Cell

Leading Edge
Perspective

The Coming Decade of Cell Death Research: Five Riddles

Douglas R. Green[1,*]
[1]Department of Immunology, St. Jude Children's Research Hospital, Memphis, TN 38105, USA
*Correspondence: douglas.green@stjude.org
https://doi.org/10.1016/j.cell.2019.04.024

Active cell death, in its many forms, is a fundamental biological process. Studies over the past several decades have explored the functions and consequences of cellular demise and elucidated several of the key cell death pathways. Here, I pose five questions, or riddles, that might provide a guide to the next decade of cell death research. Focusing mainly on four types of active cell death (apoptosis, necroptosis, pyroptosis, and ferroptosis) mainly in mammals, this Perspective explores the possible research directions that might answer these riddles, or at least prompt new ones.

Introduction

> It is a riddle wrapped in mystery inside an enigma
>
> -Winston Churchill

> My relationship with death remains the same. I am strongly against it.
>
> -Woody Allen

It never really came as a surprise that cells in a complex organism die; every living thing eventually dies. What was perhaps unexpected was that during development cells die in what appears to be a "programmed" manner (Ellis and Horvitz, 1986; Lockshin, 1969; Saunders, 1966). Although necrotic cell death was described in the 19th century, pathologists also recognized that another form of cell death occurred during embryogenesis, in response to stress, and as an aspect of tissue homeostasis. Initially based on morphological features of the dying cells, this cell death was defined and given the name "apoptosis" in 1972 (Kerr et al., 1972). By the 1980's, the first biochemical markers of apoptosis had emerged and mechanisms were sought.

The fact that apoptosis could be induced and/or predicted to occur at defined developmental stages, and the idea that it involved active participation on the part of the dying cell, led to efforts to dissect the process genetically and biochemically. Arguably, technical advances in molecular characterization of identified genes and the success of applying such approaches to fundamental biological processes (e.g., cell cycle) led to the "renaissance" in cell death research that followed. For the twenty years spanning 1990-2010, publications on regulated cell death increased exponentially to occupy a significant part of the scientific literature, a representation that plateaued (but was sustained) over the past decade (Figure 1).

As we anticipate the beginning of another decade of cell death research, it might be useful to take stock of the current state of this mature field and pose some fundamental questions with the potential to shape the advances to come. The author readily acknowledges that the queries that form the basis of the exploration before the reader are based on only one person's opinion, and if experience is a guide, likely represent at best modest predictors of the future discoveries. That said, questions drive us: a good question is half of knowledge. Herein are five. There are certainly more.

Before stepping into the unknown, it might be helpful to provide an abbreviated perspective on the state of our understanding of cell death. Here, we focus on "active" cell death (as opposed to "passive") in which a cell participates via molecular pathways in its own demise. Superficially, it has been useful (at least for this author) to parse these pathways of active cell death into (1) those that involve molecular processes that appear to have evolved to promote a form of cell death ("suicide") and (2) those that involve processes that preserve cell integrity such that, when disrupted, death occurs as a consequence of the cell's active, physiological state ("sabotage"). The ontology of sabotage is illustrative of the latter idea: removal of a railway tie (sabot) will only "kill" a train that is actively moving. For the most part, this overview focuses on three forms of suicide: apoptosis, necroptosis, and pyroptosis, and one form of cell death that appears to be by sabotage: ferroptosis. These are summarized in Box 1.

Although other distinct forms of cell death exist, some of which are mentioned in the final section, our focus on the subset listed above will help to constrain our discussion and allow comparisons between them. Further, and again for the most part, these questions are addressed in the context of cell death processes that occur in mammals. Although many of the mechanisms are conserved throughout the animal kingdom (and sometimes beyond), differences in the "wiring" of these pathways in other organisms can confuse our considerations and are (largely) avoided herein.

Riddle #1: How Deadly Is Death?

How final is engagement of active, cell death pathways, and are there consequences for survival after such engagement? Where is the "point-of-no-return" of a cell death process, after which, to

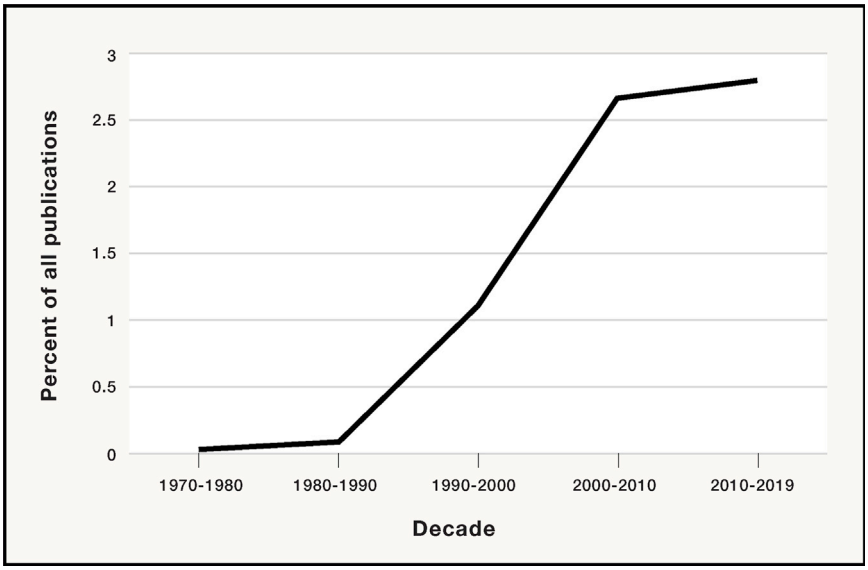

Figure 1. Publications on Cell Death by Decade

Results of Pubmed searches of the terms "apoptosis," necroptosis," pyroptosis," and ferroptosis" for each time period shown, divided by total Pubmed publications for that time period. The values are undoubtedly an underestimate.

quote the Bard, "no traveler returns"? To what extent can a dying cell be "saved"?

In the three forms of regulated necrosis considered here (necroptosis, pyroptosis, and ferroptosis), cell death occurs when the relevant effector mechanisms damage the integrity of the plasma membrane beyond repair. It follows, then, that such repair might antagonize the cell death process. In ferroptosis, lethality occurs as a result of peroxidation of polyunsaturated fatty acids (PUFAs) that self-propagate unless halted by the lipid peroxidase, glutathione peroxidase 4 (GPX4) (Stockwell et al., 2017). Indeed, ferroptosis only occurs when the function of GPX4, sustained by glutathione (GSH), is perturbed, and no other mechanism is known to prevent this death process once initiated (although it is possible that diminished GPX4 activity can be sufficient if upstream hydrogen peroxide generation and/or PUFA synthesis is constrained).

The other two forms of regulated necrosis, pyroptosis and necroptosis, are effected by engagement of pore-forming proteins, gasdermin D (GSDMD) and mixed lineage kinase domain-like protein (MLKL), respectively, that disrupt the plasma membrane (see Box 1). Components of the endosomal sorting complexes required for transport (ESCRT), especially those of ESCRT III, function in the repair of laser- or bacterial toxin-induced plasma membrane damage (Jimenez et al., 2014; Scheffer et al., 2014), and this repair is triggered by an influx of Ca^{2+} at the site of damage (Scheffer et al., 2014). ESCRT III, along with other ESCRT components, similarly antagonize the necrotic activity of active MLKL (Gong et al., 2017b) and GSDMD (Rühl et al., 2018) to sustain cell integrity upon engagement of the cell death pathways, and this repair process again appears to be dependent upon influx of Ca^{2+} (Gong et al., 2017b). As a result, ESCRT-mediated plasma membrane repair can preserve cell survival when the activity of the effectors is sufficiently low (Gong et al., 2017b; Rühl et al., 2018) or the engaged pathway is disrupted prior to lysis (Gong et al., 2017b).

Executioner caspases can induce necrosis upon cleavage of gasdermin E (also called DFNA5), which is widely expressed in epithelial and other cell types (Rogers et al., 2017; Wang et al., 2017). It is unknown whether ESCRT components similarly repair pores formed by the activated molecule, although this would appear likely. In this case, antagonizing necrosis might be expected to promote conventional caspase-dependent apoptosis in these dying cells.

Signaling events upstream of terminal effector activation in both necroptosis and pyroptosis can result in expression or processing of cytokines and chemokines within the cell, and sustained viability by ESCRT can either antagonize (pyroptosis) or enhance (necroptosis) the release of such mediators. As an example of the latter, engagement of Receptor-interacting serine/threonine-protein kinase 1 (RIPK1) upon induction of necroptosis can activate nuclear factor kappa light chain enhancer of activated B cells (NF-κB) to drive gene expression, and the resulting mediators are important for promoting appropriate antigen presentation by dendritic cells for anti-cancer immunity (Yatim et al., 2015). Disruption of the ESCRT machinery accelerates necroptotic death, reducing the production of such mediators, and thereby compromises the presentation of cell-associated antigens to T cells (Gong et al., 2017b).

In pyroptosis, processed interleukin-1β (IL-1β) and IL-18, as well as other cellular components, are released by the action of GSDMD at the plasma membrane (Kayagaki et al., 2015; Shi et al., 2014), either by diffusion through GSDMD pores (Evavold et al., 2018) or by subsequent lysis of the cell (Shi et al., 2015). The function of ESCRT III to remove the GSDMD pores and preserve plasma membrane integrity thus limits this release (Rühl et al., 2018).

The plasma membrane damage in necroptosis might itself generate signals that result in elaboration of secreted mediators. In murine cells, activation by dimerization of an N-terminal fragment of human MLKL that is unlikely to interact with other signaling molecules causes plasma membrane damage and induces the expression of CXCL1 and CXCL10 chemokines. The production of these chemokines is curtailed when ESCRT III is disrupted to promote plasma membrane lysis (Gong et al., 2017b). It is possible that MLKL-mediated membrane damage elicits a "plasma membrane stress response" that engages a gene expression signature (that includes these chemokines), the effects of which depend on the delay in lysis effected by ESCRT III (Gong et al., 2017a).

When considering the point of no return in apoptosis, the destruction of the mitochondrial outer membrane upon

Box 1. The Ways Cells Actively Die

The following serves as a primer or reminder of the four cell death pathways that form the majority of the discussions herein. For more details, the reader is referred to more extensive discussions elsewhere (Galluzzi et al., 2018; Green, 2018).

APOPTOSIS

(A) The mitochondrial pathway of apoptosis. The mitochondrial (or intrinsic) pathway is engaged by cellular stress or developmental cues that alter the expression and/or function of proteins of the Bcl-2 family (Figure 2). Proteins in this family share one or more Bcl-2 homology (BH) regions and function in the regulation of the integrity of the outer mitochondrial membrane in mammals and several (but not all) metazoan phyla. Three Bcl-2 effector proteins, Bax, Bak, and Bok, directly cause mitochondrial outer membrane permeabilization (MOMP), releasing proteins of the mitochondrial inter-membrane space into the cytosol. Each of these can act independently of the others in effecting MOMP. Active Bax and Bak are inhibited by the anti-apoptotic Bcl-2 proteins, including Bcl-2, Bcl-xL, Mcl-1, and Bfl/A1, among others. Bok appears to be constitutively active, regulated predominantly by degradation via the endoplasmic reticulum associated degradation (ERAD) machinery, and perhaps by other mechanisms independently of the anti-apoptotic Bcl-2 proteins. A third Bcl-2 subfamily, the BH3-only proteins, regulates the other two, inhibiting the anti-apoptotic Bcl-2 proteins and activating Bax and Bak to effect MOMP. Specificities and dynamic interactions among the Bcl-2 proteins in a cell determine whether or not MOMP occurs.

Proteins released to the cytosol upon MOMP include holocytochrome c, which binds to the cytosolic protein APAF1 inducing the oligomerization of the latter into the apoptosome. The "hub" of the APAF1 oligomer interacts with the initiator caspase endopeptidase caspase-9, activating it by enforced dimerization. The active caspase-9 cleaves and thereby activates the executioner caspase endopeptidases caspase-3 and caspase-7.

Caspase-9, caspase-3, and caspase-7 are bound and inhibited by X-linked inhibitor of apoptosis (XIAP). Other proteins, released upon MOMP, include Smac and Omi, which antagonize XIAP, permitting the caspases to function. The executioner caspases cleave hundreds of substrates in the cell. These include iCAD, a chaperone and inhibitor of caspase-activated DNase (CAD), and cleavage of iCAD unleashes CAD to cut inter-nucleosomal DNA. Other substrates affect the distribution of phospholipids in the plasma membrane, allowing phosphatidylserine (PS), normally constrained to the inner leaflet, to be exposed on the cell surface. PS acts as a signal to promote phagocytosis of the dying cell prior to loss of plasma membrane integrity. By cleaving substrates, the executioner caspases cause the features of apoptotic cell death, including chromatin condensation and membrane blebbing, but are not strictly required for cell death per se, as disruption of mitochondrial function upon MOMP can condemn the cell to death due to metabolic catastrophe (although such "caspase-independent cell death" does not have the features of apoptosis).

(B) The death receptor pathway of apoptosis. The death receptor (or extrinsic) pathway of apoptosis is engaged when a subset of the tumor necrosis factor (TNF) receptor family (the death receptors) are ligated. A simplified death receptor pathway exemplified by the death receptor CD95 is shown in Figure 2 (as we will see, signaling from other death receptors, such as TNFR1, can be more complex). The intracellular region of the death receptor, upon ligation, recruits the adapter molecule FADD, which in turn recruits the initiator caspase, caspase-8. Bound caspase-8 then recruits additional caspase-8 molecules, which are activated by dimerization. The active caspase-8 cleaves and thereby activates the executioner caspase-3 and caspase-7.

If XIAP is present in the cell, this inhibits the executioner caspases (as above). Caspase-8 also cleaves and thereby activates one of the BH3-only proteins, Bid, which antagonizes the anti-apoptotic Bcl-2 proteins and activates Bax and Bak, causing MOMP. Smac and Omi, released upon MOMP, inhibit XIAP, permitting the executioner caspases to promote apoptosis.

A molecule resembling caspase-8, but lacking proteolytic activity, cellular FLICE (FADD-like IL-1β-converting enzyme)-inhibitory protein (c-FLIP), disrupts the oligomerization of caspase-8. Although the caspase-8-c-FLIP dimer is an enzymatically active protease (see below), it does not promote apoptosis. Therefore c-FLIP can prevent apoptosis by the death receptor pathway.

Caspase-8 is not only activated upon ligation of death receptors. A kinase, RIPK1, can bind to FADD to promote caspase-8 activation and apoptosis. RIPK1-dependent caspase-8 activation can occur as a result of engagement of some TLRs, via the adapter molecule TRIF and by interactions with another kinase, RIPK3. RIPK1 and RIPK3 are also involved in the complex interactions that engage another active cell death mechanism, necroptosis, outlined next.

Executioner caspases, engaged by either of the above apoptotic pathways, cleave and thereby activate a pore forming protein, gasdermin E, thus promoting a "secondary necrosis" which occurs in apoptotic cells that express the protein. Such cells display characteristics of apoptosis (e.g., membrane blebbing and nuclear condensation) as well as features of necrosis (Rogers et al., 2017; Wang et al., 2017).

NECROPTOSIS

Necroptosis can be engaged by ligation of death receptors, some TLRs (via TRIF), and by an intracellular nucleic acid sensor, ZBP1 (Figure 3). Unlike apoptosis, necroptosis is a form of regulated necrosis.

(*Continued on next page*)

Box 1. *Continued*

Ligation of death receptors causes the recruitment of RIPK1 to the intracellular region of the death receptor. The ubiquitin ligases, cellular inhibitor of apoptosis protein 1 (cIAP1) and cIAP2 ubiquitinylate RIPK1 via non-degradative K63 linkages, and the ubiquitinylated RIPK1 acts in a kinase-independent manner to activate NF-κB via the I-κB kinase (IKK) complex. NF-κB prevents death-receptor-induced apoptosis, in part, by inducing the expression of c-FLIP. Deubiquitinases remove the ubiquitin chains, releasing RIPK1, which then undergoes a conformational change due to auto-phosphorylation, exposing a region (RIP homotypic interaction motif [RHIM]) that self-oligomerizes and recruits RIPK3 (which also contains the RHIM oligomerization domain). RIPK3 thus becomes activated, and this kinase phosphorylates and thereby activates the effector molecule MLKL. Phosphorylated MLKL oligomerizes and targets the plasma membrane, inducing phospholipid scrambling (exposing PS) and disrupting plasma membrane integrity, resulting in necrotic cell death.

This process, however, is disrupted by the function of the FADD-caspase-8-c-FLIP complex, which as noted above is enzymatically active. The caspase-8-c-FLIP dimer cleaves RIPK1 and RIPK3, preventing necroptosis. Inhibition or disruption of caspase-8-c-FLIP proteolytic activity therefore allows necroptosis to proceed. Inhibition of RIPK1 activity prevents its conformational change, and thereby blocks death-receptor-induced necroptosis.

RIPK3 is also directly oligomerized and activated by TRIF (upon ligation of some TLRs) and by the cytosolic nucleic acid sensor ZBP1, promoting necroptosis. The process proceeds somewhat in reverse of that described above, as the oligomerized RIPK3 binds RIPK1, recruiting in turn the FADD-caspase-8-c-FLIP complex, which destroys the RIPK3 oligomer. Again, necroptosis proceeds when the function of the caspase-8-c-FLIP dimer is inhibited or disrupted. In such settings, RIPK1 is required for the inhibition, but not the activation of RIPK3.

The complex of RIPK1, RIPK3, and MLKL, regardless of the pathway, is referred to as the necrosome.

PYROPTOSIS

Pyroptosis is a form of regulated necrosis, although in some cases the pathway can engage apoptosis. Pyroptosis occurs when the effector molecule, gasdermin D (GSDMD), is cleaved, promoting its oligomerization to form large pores in the plasma membrane, leading to cell death. Several caspases are capable of cleaving GSDMD to produce this effect: caspase-1, caspase-4, caspase-5, and caspase-11 (caspase-11 is only found in rodents, which lack caspase-4 and caspase-5). Caspase-4, caspase-5, and caspase-11 are bound by intracellular bacterial LPS and are thus directly activated via oligomerization to cleave GSDMD (Figure 4).

Caspase-1, in contrast, must be engaged by a large caspase activation platform, called the inflammasome, in order to attain its proteolytic function. There are several molecules that can form an inflammasome, but most inflammasomes include an adapter protein, ASC, that when oligomerized binds to caspase-1 and activates it by dimerization. Two types of protein function in ASC oligomerization (or in some cases, ASC-independent, direct caspase-1 binding). These include several of the intracellular NLRs and the cytosolic DNA sensor AIM2. NLRs that participate in inflammasome formation generally sense different intracellular PAMPs associated with infection, but in at least one case (NLRP3) can also respond to cellular damage induced by inert material such as engulfed asbestos, uric acid crystals, and calcium phosphate crystals, among others. Ligation of these sensors cause the NLRs or AIM1 to oligomerize, recruit ASC, and thereby bind and activate caspase-1 to promote pyroptosis via GSDMD. In addition, intracellular K^+ ions inhibit the activation of one of the NLRs, NLRP3, and an efflux of this ion can promote activation of this NLR to form an inflammasome.

In addition to cleaving GSDMD, caspase-1 (but not other pyroptotic caspases) processes two cytokines from their inactive proforms to their active mature species, IL-1β and IL-18. These lack signal sequences for secretion, and can be released through GSDMD pores or upon cell lysis.

In the absence of GSDMD, caspase-1 can cleave and thereby activate the executioner caspases, and can also cleave and activate Bid to engage MOMP. Thus, without GSDMD, apoptosis ensues upon caspase-1 activation.

FERROPTOSIS

Free intracellular iron atoms react with hydrogen peroxide (e.g., produced by the action of mitochondrial superoxide dismutatase on superoxide radicals in the mitochondria) in the Fenton reaction, which can result in the peroxidation of PUFAs (Figure 5). These oxidized lipids produce more oxidized lipids and other toxic products in the presence of oxygen, unless they are neutralized by the action of the only cellular lipid peroxidase, GPX4. The recycling of GPX4 to mediate this protective process requires glutathione, which in turn requires NADPH to recycle. Glutathione is synthesized from cysteine, which is transported into the cell through the system Xc^- transporter. Cysteine deprivation, inhibition of the system Xc^- transporter, reduction in NADPH, or inhibition of GPX4 therefore promote lipid peroxidation in cells with available free iron, hydrogen peroxide generation, and PUFAs. Unconstrained lipid peroxidation results in ferroptosis, a regulated necrosis. Sequestration of iron, scavenging of lipid peroxides and/or hydrogen peroxide, or inhibition of PUFA synthesis can protect cells from ferroptosis.

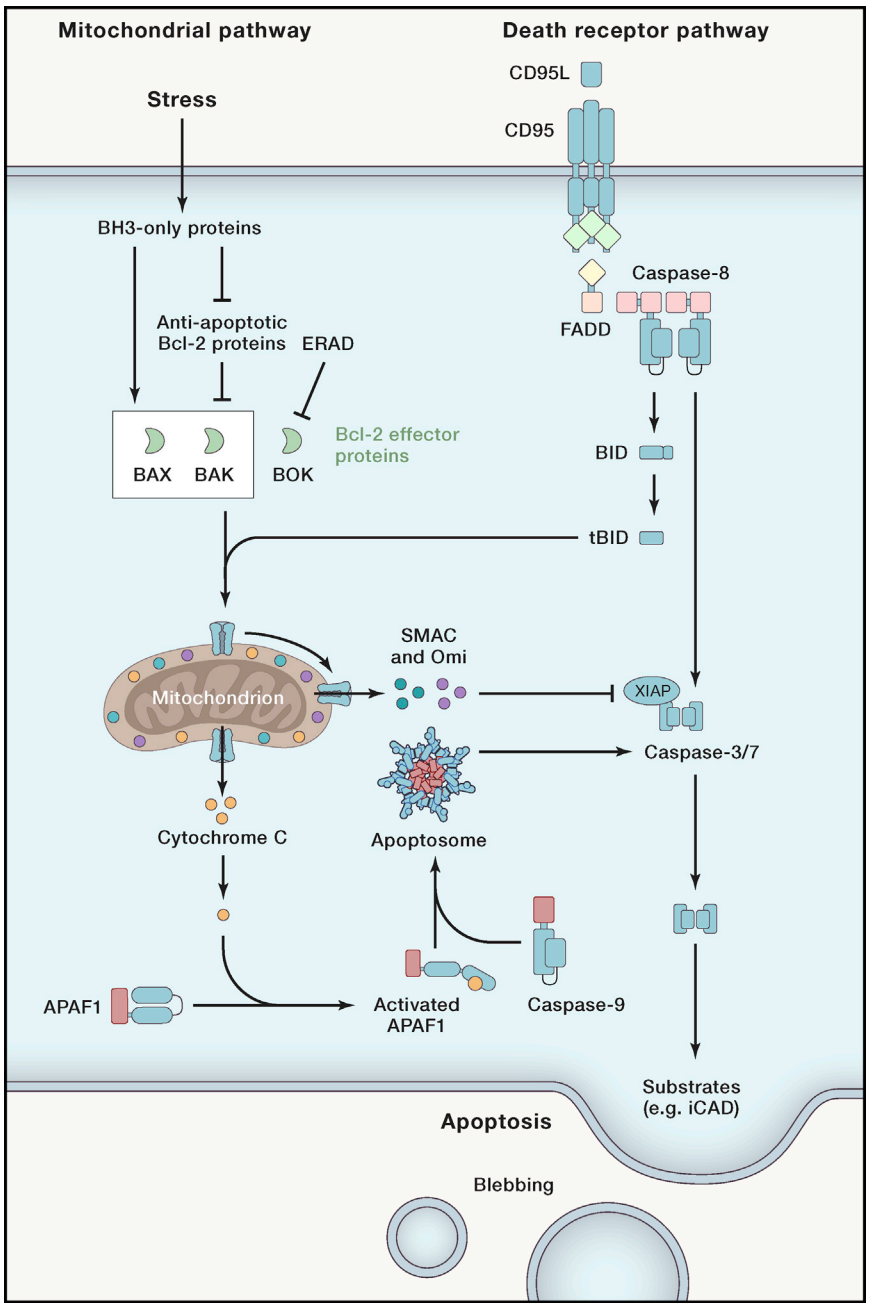

Figure 2. Apoptosis
The mitochondrial and death receptor pathways of apoptosis are illustrated. Roles for the engagement of caspase-8 in necroptosis are shown in Figure 3.

mitochondrial outer membrane permeabilization (MOMP) can commit a cell to die as a consequence of a metabolic catastrophe even if caspases are not engaged (Lartigue et al., 2009). Such death can be averted after MOMP if glycolysis is enforced and autophagic removal of damaged mitochondria allows expansion of the remaining, intact organelles (Colell et al., 2007; Tait et al., 2010). However, emerging evidence has revealed that limited caspase activation invoked by engagement of death receptors (Lovric and Hawkins, 2010) or when a minority of mitochondria undergo MOMP (Ichim et al., 2015) can result in induction of the caspase-activated DNase (CAD) and DNA damage. As a result, cells that survive the engagement of the apoptotic machinery display increased genomic instability that is dependent on both caspases and CAD activity.

Cell survival despite executioner caspase activation has been documented in several scenarios, a phenomenon that has been named "anastasis" (Ding et al., 2016; Sun et al., 2017; Tang et al., 2015), and might permit other described effects of activated executioner caspases in living cells (Nakajima and Kuranaga, 2017). An example of the latter is the putative role for active caspase-3 in the pruning of dendritic spines in the brain (Ertürk et al., 2014). Changes in gene expression in cells surviving due to anastasis might reflect the effects of the specific stressor used to induce apoptosis, or perhaps by the survival process itself. Intriguingly, cells that survive activation of MLKL in necroptosis, a process called resuscitation (Gong et al., 2017b), similarly display gene expression changes that overlap those of anastasis (Gong et al., 2017a). It is tempting to speculate that these common changes might include a general expression signature of cell survival despite induction of different core death pathways.

We do not know to what extent anastasis in apoptosis or resuscitation in necroptosis occurs in natural or therapeutic situations in mammals *in vivo*. In *Drosophila*, a probe for caspase activity suggests that anastasis might be extensive, and a majority of cells engage caspase activation during development without coincident cell death (Ding et al., 2016; Tang et al., 2015). With respect to resuscitation, kidneys from live human donors display evidence of activated, phospho-MLKL in endothelial cells after, but not prior to transplantation, without any sign of necroptosis in the healthy tissue (Gong et al., 2017b). Intriguingly, this is accompanied by induction of the expression of ESCRT components, important for preserving survival despite MLKL activation. This expression might occur as a consequence of HIF1 activation by hypoxia during the transplant (Gong et al., 2017a).

Ultimately, an analysis of cell survival despite engagement of cell death pathways will require the use of systems that

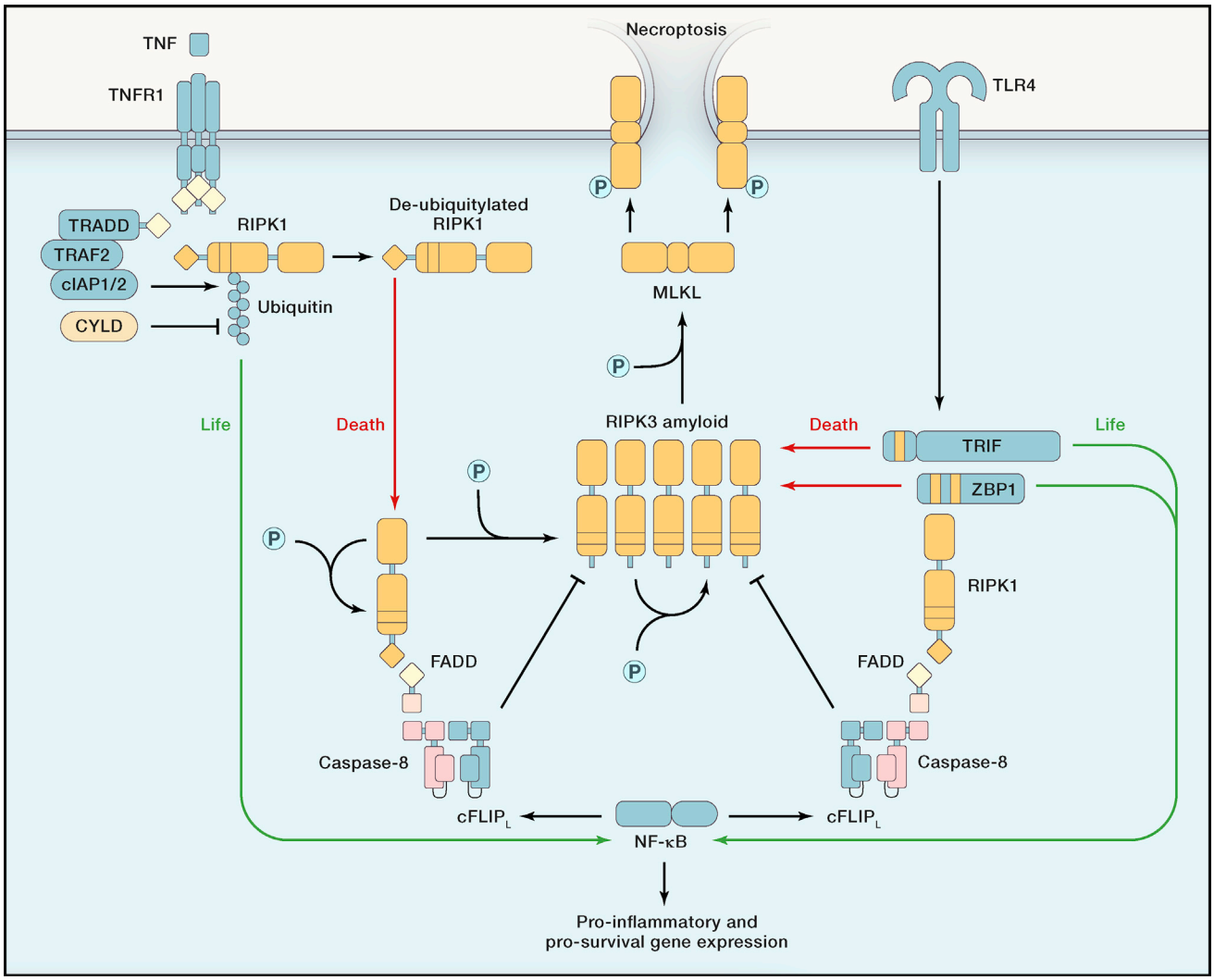

Figure 3. Necroptosis

Death receptors, TLRs (that engage TIR-domain-containing adapter-inducing interferon-β [TRIF]), and Z-DNA Binding Protein 1 (ZBP1) bind directly or via RIPK1 to activate RIPK3 and, in turn, MLKL to execute necroptosis. RIPK3 activation is antagonized by the enzymatic activity of the FADD-caspase-8-c-FLIP complex.

"mark" cells that have withstood the cell death process. One approach would be the development of sensors that activate CRE to remove a lox-stop-lox and permit expression of a fluorescent protein. Such sensors can be readily envisioned for executioner and inflammatory caspases, and perhaps for activated RIPK3 (although activated MLKL might prove more challenging).

Riddle #2: When Is a Toxin Not Toxic?

When a clonal population of cells are faced with a death-inducing stimulus, why do some cells survive when their clonemates die, and are there consequences for this state of survival? A failure to kill less than 100% of cells that are exposed to a death inducer is almost always viewed as the relative susceptibility to undergo cell death, and not the ability of some cells to withstand the death-inducing signal.

It is axiomatic that if a mutation affecting expression or function of a gene can render a cell resistant to a death-inducing treatment, such mutations will be positively selected upon application of that treatment. This idea underlies much of the thinking about relapse in cancer. More recently, however, we have come to realize that cells can survive a toxic treatment, such as a chemotherapeutic agent, by attaining a transient state called "persistence" without mutation. The surviving cells are "persisters" or "persister cells." In contrast to the positive selection of resistance mutations, progeny of such persisters are as sensitive to the stimulus as were the original cells (Hangauer et al., 2017; Viswanathan et al., 2017). One way to think about this is the concept of the lethal dose (LD) at which some proportion of a clone of cells die, e.g., LD50, at which 50% die. This simple concept has important implications beyond cell biology; indeed, survival of individuals at intermediate LD of infectious microbes

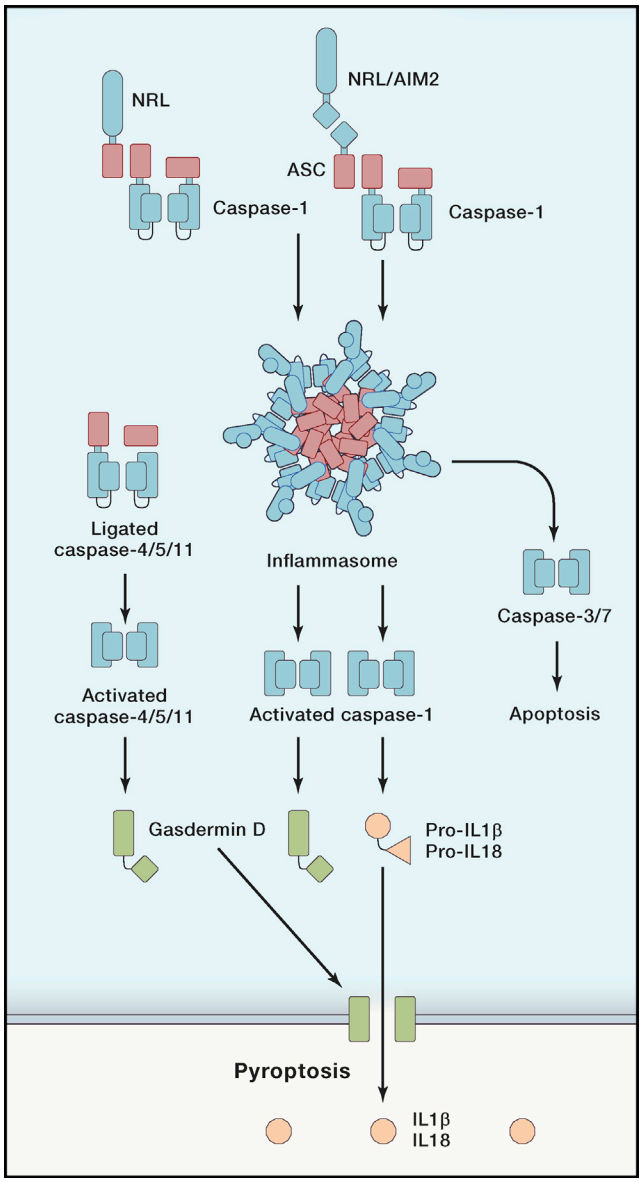

Figure 4. Pyroptosis
Several NLRs and AIM2, when activated, directly or indirectly (via apoptosis-associated speck-like protein [ASC]) bind to and activate caspase-1, which cleaves GSDMD to effect pyroptosis, and also processes the cytokines IL-1β and IL-18. Caspase-4, caspase-5, and caspase-11 are directly activated by cytosolic LPS and cleave GSDMD, but not the cytokines.

has revealed fundamental host-microbe interactions (Sanchez et al., 2018). Applying this to cells might similarly reveal novel mechanisms at the cellular level; we understand a great deal about the way in which cells die, but relatively little about those cells that survive. What are the stochastic differences between otherwise identical cells that define the persistent state, and is this state induced or does it exist prior to treatment?

Persistence is likely to be distinct from the processes outlined above for the first riddle. Although cells that engage a cell death pathway and survive (with subsequent consequences) certainly persist, there is no evidence that persistence, per se, involves engagement of core cell death processes. Instead, the "decision" to persist appears to occur upstream of such core processes. As an example, the induction of p53 by DNA damage can elicit the expression of molecules (such as BH3-only proteins; see Box 1) that promote MOMP and apoptosis, but p53 also induces cell cycle arrest and DNA repair that can preserve cell survival. Elegant single-cell dynamic studies have shown that these contrasting effects of p53 that control a decision point upstream of the cell death pathway are predicted by the nature of p53 protein stability. Cells that display oscillating p53 protein expression repair and survive, whereas those with persistent, elevated p53 engage apoptosis (Paek et al., 2016). Although informative, these observations do not themselves explain persistence; why some but not other cells in a population display a particular pattern of p53 stability remains unclear. Further, p53-deficient cells nevertheless display the persistence phenomenon.

Other stresses that can induce apoptosis also engage stress responses that can antagonize cell death, perhaps best illustrated by the unfolded protein response (UPR) during endoplasmic reticulum-induced stress (Hetz and Papa, 2018). Many cells function at the limit of their capacity for secretion, and a wide variety of conditions can cause dysfunction in this process, eliciting a UPR. These include but are not limited to nutrient deprivation, hypoxia, generation of reactive oxygen species, and disruption of calcium homeostasis. The UPR engages three distinct pathways affecting transcription of genes involved in resolving the stress, but also genes that promote apoptosis, such that cells that do not manage the stress ultimately die. Those cells that survive thus display a transient resistance to treatments that induce a UPR. Although responses such as the UPR might help to explain persistence under some circumstances, it again remains unclear why some cells but not others succeed in resolving the stress.

Persistence has been studied in some detail in the apoptotic response to the death receptor, TRAIL, at the single-cell level (Flusberg et al., 2013; Spencer et al., 2009). It appears that a threshold of caspase-8 activation exists such that cells that do not achieve this threshold survive and display a transient resistance to subsequent treatment with the ligand, or with ligands for other death receptors, such as CD95 (also known as Fas). Whether this persistent state is stochastic (that is, produced as a consequence of pre-existing levels of the relevant pathway molecules) or is induced by signals elicited by the pathway is not fully resolved. However, evidence in support of the latter exists, in that this persistence can be sustained by periodic engagement of the death receptor in the presence of caspase inhibitors and that the persistent cell state includes a signature of NF-κB-induced inflammation (Flusberg et al., 2013). It is possible that a scaffolding function for caspase-8, independent of its catalytic activity, participates in the generation of the persistent state (Henry and Martin, 2017), although experiments to test this (which would require inducible expression of the caspase) have not been described to date.

Some of the best evidence supporting the idea of a general persistent state comes from studies of emergent sensitivities of cells that survive treatments with chemotherapeutic agents

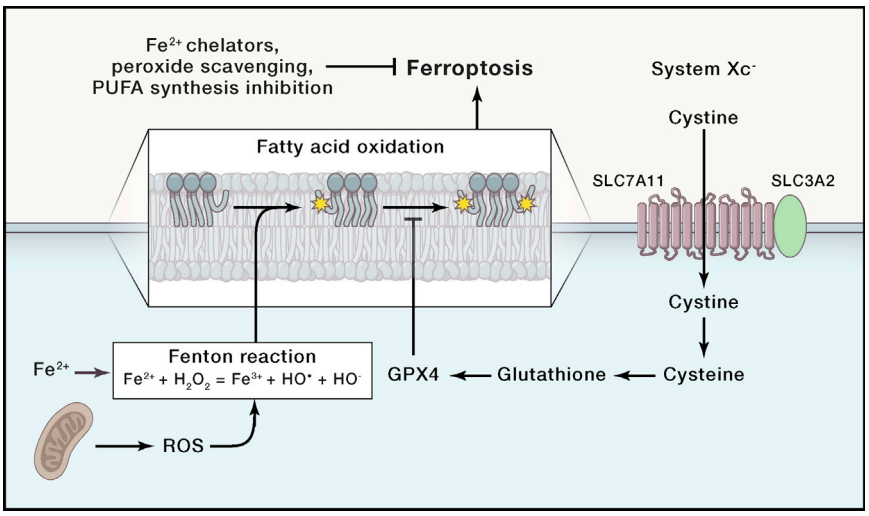

Figure 5. Ferroptosis

The Fenton reaction causes peroxidation of PUFAs, which in turn catalyze further lipid oxidation in the presence of O_2, disrupting membranes (and generating additional toxic derivatives), leading to ferroptosis. The lipid peroxidase GPX4 neutralizes oxidized lipids, but requires GSH for recycling. Disruption of GSH synthesis (by cysteine/cystine deprivation or inhibition of the System Xc^- transporter), recycling (requiring nicotinamide adenine dinucleotide phosphate [NADPH]), or inhibition of GPX4 induces ferroptosis in some cells. Ferroptosis is inhibited by sequestration of free iron, inhibition of PUFA synthesis (by acyl-coa synthetase long chain family member 4 [ACSL4]), or scavenging of reactive oxygen species. Ferroptosis is also inhibited by glutamine deprivation, perhaps due to reduction of PUFA synthesis.

(Hangauer et al., 2017; Viswanathan et al., 2017). A variety of clonal cell lines were treated with a number of different agents and surviving cells were expanded. Remarkably, in nearly every case the persistent cells were now sensitive to cell death induction by an inhibitor of GPX4, the lipid peroxidase that antagonizes ferroptosis. In contrast, the parental lines were usually resistant to GPX4 inhibition, and importantly, treatment with the GPX4 inhibitor to remove sensitive cells prior to treatment with the chemotherapeutic agent did not influence the numbers of persisters or their emergent dependence on GPX4 (Hangauer et al., 2017; Viswanathan et al., 2017). These results strongly suggest that persistence is not selection of cells with a pre-existing (if transient) state of resistance but instead is acquired in some cells in response to the stressor that engages cell death mechanisms in their non-persistent clone mates. Further, there is something about the persistent state that creates a dependence on GPX4.

Ferroptosis that occurs upon GPX4 inhibition or GSH depletion requires iron, hydrogen peroxide (which together produce superoxide as a consequence of the Fenton reaction), and PUFAs, the targets of lipid peroxidation (Dixon et al., 2012). It is likely that the hydrogen peroxide that fuels ferroptosis is produced by mitochondria (Krainz et al., 2016). Therefore, sensitivity to GPX4 inhibition in persisters might be because of changes in metabolism (mitochondrial and/or PUFA production), induced by pro-apoptotic agents, that impart a survival advantage for these cells that achieve such changes. Alternatively, the dependence on GPX4 in persisters might arise as a by-product of the persistent state, e.g., as a result of one or more transcription factors that drive both transient resistance to cell death and the metabolic state that creates GPX4 dependence. As another example, the induction of autophagy by cellular stress improves cell survival and can also sensitize cells to ferroptosis via the degradation of ferritin (Hou et al., 2016). If persister cells sustain a state of autophagic flux, this might explain the emergent dependence on GPX4.

The importance of persistence as a phenomenon depends on several factors. As noted above, we often assume that selection of pre-existing cells harboring mutations that render them resistant to a therapeutic treatment is the mechanism of relapse in tumor therapy. However, it is possible that transient persistence provides a population of surviving cells in which such mutations might arise after treatment, especially if the treatment promotes mutagenesis. The synergistic effects of combined chemotherapy and GPX4 inhibition in *in vivo* cancer models (Hangauer et al., 2017) might reflect this kind of response. Careful studies of numbers of resistant clones prior to and following treatments (essentially the "Luria Delbruck experiment" applied to tumor cells) might help to determine if and when persistence provides an opportunity for resistance mutations to arise. An understanding of persistence and its link to GPX4 dependence might therefore have profound implications for cancer therapy.

Riddle #3: How Dispensable Is Something that Is Essential?

Many papers and reviews assert that cell death, especially apoptosis, is crucial for development and tissue homeostasis. A superficial search on Google Scholar provides over 50 papers with the phrase "apoptosis is essential for development," and over 3,500 that include "apoptosis is essential." It is indisputable that apoptosis and other forms of cell death occur in metazoan development, and indeed, apoptosis is required for a specific event in *Drosophila* development (White et al., 1994). In nematodes, "normal development" requires apoptosis in that without it extra cells appear, but animals nevertheless mature (Ellis and Horvitz, 1986). In mammals, defective apoptosis is often lethal to embryonic development. But is it *essential*?

Animals lacking components of the mitochondrial pathway of apoptosis, including apoptotic peptidase activating factor 1 (APAF1), caspase-9, and caspase-3, or carrying a mutation in cytochrome *c* that permits electron transport but not efficient APAF1 activation frequently die during embryogenesis, displaying forebrain outgrowth and excess neurons. This would therefore appear to be a clear case where apoptosis is essential to remove cells in development. However, upon closer inspection, this conclusion is suspect. Properly timed closure of the

neural tube arrests proliferation of some neurons, and a delay in timing or efficiency of this closure by disruption of rapid apoptotic cell death allows this proliferation to continue, producing the observed effects (Yamaguchi et al., 2011). In some genetic backgrounds, such disruption of mitochondrial apoptosis has, at best, relatively mild effects in development (Leonard et al., 2002).

Recent studies have raised additional issues. Although animals lacking the mitochondrial pathway of apoptosis because of the ablation of the MOMP effectors Bax, Bak, and Bok (see Box 1) usually fail to survive embryogenesis (because of a failure in neural tube closure and multiple midline defects) or early life after birth (because of cleft palate defects), a small number survive to adulthood (Ke et al., 2018). These animals, although displaying excessive accumulation of lymphocytes and other cells, nevertheless appear to have mostly normal tissue and organ architecture in many tissues previously thought to depend on apoptosis for development. No compensation by other forms of cell death (such as necroptosis or pyroptosis) were observed.

Animals lacking caspase-8 or its adaptor FADD die in early embryogenesis, an effect that is dependent on RIPK3 and the necroptosis effector MLKL (Weinlich et al., 2017). Thus, caspase-8- or FADD-deficient animals that also lack either RIPK3 or MLKL develop and mature at Mendelian frequencies but eventually succumb to the expansion of an unusual T cell population and autoimmunity (autoimmune lymphoproliferative syndrome). These animals are deficient in all caspase-8-dependent apoptotic pathways, such as the death receptor pathways. Therefore, although apoptosis is undoubtedly important for the normal, efficient development of many mammalian tissues, it is not universally essential for development or homeostasis.

One prominent idea is that whereas necrosis induces inflammation, apoptosis (and perhaps other regulated cell death modes) evolved as a strategy to prevent inflammatory responses to cells that die as a consequence of developmental or homeostatic cues (Kearney and Martin, 2017; Kerr et al., 1972; Martin et al., 2012). Thus, complex organisms control inflammation by controlling the mode of cell death. Although attractive in many ways (and discussed in more detail in Riddle #4), there might be a problem with this idea. Compelling evidence exists that a functional death receptor pathway of apoptosis arose at least as early as the common progenitor of the cnidaria (corals) and the chordates (such as ourselves) (Quistad et al., 2014). Similarly, a functional mitochondrial pathway of apoptosis is shared by the platyhelminths (planaria) (Bender et al., 2012). Although molecules that function in apoptotic pathways are found throughout the animal phyla, these studies provide evidence that they function in highly conserved ways to promote apoptosis in animals that do not have (as far as we know) inflammatory cell responses. Of course, althoughit remains possible that such responses exist and are elicited by other modes of cell death (such as necrosis) in such organisms, compelling evidence is lacking.

What, then, is cell death "for"? Or more succinctly, when is cell suicide essential? From an evolutionary standpoint, active cell suicide, even at the level of single-celled organisms, is a stable strategy when it operates to restrict the spread of obligate intracellular parasites to genetically related cells (James and Green, 2002). In contrast, "altruistic suicide," say in response to limiting nutrients, is prone to defector strategies that select for suicide-resistant individuals (and hence, not stable).

That regulated cell suicide can be essential for combatting infection seems to be especially clear in the case of pyroptosis. Mice lacking caspase-1 and caspase-11, and thus lacking all pyroptotic inflammasomes, are sensitive to a variety of bacterial and viral infections, as are animals lacking specific pyroptosis-inducing sensors such as nod-like receptors (NLRs) or AIM2 (Xia et al., 2019). Indeed, caspase-11 in rodents and caspase-4 and caspase-5 in other mammals, including humans, are direct sensors of bacterial lipopolysaccharides (LPS) (Shi et al., 2014).

In keeping with this role, caspase-11 is widely expressed in murine cells. In contrast, the expression of caspase-1 is more restricted. Many invertebrates have large numbers of caspases (e.g., 31 have been annotated in sea urchins), and it is possible that many of these serve as direct sensors of intracellular pathogens.

But is it the cell death itself that controls infection? Caspase-1 processes IL-1β and IL-18 to promote immune responses, and the release of these and other mediators depends on the activation and function of GSDMD (which is also required for pyroptosis) (Kayagaki et al., 2015; Shi et al., 2014). Animals lacking only caspase-11 are more sensitive to some bacterial infections (Jorgensen et al., 2017), but caspase-11-induced GSDMD activity can also promote caspase-1 activation via the NLRP3 inflammasome, likely due to K^+ efflux (Rühl and Broz, 2015), and the extent to which this mechanism of caspase-1 activation participates in innate defense is unexplored. At this stage, we simply do not know to what extent pyroptotic death, itself, restricts infection. To conclude this, it would require identification of microbes that show increased virulence in GSDMD-deficient mice, but not those lacking IL-1β and IL-18.

Necroptosis is also thought to function predominantly in the control of infection, an idea supported by the phyletic distribution of necrosome components among the vertebrates (Dondelinger et al., 2016). Animals lacking RIPK3 (and hence, necroptosis) often show enhanced sensitivity to some viral infections, but at least in some cases, this is independent of its role in activating MLKL to promote cell death (Daniels et al., 2017; Moriwaki and Chan, 2017). As RIPK3 can trigger both necroptosis and apoptosis (via caspase-8), studies have sought to identify infections with enhanced virulence in animals lacking caspase-8 (or FADD) and MLKL to ablate both cell death pathways. Although at least one example of this exists, involving infection with an influenza strain (Nogusa et al., 2016), more extensive studies are needed to explore the roles of these cell death pathways in infection. In addition to its roles in cell death, RIPK3 can also promote inflammation, and in at least one example (West Nile Virus) it is this function of RIPK3 that appears to be important in controlling infection (Daniels et al., 2017).

An alternative way to probe the role of cell death in infection is to examine molecules produced by microbes that function to block cell death pathways. Viral and bacterial proteins that interfere with caspase-1 activation or function are described (Man et al., 2017), but as noted above, it is difficult to conclude that these evolved to limit cell death as opposed to other important functions of the inflammasome. With respect to apoptosis, several different viruses produce bona fide anti-apoptotic

Bcl-2 family proteins, or in some cases non-Bcl-2 proteins that function to inhibit Bax and/or Bak, effectively blocking MOMP and apoptosis (Galluzzi et al., 2008). Similarly, some viruses produce proteins that interfere with death receptor signaling to caspase-8 or activation of RIPK3 (Kaiser et al., 2013). In insects, the baculoviruses express two inhibitors of caspase that function to maintain cell survival until the lytic phase of viral replication (Clem, 2001). It is tempting to conclude that these mechanisms evolved to thwart cell death pathways that would otherwise eliminate virally infected cells.

Another approach to addressing the essential nature of cell death in the control of infection is to identify organisms that only effectively infect animals that are deficient in cell death mechanisms. For example, mice deficient in caspase-1 and caspase-11 can be infected by a soil bacterium that does not infect wild-type animals (Maltez et al., 2015). It will be interesting to know whether animals lacking specific effector molecules, such as GSDMD (pyroptosis), MLKL (necroptosis), or Bax and Bak (apoptosis) harbor viruses or bacteria that are not found in their wild-type littermates. Such studies are in their infancy.

Riddle #4: If a Cell Dies in the Forest of the Body, Does It Make a Sound?

How does cell death affect the physiology of surrounding cells and of the body in general? Do dead cells in the body have an "afterlife"? The death of a cell can have two general types of impact on the organism: it can induce or inhibit immune responses (innate and/or adaptive) and it can promote the proliferation of surviving cells ("compensatory proliferation"). The former has been most extensively studied in mammalian systems, whereas the latter is best described in *Drosophila* (although also observed in mammals).

It is likely that both represent aspects of wound healing in which damaged cells are removed and, in the process, the generative and resolution phases of repair are coordinated. Indeed, engulfment of dying cells has important roles in wound healing in flies (Weavers et al., 2016) and mammals (Bosurgi et al., 2017).

In general, necrotic cell death (whether regulated or not) elicits an inflammatory response, whereas apoptosis does not, and can be actively anti-inflammatory (Davidovich et al., 2014). Indeed, this is one feature that helped to define the process of apoptosis as distinct from necrosis (Kerr et al., 1972). With respect to adaptive immunity (especially the presentation of corpse-associated proteins to T lymphocytes), this simple dichotomy is replaced with another: immunogenic versus non-immunogenic cell death. The latter can be simply "silent" with respect to adaptive immunity or actively tolerogenic (preventing subsequent responses to proteins associated with the corpse). These distinctions do not simply sort with the mode of cell death; both apoptotic and necroptotic cells can promote antigen presentation to T cells, depending on the death-inducing stimulus and other environmental factors (Galluzzi et al., 2017). Understanding immunogenic cell death at a mechanistic level has clear consequences for cancer immunotherapy and vaccine development for infectious diseases.

What is it about a dying cell that triggers inflammation and immune responses? When microbes are present, pathogen-associated molecular patterns (PAMPs) engage pattern recognition receptors (PRRs), such as Toll-like receptors, and these induce inflammation and promote T cell immunity to proteins associated with the dying cells (Blander, 2016). The concept of PAMPs led to the idea that dying cells release damage- (or danger-) associated molecular patterns (DAMPs), that again are both pro-inflammatory and promote adaptive T cell immunity (Amarante-Mendes et al., 2018). It is important, however, not to equate these immune outcomes. The most commonly invoked DAMPs are high mobility group protein B1 (HMGB1) and ATP, although many others have been described, including uric acid, heat shock proteins, histones, mitochondrial proteins and nucleotides, and more.

With respect to inflammation, there is a strong case that the majority of these DAMPs do not play major roles in the inflammatory response to necrotic cells (Martin, 2016). This is based on the idea that signals that engage inflammation are generally antagonized by physiological mediators that modulate the response, thus preventing excessive damage. In a sense, this argument suggests that the existence of physiological mechanisms that counter a specific, putative DAMP can act as a guide to which DAMPs are most important. Of all generally described DAMPs, only extracellular ATP is antagonized in this way, in this case by the extracellular ATPases CD39 and CD73. Although animals lacking these ecto-ATPases show some abnormalities in lymphocyte trafficking, induction of experimental autoimmunity, and response to infectious agents (Antonioli et al., 2013), they are otherwise generally healthy, unlike the examples below. In contrast, members of the IL-1 family all have features that make them ideal candidates for DAMPs. They are processed intracellularly (by caspases or other intracellular proteases, an added regulatory mechanism that is usually required for subsequent interaction with their receptors), and all lack signal peptides for conventional secretion and thus are only released upon cell lysis or via plasma membrane pores. Many are ubiquitously expressed and responses to them are modulated by specific receptor antagonists that are required to prevent spontaneous inflammatory disease. For example, humans and mice lacking the IL-36 receptor antagonist (IL-36RA) present with catastrophic inflammation in response to normally mild tissue damage. Studies in the coming years will likely address the relative importance of DAMPs of the IL-1 family in the context of other putative DAMPs.

Similarly, the signals and mechanisms by which dying cells promote T cell immunity deserve further exploration. Immunogenic, apoptotic cell death has been described to require the release of HMGB1, ATP, and exposure of calreticulin on the cell surface (Galluzzi et al., 2017). In contrast, immunogenic, necroptotic cell death (which also releases HMGB1 and ATP and likely presents calreticulin because of cell lysis) requires NF-κB activation in the dying cell (Yatim et al., 2015). Apoptotic cells induce T cell immunity if PAMPs are present to engage TLR signaling (Torchinsky et al., 2010). If, as has been suggested, HMGB1 and other DAMPs engage TLRs, why is necroptosis, which releases such DAMPs, insufficient to promote T cell immunity when NF-κB is not engaged in the dying cell? In necroptotic cells, engagement of RIPK1 can promote NF-κB activation (Yatim et al., 2015). Is NF-κB required in dying apoptotic cells

for effective antigen presentation following engulfment? And if so, how is it induced? Engagement of death receptors leading to apoptosis can similarly engage NF-κB (Cullen et al., 2013), and after MOMP, upon engagement of the mitochondrial pathway, released inhibitor of apoptosis protein (IAP)-antagonists (such as Smac) can trigger NF-κB activation (Giampazolias et al., 2017). Whether the NF-κB-induced mediators are important in subsequent antigen presentation (or other consequences of cell death; see below) is unknown.

Dying cells can also produce or induce the production of type I interferons (IFNs) as a consequence of either the cell death process or effects in the cells that engulf them. This involves activation of the cytosolic DNA sensor stimulator of interferon genes (STING). In tumors, the activation of STING to produce type I IFNs can promote anti-cancer T cell immunity (Corrales et al., 2016). The induction of MOMP during apoptosis leads to disruption of the inner mitochondrial membrane (by an unknown mechanism) and the release of mitochondrial DNA (mtDNA) from some mitochondria to the cytosol (McArthur et al., 2018; Riley et al., 2018). This mtDNA engages STING, but only when caspase activation is disrupted or inhibited (Rongvaux et al., 2014; White et al., 2014). This might help explain previously paradoxical findings that inhibition of caspases can enhance effects of cancer therapies, although additional effects of caspases, such as CAD-induced mutagenesis and compensatory proliferation (see below) probably also contribute to this effect (Cao and Tait, 2018).

In contrast, the engulfment of apoptotic cells does not induce STING or type I IFN production in the engulfing cells. However, perturbation of processes involved in the degradation of the engulfed corpse can promote STING-dependent type I IFN production and anti-tumor immunity (Cunha et al., 2018). It is possible that lysosomal DNase II functions to prevent such STING activation, and DNase-II-deficient animals present with lethal, STING-dependent interferonopathy (Ahn et al., 2012). An understanding of how corpse-containing phagosomes possibly "leak" DNA to the cytosols of engulfing cells would clearly have implications for anti-cancer immunotherapy.

Compensatory proliferation, another consequence of apoptosis, has been documented in flies and mammals (Fogarty and Bergmann, 2017). An eicosanoid, prostaglandin E2 (PGE2), which is produced by dying or engulfing cells, has been implicated in the compensatory proliferation of tissue stem cells (Li et al., 2010). Executioner caspases cleave calcium-independent phospholipase A2 (iPLA2), resulting in production of PGE2, which in turn can promote tumor growth (Huang et al., 2011). However, this effect appears to depend on the caspase-independent activation of NF-κB and cyclooxygenase-2 (COX-2) production, and as noted above, a link between NF-κB activity and apoptosis remains somewhat unclear.

Intriguingly, production of another eicosanoid, lymphotoxin-β4 (LT-β4), by dying cells has been shown to be required for the initial neutrophil "swarm" that initiates wound repair (Lämmermann et al., 2013). How any cell death pathway engages LT-β4 production remains unexplored.

Compensatory proliferation of stem cells has direct consequences for oncogenesis. Irradiation induces both thymocyte apoptosis and the generation of thymomas (thymomagenesis), but if apoptosis is curtailed by ablation of the BH3-only protein p53 upregulated mediator of apoptosis (PUMA) radiation-induced thymomagenesis does not occur (Labi et al., 2010; Michalak et al., 2010). Similarly, PUMA is required for chemically induced generation of hepatocellular carcinoma (Qiu et al., 2011). Induction of PUMA-independent thymocyte apoptosis with glucocorticoids permits radiation-induced thymomagenesis in PUMA-deficient mice (Michalak et al., 2010). Clearly, the mechanisms and consequences of apoptosis-induced compensatory proliferation in oncogenic processes will be an important area for continued research, and virtually nothing is known about compensatory proliferation in response to other modes of cell death.

Riddle #5: Distressed Doctors Document Directed Deaths by Dastardly Deeds in Droves. How Many Dastardly Deeds Can Documenting Doctors Dictate?

How many ways can a cell actively die? Thinking about this question is aided by our earlier consideration of the concepts of cell suicide and cell sabotage. Disruption of any process essential for cell survival will result in cell death (by definition), and this will be active cell death if (1) the requirement for the process depends on the state of the cell (e.g., metabolic, proliferative, differentiation, etc.) and/or (2) the disruption is sensed by a suicide pathway to trigger death. Disruption of non-essential processes can also alter that process to become lethal (e.g., by reactive oxygen species or other toxic metabolites). In many cases, the resulting cell death might have unusual features and invite a new "osis." These are unlikely to be useful unless they can be generalized to several conditions or if the disruption is due to agents or conditions deemed to be of particular importance.

One example that might meet these criteria is active cell death by parthanatos (notably not an "osis") which is dependent on the DNA repair enzyme poly-ADP ribose polymerase. Others include cell death that involves lysosomal permeabilization (lysosomal cell death [LCD]) and netosis, a form of cell death restricted to neutrophils that functions to eject "nets" of DNA from the nucleus to the extracellular space (Vanden Berghe et al., 2014). The latter is thought to function in host defense.

Here, though, we consider another form of cell death that is frequently active on the part of the dying cell, a form that could be classified as "assisted suicide." Cytotoxic lymphocytes kill target cells, in part, by the introduction of proteases (granzymes) into cells via a perforin-mediated process. Different granzymes engage apoptosis or regulated necrosis in the target cell (Martínez-Lostao et al., 2015), processes that are important for removal of infected or transformed cells. There are several granzymes, no one of which is essential for host defense, and these perform several immunological and physiological functions that are independent of cell death mechanisms (Arias et al., 2017).

Another mechanism of assisted suicide is entosis, which occurs when a cell actively "burrows" via actin-mediated locomotion into a neighboring cell. The now engulfed cell dies because of nutrient deprivation and/or is killed upon fusion of lysosomes with the engulfed compartment (Fais and Overholtzer, 2018). Entosis occurs in epithelial cancers and in primary epithelial cells that lose matrix attachment, and other cells as

well. Entosis introduces a fundamental role for cell death that has not been extensively considered in our thinking of other cell death modalities; that of cellular competition. That cells compete for resources (growth factors, nutrients, and niches) is not surprising, but the finding that such competition has evolved as an active, molecular process might be. It has been proposed that competition within and between cell lineages was an important driver of development in multicellular organisms (Buss, 1987). Studies of cellular competition has shown that ''winners'' and ''losers'' of such competition are distinct at a molecular level and drive the fates (proliferation or cell death) of the opposing class (Bondar and Medzhitov, 2010; Bove et al., 2017).

Studies of cellular competition by entosis provide new insights into this fundamental battle of the cells. For example, upon glucose deprivation, primary cells engage entosis with winners and losers determined by a bimodal distribution of adenosine 5′ monophosphate-activated protein kinase (AMPK) activation (induced upon reduction of ATP levels) and membrane deformability (stiffness) (Hamann et al., 2017). Cells with high AMPK activation show decreased deformability and burrow into winners to die. This provides the winners with a proliferative advantage under low-nutrient conditions, perhaps also providing a source of nutrients. If such competition-associated entosis is a general feature of cells in multicellular organisms, effectively representing an altruistic suicide to preserve cells in a nutrient-deprived (or otherwise challenged) tissue, this will have major consequences for our understanding of developmental and homeostatic processes.

Conclusion

The riddles presented here are perhaps silly, but the underlying questions and the forthcoming answers are certainly not. Cell death is a fundamental biological process, and as such, its study has and will continue to have important implications for physiological and pathological processes. In an 1897 letter, Mark Twain quipped, ''The report of my death was an exaggeration.'' The same might be said of those who suggest that the study of cell death is dying. As the author hopes is apparent, investigations into the mechanisms and functions of cell death are alive, well, and thriving.

DECLARATION OF INTERESTS

D.G. is a consultant for Spiral Therapeutics and is on the scientific advisory board of SMOC Therapeutics.

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