an endogenous caspase inhibitor
Ischemia–reperfusion injury		
Stroke	Minocycline	Reduces apoptosis
Myocardial infarction	Mitochondrial ATP-sensitive potassium-channel agonist (nicorandil)	Reduces necrosis by preventing cell ionic disequilibrium; may also reduce apoptosis by acting on MPTP
	PARP inhibitors	Reduces necrosis by prevention of cell energy failure
	Cyclosporine	Reduces apoptosis by blocking opening of MPTP
Neurodegenerative disease		
Amyotrophic lateral sclerosis	Arimoclomol	Reduces apoptosis, improves elimination of misfolded proteins by heat-shock protein chaperone–mediated disposal
Huntington's disease and Alzheimer's disease	Ursodiol	Reduces apoptosis and oxidation; other effects
Parkinson's disease and Alzheimer's disease	Rasagiline	Reduces apoptosis and other effects
Other		
Hepatitis C	Caspase inhibitors IDN-6556, GS-9450	Reduces apoptosis by blocking caspases

* Details regarding the status of all clinical trials are available in Table 1 in the Supplementary Appendix and at ClinicalTrials.gov. The majority of drugs that are used in cancer trials are administered in combination with chemotherapeutic drugs, which induce apoptosis. MPTP denotes mitochondrial permeability transition pore, mTOR mammalian target of rapamycin (now known as sirolimus), PARP poly–ADP–ribose polymerase, and XIAP X-linked inhibitor of apoptosis.

apoptosis by blocking mitochondrial permeability-transition pores, can decrease the infarct size in patients with acute myocardial infarction.[67] In a pilot trial, 58 patients with acute myocardial infarction received an intravenous bolus of either cyclosporine or saline immediately before undergoing percutaneous coronary intervention. On day 5, the absolute mass of the area of infarcted tissue on magnetic resonance imaging was significantly reduced in the cyclosporine group, as compared with the control group. Patients with acute stroke who were treated within 24 hours with minocycline, an antiapoptotic compound with multiple actions, had superior neurologic outcomes, as compared with patients who were given placebo.[68]

SEPSIS

Sepsis is perhaps the most remarkable clinical setting in which apoptosis occurs. Massive apop-

tosis of immune effector cells and gastrointestinal epithelial cells develops in patients with sepsis (Fig. 2 and 3, and Fig. 1 through 4 in the Supplementary Appendix).[69-71] The profound loss of immune effector cells in sepsis inhibits the ability of the immune system to eradicate the primary infection and renders the patient susceptible to nosocomial infections. Numerous studies in animals have highlighted the role of apoptosis in aggravating sepsis. They have shown that the prevention of sepsis-induced apoptosis improves survival.[72,73]

AUTOPHAGY

DEFINITION

The word "autophagy," which is derived from the Greek "to eat" ("phagy") oneself ("auto"), was first used for structures that were observed on electron microscopy and that consisted of single- or double-membrane lysosomal-derived vesicles containing cytoplasmic particles, including organelles, in various stages of disintegration[74,75] (Fig. 5). We now understand that autophagy is the process by which cells recycle their own nonessential, redundant, or damaged organelles and macromolecular components.[12-14] It is an adaptive response to sublethal stress, such as nutrient deprivation, that supplies the cell with metabolites it can use for fuel. Autophagy also has a role in the suppression of tumor growth, deletion of toxic misfolded proteins, elimination of intracellular microorganisms, and antigen presentation.[12-14]

Three forms of autophagy have been defined on the basis of how lysosomes receive material for degradation.[12] In macroautophagy, a double-membrane structure (the autophagosome) envelops the cargo and then fuses with lysosomes. In microautophagy, an invagination of the lysosomal membrane engulfs the cargo. In chaperone-mediated autophagy, heat-shock cognate proteins deliver substrates to lysosomes.

Electron microscopy is the best way to visualize autophagosomes, the hallmark of autophagy (Fig. 5). These structures fuse with lysosomes, where acid hydrolases catabolize the ingested material into metabolic substrates. The typical whorls in autophagic vacuoles are remnants of membranes.[74-76] A complex set of autophagy-related proteins regulates the formation of autophagosomes (Fig. 5 in the Supplementary Appendix).[12,14] Among these is a complex consisting of class III phosphatidylinositol-3-kinase (PI3K) and beclin-1 (BECN1), a member of the BCL2 family with a BH3-only domain. There is additional control by mTOR (the mammalian target of rapamycin [now known as sirolimus]), a serine–threonine protein kinase that integrates input from cellular nutrients, growth factors, and cellular redox state to inhibit autophagosome formation.

The role of autophagy in cell death is controversial.[77] Despite agreement that autophagy is an adaptive response, there is no agreement that unbridled autophagy can deplete organelles and critical proteins to the point of caspase-independent cell death without signs of apoptosis (Fig. 5C). Although there are numerical increases in autophagosomes in some dying cells, it is unclear whether these structures facilitate cell death or are a feature of a cell that can no longer compensate by sacrificing vital components, a process that has been referred to as autophagy-associated cell death rather than autophagy-induced cell death. The genetic deletion of key autophagic genes accelerates rather than inhibits cell death, which emphasizes the predominant survival role of autophagy.[3,11]

CLINICAL IMPLICATIONS

Although autophagy is a survival mechanism that provides the cell with alternative sources of substrates when nutrients are limited,[12,15,77] it could also protect the cell by eliminating damaged mitochondria (which can trigger apoptosis by generating excess reactive oxygen species) or toxic misfolded proteins, including those believed to induce neurodegeneration. Drugs that activate autophagy can clear toxic protein aggregates, such as mutant huntingtin protein and mutant tau in models of neurologic disease.[78-80] Rapamycin analogues (which induce autophagy by inhibiting mTOR) decrease polyglutamine proteins and are effective in animal models of Huntington's disease; they are also being examined in acute brain injury.[78-80]

CANCER

Autophagy has a complex role in cancer.[81-83] Although current data are only associative, autophagy presumably functions as a suppressor of neoplasia. Many oncogenes (including *PI3K* and *AKT* family members, *BCL2*, and *MTOR*) suppress autophagy, whereas tumor suppressors (*PTEN*,

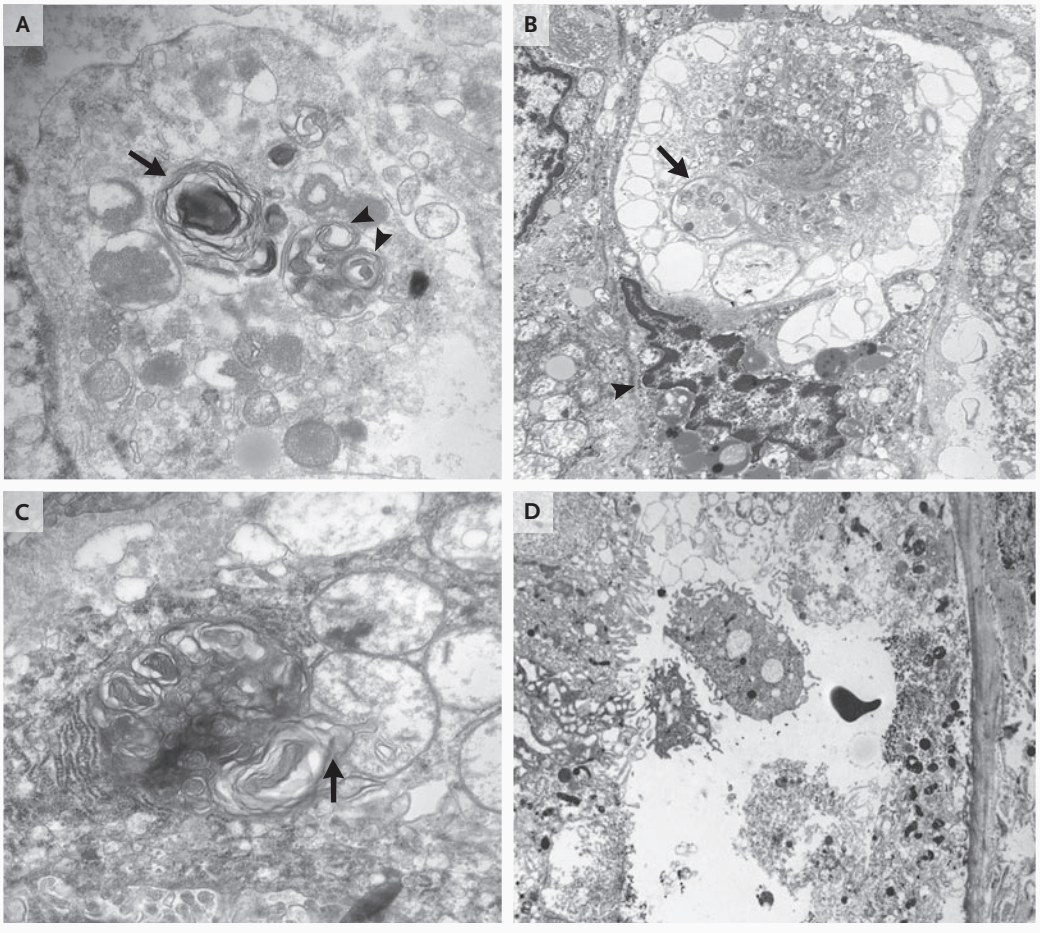

Figure 5. Electron-Microscopical Detection of Autophagic and Necrotic Cell Death.

Panel A shows two large autophagosomes, with one encompassing organelle fragments with extensive degradation (arrow) and the other containing mitochondria and other organelles in varying stages of degradation (arrowheads). The sample was obtained from an 85-year-old woman with peritonitis. Panel B shows a cell with extensive autophagic vacuolization with few remaining intact organelles, an autophagosome containing mitochondrial fragments (arrow), and a cell nucleus that has features of nuclear condensation (arrowhead) — an example of autophagy-associated cell death. Panel C shows a cell in which the autophagosomes have assumed a more complex appearance of redundant whorls of membrane-derived material. This complex lysosomal structure is juxtaposed to and focally invaginated into an adjacent mitochondrion (arrow). The specimens for Panels B and C were obtained from a 73-year-old woman with urosepsis. Panel D shows a necrotic proximal renal tubule, showing marked swelling of organelles and cytoplasm, loss of brush border, and loss of cytoplasmic detail. The specimen was obtained from a 44-year-old man with acute renal failure and a history of vancomycin toxicity and cirrhosis. (Uranyl acetate–lead citrate staining for all panels.) Panels A, B, and C are reproduced from Watanabe et al.,[75] with the permission of the publisher.

TSC2, and *HIF1A*) promote autophagy.[83] In addition, the loss of individual autophagy-related genes (especially *BECN1*, *UVRAG*, and *BIF1* [*ZBTB24*]) results in lymphomas and gastrointestinal tumors in mouse models, and these same genes are frequently mutated in human cancers, including bowel and hepatocellular carcinomas.[84-86] Thus, it is seemingly paradoxical that hydroxychloroquine, an antimalarial drug that blocks autophagy by raising intralysosomal pH, is under evaluation in cancer trials (Table 1).[81] In patients undergoing chemotherapy, autophagy can promote resistance to cell death, especially to DNA-damaging agents, and hydroxychloroquine blocks this cellular adaptive response, which results in increased tumor killing.

NECROSIS

DEFINITION

Necrosis (from the Greek "nekros," for corpse) is best defined by light or electron microscopic detection of cell and organelle swelling or rupture of surface membranes with spillage of intracellular contents (Fig. 3 and 5, and Fig. 6 in the Supplementary Appendix).[4,29,87] The term "oncosis" (Greek for swelling) is preferred by some investigators, and "oncotic necrosis" has also been used.[4] The compromise of organellar membranes allows proteolytic enzymes to escape from lysosomes, enter the cytosol, and cause cell demolition.[17,20,87-92] Necrosis usually results from metabolic failure that has coincided with rapid depletion of ATP; it classically occurs in ischemia.[26,88]

Necrosis is usually considered an accidental (i.e., nonprogrammed) form of cell death that occurs in response to acute hypoxic or ischemic injury, such as myocardial infarction and stroke. It occurs spontaneously in neoplasms when cell proliferation outpaces angiogenesis. The exposure of cells to supraphysiologic conditions (e.g., mechanical force, heat, cold, and membrane-permeabilizing toxins) also precipitates necrosis.

MEDIATORS OF PROCESS

Reactive oxygen species, calcium ions, poly–ADP–ribose polymerase (PARP), calcium-activated nonlysosomal proteases (calpains), and cathepsins mediate necrosis.[89,92] PARP is a DNA-repair enzyme that can deplete cellular ATP stores when it catalyzes the repair of multiple DNA strand breaks that occur in cell injury. In apoptosis, PARP undergoes rapid cleavage and inactivation (detection of cleaved PARP is a diagnostic test for apoptosis), so stores of ATP are preserved. ATP is necessary for numerous effector processes in apoptosis, whereas exhaustion of ATP shifts the cell from apoptosis to necrosis. PARP inhibition mitigates necrosis in ischemia–reperfusion injury and other types of damage.[93,94] Increased intracellular calcium ions, a central feature of necrosis, activates proteases that degrade critical proteins. Intriguingly, the source and amount of increased calcium ions may induce different types of cell death: the influx of calcium ions across the plasma membrane triggers necrosis, whereas the release of calcium ions from the endoplasmic reticulum more readily induces apoptosis.[17,95]

PROGRAMMED OR REGULATED?

Accumulating evidence indicates that necrosis is more ordered than was originally thought. When cells die from necrosis, damage-associated molecular-pattern (DAMP) molecules, such as high-mobility group box 1 (HMGB1) protein, enter the circulation and activate innate immune cells.[96] Thus, the first cells that die from trauma or infection may function as sentinels, alerting the host to the need for defensive or reparative responses. In addition, necrosis can be initiated by the activation of selected cell-surface receptors. For example, high concentrations of TNF induce hepatocyte necrosis.[21,26] The identification of an intracellular serpin (protease inhibitor) that prevents necrosis caused by multiple noxious stimuli indicates that necrosis can be regulated, programmed, and driven by a peptidase stress-response pathway.[20]

Other, less well characterized forms of cell death (e.g., pyroptosis and paraptosis) are discussed in the Alternative Forms of Cell Death section in the Supplementary Appendix.

CROSS-TALK BETWEEN CELL-DEATH MECHANISMS

The type and intensity of noxious signals, ATP concentration, cell type, and other factors determine how cell death occurs.[11] Acute myocardial ischemia (which precipitates rapid and profound decreases in ATP) induces necrosis, whereas chronic congestive heart failure (with more modest yet chronic decreases in ATP) induces apoptosis[97] (Fig. 3). The blockade of a particular pathway of cell death may not prevent the destruction of the cell but may instead recruit an alternative path: antiapoptotic caspase inhibitors cause hyperacute necrosis of hepatocytes and kidney tubular cells induced by TNF-α.[98-100] The overexpression of antiapoptotic proteins may allow injured cells to survive, and autophagy may assist by providing critical metabolites.[15] However, if death stimuli persist, antiapoptotic pathways and autophagy are unlikely to continue, and necrosis ensues. Furthermore, cells may be more susceptible to apoptosis if autophagy is inhibited.[101,102] Nuclear factor κB, ATG5, ATP, and PARP probably function as molecular switches that determine whether a cell undergoes apoptosis, necrosis, or autophagy.[26,88,93,103-105] Protein p53 also modulates autophagy and other responses to cell stress. Recent work indicates that basal p53 ac-

tivity suppresses autophagy, whereas the activation of p53 by certain stimuli induces autophagy and the activation of p53 by different stimuli results in the engagement of apoptosis, mediated by PUMA and NOXA.[106-108]

IMMUNOMODULATORY EFFECTS OF DYING CELLS

The effect of dying cells on immunity is an exciting area of investigation.[96,109-111] Apoptotic cells induce anergy or an immunosuppressive phenotype, whereas necrotic cells augment inflammation, in part by binding the receptor C-type lectin domain family 9 (CLEC9A) on dendritic cells.[112] The administration of apoptotic cells to mice before challenge with parasites greatly increased the number of blood parasites, as compared with control mice, whereas in mice given necrotic cells, there was greatly decreased parasitemia.[113] Similarly, mice that received apoptotic cells before the induction of peritonitis had a higher death rate than control mice, whereas mice that received necrotic cells had improved survival.[114]

FUTURE DIRECTIONS

Initial attempts at therapeutic modulation of cell death have yielded some surprising and paradoxical findings.[2,6-8,16] For example, some "pro-death" cellular proteins are also essential for cell survival. Caspase 8 and its activator, FAS-associated death domain (FADD), are essential for death-receptor–mediated apoptosis but are also critical for antigen-induced T-cell proliferation and macrophage differentiation.[27,32,115] In addition, blockade of multiple death pathways may keep susceptible cells alive, but survivors may be functionally dead and therefore useless ("zombie cells").[116,117]

A promising area of cancer therapy involves death-receptor activation. Unlike normal cells, many cancer cells are sensitive to TNF-related apoptosis-inducing ligand (TRAIL), and clinical trials studying TRAIL or antibodies against TRAIL receptor are under way in patients with colorectal cancer, non–small-cell lung cancer, and non-Hodgkin's lymphoma.[118] Proapoptotic BH3 mimetics are being tested in patients with leukemia, multiple myeloma, and other cancers.[10,57] Drugs that block endogenous inhibitors of apoptosis have been tested in clinical trials involving patients with leukemia, as well as in those with pancreatic, pulmonary, and other parenchymal cancers.[119,120] PARP inhibitors are also being tested in multiple clinical trials; these peptides dramatically sensitize cancer cells to chemotherapy by preventing DNA repair.[121]

PREVENTION OF CELL DEATH

The prevention of cell death is more technically challenging than the induction of cell death. Different forms of cell death can occur simultaneously because of the coordinated release of multiple death-inducing stimuli. In ischemia–reperfusion injury, reactive oxygen species, calcium-ion overload, and destructive protease activation may induce cell death independently. Thus, it may be necessary to target multiple death pathways or identify and block common funnel points of cell-death signaling to enable survival. Despite these challenges, meaningful clinical advances are emerging (Table 1). The inhibition of calpains and cathepsins, potent proteases that are responsible for necrosis, mitigates disease in animal models.[122,123] Nicorandil, a cardioprotective drug that acts on mitochondrial ATP-sensitive potassium channels, lowers levels of serum troponin T in patients undergoing cardiac bypass surgery.[124] Huntington's disease, Parkinson's disease, Alzheimer's disease, and amyotrophic lateral sclerosis are current investigational targets of anti-apoptotic and autophagy-enhancing drugs.[125,126] The therapeutic induction of metabolic arrest may also prove valuable. Although the means by which cells enter a "hibernating" state remain unknown, important mediators of this low-energy state are now better understood; 5′-AMP is one such mediator that allows nonhibernating animals to safely enter a hypothermic condition.[116]

SUMMARY

Answers to a number of questions remain. Why are some cells, such as neurons, much more vulnerable to ischemic cell death than most others? How does a cell select a particular type of death? How does a cell switch from a stress-recovery program to cell death? What criteria drive the selection of a cell-death pathway? When is a cell irrevocably committed to death? Answers to these and other questions will ultimately lead to a more profound understanding of cell death, an expand-

ing foundation on which increasingly effective therapeutic interventions may be modeled and introduced into clinical practice.

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