Published in final edited form as:
Mol Cell. 2014 April 24; 54(2): 273–280. doi:10.1016/j.molcel.2014.01.027.

Staying Alive: Cell Death in Anti-Viral Immunity

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Abstract

Programmed cell death is an integral part of host defense against invading intracellular pathogens. Apoptosis, programmed necrosis, and pyroptosis each serve to limit pathogen replication in infected cells, while simultaneously promoting the inflammatory and innate responses that shape effective long-term host immunity. The importance of carefully regulated cell death is evident in the spectrum of inflammatory and autoimmune disorders caused by defects in these pathways. Moreover, many viruses encode inhibitors of programmed cell death to subvert these host responses during infection, thereby facilitating their own replication and persistence. Thus, as both virus and cell vie for control of these pathways, the battle for survival has shaped a complex host-pathogen interaction. This review will discuss the multifaceted role programmed cell death plays in maintaining the immune system and its critical function in host defense, with a special emphasis on viral infections.

Keywords

apoptosis; necrosis; necroptosis; pyroptosis; caspase; RIP1; RIP3; MCMV; vaccinia virus; LCMV; Fas; TNF; granzyme

The mammalian immune system is tasked to defend the organism against foreign pathogens. Inflammatory cytokines such as interferons (IFNs) and TNF often serve as the first line of defense during infections. Both Type I IFNs and TNF are induced by pathogen associated molecular patterns (PAMPs) that stimulate toll-like receptors (TLRs) and intracellular pattern recognition receptors (PRRs). TLRs, intracellular PRRs and TNFR are potent inducers of inflammatory cytokines and chemokines that promote recruitment of leukocytes into the infected tissue milieu. Their proinflammatory functions critically depend on the latent transcription factors NF-κB, IRF3/7 and MAP kinase induced AP-1. In addition, both TNF and type I IFNs can directly signal for cell death. Moreover, type I IFNs stimulate NK

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cell cytolytic function and induce expression of MHC molecules. These innate immune defense mechanisms serve to limit pathogen proliferation and propagation before a more robust and targeted adaptive immune response is launched. As such, the host environment can be a dangerous abode for pathogens. For pathogens with a limited genome, this is an especially daunting challenge. To survive and thrive in this hostile environment, viruses have developed strategies to neutralize or co-opt the host immune responses to its advantage. Here, we will discuss the role of cell death in pathogen infection with an emphasis on viral infections.

An overview of major cell death pathways in immunity

Programmed cell death is important for development, immune homeostasis and protection against microbial infections in multicellular organisms. Since its initial description, apoptosis has been considered the main cell death mechanism used in these processes. Apoptosis can be triggered by extrinsic signals such as death cytokines, or via intrinsic stimuli that impinge on the integrity of mitochondria. Both pathways rely on the activation of cysteine aspartyl proteases known as caspases. Caspases exist as inactive zymogens that can be autoactivated for apical caspases such as caspase 8, 9 and 10, or activated as part of a proteolytic caspase cascasde for effector caspases such as caspase 3, 6 and 7. Apoptosis has a characteristic morphology that includes cell shrinkage, mitochondrial permeabilization, nuclear and chromatin condensation, DNA fragmentation, membrane blebbing and eventual breakdown into apoptotic bodies (Kerr et al., 1972). These characteristics ensure the ordered demise of unwanted or infected cells, enabling the immune system to effectively remove apoptotic cells.

The extrinsic apoptotic pathway is stimulated by death ligands in the TNF superfamily such as TNF, Fas ligand (FasL), and TNF-related apoptosis-inducing ligand (TRAIL) and their cognate cell surface receptors. Ligand binding promotes conformational change of the preassembled receptor trimer and recruitment of adaptor and effector molecules to the cytoplasmic death domains (DD) of the receptors (Chan et al., 2000). This membrane complex eventually gives rise to a second, late-forming signaling complex in the cytosol containing Fas-associated death domain (FADD), receptor interacting protein kinase (RIP) 1 and pro-caspase 8 (Micheau and Tschopp, 2003). Activation of caspase 8 in this complex, often referred to as “Complex II”, initiates the death signal by one of two different pathways. The first is executed by direct cleavage and activation of effector caspases 3, 6, and 7. Caspase activity is tightly regulated by cellular FLICE-like inhibitor protein (cFLIP) and X-linked inhibitor of apoptosis protein (XIAP). The second pathway involves an intermediate step mediated by cleavage of the pro-apoptotic Bcl-2 family member Bid (Li et al., 1998; Luo et al., 1998), triggering Bcl-2 family members Bax and Bak to orchestrate mitochondrial outer membrane permeabilization (MOMP) to initiate the intrinsic apoptotic pathway (Figure 1).

The Bcl-2 family of proteins is comprised of both pro-and anti-apoptotic members that represent an important regulatory network mediating MOMP. They are categorized by domain architecture and fall into three basic categories: pro-apoptotic effector members such as Bak and Bax that directly initiate MOMP, anti-apoptotic members such as Bcl-2, Bcl-Xl,

and Mcl-1 that inhibit apoptosis by sequestering pro-apoptotic family members, and accessory BH3-only proteins including Bid, Bad, and Bim that promote MOMP by inhibiting anti-apoptotic family members, or activating pro-apoptotic members (Tait and Green, 2010). Together, this family serves as gatekeepers for mitochondrial integrity and the initiation of intrinsic apoptosis.

The intrinsic pathway can be induced by a variety of intracellular stimuli that promote MOMP and the release of pro-apoptotic mitochondrial proteins. Among them, cytochrome c promotes the formation of the apoptosome, an oligomeric complex comprised of Apaf-1 and caspase 9. The apoptosome stimulates auto-activation of caspase 9, which cleaves and activates downstream effector caspases (Cain et al., 2000), resulting in a sequential proteolytic cascade that dismantles the cell in an ordered fashion. Additionally, mitochondrial release of second mitochondrial-derived activator of caspase (SMAC), or small molecular mimetics, inhibits or promotes the degradation of IAPs, thereby relieving inhibition of effector caspases to promote cell death (Wu et al., 2007).

Unlike apoptosis, necrosis has historically been considered an accidental from of death due to physical injury or chemical insult. However, accumulating evidence unequivocally demonstrates that necrosis can be tightly regulated, initiated by specific signals and controlled by specific genes. Diverse stimuli, including death and pathogen receptor activation, DNA damage, perturbations to intracellular calcium levels, ATP depletion, or excessive production of reactive oxygen species (ROS), can result in necrotic cell death (Moriwaki and Chan, 2013). These signals induce discrete signaling pathways leading to organelle distension and disruption, cell swelling leading to the loss of plasma membrane integrity, and the release of cytoplasmic contents. While sharing morphological characteristics with accidental or traumatic cell lysis, these regulated pathways are more commonly referred to as programmed necrosis or necroptosis.

It has long been known that the TNF can induce apoptosis in some cases, and necrosis in others (Laster et al., 1988). Studies of the TNF signaling pathway has provided significant insight into the molecular machinery leading to necrosis and the overlapping nature between apoptosis and programmed necrosis. Discovery that RIP1 is involved in death receptor-induced necrosis established the idea of necrosis as a controlled cellular response (Holler et al., 2000). A key determinant in the choice between apoptosis and necrosis lies with the activity of caspase 8 within the ripoptosome, a Complex II-like heterotypic multi-protein complex comprised of FADD, RIP1, and caspase 8. Additional recruitment of $cFLIP_L$ protects cells from death, while cFLIPs promotes apoptosis downstream of the ripoptosome (Feoktistova et al., 2011; Tenev et al., 2011). RIP3 incorporation into the ripoptosome results in formation an amyloid-like signaling complex termed the necrosome (Figure 1). Active caspase 8 homodimer or caspase 8-$cFLIP_L$ heterodimer regulate programmed necrosis, likely by cleaving RIP1, RIP3 and the deubiquitinase CYLD. In support of caspase 8's role in necrosis, cleavage resistant mutants of RIP1, RIP3 and CYLD promote necrosis without an obligate need to inhibit caspases (Moquin et al., 2013; O'Donnell et al., 2011; Zhang et al., 2009).

RIP1 and RIP3 are members of a family of kinases involved in innate immune signaling. Both contain an amino terminal kinase domain, but unlike RIP1, RIP3 lacks a DD. However, both share another crucial protein-protein interaction motif called the RIP homotypic interaction motif (RHIM). The RHIM is a small motif with a highly conserved core of hydrophobic amino acid residues that mediates binding between RHIM containing proteins (Sun et al., 2002). The RHIM is essential for necrosis-specific interaction between RIP3 and RIP1. Two other innate immune signaling adaptors contain RHIMs; the TIR-domain-containing adapter-inducing interferon-β (TRIF) (Meylan et al., 2004) and the DNA-dependent activator of interferon regulatory factor (DAI/ZBP1) (Kaiser et al., 2008). In addition to activating transcription of NF-κB and Type I IFNs in response to pathogens, both TRIF and DAI can mediate RHIM-dependent necrosis with RIP3 independent of RIP1 kinase activity (He et al., 2011; Kaiser et al., 2013; Upton et al., 2012). Whether TRIF or DAI recruitment and activation of RIP3 results in assembly of a necrosome-like complex is currently an area of active investigation. Regardless of its binding partner, RIP3 kinase activity and RHIM-mediated interaction are crucial for various pathway-specific necrosis.

Studies using chemical inhibitors of RIP1 and RIP3 or site-directed mutagenesis have established an essential role for protein phosphorylation in TNF-induced necrosis (Degterev et al., 2013; Kaiser et al., 2013). RIP1 kinase activity is required for association with and activation of RIP3 (Cho et al., 2009). Site specific phosphorylation of RIP3 was proposed to be key for recruitment of mixed lineage kinase-like (MLKL), a downstream effector of RIP3 (Chen et al., 2013; Sun et al., 2012; Zhao et al., 2012). However, phospho-mimetic mutant analysis suggests multiple regulatory phosphorylation sites on RIP1 and RIP3 mediate TNF-induced necrosis (McQuade et al., 2013). MLKL is a catalytically inactive pseudokinase that binds ATP (Murphy et al., 2013), and while required for necrosis, the precise mechanism by which MLKL functions remains unclear. One recent study suggests that MLKL recruits phosphoglycerate mutase 5 (PGAM5), a mitochondrial phosphatase that promotes mitochondrial fission and ROS production (Wang et al., 2012). However, ROS scavengers do not inhibit TNF-induced necrosis in all cell types (F.K.C, unpublished observation), and the role of PGAM5 in necrosis has been recently challenged (Murphy et al., 2013). Therefore, the relevance of PGAM5 in diverse RIP3-dependent necrosis pathways will require further experimental validation.

Another distinct mode of caspase-dependent cell death that is important for control of infectious microorganisms is pyroptosis. Initially described as caspase 1-dependent apoptosis of macrophages, it is now clear that apoptosis and pyroptosis differ significantly in morphology and mechanism. Pyroptosis is mediated by an oligomeric signaling platform called the inflammasome, which contains a cytosolic pathogen-sensing Nod-like receptor (NLR), the adaptor ASC and caspase 1 (Rathinam et al., 2012). The inflammasome is widely known to promote proteolytic maturation and release of the pro-inflammatory cytokines IL-1β and IL-18 through caspase 1. However, inflammasome activation often leads to disruption of the plasma membrane and cell death (Figure 1). The combination of inflammatory cytokines and release of “danger-associated molecular patterns” from cell rupture can act in synergy to maximize protective immunity against invading pathogens.

Autophagy is a cytoprotective process whereby cells self-cannibalize cytoplasmic materials and organelles in response to nutritional, metabolic, or infection stresses. It is an important pathway in immunity against pathogens (Deretic et al., 2013). Normally, autophagy represents a desperate effort for cells facing metabolic catastrophe to ward off cell death. However, it can promote cell death under certain situations. It is noteworthy that there is extensive crosstalk between apoptosis, programmed necrosis, pyroptosis and autophagy. For instance, pharmacologic caspase inhibitors or genetic ablation of FADD or caspase 8 leads to RIP3-dependent necrosis. FADD/caspase 8-mediated inhibition of necrosis tampers damaging necrotic cell death during embryonic development (Kaiser et al., 2011; Oberst et al., 2011; Zhang et al., 2011). Additionally, pro-IL-1β can be processed by caspase 8 in a RIP3 dependent manner (Antonopoulos et al., 2013; Vince et al., 2012). These findings emphasize the complex interplay and coordinated regulation that is necessary to control host cell death pathways in the context of infection and immunity.

Apoptotic mechanisms in anti-viral immunity

Because viruses are intracellular pathogens, neutralizing antibodies alone may not provide sufficient protection against infection. This does not mean that they enjoy a free ride inside the host, since immune effector cells are adept at detecting minute changes associated with virus infection. For example, many viruses down-regulate class I MHC as one strategy to avoid detection by the host immune system. However, this “missing self” is actually a signal that activates cytotoxic natural killer (NK) cells. In addition, viral RNAs and DNAs are recognized by intracellular PRRs such as RIG-I and MDA5 (Dixit and Kagan, 2013). These intracellular receptors are strong inducers of type I interferons, which upregulate expression of class I MHC and sensitize infected cells to TNF-like death cytokines. By presenting viral peptide epitopes on class I MHC, infected cells can be further contained by conventional cytotoxic lymphocytes.

NK cells and conventional cytotoxic T lymphocytes rely heavily on the perforin-granzyme system to eliminate virus-infected cells. Both perforin and granyzmes are released to the target cells from cytotoxic granules. Perforin oligomerizes to form a macromolecular “membrane attack complex (MAC)” through which the proteolytic granzymes are released into the cytosol of the target cell. There are 5 human granzymes, while mice encode 10 functional granzymes. Granzyme B (GzmB) is the best characterized of all granzymes. Purified perforin and GzmB induce cell death characterized by cytochrome c release, loss of mitochondrial membrane potential and membrane blebbing (Heibein et al., 1999; MacDonald et al., 1999). GzmB is unique among serine proteases in that it cleaves substrates after an aspartic acid residue (Odake et al., 1991). As such, caspase 3, 6, 8 and 10 have all been shown to be direct substrates of GzmB . In addition, GzmB can cleave Bid to activate mitochondrial release of SMAC, which binds XIAP to relieve caspase 3 from inhibition (reviewed in (Ewen et al., 2012).

Although the evidence supporting a caspase-dependent mechanism of cell killing by GzmB is quite compelling, many other granzymes are insensitive to inhibition by pan-caspase inhibitors. For example, GzmA enters the mitochondria to cleave NDUFS3 in Complex I, increasing ROS production and driving the ER-associated SET complex to the nucleus

(Martinvalet et al., 2008). Endonucleases in the SET complex then induce single-stranded DNA damage. In addition, GzmA cleaves histones, lamins and heterogeneous nuclear ribonucleoproteins (hnRNPs) to breakdown the nuclear architecture (Rajani et al., 2012). Despite differences in molecular mechanisms and targets, most granzymes ultimately elicit apoptotic features such as exposure of phosphatidyl serine, chromatin condensation and DNA fragmentation (Ewen et al., 2012). However, because caspases are not required for the action of many granzymes, the possibility that granzymes may induce non-apoptotic cell death should not be ignored.

At the organismal level, granzymes have overlapping and redundant functions in anti-viral immunity. For example, GzmA$^{-/-}$ and GzmM$^{-/-}$ mice exhibited mild defects in clearance of ectromelia virus and murine cytomegalovirus (MCMV) respectively (Mullbacher et al., 1996; Pao et al., 2005). However, the mildly increased susceptibility of GzmB$^{-/-}$ mice to gammaherpevirus was further exacerbated by deficiency in GzmA or GzmC and GzmF (Loh et al., 2004). GzmA$^{-/-}$ B$^{-/-}$ mice were much more susceptible to ectromelia virus infection than single knock-out mice (Mullbacher et al., 1999). Hence, despite their distinct mechanisms of action, granzymes appear to have somewhat redundant functions *in vivo*.

Besides perforin/granzymes, cytotoxic lymphocytes also use Fas ligand (FasL) to eradicate virus-infected cells. Residual cytolytic activity in perforin deficient T cells was abolished when Fas was also inactivated (Kagi et al., 1994; Lowin et al., 1994). However, *lpr* or *gld* mice, which harbor mutations in Fas and FasL respectively, cleared lymphocytic choriomenigitis virus (LCMV) infection normally. This is in contrast to perforin deficient mice, which failed to clear the virus (Kagi et al., 1995). Similarly, Fas was dispensable for control of MCMV induced retinitis (Dix et al., 2004). In fact, *lpr* mice were more efficient at controlling vaccinia virus replication (Seedhom et al., 2012). Hence, although cytotoxic lymphocytes can use perforin/granzyme or Fas/FasL pathways to eliminate target cells, *in vivo* evidence indicates that Fas-FasL pathway has a modest role in cytolysis of virus-infected cells.

To evade this barrage of attack from the host, viruses often encode apoptosis inhibitors in their genomes. One group of well known inhibitors are those that target caspases, such as the cowpox virus cytokine modifier A (CrmA). CrmA was originally identified as an inhibitor of the IL-1 converting enzyme (ICE), now more commonly known as caspase 1 (Ray et al., 1992). CrmA is unique among serpins as it inhibits serine as well as cysteine proteases. The first hint that CrmA also functions as an apoptosis inhibitor came from the realization that IL-1 converting enzyme (ICE)/caspase 1, a homolog of the *C. elegans* cell death effector CED3, caused apoptosis when over-expressed in fibroblasts (Miura et al., 1993). Later, CrmA was shown to be a high affinity inhibitor for caspase 8 and GzmB (Quan et al., 1995; Zhou et al., 1997). Since then, other viral caspase inhibitors have been identified, especially in herpeviruses (Brune, 2011).

Besides caspase inhibitors, many viruses encode Bcl-2 homologs that are direct inhibitors of Bax and Bak (Galluzzi et al., 2008). These include the adenovirus E1B-19K, and Epstein Barr virus BALF1 and BHRF1. However, the gamma-herpevirus 68 Bcl-2 homolog M11 does not inhibit Bax and Bak, but instead interferes with autophay via binding to Beclin-1

(Sinha et al., 2008). On the other hand, viral mitochondria inhibitor of apoptosis (vMIA) from cytomegalovirus does not share sequence homology with Bcl-2, but is nonetheless a potent inhibitor of Bax and Bak (Goldmacher, 2005). Thus, a common theme is emerging that viruses often interfere with multiple host cell signaling pathways to maximize their chance for survival and successful dissemination.

Necrosis in anti-viral immunity

Inhibitors of apoptosis are critical tools in the arsenal of viral pathogens. However, apoptosis inhibition can trigger another host defense response, anti-viral necrosis. Vaccinia virus encodes a CrmA ortholog, B13R, whose expression is required for pathogenesis in mouse infection (Legrand et al., 2004). While B13R protects infected cells from apoptosis (Dobbelstein and Shenk, 1996; Kettle et al., 1997), its expression during infection actually sensitizes infected cells to TNF-mediated, RIP1-RIP3 dependent necrosis (Chan et al., 2003; Cho et al., 2009; Li and Beg, 2000) (Figure 2A-B). As a result, RIP3$^{-/-}$ mice show reduced innate inflammation and succumb to the infection due to overwhelming viral replication (Cho et al., 2009). Thus, programmed necrosis is a potent cellular response that limits viral replication when apoptosis is blocked by viral caspase inhibitors.

In many regards, viral inhibition of caspase 8 may stimulate anti-viral necrosis (Figure 2). As such, some viruses encoding inhibitors targeting caspase 8 must concurrently have a strategy for dealing with host cell necrosis. MCMV encodes multiple inhibitors of apoptosis, including the viral inhibitor of caspase activation (vICA), which directly targets pro-caspase 8 to prevent its association with FADD (McCormick et al., 2003; Menard et al., 2003; Skaletskaya et al., 2001). Indeed, vICA expression alone was sufficient to cause necrosis in L929 cells (Kaiser et al., 2011). However, unlike vaccinia virus, MCMV infection does not sensitize or induce necrosis (Mack et al., 2008). Instead, MCMV prevents RIP3-dependent necrosis by the expression of the viral inhibitor of RIP activation (vIRA), a product of the M45 gene. Interestingly, vIRA is a RHIM-containing inhibitor that directly targets RIP3 to prevent its association with and activation by DAI (Upton et al., 2008, 2010, 2012). Recombinant virus encoding a mutant vIRA lacking its RHIM was severely attenuated and failed to establish productive infection even in immuno-compromised mice. However, productive infection by the mutant virus was restored in RIP3$^{-/-}$ mice (Upton et al., 2010). This indicates that suppression of host cell necrosis is a vital survival strategy of the virus (Figure 2C-D).

Many other viruses, particularly the poxvirus and herpesvirus families, encode viral FLIP-like molecules that, like cFLIP, contain two tandem death effector domains (DED) and are strong apoptosis inhibitors (Mocarski et al., 2012). However, a subset of these vFLIP molecules including MC159 from Mollulscum contagiosum virus, E8 from equine herpesvirus 1, and K13 from Kaposi's sarcoma associated herpesvirus can also inhibit TNF-induced programmed necrosis (Chan et al., 2003) (Figure 2E). It is noteworthy that in addition to cell death inhibition, vFLIPs and vIRA can also modulate NF-κB signaling, (Challa et al., 2010; Fliss et al., 2012) although it is unclear whether these activities influence inhibition of necrosis. It will be interesting to see if inhibition of necrosis is a common strategy employed by viruses to evade the host immune system.

Lymphocyte cell death in anti-viral responses

While killing of infected cells is no doubt a critical defense mechanism against viruses, cell death of uninfected cells, especially lymphocytes, can also greatly impact the overall quality of immune responses against viruses. In response to antigenic stimulation, T and B cells undergo massive clonal expansion and functional differentiation. Prior to this expansion, there is a transient contraction of CD8+ T cells that is thought to create “space” to accommodate the ensuing clonal expansion (Welsh et al., 2012). Studies with LCMV indicate that this early wave of cell death affects mostly memory cells and is dependent on type I interferons, but not TNF or FasL (Bahl et al., 2006).

Rapidly dividing lymphocytes are prone to death signals. When restimulated through the antigen receptor, they undergo activation-induced cell death through autocrine expression of TNF and FasL. Failure to do so can result in immunopathology or worse yet, autoimmunity. In human, mutations in Fas or FasL cause a systemic autoimmune disease called the ALPS (Autoimmune Lymphoproliferative Syndromes) (Su and Lenardo, 2008), which is recapitulated in *lpr* and *gld* mice. As discussed above, *lpr* and *gld* mice are generally competent in controlling different viral pathogens, however the persistence of activated lymphocytes in *lpr* and *gld* mice often led to severe immunopathology (Balkow et al., 2001; Olson and Varga, 2009; Zhang et al., 2000). Thus, while perforin and granzymes are more important for cytolysis of virus-infected cells, it appears the Fas-FasL pathway serves to minimize the debilitating immunopathology from virus infections.

Mice deficient in TNF, TNF receptor 1 (TNFR-1) or TNFR-2 do not develop systemic autoimmune disease. On face value, it appears to suggest a subsidiary role for TNF in lymphocyte homeostasis. However, $TNF^{-/-}$ mice developed higher number of CD8+ memory T cells against LCMV (Singh and Suresh, 2007), suggesting that TNF and TNFR interaction also has a minor role in lymphocyte cell death. Thus, while innate production of TNF can have direct cytotoxic effects on virus-infected cells, TNF has a modest role in lymphocyte cell death during the adaptive phase of immune reactions. These results highlight another important concept in anti-viral immunity: that the biological consequence of cytokine signaling is temporally controlled and context-dependent.

After clearance of a pathogen, the absence of antigen reduces production of the trophic factor IL-2. This cytokine withdrawal primes lymphocytes for “intrinsic” cell death controlled by Bim and related Bcl-2 family members. The majority of the expanded effector lymphocytes are eliminated through this process. Consistent with this, $Bim^{-/-}$ mice developed lymphoproliferative disease, and effector T cells from $Bim^{-/-}$ mice are refractory to cell death (Bouillet et al., 1999). $Bim^{-/-}$ *lpr* mice exhibited much higher effector CD8+ T cells responses to LCMV (Weant et al., 2008), indicating that Bim collaborates with Fas to control lymphocyte homeostasis. Effector T cells from $Mcl\text{-}1^{-/-}$ mice undergo rapid Bax/Bak-dependent cell death in response to LCMV infection (Tripathi et al., 2013). Thus, pro-survival and pro-apoptotic Bcl-2 family members have important functions in maintaining clonal expansion and immunologic memory.

Besides T and B cells, cell death of other immune effectors can also impact the efficacy of anti-viral immune responses. Dendritic cells are powerful antigen presenting cells that prime and activate antigen-specific lymphocytes. Human patients with mutations in perforin developed a fatal disease called hemophagocytic lymphohistiocytosis (HLH). LCMV and MCMV infection of perforin-deficient mice led to a similarly fatal reaction due to failure of cytotoxic T cells to eliminate dendritic cells, which in turn caused sustained T cell activation and the associated pathology (Terrell and Jordan, 2013; van Dommelen et al., 2006).

Concluding remarks

As we have illustrated here, different cell death mechanisms can have distinct effects on the quality of immune responses against viruses. While apoptosis is usually non-immunogenic, it has a vital role in eliminating virus-infected cells. For viruses that successfully resist apoptosis, programmed necrosis and pyroptosis may be vital to eliminate the viral factory before adaptive immunity is engaged. It is striking that many innate immune signaling pathways can promote inflammatory cytokine expression and cell death. However, the signaling outcome is dependent on many factors such as when the cytokines are induced, whether the cells are infected, and the presence of other synergizing or inhibitory cytokines. This reinforces the notion that these signaling pathways can synergize with each other to achieve containment of the pathogen and to shape the outcome of host-pathogen interactions.

Acknowledgments

We apologize to our colleagues whose work we could not reference due to space limitation. This work is supported by NIH grant AI083497 (F.K-M.C.) and by Cancer Prevention & Research Institute of Texas (CPRIT) Scholar award R1202 (J.W.U).

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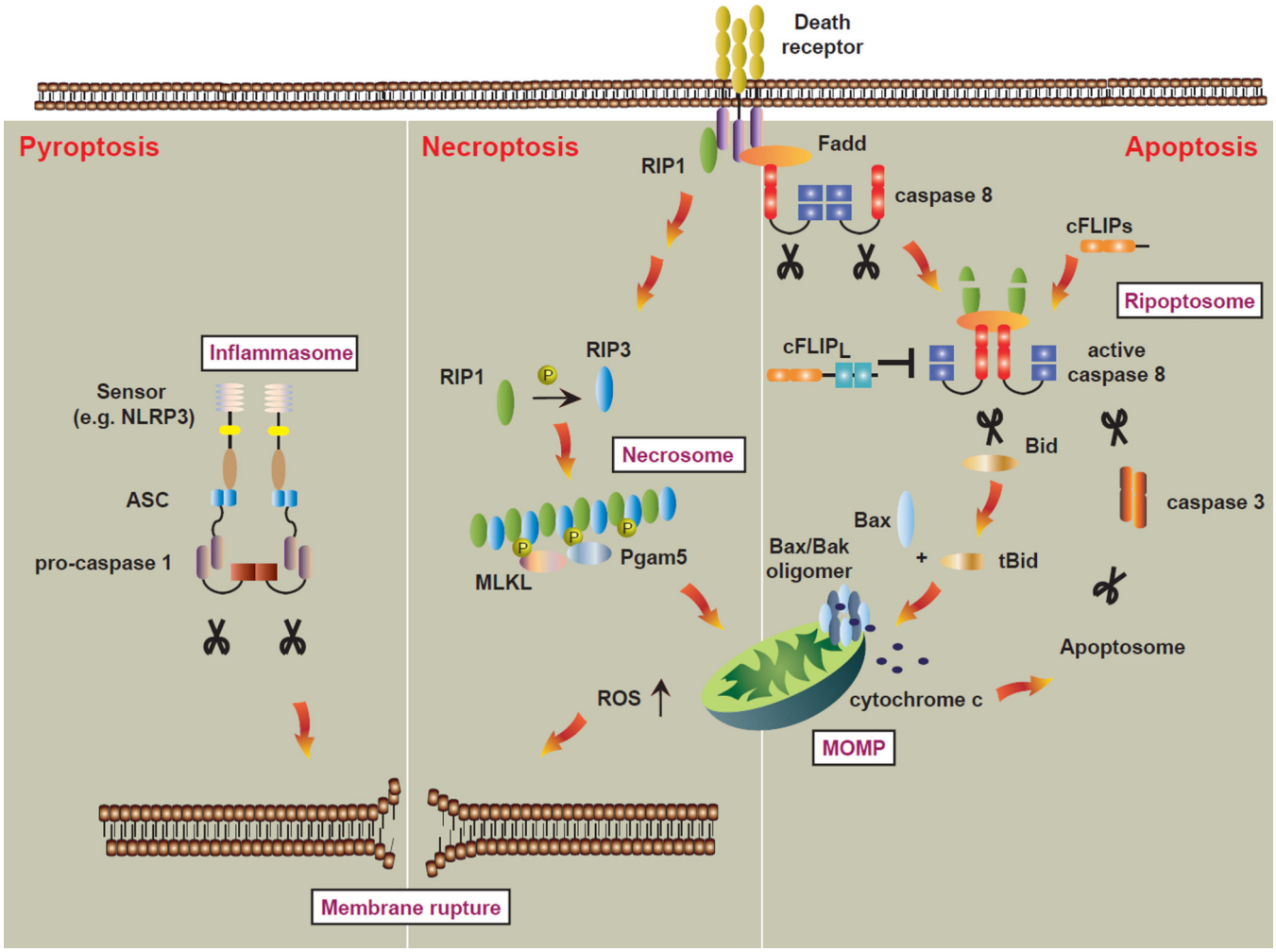

Figure 1.

The major cell death pathways at a glance.

The extrinsic (death receptors) and intrinsic (mitochondria) apoptosis pathways intersect at the mitochondria and cumulate in the formation and activation of the apoptosome. In some cell types, death receptors can bypass the mitochondria to directly engage downstream effector caspases. When caspase 8 is inactivated, RIP1 forms an amyloid-like signaling complex with RIP3 to recruit downstream effectors such as MLKL and PGAM5. ROS may act upstream to strengthen necrosis signaling, or downstream to induce cellular damages such as membrane rupture for the execution of necrosis. Pyroptosis, which is triggered by the inflammasome, is also marked by rapid membrane rupture. Whereas apoptotic cells are rapidly engulfed and cleared by phagocytes such as macrophages, necrosis and pyroptosis promote inflammation through leakage of cellular “danger signals”.

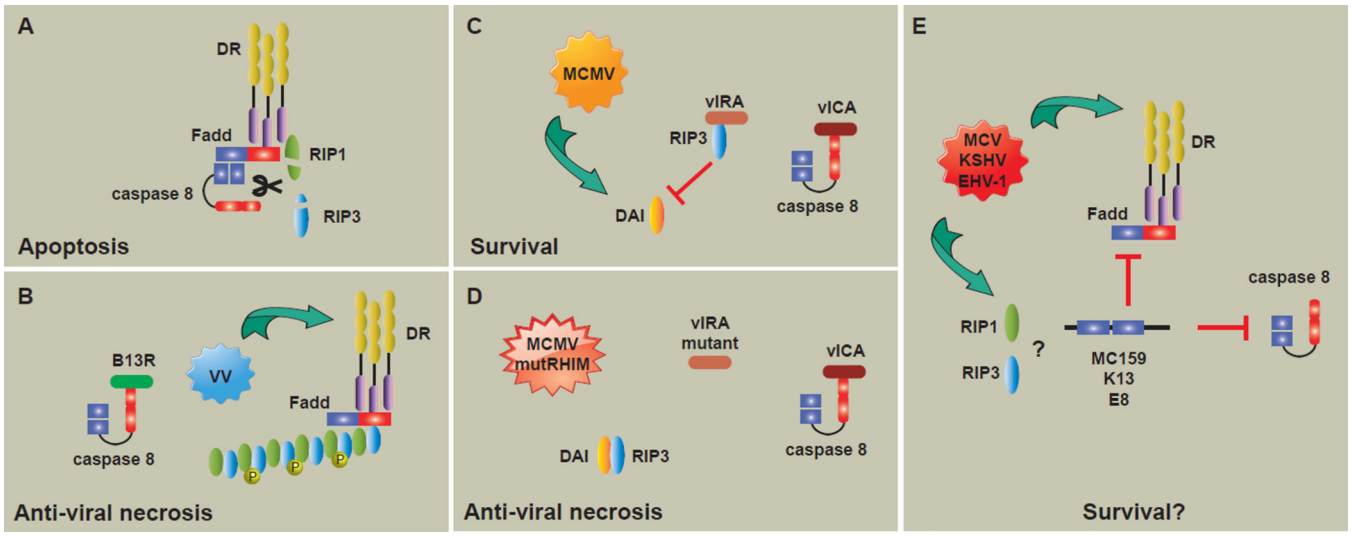

Figure 2.

Interaction of viral inhibitors with host cell death machineries.

(A) Ligation of death receptors (DRs) induces recruitment and association of FADD, RIP1 and caspase 8. The activity of caspase 8 negatively regulates necrotic signaling by cleavage of RIP1 and RIP3 as well as initiates the proteolytic cascade of apoptosis. **(B)** Infection of cells with vaccinia virus (VV) leads to expression and release of DR cytokines. VV encodes the B13R gene product, which potently inhibits caspase 8. Signaling via the DR proceeds in the absence of caspase 8 activity results in necrosome formation and anti-viral necrosis. **(C)** Murine cytomegalovirus (MCMV) encodes inhibitors of apoptosis and necrosis to promote survival and viral replication. MCMV expresses the viral Inhibitor of Caspase Activity (vICA), which binds and inhibits capsase 8. This normally sensitizes cells to necrosis through DAI, a RIP3 interacting partner. However, MCMV also expresses the viral Inhibitor of RIP Activation (vIRA) that directly targets RIP3 to prevent association with DAI and initiation of necrotic signaling. **(D)** Functional inactivation of the vIRA RHIM releases RIP3 from inhibition, resulting in antiviral necrosis. **(E)** Some poxvirus- and herpesviruses-encoded vFLIPs (e.g. MC159, K13, E8) are strong inhibitors of apoptosis and necrosis. Although the underlying mechanism remains unclear, the net result is dual-functioning cell death inhibitors capable of preventing both apoptosis and necrosis to promote cell survival and viral persistence.