DEATH BY FINE TUNNING

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ABSTRACT

Since the first evidence that physiological cell death is a normal feature of cell life, there has been considerable effort in trying to define the mechanisms, regulation and morphology of cell death events. In nearly four decades of investigation, several types of cell death have been described in a wide range of organisms and cells, and this has led to great deal of confusion regarding the morphology and regulation of these processes. Historically, cell death has been characterized as physiological or accidental (*necrosis*). However, in the past five years, several attempts have been made to define the types of cell death based on mechanistic and morphological criteria. Currently, at least three types of cell death apoptosis, autophagy and necrosis are recognized and share some mechanisms in common. Thus, cell death is more than simply being a caspase-mediated phenomenon. The aim of this review is to discuss recent findings on how cells choose to die in different biological contexts, and to consider the morphological changes associated with each cell type of cell death.

Key words: Apoptosis, autophagy, necrosis, programmed cell death, programmed necrosis

INTRODUCTION

The term cell death, which is generally used to refer to cell elimination in a variety of situations, is attractive to biologists. Biologically, cell death is not simply "the terminal point of cellular life and function", but plays a major role in several processes during embryonic and post-embryonic development, including morphogenesis. Throughout adult life, cell death balances cell proliferation to maintain the homeostasis of tissue and organs. Cell death also occurs when undesirable or damaged cells need to be eliminated, as well as in response to viral infections. Defects in the regulation of cell death are widely recognized as the molecular basis of several human diseases, such as autoimmune and degenerative diseases, diabetes and cancer [23,32,45].

The study of cell death advanced significantly after the report by Kerr *et al.* in the 1970s [36] in which this process was referred to as apoptosis in reference to the loss of leaves by trees during fall. This term was subsequently applied indiscriminately to all sorts of cell death processes, although apoptosis is now widely recognized as the major, and best understood process of cell death [18,19,36,44,46]. However, the question as to whether all cell death processes are very much like apoptosis remains to be resolved and will be discussed below.

An understanding of the processes involved in cell death can provide insights into cellular function in a "social context" within tissues and organs, and also helps to explain cell aging and differentiation [11,12,22]. The biological implications of cell death have lead to intense efforts to unravel the molecular basis and morphology of this phenomenon [29]. The rapid development of the field of cell death in the past 20 years has resulted in the description of many processes with varying morphologies and machineries in widely different organisms such as nematodes and mammals. While apoptosis is still generally used to define many cell death processes, a new paradigm has arisen, in which cells can also die by two distinct non-apoptotic mechanisms, namely autophagic cell death and programmed necrosis [25,35,37,48,60].

Despite the rapid progress in this field, many of the early questions are remain unanswered, e.g. Are cell death processes always physiological? Is necro-

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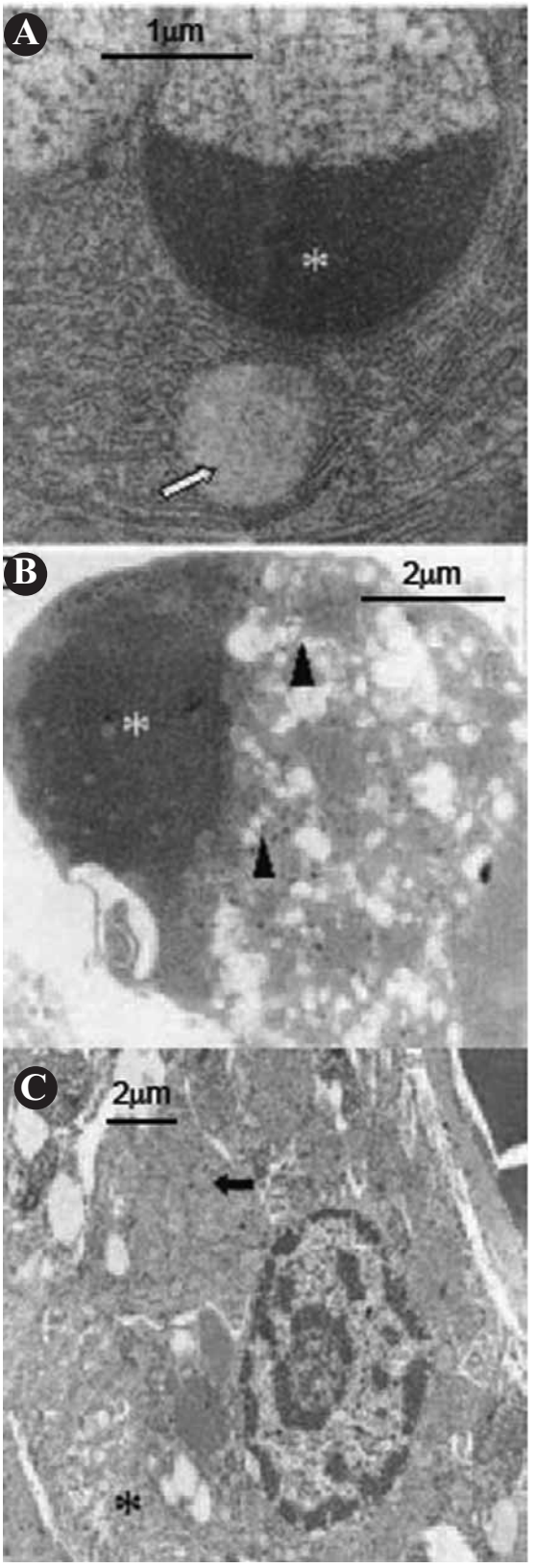

sis accidental cell death? Is autophagy an alternative way of killing cells? How do organisms select the best way to kill cells? How are the distinct types of cell death related to diseases? and Could an understanding of the cellular and molecular bases of these different forms of cell death provide the basis for new therapies? As the cell death machinery becomes better understood and conserved evolutionary relationships are established answering these questions has become a major focus in the study of cell death.

The main goal of this review is to summarize recent data on the major types of cell death in terms of their concepts and occurrence rather than to discuss detailed molecular mechanisms. The reader is referred to others reviews in the literature for a better understanding of the cell death machinery [19,24,66].

Reborn from an accident

For many years, the starting point for discussing cell death was to consider its division into physiological and accidental cell death. As far as accidental cell death is concerned, necrosis was the process that best fitted this concept, at least until ~5 years ago.

Necrosis has been described as uncontrolled death caused mainly by ATP depletion and osmotic stress leading to a recognizable morphology of cellular and organelle swelling followed by cell lysis that triggers inflammatory responses (Fig. 1) [47]. As will be discussed below, this concept is controversial since recent data have shown that necrosis can be considered as a physiological and regulated form of cell death known as *programmed necrosis* [60,75].

On the other hand, physiological cell death is

Figure 1A-C. A- An apoptotic mouse pancreatic acinar cell two days after ligation of the secretory ducts. A large number of these cells is seen under these conditions, their major characteristic being the very compacted chromatin and its peripheral distribution (**asterisk**). Although these cells are apoptotic, they still have very well preserved ER cisternae as well as swollen mitochondrion (**arrow**). Bar = 1 µm. **B**- Autophagic PC12 cell with a cytoplasm rich in electron-lucent autophagic vesicles (**arrowheads**) and a pycnotic nucleus (**asterisk**). This autophagic morphology is seen after 16 h in culture medium deprived of fetal bovine serum (Bar = 2 µm). Before this time, cell death was predominantly apoptotic. **C**- Proximal renal tubule cell in initial necrosis characterized by a large number of swollen mitochondria with electron-dense amorphous spots (**arrows**) and microvillar collapse (**asterisk**). This cell death was seen 1 h after ligation of the renal artery. Bar = 2 µm. Electron micrographs by courtesy of Dr. Antonio Sesso (ICB-USP).

a normal feature of cellular function that can be activated or blocked physiologically. Like many other cellular processes, a specific set of gene products is required for physiological cell death. This definition fits the term *programmed cell death* very well because of the genetic potential of cells to die in a regulated mode, i.e. cell death as normally seen during development, such as in the elimination of larval tissues, during eye development in insects, tadpole tail regression and in thymocyte negative clonal selection [7,14,39,63]. This programmed cell death can be selectively blocked by repressing mRNA and protein synthesis [55].

Programmed cell death (PCD) is not necessarily linked to death processes associated with development. This fact led to the identification of genes that are evolutionarily conserved and are used alternatively during cell life. Thus, a cell carries a suicide program of that can be activated in physiological or pathological conditions and may be potentially deleterious to the organism [14,21].

This concept increases the complexity of signal transduction and the regulation of cell death such that cell death can no longer be considered solely as physiological or necrotic/accidental [57]. If one excludes programmed necrosis as a partially physiological process, then there are at least two distinct types of physiologically and genetically regulated cell death, namely, apoptosis and autophagy [25]. These terms are not new, especially autophagy, but define two cell death processes that show similarities and peculiarities, as well as evolutionary conservation and an overlap in their effector machinery (Fig. 1).

Morphologically, there are major differences in the cytoplasm (presence of large vacuoles and organelle swelling, not seen in apoptosis), in the elimination of the cellular debris and in the occurrence of apoptosis and autophagy [7,8,71]. Apoptosis and autophagy are also known as type I and type II cell death, respectively. However, this classification is controversial, mainly because both types of cell death share similar morphologies and mechanisms (DNA degradation, caspase activation and phosphatidylserine exposure in the outer membrane) [7,14,18,41]. Hence, morphology by itself is not the best way to define similarities and differences between forms of cell death [28]. However, the search for cell death genes has provided more clues about how cells select ways of dying during development. This is seen in fruit fly development where different repertoires of genes are used to regulate cell death in the larval salivary glands, the larval midgut and the compound eyes [29,41,71].

In the case of the *Drosophila* retina, the cell death machinery must be exquisitely regulated to allow the removal of a comparatively small but fairly constant number of surplus interommatidial cells in a few hours during the first third of pupal development. If too many or too few cells are removed, the highly ordered, crystal-like organization of the compound eye is severely disrupted and its function greatly impaired. This regulation is achieved by sorting out the as yet unspecified interommatidial cells around each developing ommatidial cluster. This cell sorting, which depends on the function of the *roughest* gene (*rst*), apparently allows cells to compete for different signals coming from already specified cells in the ommatidial cluster that contain the epidermal growth factor (EGF) receptor kinase signal pathway [3,27,61]. Cells that do not compete efficiently for these signals and that do not differentiate are then removed by standard apoptotic cell death [69, 70].

Apoptosis, a thousand and one uses

Cell death by apoptosis generally involves a set of regulated nuclear and cytoplasmic changes mediated mainly by the action of cysteine-proteases (caspases). These changes include cell shrinkage, blebbing, and DNA condensation that lead to cell disintegration by nuclear and cytoplasmic fragmentation into membrane-bound vesicles known as apoptotic bodies; these vesicles are ultimately recognized and engulfed by neighboring cells or macrophages (Fig. 1) [23].

The genetic machinery of apoptosis has been extensively studied in model organisms such as *Drosophila*, the nematode *Caenorhabditis elegans* and mammals. These studies have shown that apoptosis involves a sophisticated program of gene action based on the ability to recognize a wide range of stimuli that activate a common set of cell death effectors (based mainly on caspases and proteins of the Bcl-2 family), with an important role for mitochondrial apoptogenic proteins in generating and regulating cell death through the apoptosome [reviewed in 1,4,14,15,20,21].

Apoptosis is a common phenomenon during normal life, and serves to eliminate cells with unrepairable damage, to maintain tissue homeostasis

by controlling tissue turnover, and to eliminate cells infected with pathogens [1,47]. An important aspect of apoptosis, and one of the major differences between apoptosis and developmental cell death, is that protein synthesis is not necessarily a crucial step in its activation since apoptosis can occur even in the presence of cycloheximide. This characteristic reflects the fact that caspases (apoptosis effector proteins) are present in cells as inactive precursors that are rapidly activated by appropriate stimuli to trigger a cascade of intracellular pathways [reviewed in 2,48,50].

Apoptosis is thus a caspase-dependent process that occurs mainly in maturating or mature cells with a program of self-destruction that is controlled by a complex regulatory machinery mediated by extrinsic (death receptors) or intrinsic (mitochondrial depolarization) pathways; the activation of these pathways leads to rapid cell death when normal cell function is disrupted (Fig. 2). This phenomenon of adjustable apoptosis has recently been referred to as partial apoptosis since the cells are partially destroyed as an adaptive response to specific physiological conditions [2,47]. However, the regulation of this adjustable apoptosis has yet to be understood. The various characteristics mentioned above indicate that apoptosis is the "Handy Andy" of cell death processes in a wide variety of situations.

Autophagic cell death: a real deal?

Autophagy, also termed type II cell death, was known before the discovery of caspases and the introduction of the term apoptosis. Initially, cell death was mainly recognized as lysosomal death because of the central role of lysosomes as compartments for organelle destruction (Fig. 1) [64]. Autophagy, which does not necessarily imply cell death and differs from autophagic cell death, is a normal physiological process that occurs under certain conditions such as organelle turnover and cell survival during nutrient deprivation or limited availability, as shown in yeast [7,38,43]. Indeed, autophagy is one of the most prominent cellular mechanisms for protein and organelle turnover (Fig. 2) [54,72].

Protein and organelle turnover in cells can be classified as bulk turnover or fine turnover that are regulated by autophagy and ubiquitin-mediated degradation, respectively. In terms of cytoplasmic degradation, autophagy eliminates long-lived proteins by targeting them to lysosomes, whereas most short-lived proteins are targeted for degradation by the ubiquitin/proteasome pathway [7,58,69]. Various stimuli can activate autophagy, including hormone deprivation, starvation and protein abnormalities, and lead to the accumulation of mitochondria in autophagic vacuoles. These observations indicate that autophagy is a survival strategy for cells during metabolically and energetically difficult periods.

How can autophagy be considered a cell death process? The term autophagic cell death has evolved in the past five years, and many recent papers have shown that autophagic-like dying cells are present in diseases such as Parkinson´s disease and Alzheimer´s disease. As in mammals, many tissues in insects die during development, either by apoptosis or by a steroid-regulated autophagic-like process, with the main examples being larval salivary glands, intersegmental muscles and gut [7,8,30,39,40,71,74].

The major features of autophagic cell death have been elegantly depicted by Baehrecke [7], who showed that during autophagic cell death the cytoplasm and organelles are restricted to an acidic vesicle derived from ER cisternae and referred to as an autophagic vacuole/vesicle (Fig. 2). These vacuoles subsequently fuse with lysosomes in which the contents of these vacuoles are ultimately degraded [7,39,40]. However, not all workers in this field consider autophagy to be an alternative process of cell death, for various reasons. Thus, since autophagy has been related to cell survival (see above), there is a need to explain how cells switch from normal autophagy involved in survival to autophagic cell death. In addition, it is still unclear whether autophagic cell death be an alternative endpoint for autophagy and whether these two processes share common stimuli. These and other aspects require further investigation, although recent reports have improved our understanding of signaling during autophagy [4,47].

Autophagy may be an initial step to reduce cellular mass prior to apoptosis. This possibility and other aspects have been reviewed by Lockshin and Zakery [47], who suggested that autophagic cell death could be an "open-ended" form of autophagy in which, if cells are unable to recover from survival signals, they can engage in autophagic cell death by unknown mechanisms. Additional possible crosstalk between autophagy and apoptosis was investigated by Mills *et al.* [53], who found that the pro-apoptotic protein TRAIL participated in the modulation of

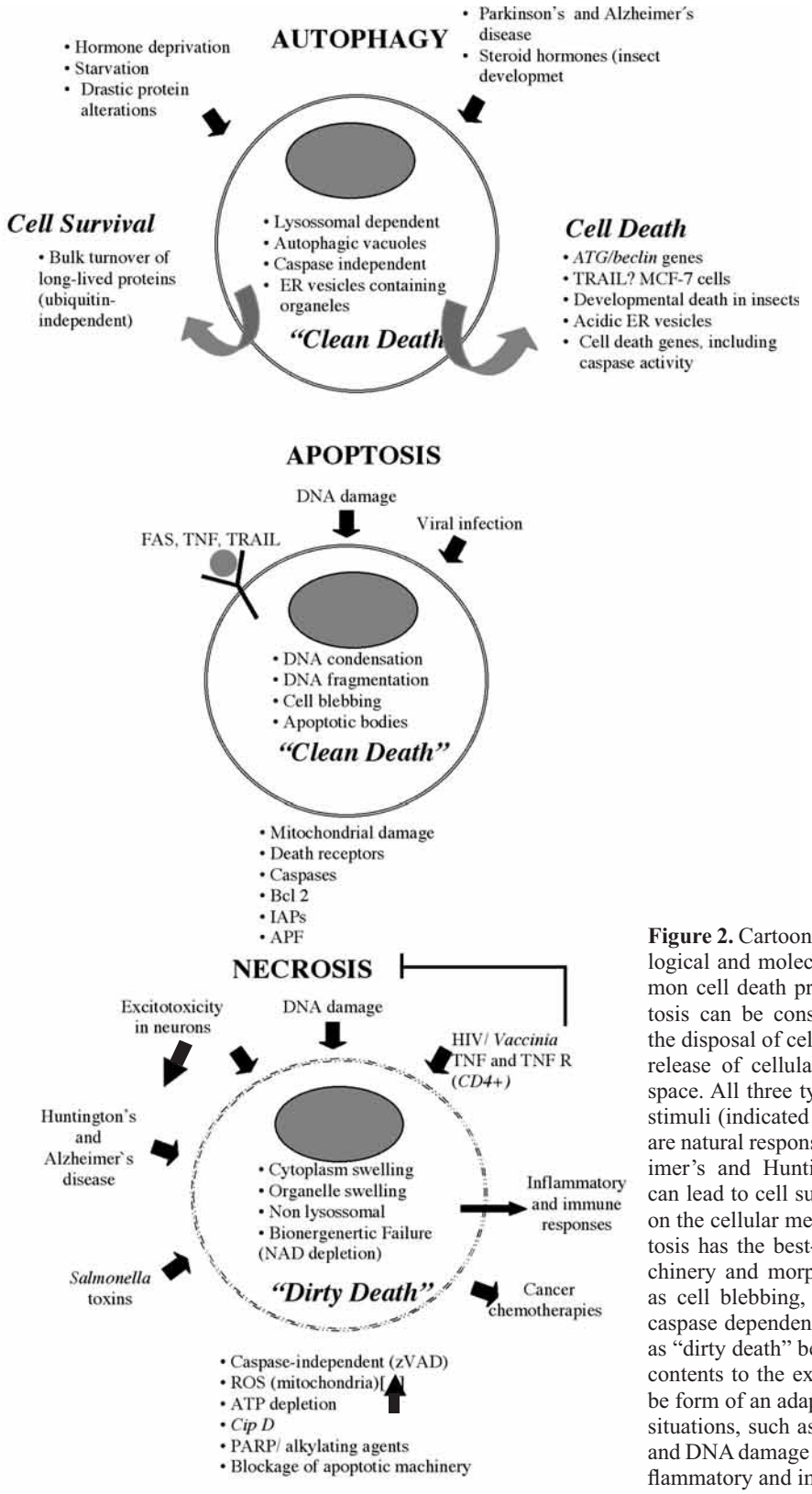

Figure 2. Cartoons comparing the major morphological and molecular features of the most common cell death processes. Autophagy and apoptosis can be considered as "clean death" since the disposal of cell remnants does not include the release of cellular contents to the extracellular space. All three types of death also have several stimuli (indicated by arrows outside the cells) or are natural responses to conditions such as Alzheimer's and Huntington's dieseases. Autophagy can lead to cell survival or cell death, depending on the cellular metabolism (cell turnover). Apoptosis has the best-known genetic cell death machinery and morphological characteristics, such as cell blebbing, apoptotic-body formation and caspase dependency. Necrosis can be considered as "dirty death" because of the release of cellular contents to the extracellular space. Necrosis can be form of an adaptative cell death under specific situations, such as viral and microbial infections and DNA damage resulting in the activation of inflammatory and immune responses.

autophagy during acinar formation in MCF-7 breast cancer cell lines and that blocking TRAIL inhibited autophagy. However, it remains to be determined whether blocking autophagy in the presence of TRAIL will result in luminal fillling during acinar formation. If this were found to be true then, under special conditions, autophagy could function as an alternative to apoptosis for cell death. In neural precursor cells deprived of growth factor, cell death involves autophagic-like characteristics, but this process can be blocked by the anti-apoptotic protein Bcl-2 [16].

In insects such as *Manduca sexta* and *Drosophila*, crosstalk also occurs in the elimination of neurons and salivary glands, respectively [8,39,52,68], with several cell death genes and the caspase machinery being involved in cell death processes. For many years, autophagic cell death has been considered the preferred means of eliminating entire tissues during insect development [reviewed in 4,5,7]. However, not all insects use this pathway. The best example of this exception is cell death in the salivary glands of the inferior dipteran *Bradysia hygida.* The large salivary glands of this species die during the pupal stage via an apoptotic pathway involving cell shrinkage, blebbing and apoptotic body formation, with the involvement of caspase-like activity [65].

Many important aspects of autophagy and apoptosis that are controlled by steroid hormones during insect development remain to be understood (Fig. 2). In particular, the mechanisms by which these organisms choose to die, how this choice is made, and the existence of crosstalk among the various types of cell death remain to be established.

Central to the demonstration of crosstalk between apoptosis and autophagy is the question of whether autophagy precedes apoptosis and, if so, in which cases, e.g., only during development? The answer to this question will depend on an improved knowledge of the molecular basis of autophagic cell death and on the identification of specific markers for this process to determine whether autophagy is an alternative pathway for saving or destroying cells. Although the genes involved in this selection remain poorly understood, some progress has been made through studies in yeast, mammals and insects [7,8,73].

In yeast, autophagy is based on the activity of ubiquitin-like genes (ATG/beclin-1) that are involved in the formation of autophagic vacuoles [reviewed in 6,7,23]. The Atg/beclin-1 genes are required for autophagy induced by the caspase inhibitor Z-VAD (necrosis initiator). This pathway, along with that demonstrated for insects (discussed below), may be a putative program for autophagic cell death [25,26,73]. The use of RNAi to knockout ATG genes resulted in marked inhibition of Z-VAD [73]. A similar approach to study the role of *Beclin-1*, the mouse homolog of the yeast *ATg 6* gene, in Z-VAD-mediated non-apoptotic cell death indicated that the ATg/beclin-1 genes are required for this type of cell death and that this requirement is evolutionarily conserved.

In summary, there is still no general consensus that autophagy is a form of programmed cell death, i.e., that it is more than just a survival process that can be directed to cell death if no cell recovery is possible during drastic conditions. However, the intricate crosstalk between apoptosis and autophagy, its steroid-based regulation in insects and its stage and tissue-specific characteristics strongly suggest that there is an autophagic-like cell death in insects and yeast, especially during development.

Necrosis versus programmed cell necrosis: an accident turned into hope?

As discussed above, classifying cell death simply as accidental (necrotic cell death) or physiological (autophagy and apoptosis) is becoming increasingly more difficult, not only because of the many different ways of killing cells currently recognized, but also because the three major forms of cell death overlap molecularly, biochemically and/or morphologically.

Although numerous studies have shown similarities between autophagy and apoptosis, the idea that necrosis is not simply accidental, uncontrolled cell death has developed quickly. This development is reflected in a recent review by Zong and Thompson [76] in which these authors discuss many features of programmed cell necrosis as an alternative means of killing cells. Historically, until the 1970s, all cell death was referred to as necrosis, but the studies by Kerr *et al.* [36] revealed another form of cell death, namely, apoptosis, which is currently the best example of physiological cell death. Although there has been indiscriminate use of the term apoptosis, it is generally accepted that not all cell death is apoptotic and that there are alternative ways of killing cells in a controlled, tightly regulated manner, e.g., by autophagy (discussed above). In addition, in the past three years,

there has been increasing recognition of necrosis as a regulated form of cell elimination that is of potential clinical use in diseases such as cancer [23,57,75].

Whereas apoptosis is considered as programmed cell death that requires a series of ATP-dependent events, genetically-regulated machinery and an array of stimuli, necrosis has been labeled as an uncontrolled default form of cell death that is based mainly on irreversible bioenergetic collapse and consequent cell lysis inducing strong inflammatory responses. Necrosis, which can be activated by heating, chilling, or mechanical forces, may also be important in mediating immune responses and in healing damaged tissues [23,57,60,76].

A major evidence that necrosis is a form of regulated cell death (programmed cell necrosis) is the observation that after exposure to apoptotic stimuli in the presence of a broad-spectrum caspase inhibitor (Z-VAD) cells die with all of the hallmarks of necrosis [35,48]. However, this necrosis is seen only if apoptosis is genetically or chemically impaired. The finding that activation of the apoptotic machinery suppresses programmed necrosis suggests that necrosis is under the control of apoptosis [34,67,75]. In addition, the molecular machinery underlying regulated cell death by necrosis remains poorly understood. Recent studies have exploited the biological importance of necrosis in response to viral and microbial infections and its potential clinical use in cancer therapy [17,23,75,76].

To be considered as programmed or regulated cell death, necrosis should fulfill two basic requirements, namely, there should be direct participation by the cell through the activation of genes capable of inducing irreversible changes in the bioenergetic balance in the cytoplasm and membrane, and the cells involved in this process should release signaling molecules to trigger specific tissue responses [76].

The adaptive nature of programmed necrosis means that this phenomenon can be activated when cells or tissues are damaged or invaded by pathogens, i.e., when there are external stimuli. However, internal stimuli may also result in programmed cell necrosis, particularly when an internal stimulus challenges the survival of the organism as a whole. The latter idea is based mainly on studies in which cell death by necrosis occurs in response to neurodegenerative, inflammatory and vascular occlusive diseases, as well as cancer and other pathologies [49,59,74].

Physiologically, the major mediators of necrosis are the concentration of intracellular calcium and the level of reactive oxygen species (ROS), both of which can result in a general bioenergetic shutdown and a loss of plasma membrane integrity (Fig. 2). Although these two mediators also occur during apoptosis, many studies have shown that necrotic dying cells participate directly in this process, mainly through an increase in ROS production by mitochondria, an increase in calcium uptake, the activation of non-apoptotic proteases and the enzymatic destruction of cofactors required for ATP production [reviewed in 76]. These mediators can be induced simultaneously, with cell death being propagated from dying cells to neighboring cells. In vascular occlusive diseases, necrotic cell death involves the activation of genes such as *cyclophilin D* (*Cip D*) [9,10,56,62].

Cell death is a normal feature during development of the central nervous system (CNS), and at least two types of cell death can occur, depending on the stimuli. Apoptotic-like cell death induced by growth factor deprivation is responsible for the elimination of excessive neurons. In mature neurons, excessive excitation leads to cell death that can occur by necrosis rather than apoptosis if the genes of major cell death regulators such as Bax and Bak are deleted. Excitotoxicity is important in diseases of the central nervous system, including neurodegenerative disorders such as Alzheimer's and Huntington's diseases [reviewed in 76].

Necrosis can be the preferred form of cell death during bacterial and viral infections, such as described for the HIV-1 virus, which kills CD4(+) T lymphocytes by necrosis rather than apoptosis [13,42]. Chan *et al.* [17] demonstrated that necrosis can be activated by infection with the vaccinia virus through anti-apoptotic proteins encoded by this virus. This activation occurs when the anti-inflammatory cytokine TNF is present. This virus also produces proteins capable of suppressing necrosis (Fig. 2). In agreement with these findings, knockout mice for TNFR2 (necrosis deficient) show a decrease in their inflammatory response and in clearance of the virus [17,23]. In macrophages infected with bacteria such as *Salmonella* species, the release of bacterial toxins results in rapid cell death with features of necrosis, although there is a requirement for the activation of host-cell caspase-1 [31].

DNA damage can also result in the activation of a regulated form of necrosis. Although DNA

damage is a classic trigger of apoptosis when the apoptotic machinery is intact, cells can still be eliminated when apoptosis is impaired [75]. Programmed necrosis rather than apoptosis may be activated when alkylating agents are used to produce DNA damage. Exposure to these agents activates the DNA repair enzyme poly(ADP-ribose) polymerase (PARP) which results in the rapid depletion of NAD and, consequently, of ATP, leading to necrotic cell death during active proliferation. The final response depends on the level of cellular activity since proliferating cells are unable to sustain ATP production because of the dependance on glycolysis, which is impaired by NAD depletion. Consequently, energy will need to be obtained from alternative sources such as fatty acids and amino acids that are used for protein and membrane synthesis in proliferating cells. If the cells are in vegetative stage, they will be able to maintain sufficient levels of ATP by oxidative phosphorylation and will be able to repair their DNA and survive [17,23,75].

The studies discussed above indicate that cells can ultimately choose to die by necrosis instead of apoptosis, although in some situations the induction of necrosis depends on impaired apoptosis. The occurrence of necrosis in viral infections and as the means of cell death in some diseases indicate that necrosis is somehow regulated. Modulation of the mechanisms involved in this regulation may be of potential clinical use in the treatment of cancer.

Cancer is a genetic disease characterized by the subversion of various cellular mechanisms, such as control of the cell cycle, cell adhesion, and cell motility, with the modulation of these activities leading to cell proliferation, tissue invasion and the disruption of homeostasis in multicellular organisms. One of the main systems in cellular protection is the activation of cell death as a tumor suppressor device, mainly by apoptosis. However, many cancerous cells can shutdown apoptosis in order to allow their uncontrolled growth [33]. The major mutation involved in this phenomenon in many human cancers occurs in the *p53* gene, which regulates the cell cycle arrest and entry into apoptosis when there is DNA damage or exposure to mitogenic conditions. In addition to p53, the Bcl-2 family of proteins is also commonly mutated in cancerous cells. Tumor cells in which p53 and Bcl-2 family proteins are mutated generally show a reduced response to antineoplastic agents that rely on the activation of apoptosis, thereby limiting the clinical use of such drugs [20,51].

One of the major areas of clinical investigation is how to overcome defects in the activation of apoptosis, with the main complication being the diversity of pathways and components involved in the regulation of this phenomenon. This diversity creates the need for an almost personalized approach for each type of cancer and patient. Based on the findings discussed above and on the fact that necrosis can substitute for apoptosis under certain conditions in which the latter is impaired, some researchers have argued that necrosis, in combination with other therapeutic approaches, may be used to treat cancer. In this regard, Zong *et al.* [75] used DNA alkylating agents to shown that necrosis is induced in a Bax- and Bak-independent manner, as also reported for other cytotoxic agents, including cisplatin [28,60]. In their work, Zong *et al.* [75] identified the molecular cues that sensitize cells to necrosis instead of apoptosis. As described earlier in this section, the metabolic state of the cell is important since proliferating cells will respond to alkylating agents by inducing necrosis, whereas vegetative cells will not. This observation provides an important link between the phenotype of cancer cells and their sensitivity to chemotherapeutic drugs. However, the potential usefulness of manipulating necrosis as an alternative to chemotherapy for the treatment of cancer requires further investigation.

CONCLUDING REMARKS

In summary, the evidence discussed in this review indicates that physiological cell death is more complex and wide-ranging than simply apoptosis. There are currently at least three recognizable types of cell death (apoptosis, autophagy and necrosis) that can be selected under special conditions. Crosstalk among these forms of cell death is possible and the metabolic state of the cell and type of stimuli are indispensable information that directs the cells to choose a given type of cell death. The major advances in characterizing autophagy and necrosis have been in defining the molecular machinery that is activated during developmental autophagic death in insect tissues and during necrotic cell death of tumor cells in response to DNA-damaging agents. Although autophagy is caspase-independent, several apoptotic genes are nevertheless involved in this process, indicating that some pathways are shared with apoptosis.

The different and specific contexts in which these types of cell death occur is an exciting field because of their potential clinical use to treat important cancer, as well as autoimmune and degenerative diseases. However, we are only at the beginning of such investigations and a better understanding of the molecular basis of the activation/regulation of cell death is necessary in order to exploit the benefits of selecting alternative ways of killing cells in multicellular organisms.

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