

Cell death by necrosis: towards a molecular definition

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Necrosis has been defined as a type of cell death that lacks the features of apoptosis and autophagy, and is usually considered to be uncontrolled. Recent research suggests, however, that its occurrence and course might be tightly regulated. After signaling- or damage-induced lesions, necrosis can include signs of controlled processes such as mitochondrial dysfunction, enhanced generation of reactive oxygen species, ATP depletion, proteolysis by calpains and cathepsins, and early plasma membrane rupture. In addition, the inhibition of specific proteins involved in regulating apoptosis or autophagy can change the type of cell death to necrosis. Because necrosis is prominent in ischemia, trauma and possibly some forms of neurodegeneration, further biochemical comprehension and molecular definition of this process could have important clinical implications.

Introduction

The death of a cell can be defined as an irreversible loss of plasma membrane integrity [1]. Historically, three types of cell death have been distinguished in mammalian cells by morphological criteria. Type I cell death, better known as apoptosis, is defined by characteristic changes in the nuclear morphology, including chromatin condensation (pyknosis) and fragmentation (karyorrhexis); minor changes in cytoplasmic organelles; and overall cell shrinkage, blebbing of the plasma membrane and formation of apoptotic bodies that contain nuclear or cytoplasmic material. All of these changes occur before plasma membrane integrity is lost. Type II cell death is characterized by a massive accumulation of two-membrane autophagic vacuoles in the cytoplasm. Type III cell death, better known as necrosis, is often defined in a negative manner as death lacking the characteristics of the type I and type II processes. A classical positive definition of necrosis based on morphological criteria (early plasma membrane rupture and dilatation of cytoplasmic organelles, in particular mitochondria) [1,2], can now be updated and refined.

The distinction between cell death types is important, particularly because necrosis is often associated with unwarranted cell loss in human pathologies [3–5] and can lead to local inflammation, presumably through the

liberation of factors from dead cells that alert the innate immune system [2,3,6]. In addition, it seems that clearance of apoptotic cells operates differently from that of necrotic cells [7]. Whereas apoptotic cells (which shrink) are engulfed completely by phagocytes, necrotic cells (which swell) are internalized by a macropinocytotic mechanism, meaning that only parts of the cell are taken up by phagocytes [8].

Our intention here is to discuss recent results that might facilitate a shift from the negative definition of

Glossary

AIF: apoptosis-inducing factor, a flavoprotein normally located in the mitochondrial intermembrane space that can translocate to the nucleus on induction of cell death. Mitochondrial AIF participates in local redox homeostasis, whereas nuclear AIF can contribute to chromatin condensation and degradation.

Akt: a survival kinase (also called protein kinase B or PKB) that, when activated, indirectly enhances glucose metabolism and suppresses autophagy through the mTOR kinase.

Atg: a family of evolutionarily conserved genes, whose products are essential for different steps of the autophagic process. One of these genes encodes Atg6, which is also known as Beclin-1.

Bcl-2: the prototypic anti-apoptotic protein of the Bcl-2 family. The Bcl-2 protein is inserted in the outer mitochondrial membrane and protects mitochondria against MMP.

Bax and Bak: the pro-apoptotic multidomain proteins of the Bcl-2 family. Bax and/or Bak are often required for the apoptosis-specific outer MMP. Whereas Bak is pre-inserted in the outer mitochondrial membrane, Bax has to translocate from the cytosol to mitochondria to mediate MMP.

LMP: lysosomal membrane permeabilization, a process leading to leakage of catabolic enzymes from the lysosomal lumen, is induced by ROS, sphingolipids and lysosomotropic agents.

MMP: mitochondrial membrane permeabilization, a process affecting both mitochondrial membranes to a variable extent, leading to disruption of mitochondrial structure and function. Outer MMP leads to leakage of intermembrane proteins from mitochondria. Inner MMP is linked to bioenergetic failure caused by loss of the inner mitochondrial transmembrane potential.

ROS: reactive oxygen species, a side product of normal oxidative phosphorylation that need to be scavenged by the anti-oxidant system of the cell. ROS can be overproduced during deleterious and pathological processes.

NCX: a plasma membrane Na⁺/Ca²⁺ exchanger required for maintaining physiological low levels of Ca²⁺ in neurons.

Permeability transition: a process leading to the permeabilization of the inner mitochondrial membrane to solutes up to 1500 Da, causing dissipation of the inner mitochondrial transmembrane potential, colloid osmotic swelling of the mitochondrial matrix and physical disruption of the outer mitochondrial membrane.

RIP1: a specific kinase that is recruited to the death-inducing signaling complex after occupation of the TNF-R1, and that on activation can mediate various effects including the induction of necrosis.

TNFα: tumor-necrosis factor-α, an inflammatory cytokine that can cause cell death by acting on a specific cell-surface receptor.

Table 1. Examples of necrotic cell death and events therein

Cells	Inducers	ROS in mitochondria	Swelling of mitochondria	ATP depletion	Cytoplasmic Ca ²⁺ increase	Peri-nuclear clustering	Calpain required	Cathepsin required	Lysosomal alteration	Refs
L929	TNF	Yes	–	–	–	Yes	–	–	–	[61–64]
Primate neuronal cells	Ischemia	–	Yes	–	–	–	Yes	Yes	Yes	[48,65]
Rabbit kidney cells	AntimycinA	–	–	Yes	Yes	–	Yes	–	–	[66,67]
Human T cell lines	Anti-Fas and others	–	–	Yes	–	–	–	–	–	[23,68]
<i>C. elegans</i> neuronal cells	<i>mec-4(d)</i> and other mutants	–	–	–	Yes	–	Yes	Yes	Yes	[47,69,70]
<i>Dictyostelium discoideum</i>	Developmental signals in <i>atg</i> [–] mutants	Yes	Yes	Yes	–	Yes	–	–	–	[25,26]

necrosis (which phenomenologically is neither apoptosis nor autophagy, and biochemically requires neither caspases nor autophagy) to a more positive definition of this particular modality of cell death. In short, could necrotic cell death be programmed in the sense that it would constitute a stereotyped, evolutionarily designed, sequence of biochemical events?

Approaching a programmed course of necrotic cell death

For the purpose of our discussion, we consider a few well-characterized experimental systems in which elements of the necrotic pathway have been described and can be condensed into a cumulative rather than consensual sequence of events. The six experimental systems considered (Table 1) are heterogeneous in terms of cell types and species (several mammalian cell types, the nematode *Caenorhabditis elegans* and the slime mold *Dictyostelium discoideum*) and death inducers (from ischemia to ligands of cell-surface receptors); however, most of them are similar in that they manifest early plasma membrane rupture but no sign of apoptosis and no overt autophagy. They also each show several traits that, when considered in total, might delineate a sequence of intracellular events specific to necrotic cell death. Such a sequence includes early signs of mitochondrial dysfunction; namely, production of reactive oxygen species (ROS; see Glossary) by mitochondria and swelling of mitochondria; ATP depletion; failure of Ca²⁺ homeostasis; perinuclear clustering of organelles;

activation of a few proteases, in particular calpains and cathepsins; lysosomal rupture; and ultimately plasma membrane rupture (Figure 1).

Strengthening the idea of conservation of a necrotic cell death mechanism across eukaryotes [9], recent results in plant cells emphasize the existence of a possibly similar cell death pathway, including generation of ROS, ATP depletion [10], and changes in the actin cytoskeleton [11]. The chronological and molecular order of the events accompanying necrosis remains elusive. For example, the relationships among Ca²⁺, ATP and ROS can be complex, presumably because of the existence of self-destructive feed-forward loops [12]. Although taken alone each of these events is not specific to necrosis (and indeed some might be shared with apoptosis [13]), it is the possible accumulation of these events in an organized, programmed cascade of self-destruction that might define necrosis.

Programmed occurrence of necrotic cell death?

Obviously, in harsh conditions such as detergent stress or freeze-thawing, cells die through a non-regulated, poorly defined, necrotic process. However, at variance with the classical textbook notion that it is merely an accidental consequence of non-physiological stress, necrosis might be programmed in terms of both its course and its occurrence. The cascade of events shown in Figure 1 suggests that there is a programmed ‘course’ of events (i.e. how necrosis manifests) within the necrotic dying cell. Programmed ‘occurrence’ of necrotic cell death (i.e. whether necrosis

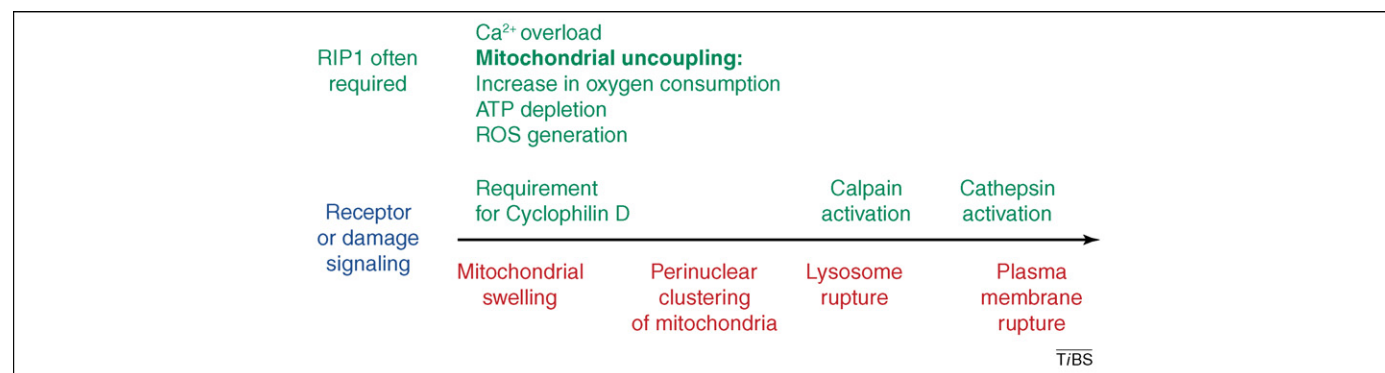


Figure 1. Tentative cascade of events in necrotic cell death. This scheme was established on the basis of the data summarized in Table 1 and the information in the main text. Note that the scheme is a cumulative regrouping of steps (mostly morphological, red; molecular, green; signaling, blue), with the main caveats that non-necrotic examples of cell death might have been wrongly enlisted and that necrotic cell death might be mechanistically heterogeneous.

occurs) has been previously noted [14]. The concept of both programmed course and programmed occurrence is supported by several data.

First, necrosis can occur during development (e.g. the death of chondrocytes controlling the longitudinal growth of bones) [15] and in adult tissue homeostasis (e.g. in intestinal epithelial cells) [16]. Second, necrosis can be triggered by the occupation of specific plasma membrane receptors by their physiological ligands (see later). This observation implies that specific signal transduction pathways are connected to the induction of necrosis rather than to the induction of other types of cell death. Third, susceptibility to necrotic death can be regulated by genetic and epigenetic factors. For example, mouse strains vary in their susceptibility to brain ischemia, and important differences are dictated by the age of the mice and by distinct brain regions [17]. Fourth, the inhibition of some enzymes and processes can prevent necrosis, meaning that these enzymes and processes have an active, decisive role in the lethal process (see below). Fifth, inhibition of caspases (which are often required for the morphological manifestation of apoptosis) can change the morphological appearance of cell death from type I to type II or III cell death [9,14,18]. Thus, the same upstream signal can produce different types of cell death as a function of, in particular, the activation or inhibition of catabolic enzymes in the cell.

Programmed necrotic cell death as a default pathway

The idea that necrosis constitutes a (or even the) default cell death pathway is supported by the observation that inhibition of essential apoptotic events plus inhibition of autophagy can induce necrosis in, for example, immortalized baby mouse kidney epithelial (iBMK) cells that undergo cell death in response to hypoxia *in vitro* and *in vivo* [19]. In this model, suppression of mitochondrial membrane permeabilization (MMP) by the simultaneous knockout of the pro-apoptotic proteins Bax and Bak, or by overexpression of the anti-apoptotic protein Bcl-2, causes a shift from type I to type II cell death, whereas additional inhibition of autophagy by transfection with constitutively active Akt protein kinase or knockout of one of the alleles encoding the pro-autophagic Beclin-1 protein determines a shift from type II to type III cell death [19]. Only the simultaneous inhibition of MMP and inhibition of autophagy induces necrosis in this model. This pattern of death has also been observed in mouse embryonic fibroblasts (MEF) responding to etoposide, where the simultaneous knockout of Bax and Bak coupled with the depletion of essential autophagy proteins such as Atg5 by short interfering (siRNA) leads to the induction of delayed necrosis by etoposide [20].

In other cellular models, by contrast, it is sufficient to inhibit caspases to induce necrosis. For example, interdigital cells contained in the limb anlage from mice that lack the essential caspase activator Apaf-1 undergo a non-apoptotic, non-autophagic cell death in which chromatin condensation is delayed and partial [9,14,21]. These observations have also been made *in vitro*, by adding the caspase inhibitor Z-VAD-fmk (*N*-benzyloxycarbonyl-Val-Ala-Asp-fluoromethylketone) to explanted limb anlagen [14]. It is important to note that in these developmental

circumstances necrotic cell death follows the same spatio-temporal pattern as apoptosis, leading to the suppression of interdigital webs and the formation of normal digits. Thus, necrosis simply substitutes for failed apoptosis.

In other situations, caspase inhibition can even sensitize cells to the induction of necrosis, thereby reducing the dose of tumor-necrosis factor- α (TNF α) required to kill some cell lines [22]. This observation implies that specific elements of the apoptotic signaling cascade might inhibit necrosis in an active manner. Depletion of ATP can also favor a switch from apoptotic to necrotic cell death, in part because ATP is required for optimal activation of caspase [23]. Nitric oxide, which can inhibit caspases by covalently modifying the prosthetic cysteine group, can also induce a switch from apoptosis to necrosis [24]. Similarly, in the protist *Dictyostelium*, inactivation of the *atg1* autophagy gene converts development-associated cell death from an autophagic to a necrotic morphology, giving evolutionary weight to this unmasking of a necrotic cell death pathway [25,26].

In summary, depending on the context necrosis might be fully unregulated or, on the contrary, 'programmed'. Necrosis can occur as a result of the activation of specific signal transduction cascades and subsequently can be overt or revealed only on inhibition of apoptosis and/or autophagy. Such a default occurrence of necrotic cell death and its unmasking by inhibition of autophagy and/or apoptosis might well reflect its early emergence in evolution, perhaps as the primordial eukaryotic cell death pathway, with subsequent additions of other layers of cell death mechanisms such as apoptosis and autophagy [9] (Figure 2).

Towards necrosis-specific molecular processes?

Mutagenesis studies have identified genes that are required for apoptotic cell death, such as *ced3*, *ced4* and *ced9* in *C. elegans* and their homologs in other species [27]. An unambiguous definition of necrotic cell death would considerably benefit from the identification of molecules or processes that are specifically required for necrosis. Such a discovery would also further upgrade necrotic cell death from a programmed pathway to a specific function. Some candidate molecules have been recently identified.

Receptor-induced necrosis and RIP1

One of the best-studied models of receptor-induced necrotic cell death is the L929 mouse fibrosarcoma cell line treated with TNF α . In this model, cells initiate a complex multi-step signal transduction pathway in which Fas-associated death domain (FADD) is recruited to the (possibly endocytosed) TNF receptor 1 (TNF-R1) and induces necrosis, presumably through its death domain [3]. In L929 cells, TNF α -induced necrosis is accompanied by a rapid burst in mitochondrial ROS production that can be dampened by rotenone, an inhibitor of the respiratory chain complex I [28]. Both rotenone [28] and lipophilic antioxidants [29] prevent necrotic cell death in this model [29]. Moreover, Bcl-2 can reduce TNF-induced necrosis, presumably by maintaining mitochondrial integrity. Because there is no permeabilization of the outer mitochondrial membrane in L929 cells treated with TNF α , Bcl-2 must act on other aspects of mitochondrial function [3]. Knock down of

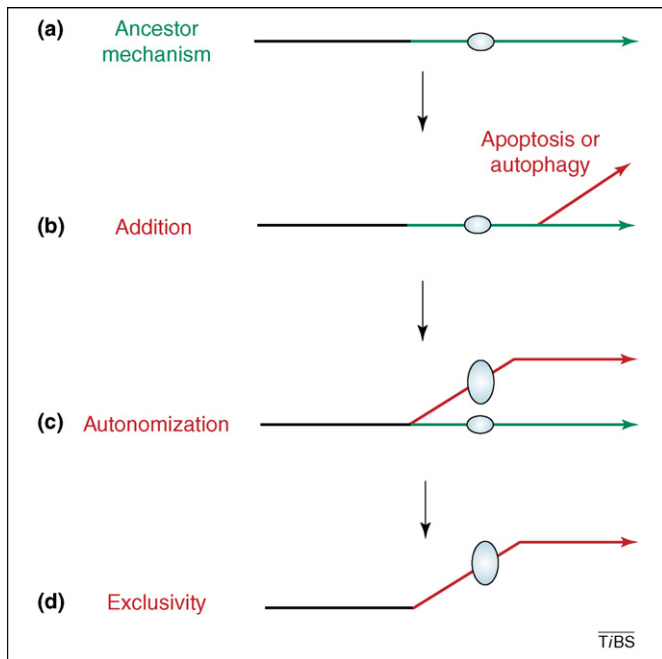


Figure 2. Hypothetical evolution of cell death. (a) Primordial necrotic mechanism of cell death and dismantling. The oval indicates the ‘point of no return’ of the process. (b) Caspase activation or autophagy are recruited into the system for optimal dismantling of dying cells after the point of no return has been trespassed. (c) Two effector mechanisms are now available, as in death receptor signaling in mammalian cells, leading to either caspase activation or necrosis. Note that the process leading to caspase activation or autophagy (red line) has acquired the capacity to ‘decide’ the cell death process and thus starts upstream of the point of no return. (d) Only one effector mechanism is left, as in *C. elegans*, where developmental death of somatic cells depends exclusively on caspases. Black lines indicate the signaling stage, green and red lines indicate the effector and dismantling stages (green line, primordial necrotic mechanism; red lines, superadded apoptotic and/or autophagic mechanisms). Adapted, with permission, from Ref. [9].

expression of the kinase RIP1 prevents the mitochondrial manifestations of TNF α -treated L929 cells [3].

RIP1-deficient Jurkat cells are refractory to the propagation of necrosis induced by the Fas/TNF-R/TRAIL-R pathway, again implicating RIP1 in lethal signaling [30,31]. In several types of cell (e.g. Jurkat, U939, THP1), death receptor signaling leads to the activation of caspase-8 (which, in TNF α -induced death, depends on a homotypic interaction between the death effector domains of caspase-8 and FADD), which cleaves RIP1, thereby inactivating the pro-necrotic activity of this kinase [31–33]. Inhibition of caspase-8 by Z-VAD-fmk or siRNA, in the absence of death receptor ligation, is sufficient to cause cell death in, for example, L929 cells and Jurkat cells [34]. This cell death follows a slower kinetics and is accompanied by the accumulation of autophagic vacuoles; nonetheless, it is also dependent on RIP1 expression [34,35]. This observation suggests that RIP1 has a pleiotropic function that is highly context dependent. RIP1 has been involved in other models of necrotic signaling. In necrosis induced by alkylation of DNA and activation of poly(ADP-ribose)polymerase (PARP), for example, knock down of RIP1 expression can interrupt the lethal signal transduction cascade [36,37].

How activation of RIP1 causes necrosis is a matter of debate. In DNA-damage-induced necrosis, Jun N-terminal kinase seems to be required as a downstream effector of RIP1 for the permeabilization of mitochondrial membranes

[35]. RIP1 might also be required for the accumulation of pro-necrotic ceramides [38]. According to one report [32], RIP1 is needed to inhibit the enzymatic activity of the adenine nucleotide translocase (ANT) induced by a combination of TNF α and Z-VAD-fmk. This inhibition might involve a direct interaction between ANT and Z-VAD-fmk [32], suggesting that the pro-necrotic effect of Z-VAD-fmk might involve an off-target effect. Irrespective of the details, it seems that RIP1 can contribute to necrosis as an upstream signal in several models.

Cyclophilin D

Cyclophilin D (CypD) is a mitochondrial matrix protein that can interact with inner membrane proteins including ANT, and that participates in the so-called ‘permeability transition’ – the opening of non-specific channels causing dissipation of the inner mitochondrial transmembrane potential. The strongest stimulus of permeability transition is cytosolic Ca $^{2+}$, but additional conditions, including ROS, depletion of ATP and the production of lipid stress signals (e.g. ceramide, ganglioside GD3), favor permeability transition [39]. Knockout of the gene encoding CypD induces resistance to necrotic cell death induced by ROS or Ca $^{2+}$ overload in, for example, hepatocytes and fibroblasts [40–42]. In addition, CypD-deficient mice are resistant to cardiac injury by ischemia–reperfusion [40,41] and focal cerebral ischemia [42]. CypD is the mitochondrial target of cyclosporin A, which has also been shown to reduce cell loss induced by necrotic stimuli in, for example, TNF α -treated hepatocytes [43].

By contrast, it seems that apoptotic mitochondrial membrane permeabilization mediated by Bax or Bak is not regulated by CypD [40–42], suggesting that there are two mechanisms of MMP – one that is strongly connected to necrosis (and is CypD dependent) and another that is linked to apoptosis (and is mediated by pro-apoptotic members of the Bcl-2 family such as Bax and/or Bak). In some situations, however, cyclosporin A can inhibit apoptotic death and anti-apoptotic proteins of the Bcl-2 family can inhibit permeability transition, suggesting that there is considerable crosstalk between the two pathways [39]. Although the link between necrosis and CypD is impressive, CypD might not be involved in all instances of necrosis and might be involved in some cases of apoptosis (e.g. Ref. [44]).

Non-caspase proteases

During ischemic or hypoglycemic episodes affecting the central nervous system, the plasma membrane potential of presynaptic neurons is reduced, resulting in massive release of the excitatory neurotransmitter glutamate into the synaptic cleft. Overstimulation of two glutamate receptors, the glutamate NDMA (*N*-methyl-D-aspartate) receptor and the AMPA (α -amino-3-hydroxy-5-methyl-4-isoxazole propionic acid) receptor channel, then triggers the excitotoxic cell death of postsynaptic neurons. This process involves immediate overload of Ca $^{2+}$ and Na $^{+}$, followed by secondary activation of voltage-gated Ca $^{2+}$ channels and calpain-mediated cleavage of the plasma membrane Na $^{+}$ /Ca $^{2+}$ exchanger (NCX), resulting in an inactivation of homeostatic mechanisms [45]. Calpains (a family of Ca $^{2+}$ -dependent cysteine proteases) and lysosomal cathepsins (the aspartyl protease cathepsin D

and the cysteine proteases cathepsin B, H and L) are then likely to 'execute' cell death [46].

In *C. elegans*, necrotic neurodegeneration can be caused by the *deg-3(d)* allele, which encodes a hyperactive acetylcholine receptor Ca^{2+} ion channel, or the neurotoxic gain-of-function *mec-4(d)* allele, which encodes a hyperactive ion channel subunit that is normally required for mechanosensitization. Lysosomal proteases seem to have an important role in this necrotic death, because lysosomes fuse among each other, cluster around the swollen nucleus and ultimately rupture, releasing their content, perhaps owing to activation of two calpain proteases (CLP-1 and TRA-3). Alkalinization of the lysosomal compartment with NH_4Cl or acridine orange or by knock down of the vacuolar H^+ -ATPase, and knock down of lysosomal aspartyl proteases (*asp-3*, *asp-4*) both reduce neuronal cell death induced by *mec-4(d)* or *deg-3(d)* [47]. Thus, the data obtained in *C. elegans* suggest that there is a process of necrotic neurodegeneration in which altered ion homeostasis causes activation of calpain, followed by lysosomal rupture and cathepsin-mediated cell death [46,47], in agreement with a hypothesis of calpain–cathepsin-mediated necrotic cell death [48].

In mammalian cells, Ca^{2+} overload induces permeability transition but can also stimulate calpains, which in turn can induce cell death by several mechanisms – namely, by activating pro-apoptotic members of the Bcl-2 family [49,50], by facilitating the release of apoptosis-inducing factor (AIF) from mitochondria (presumably through proteolytic cleavage of a membrane anchor that retains AIF on the inner mitochondrial membrane) [51], by cleaving essential components of the cytoskeleton, by inactivating NCX [45], and by inducing the release of lysosomal cathepsins [46]. In several examples where cell death is caspase independent and shares features of apoptosis and necrosis (with partial chromatin condensation), depletion of AIF (by siRNA or by using a hypomorphic mutant) reduces cell death, suggesting that this factor might be important, especially in neuronal cell death [52,53]. Manipulation of calpain activation *in vivo*, by either gene knockout or transgenic overexpression of the endogenous inhibitor calpastatin, has confirmed that calpains make an essential contribution to the excitotoxic effects of the neurotoxin kainite [54].

Lysosomotropic toxins can cause both apoptosis (at low doses) and necrosis (at high doses) through the induction of lysosomal membrane permeabilization (LMP). ROS can also kill cells by LMP induction [55]. LMP causes spillage of catabolic enzymes from the lysosomal lumen into the cytosol. Although most of these enzymes function optimally only at a low pH (which is normally maintained in the lumen of lysosomes), some of them, including cathepsins B and D, conserve their activity in the cytosol. The susceptibility to undergo LMP might increase with aging, perhaps as a result of the lysosomal accumulation of Fe^{3+} (which, through the Fenton reaction, enhances the local production of ROS), and this process has been implicated in age-associated neurodegeneration [56].

Therapeutic manipulation of necrosis

Necrosis induced by non-specific stimuli, such as ischemia, trauma or infection, often involves receptor-mediated cell

death, for example, as a consequence of either $\text{TNF}\alpha$ production or excitotoxicity. As a result, blockade of surface receptors or interruption of the signals that emanate from such receptors might be a prime choice for the therapeutic inhibition of necrosis. As discussed earlier, several enzymes have a rate-limiting role in receptor- or damage-induced necrosis, suggesting that their inhibition might have a therapeutic effect *in vivo*. Preclinical experiments indicate that inhibition of PARP, RIP1, CypD, calpains and cathepsins can inhibit necrotic cell death *in vivo* in models of acute cell loss [18]. In addition, a drug screening approach has led to the identification of 'necrostatins' that can inhibit $\text{TNF}\alpha$ -induced cell death *in vitro* in FADD-deficient Jurkat cells and also has a beneficial effect in a mouse model of stroke [57]; the target of necrostatins has not yet been identified. Conversely, it might be therapeutically desirable to trigger necrotic cell death in tumor cells that might have been selected to resist apoptotic cell death.

Another therapeutic goal might be to deviate the necrotic pathway to an apoptotic one with the rationale that substitution of a pro-inflammatory death modality (necrosis) by a non-inflammatory one (apoptosis) might reduce secondary tissue damage. Caspases might inhibit the necrotic pathway, meaning that in some circumstances their inhibition might enhance cell necrosis, as occurs, for example, in hepatocytes responding to $\text{TNF}\alpha$ *in vivo* [58]. Conversely, facilitating caspase activation (by blocking cellular caspase inhibitors) can cause a switch from necrotic to apoptotic death. In an *in vivo* model of acute pancreatitis, for example, blocking the X-linked inhibitor XIAP with embelin facilitates caspase activation and simultaneously attenuates necrosis and inflammation, which correlates with an increase in caspase-mediated cleavage of RIP1 [59].

Alternatively, and probably more frequently, we might wish to prevent cell death altogether. Considering that several types of cell death pathway can coexist in the same cell and substitute for each other, preventing cell death would require inhibition of all of them. Thus, when caspase inhibition is, by itself, insufficient to prevent cell death (because it causes a shift from apoptosis to other cell death modalities including necrosis), only the combined inhibition of caspases (or their upstream activators) and essential ingredients of the necrotic cascade would truly prevent cell death.

Concluding remarks

As we have discussed, accumulating evidence supports a 'sequence' of events that characterize necrotic cell death at both the phenomenological and the biochemical level, thereby reflecting a programmed course of events in the dying necrotic cell and contributing to a definition of necrotic cell death. In addition, in some circumstances the 'occurrence' of necrotic cell death is programmed. Does this mean that necrotic cell death can be considered a *bona fide* programmed type of cell death in terms of both its course and its occurrence?

To answer this question emphatically, further studies will be required. For example, whereas genes have been found to encode molecules that seem to be specifically required for apoptosis (leading to the concept of apoptotic

cell death as a cell function in itself), no corresponding genes have been identified for necrotic cell death (although RIP1 and CypD might come close in some instances).

We anticipate that future studies will identify more processes and individual molecules that contribute to the necrotic pathway. As a caveat, it should be noted that many proteins that were initially implicated specifically in apoptosis have turned out to fulfill normal cellular functions in, for example, metabolic control, differentiation and signal transduction [60]. Thus, it seems unlikely that a particular set of molecules will be found to be exclusively involved in necrotic cell death; it is more likely that the specific action of some factors in a peculiar cell death process will shape the molecular definition of necrosis.

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