Immunity
Forum

# The End and After: How Dying Cells Impact the Living Organism

Douglas R. Green[1,*]
[1]Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA
*Correspondence: douglas.green@stjude.org
DOI 10.1016/j.immuni.2011.10.003

All cells die, but the manner of death dictates interactions with living cells and consequences for the organism, especially with respect to the immune response. Here we discuss the different modes of cell death as they relate to this rapidly evolving field.

## Introduction

The study of cell death represents something of a paradigm shift in recent years. Cell death was first described in the 19th century in the writings of Virchow and Ramon y Cajal, and the theme re-emerged sporadically throughout much of the 20th century. Research in the field was languid, however, even after apoptosis was defined as a distinct and active process, a biochemical marker (DNA fragmentation) was identified, and it was demonstrated that developmental cell death is genetically controlled. It was not until the 1990s that the study of the molecular basis of cell death flourished, and over the past 20 years we saw an explosion of interest in the subject. In a sense, the slow paradigm shift was from "obvious" (cells die) to "somewhat interesting" (cells seem to die on a schedule) to "profound" (novel, regulated molecular processes mediate and define distinct pathways of cell death). Known throughout was that dying cells only sometimes elicit inflammatory responses, and it was not until different forms of cell death were distinguished that we realized that the type of death actually matters to the physiology and pathology of the organism.

The reviews in this issue focus on cell death and the immune system, or more precisely, on how dying cells impact physiology through their interactions with components of the immune system. These reviews explore four areas of intensive investigation revolving around these interactions and their consequences in mammals. The rapid engulfment of dying cells and the molecular events controlling their clearance is discussed in this issue by Ravichandran. The impact of such clearance on the adaptive immune response is then considered by Griffith and Ferguson, and the resulting impact on the response to infection, and in turn, the coevolution of evasion mechanisms by infectious organisms is outlined by Yatim and Albert. Finally, Kuraishy et al. consider how dying cells, and the altered inflammatory milieu, contribute to oncogenesis.

### *Active versus Passive Cell Death*

Three types of cell death, based on the morphologies of the dying cells, have been broadly recognized. While other types of cell death have been suggested, a recent survey has proposed that these three be generally adopted. These are Type I, or apoptotic cell death; Type II, or "autophagic" cell death; and Type III, or necrotic cell death (a fourth type, cornification, is also recognized, but is restricted to a single cell type, the keratinocyte). Until recently, the first two types were considered "active," that is, controlled by molecular processes that not only dictate the morphology but also determine the fate of the cell. Necrosis (Type III) was considered "passive," a consequence of damage so extensive that the cell cannot survive. As we will see, we now realize that there are forms of active necrosis as well.

Another attempt at classification relies on the consequences of cell death, and again, until recently, necrosis was viewed as "inflammatory and immunogenic" whereas apoptosis was considered immunologically "silent, or actively tolerogenic." More current evaluations have modified this simple framework (Ullrich et al., 2008) as we have come to realize that the specific triggers for cell death or the presence of microbial products can result in immunogenic apoptosis. The immunologic consequences of Type II cell death are less well understood, in part because (as discussed below) the nature of this type of cell death itself is only poorly elucidated in mammals.

## Pathways of Cell Death

### *Type I. Apoptosis*

Apoptosis is characterized by chromatin condensation and often involves plasma membrane blebbing and fragmentation of the cell into membrane-enclosed bodies. This process is orchestrated by a set of proteolytic enzymes, cysteine proteases with specificity for aspartic acid residues in their substrates, or caspases. The executioner caspases (caspases-3, -6, and -7) cleave at least 1,000 substrates in the cell, and it is the cleavage of such substrates that causes the changes associated with apoptosis. These executioner caspases are inactive dimers in the cell until cleaved at specific sites, and this cleavage is mediated by the initiator caspases. In contrast to the executioner caspases, the initiator caspases are activated by adaptor molecules that bring the inactive caspase monomers into close proximity on "caspase activation platforms." The specific adapters and initiator caspases, and the resultant activation platforms, define the pathways of apoptosis.

Simplified schemes for four different (but often interconnected) apoptotic pathways are shown in Figure 1. More detailed descriptions of these pathways are reviewed elsewhere (Green, 2011). The mitochondrial pathway, the most prevalent route to cell death in mammals, is controlled by proteins of the BCL-2 family, a family of proteins that as their primary function regulate the integrity of the mitochondrial outer membrane. These proteins are expressed and/or modified in

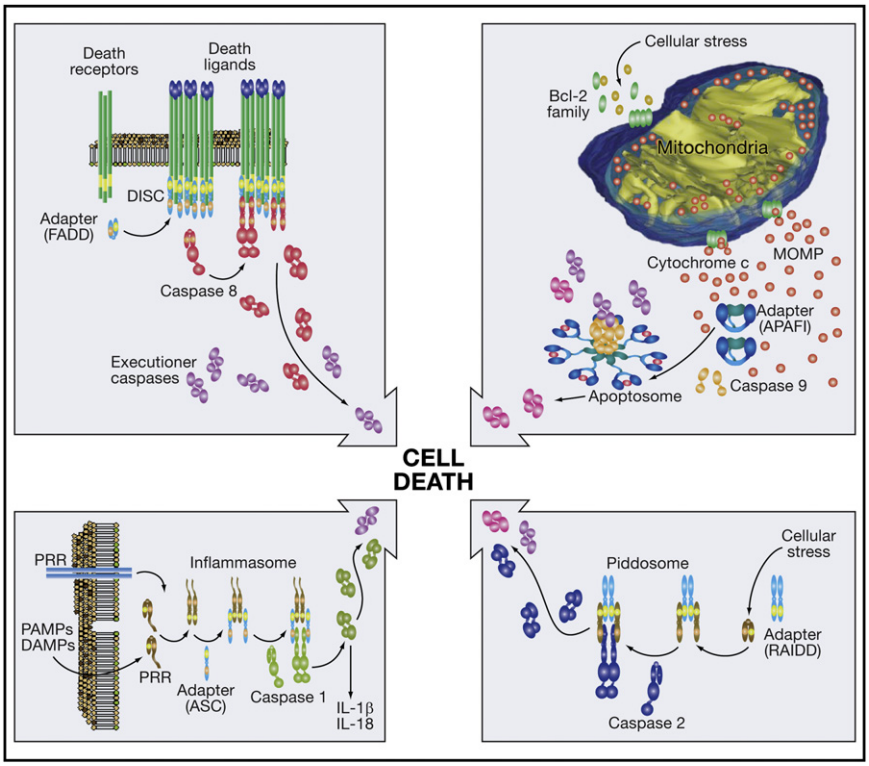


**Figure 1. Pathways to Apoptosis**

Four routes to caspase activation that can result in apoptosis are shown. In each, the formation of a caspase activation platform brings an initiator caspase monomer into proximity, promoting their activation. In the death receptor pathway (top left), ligation of death receptors of the TNFR superfamily recruits an adaptor molecule, FADD, forming an activation platform for caspase-8, called the death inducing signaling complex (DISC). In the mitochondrial pathway (top right), a variety of cellular stresses induce expression and/or modification of proapoptotic proteins of the BCL-2 family, which promote mitochondrial outer membrane permeabilization (MOMP). Cytochrome c, released to the cytosol, triggers the oligomerization of an adaptor protein, APAF1, forming an activation platform for caspase-9, called the apoptosome. In the caspase-1 pathway (bottom left), pathogen- or damage-associated molecular patterns (PAMPS, DAMPS) engage cell surface or intracellular pattern recognition receptors (PRR), which signal to or directly promote the assembly of adaptor proteins, such as ASC, into an activation platform for caspase-1. This is called the inflammasome. In addition to potentially promoting apoptosis, caspase-1 also processes the cytokines IL-1β and IL-18 and promotes secretion of these and other inflammatory mediators. In the caspase-2 pathway (bottom right), some cellular stresses induce the assembly of an adaptor protein, RAIDD, into an activation platform for caspase-2, called the piddosome (as it often contains another protein, PIDD). If activated in sufficient amounts, the initiator caspases can process and thereby activate executioner caspases, resulting in apoptosis. Alternatively, caspases-1, -2, and -8 often must engage the mitochondrial pathway through cleavage and activation of a BCL-2 family protein, in order to promote apoptosis.

response to a variety of cellular stresses and interact to cause outer mitochondrial membrane permeabilization (MOMP). Proteins released from the mitochondrial intermembrane space include cytochrome c (which induces oligomerization of APAF-1, the adaptor protein in this pathway), engaging and activating the initiator caspase, caspase-9 (other released proteins facilitate caspase activation). The death receptor pathway involves a subset of the tumor necrosis factor receptor (TNFR) superfamily, which upon ligation induce oligomerization of another adaptor, FADD, which activates the initiator caspase-8. Another pathway is engaged in response to pathogen- or damage-associated molecular patterns, "PAMPS" and "DAMPS," respectively, triggering the assembly of adaptor proteins for caspase-1, thereby activating it (there are several adaptor proteins for caspase-1, the major one being ASC). Finally, some cellular stresses, including heat shock, microtubule disruption, and some forms of metabolic stress, engage an adaptor molecule (RAIDD) for the activation of caspase-2. While caspase-8, caspase-1, and perhaps caspase-2 can directly cleave and activate executioner caspases, in many cells they must engage the mitochondrial pathway to do so effectively (which they do by cleavage and activation of one of the BCL-2 family proteins).

For our purposes here, three distinct "modes" of apoptosis should be noted. Apoptosis can proceed with or without MOMP, which exposes the mitochondrial inner membrane to proteolysis, thereby disrupting the organelle to release and/or modify DAMPs (discussed in more detail by Griffith and Ferguson in this issue). In addition to these two modes, a third mode includes the activation of caspase-1, which can cause cell death, but can independently function to process the inflammatory cytokines interleukin-1 and interleukin-18 and engage a nonclassical secretory pathway for the release of these and other inflammatory mediators. (Another mode, that of caspase-2 activation without MOMP, has not been analyzed in terms of immunologic consequence.)

### *Type II. "Autophagic" Cell Death*

Type II cell death is triggered in response to certain drugs, including agents that disrupt lysosomes, and can also occur in response to developmental and tumor-suppressor signals. It is morphologically defined as distinct from apoptosis, with cells displaying large vacuoles (probably enlarged lysosomes) and engagement of autophagy. Autophagy is the process of "self eating" in which double membrane vesicles form de novo, engulfing cytoplasmic components for degradation upon fusion with lysosomes. In general, the autophagic process (reviewed in detail elsewhere, e.g., Singh and Cuervo, 2011) promotes cell survival, and inhibition of autophagy causes more cell death under Type II conditions. Thus, this form of cell death is often suggested to be "cell death with accompanying autophagy" (Maiuri et al., 2007).

In addition, Type II cell death that actually *depends* on elements of the autophagy pathway has been described. This is best characterized in *Drosophila* metamorphosis (Ryoo and Baehrecke, 2010), although examples in mammalian cells also exist (Elgendy et al., 2011). At this point, it may be simplistic to suggest that this form of cell death occurs as a consequence of "too much autophagy," that is, involvement of elements of the autophagy pathway does not necessarily

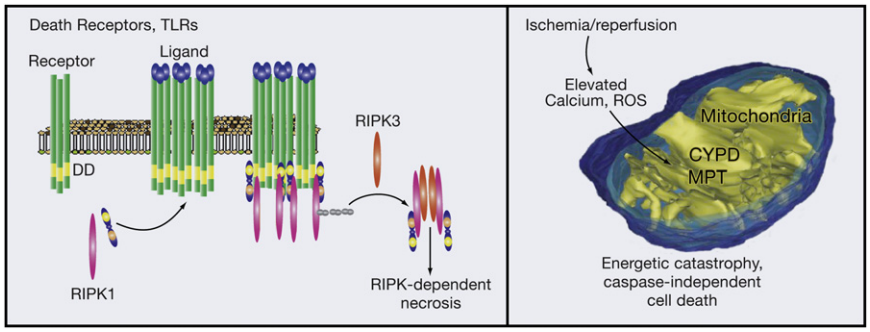


**Figure 2. Pathways to "Programmed Necrosis"**
Necrosis can be passive, occurring as a consequence of excessive cell damage or catastrophic loss of energy. However, necrosis can also be an active process. Two such mechanisms are illustrated. In RIPK-dependent necrosis (left), ligating death receptors or some TLRs (TLR3 or TLR4) activates a kinase, RIPK1, which in turn binds to a related kinase, RIPK3, triggering necrosis. Precisely how RIPK3 causes necrosis is not known. In the mitochondrial pathway of necrosis (right), injury (such as ischemia plus reperfusion) causes elevation of calcium and/or reactive oxygen species (ROS). These impact the mitochondria to cause a sudden change in permeability of the inner membrane to small solutes (mitochondrial permeability transition, MPT). Efficient MPT requires the matrix protein cyclophilin D (CYPD). As a consequence of MPT, mitochondrial transmembrane potential is lost and the matrix swells, damaging the mitochondria. Death ensues as a consequence of energetic catastrophe or through active damage by mitochondrial products or contents. Two modes of active necrosis, not shown here (see text), are caspase-independent cell death (CICD, a consequence of MOMP induced by BCL-2 proteins, proceeding in the absence of caspases) and secondary necrosis, a consequence of failure to clear apoptotic cells.

mean that it is autophagy, per se, that kills the cell in this setting. The precise mechanisms responsible for such cellular demise remain to be elucidated.

From our perspective here, the paucity of information on immune consequences of Type II cell death somewhat precludes further discussion. It should be noted, however, that autophagy appears to impact inflammatory (Levine et al., 2011) and adaptive immune responses as discussed by Yatim and Albert in this issue. This may or may not relate to Type II cell death.

### *Type III. Necrosis*

Necrosis is characterized by a loss of plasma membrane integrity, accompanied by organellar (e.g., endoplasmic reticulum and mitochondria) swelling, usually without nuclear condensation (except in one case, discussed below). It can be caused by excessive damage and/or catastrophic energy loss, and thus can be a passive process, as we noted above. Recent evidence has shown, however, that necrosis can be the result of regulated processes and therefore, in a sense, "programmed." Two pathways of programmed necrosis are outlined in the simplified scheme shown in Figure 2. These and other pathways of necrosis are reviewed in more detail elsewhere (Green, 2011).

One active process involved in some forms of necrosis is the mitochondrial permeability transition (MPT), which is distinct from MOMP in apoptosis. Although only partially understood, MPT involves the mitochondrial matrix protein cyclophilin D (CYPD) and results in sudden loss of mitochondrial transmembrane potential and matrix swelling. It can result from high levels of calcium and/or reactive oxygen species, such as are seen in ischemia/reperfusion injury. Indeed, animals deficient in CYPD, although displaying no defects in apoptosis, are resistant to necrosis induced by ischemia/reperfusion (Leung and Halestrap, 2008).

Another form of programmed necrosis occurs via the action of two kinases: receptor interacting protein kinase-1 (RIPK1) and RIPK3. These are engaged by ligation of death receptors (see above) or by Toll-like receptor 3 (TLR3) and TLR4. It can also occur as a consequence of DNA damage, apparently independently of surface receptors (Green et al., 2011). They are inhibited by some caspases (in particular, caspase-8) and therefore this form of necrosis can manifest under some conditions of caspase inhibition. The mechanisms by which RIPK1 and RIPK3 cause necrosis are under active investigation. This RIPK-dependent necrosis (sometimes called "necroptosis") is frequently equated with Type II (autophagic) cell death. However, no role for autophagy (as inducer, effector, or regulator of this form of necrosis) has been unambiguously elucidated (Maiuri et al., 2007).

Necrosis also occurs if MOMP, regulated by the BCL-2 proteins (see above), proceeds in the absence of caspase activation (as a consequence of inhibitors or ablation of the downstream pathway). This caspase-independent cell death (CICD) appears to be the result of a loss of mitochondrial function or may be caused by other mediators released from the mitochondria (reviewed elsewhere; Green, 2011).

Importantly for our discussion here, necrosis also occurs if a cell dying by apoptosis is not rapidly engulfed and cleared. This "secondary necrosis" is distinct from other forms of necrosis in that the chromatin condensation, characteristic of apoptosis, is seen in this form of death. Failure to clear apoptotic cells, and the ensuing secondary necrosis, is thought to contribute to autoimmune diseases such as systemic lupus erythematosis.

Although necrotic cells and their associated DAMPs are generally thought to be proinflammatory and immunogenic, it is not known whether the pathway leading to necrosis affects the inflammatory or immunologic outcome.

## Coda: In Memorium

Dying cells impact the organism through their interactions with living cells. Our focus, in large part, is on those interactions that affect the immune system and consequences of this interaction in nonimmune tissues, such as occurs in cancer.

While considering these effects, we should be aware that other types of interactions between dying and living cells may well exist. In arthropods, a process called "compensatory proliferation" has been elucidated, in which dying cells stimulate proliferation of neighboring cells (Fan and Bergmann, 2008). This process depends on caspase activity in the dying cells and appears to be mediated via TGF-β and WNT family proteins. Although not extensively described in mammalian systems, evidence supports the idea that dying cells can induce stem cell differentiation (Li et al., 2010). The role of the immune and/or inflammatory response in such effects may prove interesting.

Another area worth considering is the impact on the immune system of cells that have not died but have undergone senescence. Such cells, like dying cells, are cleared from the body and produce mediators that facilitate such clearance (e.g., Coppé et al., 2008). It is not unlikely that they influence immune responses in the process.

In any case, it is clear that when cells die they are not "forgotten." How dying cells impact their surroundings is a rich field of investigation, as the reviews that follow amply illustrate.

## ACKNOWLEDGMENTS

This piece is dedicated to the memory of a great friend and brilliant scientist, J. Tschopp, whose work enlightened the fields of cell death and immunology. His passing earlier this year has left an unfillable hole in these research areas and in those who knew him. He is sadly missed. D.R.G. is supported by grants from the U.S. National Institutes of Health and by the American Lebanese and Syrian Associated Charities.

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