CHAPTER 1

Cell Injury and Cell Death

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WHEN CELLS are damaged, as often occurs during trauma and metabolic stress, the organism has to choose whether to repair the damage by promoting cell survival or remove irreparably injured cells. Cell injury occurs when an adverse stimulus reversibly disrupts the normal, complex homeostatic balance of the cellular metabolism. In this case, after injury the cells attempt to seal breaks in their membranes, chaperone the removal or refolding of altered proteins, and repair damaged DNA. On the contrary, when cell injury is too extensive to permit reparative responses, the cell reaches a "point of no return" and the irreversible injury culminates in programmed cell death (PCD). Specific properties or features of cells make them more or less vulnerable to external stimuli, thus determining the kind of cellular response. In addition, the characteristic of the injury (type of injury, exposure time, or severity) will also affect the extent of the damage.

We present a short overview of the best-known PCD pathways. We emphasize the apoptotic pathway, considered for years the hallmark of PCD, and the different stimuli that produce cell injury.

CELL INJURY

The survival of multicellular organisms depends on the function of a diverse set of differentiated cell types. After development is complete, the viability of the organism depends on the maintenance and renewal of these diverse lineages. Within each lineage homeostasis is maintained through a delicate balance between cell proliferation and cell death.[1] Disorders of either process have pathological consequences and can lead to disturbed embryogenesis, neurodegenerative diseases, or the development of cancer.[2] Therefore, the equilibrium between life and death is tightly controlled, and faulty elements can effectively be eliminated by PCD, a term that well defines the planned sequence of physiological cellular autodestruction, which requires both energy expenditure and a specific enzymatic network. Cell death is an essential strategy for the control of the dynamic balance of the living system, and it is the ultimate result of most physiological as well as pathological processes. Skulachev aptly described the concept of cell death using the metaphor of the "Samurai law of biology" (i.e., it is better to die than be wrong), showing that the suicide program is a way to purify cells of damaged organelles and tissues of unwanted cells that use up valuable substrates and nutrients.[3,4] Likewise, cell death also has value for the species, as it provides a mechanism for eliminating terminally injured individuals who consume necessary society resources or harbor toxic pathogens.[3,5] Death, therefore, appears as the unique solution to eliminate what is unwanted or dangerous to the "community."

In past decades, PCD was mainly associated with apoptosis, a death process characterized by morphological changes such as shrinkage of the cell, condensation of chromatin, and disintegration of the cell into small fragments (so-called "apoptotic bodies") that are removed by phagocytosis. On the contrary, necrosis was considered as an alternative passive cell death occurring in an accidental, violent, or chaotic way.[6] Necrosis, however, has been recently recognized as a specific form of cell death with distinct morphological features.[7,8] It is now known that cell death cannot readily be classified as "apoptosis" or "necrosis," and alternative types of PCD have been described.[9–11] Different PCD pathways exist, either mediated by caspases (a specific family of cysteine proteases, as in apoptosis) or caspase-independent (such as autophagic cell death [ACD], paraptosis, and programmed necrosis).[1] Death patterns may overlap or integrate, reflecting the high flexibility in cell responses to various circumstances and stimuli (Figure 1.1).

Cell injury occurs as a result of physical, chemical, or biological insults or as a result of vital substrate deficiency. The cellular response to injury can be adaptive when it is designed to restore homeostasis and protect the cell from further injury. In this context, the gene transcriptional activity is modified in favor of vital genes.[5] If the genetic and metabolic adaptive responses are inadequate

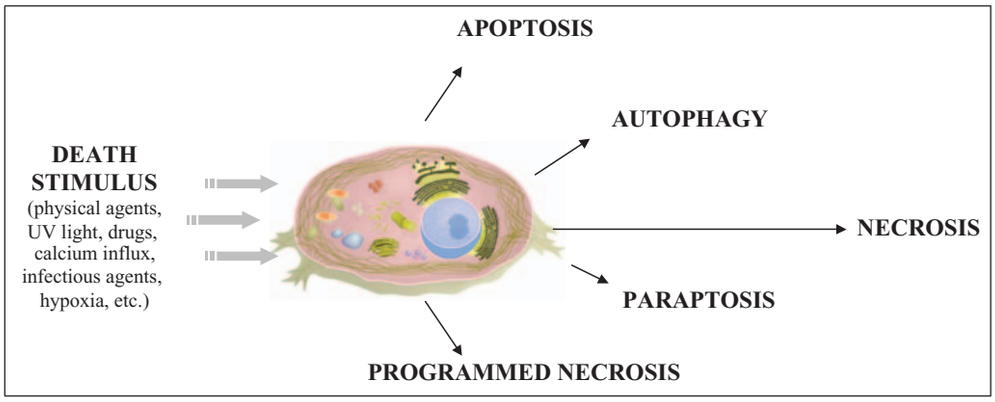

FIGURE 1.1: *Various models of cell death (© Quill Graphics, www.cellsalive.com).*

for a given injury, or if injury accumulation reaches a critical level, the damaged cells commit suicide.[3] Cell injury can, therefore, be reversible (sublethal) or irreversible (lethal). Cells may be reversibly injured, but if severely injured, they may be unable to recover and cell death will occur. The death stimuli are diverse and include normal physiological signals, such as hormones that trigger deletion of cells during differentiation or involution of tissues and organs, maturation of organ systems as, for example, in the immune system, and removal of cells that have sustained some form of damage.[2] Alternatively, cells already may be primed to undergo cell death, with the withdrawal of important extracellular components, such as serum or growth factors, providing the signal.[12] Other death stimuli also are important from a biomedical perspective. These include physical agents (ultraviolet [UV] light causing damage to the skin, hyperthermia, cold, and trauma), cytotoxic drugs, calcium influx, glucocorticoids, infectious agents (bacteria, virus, yeast), and hypoxia. The stimuli that initiate the death pathways vary widely with the affected cells.[13] In particular, various stimuli (e.g., cytokines, heat, irradiation, pathogens) can cause both apoptosis and necrosis in the same cell population (Figure 1.1). Apoptosis can be induced by a lower concentration or level of almost all the stimuli that cause necrosis.[14] This means that the mechanism of self-destruction can be activated by a relatively mild stimulus. Whereas mild hypoxia produced symptoms of apoptosis, severe hypoxia produced infarction and necrosis;[15] similarly, exposure to temperatures between 37°C and 43°C induced apoptosis in lymphocytes, and exposure to higher temperatures induced necrosis.[16] Therefore, the character of the injury will determine the pattern of cell death evoked. This aspect is important to highlight. The three main features of injury are type of injury, exposure time, and severity.

Type of Injury

The injury can be, for example, physical, chemical, or toxic, but the response will be different for different cell types. In fact, some cells will be more susceptible than others to agents (heart muscle cells are more susceptible than connective tissue cells to oxygen depletion).

Exposure Time

The length of exposure to a particular stimulus will affect the chances of cell survival. Relatively resistant cells will be damaged if the duration of exposure is prolonged.

Severity

The ability of a cell to survive an injury also will depend on its severity; if the withdrawal of growth factor is partial, the cell is still able to survive for a long period (depending on cellular resistance), but if it is complete, cell death occurs in a very short time with modalities that vary from cell to cell.

We now describe some models of cellular death, taking into consideration that a clear-cut definition cannot be given because of the overlapping of the different programs of cell death.

Apoptosis. Cells have different ways of committing suicide and may select the fastest and most effective of the options available. Apoptosis has been considered for years as the PCD paradigm and is still considered one of the main pathways activated during stressful conditions, although alternative pathways were recently identified.[1] The term "apoptosis" derives from the ancient Greek word used to describe the "falling off" or "dropping off" of petals from flowers or leaves from trees, to emphasize the normal physiological nature of the process.[17] In 1972 this kind of cell death was first described[17] and noted that it was truly distinct from necrosis, underscoring the importance of apoptosis in human medicine. As part of the immune response, apoptosis allows the elimination of virally infected and cancer cells or the deletion of unnecessary or potentially dangerous lymphocytes.[18] Defects of apoptotic cell death may promote tumor or autoimmune disease development. The apoptotic process has been shown to proceed

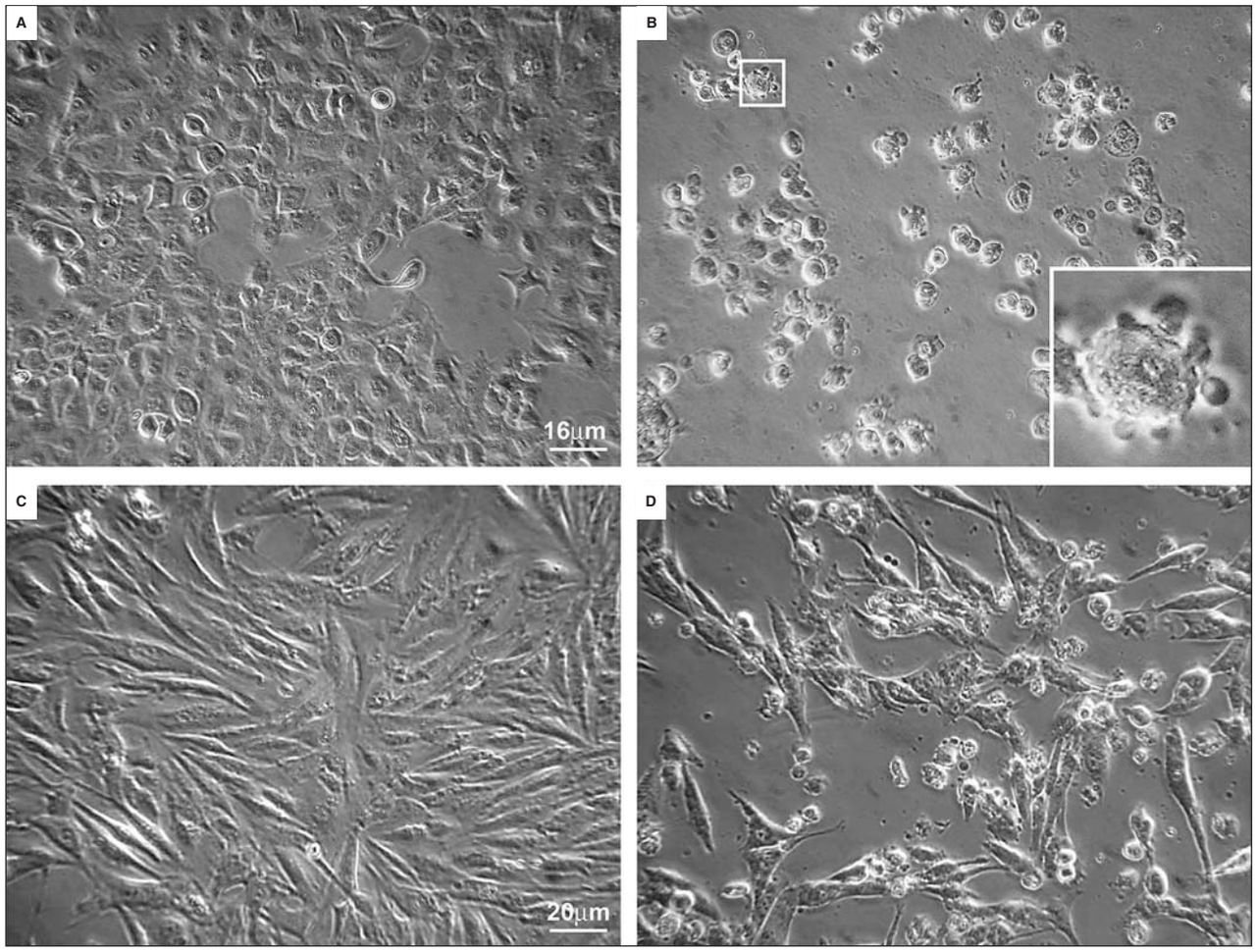

FIGURE 1.2: ***Morphological changes occurring in A549 (human carcinoma lung cell line) (panel B) (R. Nicoletti, E. Buommino, A. De Filippis, M. P. Lopez-Gresa, et al. World J Microbio and Biotechnol. 2008; 24: 189–95) and NCI (human mesothelioma cell line) (panel D) cells (E. Buommino, I. Paoletti, A. De Filippis, R. Nicoletti, et al. Cell Prolif. forthcoming 2010), treated with proapoptotic metabolites, with a particular of membrane budding shown in panel B; panel A and C show the morphology of A549 and NCI untreated cells, respectively. Magnification: 20X.***

via a number of discrete steps. Cells undergoing apoptosis are characterized morphologically by cell shrinkage, chromatin condensation, loss of contact with neighboring cells and the extracellular matrix (Figure 1.2), [19] actin cleavage,[20] and biochemically by DNA laddering (Figure 1.3).[19] The last is a peculiarity of most apoptotic pathways. The double-stranded linker deoxyribonucleic acid (DNA) between nucleosomes is cleaved at regularly spaced internucleosomal sites, giving rise to DNA fragments representing the length of nucleosomes (180–200 base pairs).[13] Molecular characterization of this process identifies a specific DNase (caspase-activated DNase) that cleaves chromosomal DNA in a caspase-dependent manner.[21] Other features of apoptosis are early depolymerization of cytoskeletal proteins, loss of phospholipid symmetry in plasma membrane with the outer layer exposure of phosphatidylserine (PS) residues, and the appearance of a smooth-surfaced protuberance of the plasma membrane with its preserved integrity. The fragmentation of both nucleus and whole cell then produces membrane-bound bodies in which the organelles are intact to form apoptotic bodies (Figure 1.2 [inset]).[22] This is also called the "budding phenomenon" and should not be confused with blebs, fluid-filled structures typically devoid of organelles.[6] The apoptotic bodies are cleared from tissues by professional phagocytes, such as macrophages, but also epithelial cells and even fibroblasts have been shown to clear apoptotic bodies.[23,24] Phagocytosis is initiated by the exposure of the PS receptor located on the membrane of the phagocytes and vitronectin receptors, resulting in a cell-signaling response.[22] The apoptotic pathway and the engulfment process are part of a continuum that helps ensure the noninflammatory nature of this death paradigm. Studies in mammals have highlighted the importance of proper disposal of apoptotic bodies by phagocytic cells.[24] The suppression of proinflammatory factors is necessary during apoptotic

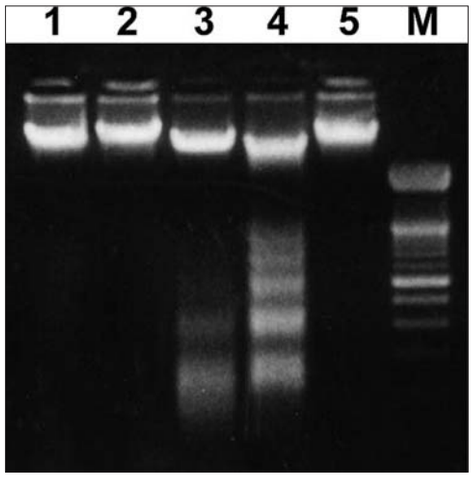

FIGURE 1.3: DNA feature of HeLa cells treated or not with 3-O-methylfunicone. DNA fragmentation induced in OMF treated cells after 48 and 72 h (lane 3 and 4, respectively). Lane 1, untreated cells; lane 2, cells treated for 24 h; lane 5, negative control (absolute ethanol). M, 100 bp ladder (Roche Diagnostics) used as MW-marker. (E. Buommino, R. Nicoletti, G. M. Gaeta, M. Orlando et al. Cell Proliferation. 2004; 37:413–26)

body clearance. This suppression is accomplished at least in part by a release of antiinflammatory factors including transforming growth factor β and IL-10 by macrophages engaged in corpse engulfment. Furthermore, regulatory mechanisms help ensure that, when phagocytosing dendritic cells present peptides from apoptotic bodies to T cells, no immune reaction against self-peptides is initiated. Defects in the clearance of corpses are predicted to create a proinflammatory milieu that may predispose to autoimmune disorders.

A cascade of genes is activated as a consequence of the induction of a defined genetic program in which caspases have a prominent role. Caspases are cysteine proteases (preexisting as inactive zymogen precursors in the cell) that cleave substrates at critical aspartic acid residues.[18] Activation of caspases is the central event in apoptosis, leading to the cleavage of numerous proteins involved in the cell structure, cell-cycle control, and DNA synthesis and repair. The initiator caspases (caspase-2, -8, -9, and -10) are activated by interaction with caspase adapters, whereas the effector caspases (caspase-3, -6, and-7) are downstream of the activator caspases and act to cleave various cellular targets and substrates and induce cell death.[18] The enzyme poly(adenosine diphosphate [ADP]-ribose) polymerase, or PARP, was one of the first proteins identified as a substrate for caspases. PARP is involved in the repair of DNA damage. It functions by catalyzing the synthesis of PARP and by binding to the DNA strand breaks and modifying nuclear proteins.[25] The ability of PARP to repair DNA damage is prevented following cleavage of PARP by caspase-3. The inflammatory caspases are involved in cytokine activation and are represented by caspases-1, -4, -5, -11, -12, -13, and -14.

Caspases can be activated through three main pathways: an "extrinsic" death receptor (DR)–mediated process and two "intrinsic pathways," a mitochondria-mediated– and an endoplasmic reticulum (ER)–mediated pathway (Figure 1.4).[1,18]

The extrinsic pathway involves the surface DRs, a subfamily of the tumor necrosis factor receptor (TNF-R) superfamily activated in response to specific extracellular signals.[26] To date, eight DRs have been identified, namely, Fas (CD95, Apo-1), TNF-related apoptosis-inducing ligand (TRAIL)-receptors 1 (TRAIL-R1) (DR4) and 2 (DR5, Apo-2), TNF-R1, TRAMP (WSL-1, Apo-3), EDAR, p75 neurotrophin receptor (p75NTR), and DR6.[26] Despite their name, not all of these receptors induce apoptosis, but they may trigger specific signaling pathways that result in a variety of cellular outcomes. The DRs comprise three domains: an extracellular cysteine-rich domain for ligand binding, a transmembrane domain, and an intracellular death domain (DD), which is required for apoptotic signal transduction.[26] The DR TNF ligand (TNF-L), Fas ligand (FasL), and TRAIL induce apoptosis by binding to their cell membrane receptors. Following ligand binding, a conformational change in the intracellular domains of the receptors reveals the presence of a "death domain," which allows the recruitment of various apoptotic proteins to the receptor. This protein complex is known as the death-inducing signaling complex (DISC). The final step in this process is the recruitment of one of the caspases, typically caspase-8, to the DISC. This recruitment results in the activation of caspase-8 and the initiation of apoptosis.

Interestingly, there are also decoy receptors (DcRs) that compete to bind ligands to DRs, allowing the cell to escape death ligand–induced killing. DcR1 and DcR2 compete with DR4 or DR5 to bind to TRAIL. DcR3 competes with Fas to bind to the FasL.

The intrinsic cell death pathway involves the mitochondria and ER. The mitochondria-mediated pathway is induced by lethal intracellular signals such as oncogenic transformation and DNA damage. Mitochondria contain many proapoptotic proteins such as apoptosis-inducing factor (AIF), cytochrome *c*, and Smac/DIABLO.[27] The last is a protein that directly neutralizes inhibitors of apoptotic proteins (IAPs), such as survivin, originally described as an inhibitor of apoptosis proteins with a cell-cycle–specific function.[28] AIF, cytochrome *c*, and Smac/DIABLO are released from the mitochondria following the formation of a pore in the mitochondrial membrane called the permeability transition (PT) pore. These pores are thought to form through the action of the proapoptotic members of the bcl-2 family of proteins, which in turn are activated by apoptotic signals such as cell stress, free radical damage, or growth factor deprivation.[29] In particular, AIF and Smac/DIABLO were also reported to be involved in the mitochondrial death pathway related not to apoptosis but to the apoptosis-like death pathway.[30] The release of cytochrome *c* from the mitochondria is a

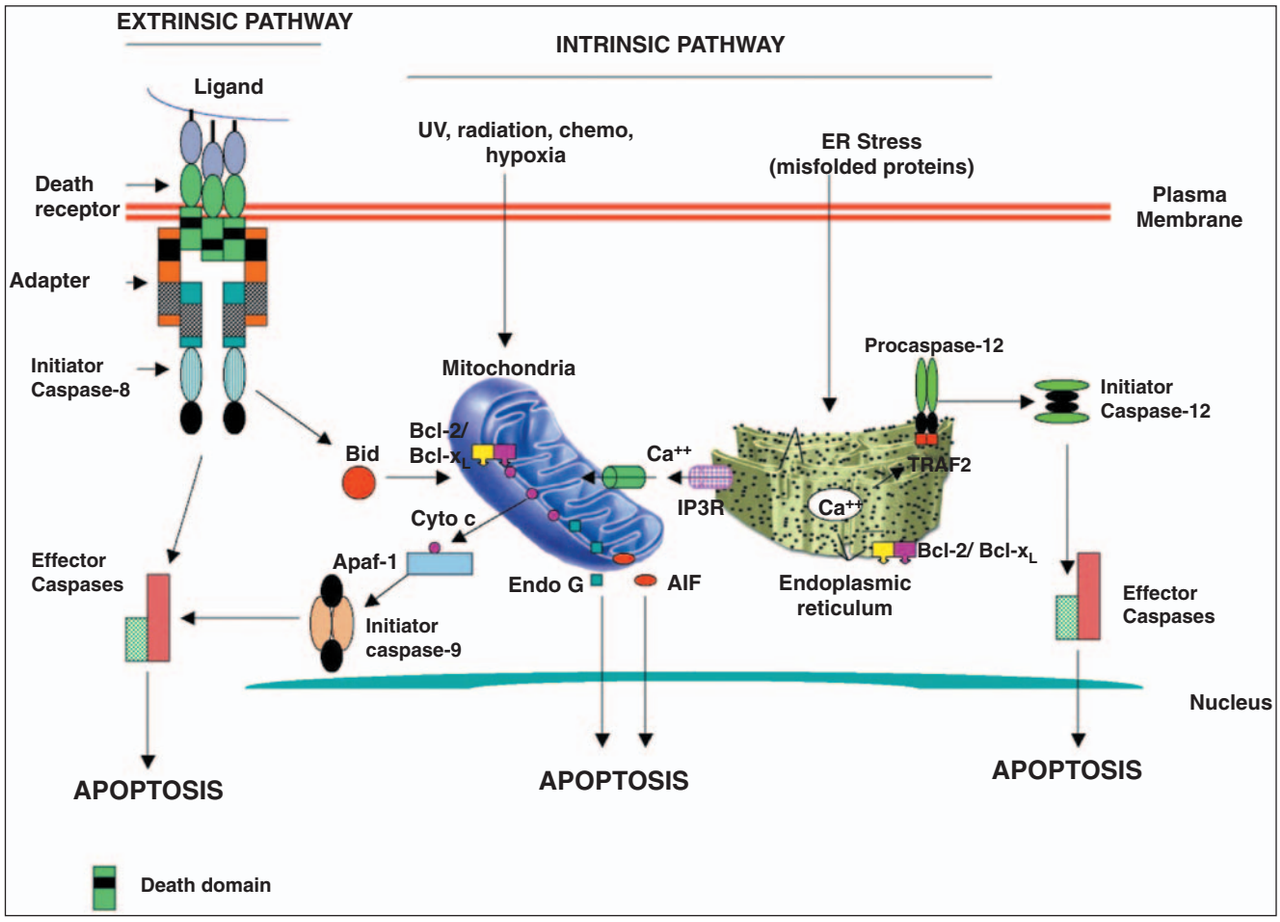

FIGURE 1.4: ***The two main pathways for the initiation of apoptosis: the extrinsic pathway and the intrinsic pathway (S. Gupta, A. Agrawal, S. Agrawal, H. Su et al. Immunity & Ageing 2006; 3:5 doi:10.1186/1742-4933-3-5).***

particularly important event in the induction of apoptosis. When cytochrome *c* has been released into the cytosol it interacts with a protein called Apaf-1. This interaction leads to the recruitment of procaspase-9 into a multiprotein complex with cytochrome *c* and Apaf-1 called the apoptosome. Specifically, adenosine triphosphate (ATP) is required for the formation of the apoptosome, necessary for the activation of caspase-9 and the induction of apoptosis. If damage to the mitochondria is such that the ATP levels are insufficient to complete the apoptotic process, the mode of death may be directed toward necrosis.

The bcl-2 proteins are a family of proteins involved in the response to apoptosis. Some of these proteins (such as bcl-2 and bcl-X_L) are antiapoptotic, whereas others (such as Bad, Bax, or Bid) are proapoptotic. The sensitivity of cells to apoptotic stimuli can depend on the balance of pro- and antiapoptotic bcl-2 proteins. When there is an excess of proapoptotic proteins, the cells are more sensitive to apoptosis, but when there is an excess of antiapoptotic proteins, the cells will tend to be more resistant. An excess of proapoptotic bcl-2 proteins at the surface of the mitochondria is thought to be important in the formation of the PT pore.[29] The proapoptotic bcl-2 proteins are often found in the cytosol, where they act as sensors of cellular damage or stress. Following cellular stress they relocate to the surface of the mitochondria, where the antiapoptotic proteins are located. This interaction between proapoptotic and antiapoptotic proteins disrupts the normal function of the antiapoptotic bcl-2 proteins and can lead to the formation of pores in the mitochondria and the release of cytochrome *c* and other proapoptotic molecules from the intermembrane space. This in turn leads to the formation of the apoptosome and the activation of the caspase cascade. The *bcl-2* gene has been shown to be transcriptionally repressed by *p53*.[31] The *p53* tumor suppressor gene codes for the p53 protein and plays an important role in the control of the cell cycle, apoptosis, senescence, differentiation, and accelerated DNA repair.[32] DNA damage caused by exposure to ionizing radiation, UV light, or some exogenous or endogenous chemical mutagens, which results in DNA strand breakage, can trigger an accumulation of *p53*. This

TABLE 1.1: Different Characteristics of the Cell Death Pathways

Types, characteristics	Apoptosis	Autophagic cell death	Paraptosis	Programmed necrosis	Necrosis
Triggers	Death receptors, trophic factor withdrawal, DNA damage, viral infections, etc.	Serum amino acid starvation, protein aggregates	Trophotoxicity	Ischemia, excitotoxicity	Excessive damage by physical or chemical injury, high intensities of pathological insult
Plasma membrane	Membrane-bound apoptotic bodies, blebbing	Elongation and invagination, blebbing	Shrinkage	Rapid loss of plasma membrane integrity	Rapid disintegration
Nucleus	Chromatin condensation, internucleosomal DNA cleavage (ladder)	Pyknosis in some cases, but neither prevalent nor striking, no DNA laddering	Late disintegration	No chromatin condensation, in some cases chromatin clustering to loosen speckles	Karyolysis
Cytoplasm	Condensation and shrinkage, cytoskeleton collapse	Vacuolization, autophagosome and autolysosome formation	Vacuolization	Swelling, extensive vacuolization	Condensation, loss of structure, fragmentation, swelling
Organelles	Preservation	Enwrapped by membrane sac. Autodigestion	Swelling	Swelling	Condensation and final disintegration

gene can activate transcription of growth regulatory genes such as *p21 WAF1/Cip1*, *GADD-45*, and *cyclin G*, resulting in G_1 growth arrest, presumably to allow for repair of damaged DNA. If irreparable DNA damage exists, the cell becomes committed to the apoptosis pathway and is deleted by the system. For this reason, p53 is known as the "guardian of the genome." Mutant p53 proteins may allow an escape from this surveillance mechanism and generation of a malignant phenotype.

The ER is another important sensor of cellular stress that can withhold protein synthesis and metabolism to restore cellular homeostasis. Misfolded proteins are constantly produced; these proteins trigger a protective stress response, known as the unfolded protein response. Although this response may put off a cellular catastrophe for a short time, if the damage to ER is too extensive, the damage can initiate PCD via the unfolded protein response or via release of calcium into the cytoplasm.[33] Thus caspase-12 is activated, which then engages caspase-9 and leads to the effector cascade recruitment.[34] In addition, an intracellular calcium influx caused by ER stress induces the activation of a family of cytosolic proteases, the calpains (calcium-activated neutral proteases), which normally reside in the cytosol as inactive zymogenes.[35,36] Calpains, kept in control by their natural inhibitor calpastatin, have been shown to act downstream of caspase. In fact, it has been demonstrated that vitamin D compounds trigger cell death in MCF-7 cells via calpains and independent of caspase activation, thus indicating a role of ER in certain types of caspase-independent cell death.[37] Disorders such as Alzheimer disease, Parkinson disease, Huntington disease, amyotrophic lateral sclerosis, and prion protein disease all share the common features of accumulation and aggregation of misfolded proteins.[38]

OTHER FORMS OF PCD

Compared to apoptosis, relatively little is known about autophagic PCD and paraptosis, and even less is known about other nonapoptotic forms of PCD. Most of what is known is based on morphological descriptions. The exact phenotype of a dying cell is certainly dependent on many different factors that include the cell type, the cellular context, and the specific death stimulus.[30] Characteristic changes that differ in the various forms also include modifications of the cell shape and architecture, such as alterations of the cytoskeleton (Table 1.1). Any questions about the other forms of cell death remain unanswered: How important is the activation of the different PCD for the organism, and are all the mediators that trigger one type of death or another known? To these and other questions we will try to give an answer.

ACD

ACD is a long-known nonapoptotic cell death modality, also called type II cell death (to distinguish it from apoptosis or type I cell death).[39] Phagocytosis and autophagy

are two well-known processes involved, respectively, in the removal of extracellular organisms and the destruction of organisms in the cytosol. Autophagy, for either metabolic regulation or defense, involves the formation of a double membrane called the autophagosome, which then fuses with lysosomes to degrade the contents, a process that has similarities to phagosome maturation. Autophagy is, in fact, normally activated during starvation by nutrient sensors to allow the recycling of substrates and organelles and to ensure the metabolic precursor.[40] Autophagy is also a means to eliminate dysfunctional organelles and allow a turnover of long-living proteins, thus preventing their pathological accumulation in the cells. Consequently, the cell "cannibalizes itself" from the inside (autophagy = "self-eating" in Greek). When this self-eating reaches excessive levels it may progress toward ACD, occurring in response to prolonged deprivation or stress, during embryogenesis, in adult tissue remodeling, in human diseases, or during cytotoxic drug treatment.[39] Autophagy is often observed when massive cell elimination is needed or when phagocytes do not have easy access to the dying cells. ACD is differentiated from apoptosis by certain peculiarities, including autophagosome and/or autolysosome formation, a vast autodigestion of organelles, a preserved nucleus until late stages (with the absence of DNA laddering), and cytoskeleton preservation until the final stages. In contrast to apoptosis, ACD occurs in a caspase-independent pathway. Interestingly, autophagy can be a factor in both the promotion and prevention of cancer, and its role may be altered during tumor progression.[41] The first autophagy gene identified in humans was *Beclin 1*. The heterogeneous disruption of this gene leads to increased tumorigenesis in mice.[42] Beclin 1 is inhibited by its interaction with Bcl-2, which thus not only functions as an apoptotic suppressor, but also as an antiautophagic factor.[43] Another aspect to be considered is that some malignant cell types respond to anticancer agents by triggering autophagy, indicating the potential utility of ACD induction in cancer therapy. Cancer cells may need autophagy to survive nutrient-limiting and low-oxygen conditions, and autophagy may protect cancer cells against ionizing radiation by removing damaged elements. The precise role of this cell death is, therefore, not yet fully understood, but it is important to underline that autophagy and apoptosis can be observed simultaneously in the same tissue, and, in some cases, autophagy may precede and later trigger apoptosis when the autophagic capacity is overwhelmed.[39] In other settings, autophagy has been observed to delay or antagonize apoptosis, and there are also examples in which the two processes can be mutually exclusive.[39]

PARAPTOSIS

Recently, a novel nonapoptotic PCD process designated paraptosis was described by Sperandio and colleagues.[10] The features of paraptosis differ from those of apoptosis and involve cytoplasmic vacuolation, mitochondrial swelling, the absence of caspase activation, and typical nuclear changes including pyknosis and DNA fragmentation.[10] There is increasing evidence that this alternative, nonapoptotic PCD exists in parallel with apoptosis. The neuropeptide substance P and its receptor, neurokinin-1, mediate a nonapoptotic form of PCD resembling paraptosis in some cases.[44] Activated microglia trigger neuronal cell death with ultrastructural characteristics of marked vacuolation and slightly condensed chromatin following the blockage of the caspase cascade.[45] In addition, ceramide induces nonapoptotic PCD with necrosis-like morphology in human glioma cells in the presence of pan-caspase inhibitors or during overexpression of bcl-X_L.[46] These examples support the theory that cells have other intrinsic programs for death that are distinct from apoptosis. This death program can be mediated by mitogen-activated protein kinases and can be triggered by the TNF-R family member *TAJ/TROY*, capable of inducing apoptosis independent of DNA fragmentation and caspase activation, and the insulin-like growth factor I receptor.[1,47] The idea that PCD might be induced by hyperactivation of a trophic factor receptor (trophotoxicity) is compatible with an earlier observation that some trophic factors may increase neuronal cell death, for example, that induced by excitotoxicity.[48] Such an effect might be protective against neoplasia in that it may eliminate cells that would otherwise undergo autocrine loop–stimulated oncogenesis. The resulting program would necessarily be nonapoptotic because trophic factors inactivate apoptotic signaling.

NECROSIS AND PROGRAMMED NECROSIS

For a long time necrosis was considered as an alternative to apoptosis.[7,8] Recently, necrosis, once thought of as simply a passive, unorganized way to die, has emerged as an alternative form of PCD, the activation of which might have important biological consequences, including the induction of an inflammatory response.[49] The term necrosis has, therefore, been wrongly used for years to define an alternative mode of cell death. It is now evident that we cannot refer to necrosis to mean a particular program of death and that this term should be used to describe what happens after a cell is dead. It is, therefore, more correct to use the term "programmed necrosis" or "necrosis-like PCD" when we describe certain kinds of cell death governed by a specific genetic program and quite different from classical apoptosis or not falling within the cell death pathways described earlier in this chapter.[6] There are many examples of programmed necrosis being a normal physiological and regulated (programmed) event. Signaling pathways (e.g., DRs, kinase cascades, and mitochondria) participate in both processes, and, by modulating these pathways, it

is possible to switch between apoptosis and programmed necrosis. Moreover, antiapoptotic mechanisms (e.g., bcl-2/bcl-x proteins, heat shock proteins) are equally effective in protecting against apoptosis and programmed necrosis. There are several examples of necrosis during embryogenesis, normal tissue renewal, and the immune response.[7] The core events of programmed necrosis are bioenergetic failure and rapid loss of plasma membrane integrity. These events can result from specific molecular events that occur in the dying cell, including increased mitochondrial reactive oxygen species production, channel-mediated calcium uptake, activation of nonapoptotic proteases, and/or enzymatic destruction of cofactors required for ATP production. Karyolysis of the nucleus occurs as a consequence of the complete dissolution of the chromatin due to the activity of specific DNase. In addition, these necrotic mediators are often induced in the dying cell simultaneously and enhance each other's ability to initiate the demise of the cell.[50] Calpain and lysosomal cathepsin activation have been shown to contribute to necrotic cell death. Due to the immunogenic potential of the necrotic cell debris removal, the target induction of programmed necrosis is gaining attention among immunologists and oncologists in cancer immunotherapy.[51] At this point, it is important to note that, to complicate the intricate net of terminologies used to define the area of apoptosis versus necrosis, the term "oncosis" (from the Greek word for swelling), a form of cell death activated by ischemia, has also been used through the years to define all the situations in which marked cellular swelling occurred. Extensive literature on the morphological criteria for oncosis exists, but the biochemical pathway(s) of oncosis has not yet been described. Oncosis is thought to be mediated by a failure of plasma membrane ionic pumps. One potential mediator of oncosis is a calpain-family protease (possibly a mitochondrial calpain), which suggests that oncosis may turn out to be related to, or synonymous with, a calcium-activated programmed necrosis cell death. Majno and Joris proposed this term for designating any programmed cellular suicide characterized by a marked swelling, whereas the term "necrosis" refers to the features that appear after the cell has died.[6] Necrosis may be either oncotic or apoptotic in origin. In this context, oncosis comprises the prelethal changes leading to ischemic or coagulation necrosis, whereas necrosis describes a morphology but not a process, thus underscoring the final feature of a dead cell.

APOPTOSIS AND HUMAN DISEASES

Nonregulated apoptosis involves different pathophysiological situations such as malignant and premalignant conditions, neurological disorders (e.g., Alzheimer disease, prion-associated disorders), heart disease (ischemic cardiac damage, chemotherapy-induced myocardial suppression), immune system disorders (e.g., acquired immune deficiency syndrome [AIDS], type I diabetes, systemic lupus erythematosus [SLE], Sjögren syndrome), intestinal disorders, and kidney disease.[2] In particular, diseases characterized by the accumulation of cells include cancer, autoimmune diseases, and certain viral illnesses. Cell accumulation can result from either increased proliferation or the failure of cells to undergo apoptosis in response to appropriate stimuli.

Cell Death in Cancer

Tumor growth occurs when the cellular birth rate exceeds the death rate. Control of cell growth is important in the process of normal development and tissue homeostasis, and in pathological conditions such as neoplasia. Growth arrest and cell death are also important in normal and neoplastic growth.

Inactivation of apoptosis is a hallmark of cancer, an obligate ritual in the malignant transformation of normal cells. By inactivating apoptosis, cancer cells enhance their chances of survival and increase their resistance to chemotherapeutic agents. Because apoptosis is a gene-controlled process, it is susceptible to genetic manipulation for therapeutic purposes, such as in cancer treatment. The acquisition of resistance to apoptosis is important in the transition from normal melanocyte to melanoma. Apoptosis is, in fact, critical for epidermal homeostasis, representing a key protective mechanism removing premalignant cells that have acquired mutations.[52]

Melanoma is the most aggressive form of skin cancer, notoriously resistant to current modalities of cancer therapy and known to be a tumor with an elevated metastatic ability.[53] Although today melanoma is more often diagnosed in an early stage of disease and therefore shows a better overall survival, when tumor cells are detected in the regional lymph node, the patient has a poorer prognosis. One of the earliest events in melanoma progression involves the unregulated proliferation of melanocytes. In this stage of melanoma progression, the cells lose their ability to maintain the cell-cycle controls that function in normal unstimulated melanocytes. This loss of cell-cycle control can lead to sustained proliferation, decreased apoptosis, or both. It also has been reported that melanocytes displayed a broad expression of apoptotic inhibitors to maintain their longevity, at the cost of the nonelimination of damaged cells, thus resulting in a high probability of developing melanoma.[52] In contrast, keratinocytes are more prone to undergoing apoptosis to ensure a rapid turnover and efficiently remove damaged cells and meet their functional needs in the skin. Melanoma cells are resistant to a wide range of antineoplastic treatments because of their ability to evade the cytotoxic action of different insults such as DNA damage, microtubule destabilization, or topoisomerase inhibition,[53] showing, in contrast, strong resilience. In fact, melanoma cells in vivo demonstrate low

levels of spontaneous apoptosis compared with other tumor cell types, and resistance to apoptosis is associated with increased resistance to chemotherapeutic agents.[54] The knowledge acquired about the altered apoptotic mechanism in melanoma has focused the attention of researchers on molecules able to compensate for or bypass the cell death defects and on the development of new chemotherapeutic strategies that facilitate the death of cancer cells.

Cell Death and Autoimmune Disorders

Physiological regulation of cell death is essential for the removal of potentially autoreactive lymphocytes during development and for the removal of excess cells after the completion of an immune response. Failure to remove autoimmune cells that arise during development or that develop as a result of somatic mutation during an immune response can result in autoimmune disease.[55] Upregulated levels of soluble Fas, which might competitively inhibit FasL–Fas interactions, have been documented in many autoimmune disorders such as rheumatoid arthritis, SLE, and pemphigus vulgaris (PV).[56] PV is a chronic autoimmune cutaneous disease characterized by circulating autoantibodies that cause blisters and erosions on the skin and mucous membranes.[57] Circulating autoantibodies bind with the epidermal cell membrane and cause cell–cell detachment (acantholysis), leading to epidermal tissue damage. In recent years, the idea that apoptosis might play a central role in the induction of acantholysis has gained momentum. In support of this supposition, a study by Weiske and colleagues demonstrated the proteolytic cleavage of desmoglein 3 (PV antigen) by caspase-3 during apoptosis, thereby causing desmosome disruption only after the induction of apoptosis.[58]

In a past study, we showed the ability of pemphigus serum and captopril to induce apoptosis in human keratinocytes.[59] In particular, the authors demonstrated that a drug (captopril) or antibodies (PV serum) acting, respectively, by a biochemical or immunological mechanism induced acantholysis through the same genetic program leading to PCD. Of interest is a contribution published by Arredondo and colleagues demonstrating the therapeutic action of intravenous immunoglobulin (IVIg) in PV.[60] In the plethora of biological effects exerted by IVIg administration (acceleration of the clearance of autoantibodies, modulation of serum levels of proinflammatory cytokines, induction of immunocompetent cell death), an array of antiapoptotic effects should also be mentioned. IVIg inactivates FasL, protects target cells from apoptosis by upregulating Bcl-2 expression, interferes with TNF-α and interferon-γ signaling pathways, and increases sensitivity to corticosteroid action, thus strengthening the idea that apoptosis may play an important role in the onset of the disease.

CONCLUSIONS

Even in diseases in which the affected cells have been shown to die with "apoptotic morphology," one cannot exclude the possibility that a caspase-independent cell death program occurs in concert with a caspase-dependent program.[61] The knowledge of the genetic program underlying the onset of the disease might help the researcher to use the appropriate genetic therapy by inhibiting one pathway or another. In such cases, it is important to understand whether a program of death is controlled by caspase activation. The inhibition of the caspase cascade to control apoptosis induction in some degenerative diseases can delay (but not prevent) the progression of the disease if some other caspase-independent program of death is operating. The occurrence of one or another of the different programs of cell death is an important aspect to take into account. In fact, one of the cancer therapy approaches is to kill cancer cells by apoptosis. It is also known that cancer cells are selected for their acquired resistance to apoptosis. It is, therefore, important to be able to exploit other genetic programs to complement or integrate apoptosis and perhaps open new frontiers for tumor therapy.

Despite the numerous models proposed to categorize PCD, it is difficult to give one single definition, and probably also incorrect, due to the overlap and shared signaling pathways of the different death programs. It has, therefore, been postulated that the dominant cell death phenotype triggered by cytotoxic agents is determined by the most readily available death program.[62] Besides caspases, a broad spectrum of proteases can carry out PCD, with the participation of different cellular organelles, including mitochondria, lysosomes, or ER, which can act independently or actively collaborate with each other. The multicellular organism can take advantage of the existence of multiple death pathways because they offer protection, for example, against the development of malignant diseases. Many difficulties and obstacles have to be overcome before a cell becomes a tumor cell, and this in part explains the rarity of cancer, considering the number of cell divisions and mutations that occur during human life. The control of PCD may ultimately offer a new perspective in cancer immunotherapy, but also in the treatment of autoimmune diseases and neurodegenerative disorders.

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