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Viral modulation of programmed necrosis

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Summary

Apoptosis and programmed necrosis balance each other as alternate first line host defense pathways against which viruses have evolved countermeasures. Intrinsic apoptosis, the critical programmed cell death pathway that removes excess cells during embryonic development and tissue homeostasis, follows a caspase cascade triggered at mitochondria and modulated by virus-encoded anti-apoptotic B cell leukemia (BCL)2-like suppressors. Extrinsic apoptosis controlled by caspase 8 arose during evolution to trigger executioner caspases directly, circumventing viral suppressors of intrinsic (mitochondrial) apoptosis and providing the selective pressure for viruses to acquire caspase 8 suppressors. Programmed necrosis likely evolved most recently as a “trap door” adaptation to extrinsic apoptosis. Receptor interacting protein (RIP)3 kinase (also called RIPK3) becomes active when either caspase 8 activity or polyubiquitylation of RIP1 is compromised. This evolutionary dialogue implicates caspase 8 as “supersensor” alternatively activating and suppressing cell death pathways.

Overview of intrinsic apoptosis, extrinsic apoptosis and programmed necrosis

Apoptosis is executed by two distinct signaling pathways that converge on the activation of executioner caspase (Casp)3 and Casp7, called intrinsic and extrinsic apoptosis. Either drives characteristic morphological changes (cell shrinkage, membrane blebbing, nuclear condensation and DNA fragmentation) dependent on caspase activity [1,2]. The intrinsic pathway relies on pro- and anti-apoptotic B cell lymphoma 2 (BCL2) family proteins [3] at mitochondria to sense stress, signal and execute dismantling of the cell. Two key pro-apoptotic players, BCL-2-associated X protein (BAX) and BCL-2 antagonist/killer (BAK), form a pore that releases pro-apoptotic factors (e.g. cytochrome *c*, SMAC/DIABLO) to form an Apaf1-dependent apoptosome responsible for Casp9-dependent activation of Casp3 and/or Casp7 [1,2]. Large DNA viruses such as adenoviruses, herpesviruses and poxviruses block apoptosis by elaborating anti-apoptotic functions that mimic BCL2 proteins [4–6]. These inhibitors have subtle effects in cultured cells but maintain cell viability and facilitate viral dissemination within monocyte-macrophage lineage cells during herpesvirus [7–10] and poxvirus [11] infections. For example, human cytomegalovirus (CMV) targets both BAX and BAK by expressing potent viral mitochondrial inhibitor of apoptosis (vMIA), encoded by the UL37x1 gene [12–16]. The biologically related murine CMV splits the job using two proteins: vMIA (m38.5 gene product) targeting BAX [17,18] and viral inhibitor of BAK oligomerization (vIBO), the m41.1 gene product [19]. Human CMV vMIA modulates

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apoptosis as well as a caspase-independent death pathway radiating from mitochondrial serine protease HtrA2 [16,20]. Murine CMV vMIA and vIBO facilitate dissemination by supporting viability of infected peripheral blood monocytemacrophages [7–10]. Virus-encoded cell death suppressors also influence respiration, metabolism, and morphology [16,21–25], working in tandem with interferon (IFN)-activated mediator, viperin [26] as well as non-coding RNA [24]. Virus-encoded suppressors of mitochondrial steps in apoptosis are numerous [6], but their natural roles are subtle.

This review focuses on recent advances in understanding the complex interplay of extrinsic cell death pathways where the suppressors encoded by large DNA viruses have striking roles in controlling cell fate, providing key insights to recently evolved host defense pathways. Understanding of extrinsic cell death comes largely from tumor necrosis factor (TNF) family death receptor studies. Extrinsic death is triggered via death receptors and pathogen sensors, which have evolved as components of the pathogen-host arms race. Extrinsic death pathways are a crucial first line of host defense, serving where intrinsic apoptosis has been compromised [27] (Fig. 1). A complex composed of Casp8, FAS-associated protein with death domain (FADD) and FLICE (e.g., Casp8) inhibitory protein (FLIP), controls extrinsic death pathways (Fig. 2). Casp8 inhibitors, either vFLIP-like proteins encoded by herpesviruses and poxviruses [28,29], or cellular FLIP [5,30,31], provide regulation of extrinsic death, particularly in macrophages and dendritic cells [16,27,32,33]. For example, the viral inhibitor of Casp8 activation (vICA) conserved in human and murine CMV [31] supports interaction with monocytemacrophages [34,35], aiding efficient dissemination of virus by maintaining viability of the infected monocyte-macrophage reservoir [36]. Although viral mimics override host regulation, cellular FLIP in the Casp8-FADD-FLIP complex dictates whether Casp8 homodimerization and self-activation occurs. Specifically, the long form of cellular FLIP (FLIP_L) is catalytically inactive caspase paralog that heterodimerizes with unprocessed Casp8 to block self-activation (Fig. 2). Basal activity from this Casp8-FLIP_L heterodimer suppresses RIP3 kinase-dependent pathways, preventing programmed necrosis [27]. When levels of FLIP_L are too low, Casp8 homodimerization and self-activation triggers extrinsic apoptosis [27], following either of two pathways: (i) direct cleavage of Casp3 to trigger processing of the effector caspases or (ii) cleavage of pro-apoptotic BCL2 family member BH3-interacting domain (BID) to truncated (t)BID, which in turn triggers BAX-BAK activation at mitochondria, merging into intrinsic signaling [37,38].

Three potential outcomes emanate from death receptor activation [39,40]: (I) proinflammatory cytokine activation, (II) extrinsic apoptosis and (III) programmed necrosis. Importantly, a central Casp8-FADD-FLIP complex (Fig. 2) regulates outcome choices, but dictates Outcomes II and III. We have posited [27] that evolution of alternate death pathways have been driven by host adaptation to virus-encoded cell death suppressors (Fig. 1), such that the inflammatory consequences resulting from extrinsic cell death is the cost of effective innate host defense. Although pathogen sensors are recognized as contributing to innate NF- κ B (Outcome I) and IFN regulator factor (IRF)3 and IRF7 activation [41], sensors also activate extrinsic cell death (Outcomes II and III) via Casp8 in a manner paralleling death receptors [27,42]. In most vertebrates, Casp10 (not found in mice) also mediates extrinsic death. Human Casp8 and Casp10 bypass the mitochondria [43] and activate executioner caspases 3 and 7 directly, or merge with intrinsic apoptosis [1,43] by processing BID to tBID when x-linked inhibitor of apoptosis protein (XIAP) levels are sufficient [44]. In humans, Casp10 is downstream of FAS, such that autoimmune lymphoproliferative syndrome [45] is observed in Casp10 or FAS-deficient individuals [46], reminiscent of lymphoid hyperplasia in FAS-signaling deficient mice [47]. It remains unclear whether Casp10 holds RIP3-dependent necrosis in check; however, phenotypes of humans with

Casp8 or Casp10 deficiency suggests Casp10 sits in exclusive control of extrinsic apoptosis, whereas Casp8 regulates alternate activation of apoptosis and necrosis [27,48].

Nuts and bolts of extrinsic death pathways

TNF family receptors contribute to inflammation by activating NF- κ B and MAPK pathways, producing cytokines [27,49] independent of DD interactions [41,50,51]. Cytokine activation is subjected to modulation by virus-encoded suppressors [5,16,27,52,53]. Human death receptors (TNFR1, FAS, TRAIL-R1 and TRAIL-R2) signaling occurs via a death domain (DD) to FADD followed by self-cleavage activation of Casp8 or Casp10 [40,54–57]. Murine death receptors (TNFR1, FAS, TRAIL-R) all signal via Casp8 where death receptor signaling feeds into a Casp8-FADD-FLIP_L-RIP1-RIP3 complex forms (Fig. 2). FADD-Casp8-FLIP_L complex requires death effector domain (DED) interactions. Cellular FLIP_L is anti-apoptotic, preventing homodimerization and self-activation of Casp8 [43,58]. It is the DD-containing subset of TNF family receptors that signal via the FADD-Casp8-FLIP_L complex, and that recruit RIP1 to FADD via its DD. This leads to two alternatives: activation of NF- κ B independent of RIP1 kinase activity [39] and triggering necrosis dependent on kinase activity [58,59]. An analogous cytosolic complex, called the ripoptosome, is triggered by toll-like receptor (TLR)3 signaling [60] as well as following genotoxic stress [61]. No matter how activation occurs, the ripoptosome controls cell fate (Outcomes II and III) [49,62], relying on DD-dependent association of RIP1 and FADD [39] and RIP homotypic interaction motif (RHIM)-dependent RIP1-RIP3 association. Death receptors and pathogen sensors rely on sustained basal Casp8 activity emanating from the FADD-Casp8-FLIP_L complex to prevent RIP1-RIP3 kinase-dependent necrosis [27,63–66] (Fig. 2). Virus-encoded FLICE-inhibitory protein (vFLIP)-like proteins block this complex, preventing Casp8 homodimerization and self-activation [28–30]. Most vFLIPs act analogous to the short form cellular protein, FLIP_S and completely shut down Casp8 homodimer activity [32,67]. This contrasts the more subtle role of FLIP_L-Casp8 heterodimer, suppressing selfactivation while retaining basal caspase activity to suppress necrosis [68].

In addition, the FADD-Casp8-FLIP_L-RIP1-RIP3 signaling platform (Fig. 2) [27] is tightly regulated by polyubiquitylation via cellular inhibitor of apoptosis protein (cIAP)1 and cIAP2 E3 ubiquitin (Ub) ligases [69] as well as by the linear Ub assembly complex (LUBAC) [70]. RIP3 is a potential substrate for cIAP1 or cIAP2 polyubiquitylation [71], although much remains to be clarified in this area. However, when Casp8 [72], cIAP1/cIAP2 E3 Ub ligase [69] or LUBAC [70] become compromised, de-ubiquitinases (DUBs), possibly A20 or cylindromatosis (CYLD), remove poly-Ub side chains decreasing the contribution of RIP1 activity in NF- κ B activation [72–74] while also unleashing RIP1 and RIP3 kinase-dependent necrosis. These events influence TNF-induced extrinsic apoptosis, RIP1-RIP3 necrosis (called necroptosis) and inflammation [70]. The necroptosis ‘trap door’ involves RIP1-RIP3 oligomerization [75] via autophosphorylation of RIP3 at S227 and activation of MLKL kinase [76] by phosphorylation at T357 and S358. MLKL, recognized as a potent cell death mediator [77] prior to its implication in RIP3 necrosis, apparently acts in complex with RIP3 [78] (Fig. 2). The downstream execution of necrosis may proceed via PGAM5 and Drp1 recruitment to mitochondria [79], or via a reactive oxygen species (ROS) intermediates [80] (Fig. 2).

Consequences of dysregulating cell death pathways during development

Given that individual death receptors are dispensable for mammalian development, midgestational death of germ line Casp8 [81], FADD [82,83] or FLIP [84] deficient mice came as a surprise; however, the common embryonic day 10.5 (E10.5) lethality supported a coordinated FADD-Casp8-FLIP_L contribution. Mice with endothelial cell-specific

disruption of Casp8 exhibit the same E10.5 demise [85]. Normal development required enzymatic activity of Casp8, but not self-processing, eliminating the possibility of extrinsic apoptosis as the cause of death [86]. A caspase-independent function of Casp8 was suspected, and emerging experiments on RIP1–RIP3 necroptosis as well as demonstrating ability of viral suppressors to trigger [87,88] or block [89] this process opened understanding of FADD-Casp8-FLIP_L complex-dependent host defense [27], harnessing machinery best understood in death receptor signaling Fig. 2.

Significant insight emerged when crossing Casp8- and RIP3-deficient mice produced viable *Casp8*^{-/-}*Rip3*^{-/-} mice [63,90] that became fertile adults able to mount immune control over virus infection [90]. *Fadd*^{-/-}*Rip3*^{-/-} and *Fadd*^{-/-}*Rip3*^{-/-}FLIP^{-/-} mice [91] also develop into viable adults. Thus, the embryonic lethality of Casp8, FADD or FLIP deficiency results from dysregulated RIP3 function, confirming the contribution of the FADD-Casp8-FLIP_L platform in restricting RIP3 pathways during development. Importantly, embryonic lethality due to germ line Casp8 deficiency in mice depends on RIP1 and RIP3 [63,90,92] and is not influenced by germ line elimination of the other RHIM-dependent interaction partners, DNA-dependent activator of IRFs (DAI) [93] or TIR-domain-containing adapter-inducing IFN (TRIF) [94]. *Casp8*^{-/-}*Rip3*^{-/-} mice also revealed that, once embryonic lethality is suppressed, the importance of Casp8 downstream of FAS becomes apparent. Abnormal B220+ T cells accumulate in aging mice similar to FAS signaling-deficiency [47]. Mice deficient in FADD and RIP1 (*Fadd*^{-/-}*Rip1*^{-/-}) survive to birth [92], further implicating FADD-Casp8-FLIP_L signaling linked to RIP1- RIP3 in death [27,95]. The demonstration that germ line disruption of Casp8 unleashes RIP3 death provides clarification to disparate observations made in the fields of immunology, cell biology, development and signal transduction implicating the FADD-Casp8-FLIP_L complex as a vital player in diverse cell cycle, NF B activation, autophagy, cell adhesion and migration, and suppression of inflammation processes, particularly as relates to immunology [48,95,96]. Thus, Casp8 provides vital activity silencing RIP3-dependent pathways during development (Fig. 2), revealing gross dysregulation of normal processes as a cost of the evolutionary pressure to eliminate pathogen-infected cells [27] via RIP3 pathways (Fig. 3).

Programmed necrosis contributes to an array of serious defects and inflammatory consequences that arise from germ line [90] as well as cell-type-specific disruption of Casp8 or FADD in mice [49,55,85,86,97–109]. The phenotypes of mice carrying such disruptions have spawned debate as to whether necrosis contributes to inflammation or inflammation drives necrosis [42,49]. Pathologies associated with tissue-specific Casp8- or FADD-deficient mice are uniformly reversed when crossed into the *Rip3*^{-/-} background, clearly showing that dysregulating RIP3 underlies tissue damage [42,91,105,110–114]. An array of different viral cell death suppressors [16,27,32,33] has clearly contributed to the evolution of the RIP3 ‘trap door’. This arms race (Fig. 1) has produced novel pro-death strategies to counterbalance virus-encoded anti-death functions, which in turn, contributes to inflammatory disease as the collateral damage of unleashed death intended to eliminate pathogens [27,115]. Acute and chronic diseases linked to viral infection are likely to radiate from such processes, particularly when the pathogen persists in the host.

Host defense value of programmed necrosis

In addition the interaction of RIP1 [116] and RIP3 [87,117,118], programmed necrosis follows RHIM-dependent association of RIP3 with DAI or TRIF, as depicted in Fig. 3. No matter whether RIP3 kinase is activated by RIP1, DAI or TRIF, an oligomeric complex forms and RIP3 kinase acts in collaboration with MLKL [94] (Fig. 2). Thus, studies that initially centered on RIP1 [69,72] paved the way to discovery of RIP3 [87,117,118] as well as to a conceptual framework where RIP3 in complex with MLKL is now recognized as the

central mediator of programmed necrosis [27,89,93,94,119]. Vaccinia virus is highly susceptible to TNF-induced RIP1-RIP3 necroptosis [120], with cell death in infected organs dependent on TNFR1 and TNFR2 [87,88]. *Rip3*^{-/-} mice are highly susceptible to vaccinia [87] and succumb to a dose of virus that WT C57BL/6 mice resist. Thus, necroptosis provides key antiviral control and tissue pathology [87,88]. Vaccinia-encoded B13R is a caspase inhibitor that blocks apoptosis [121,122]. Based on the increased susceptibility of *Rip3*^{-/-} mice to WT vaccinia and the increased size of skin lesions formed by B13R mutant virus [123], vaccinia infection opens the necroptosis trap door by inhibiting caspases (Fig. 1). While highly susceptible to vaccinia, *Rip3*^{-/-} mice are no more susceptible than WT mice to infection with murine CMV [89,93], lymphocytic choriomeningitis virus [96] or murine hepatitis virus [124]. Murine CMV encodes suppressors of both Casp8 and RIP3 [16,27,125]. RNA viruses are not known to actively suppress cell death pathways. Thus, the RIP3 pathway appears selective and specific regarding control of different viruses.

As large DNA viruses, both vaccinia and CMV encode a wide variety of immunomodulatory and cell death suppression functions that have helped to define host defense pathways crucial to the virus infection [13,16,27,125–127]. Whereas vaccinia encodes B13R caspase inhibitor that apparently opens the necroptosis ‘trap door’, murine CMV deploys viral inhibitor of RIP activation (vIRA) as a potent RHIM-dependent inhibitor of programmed necrosis to counteract the consequences of Casp8-inhibitor vICA [90] (Fig. 3). Infection with any large DNA virus is complicated by the overlapping nature of host defense as well as the myriad virus-encoded suppressor activities. Both vaccinia and murine CMV deflect many aspects of pathogen sensing, including activation of NF- κ B [53,128,129], IFN [130,131] and PKR [131–133]. Murine CMV vIRA allows this virus to evade RIP1-RIP3 as well as DAI-RIP3 complex-dependent programmed necrosis (Fig. 3) [89,93]. In contrast, vaccinia is sensitive to necroptosis induced by TNF and causes histopathology consistent with widespread tissue necrosis *in vivo* [87,88]. Murine CMV is species specific and has evolved in dialogue with its host continuously for approximately 100 million years. Vaccinia infects a variety of mammalian hosts but its natural host is unknown [27,32]. The reasons underlying susceptibility of vaccinia to RIP1-RIP3 and murine CMV M45 mutant to DAI-RIP3 necrosis may stem from differences in modulation of mouse pathogen sensors. The Z-DNA-binding motifs of DAI [134] can replace the aminoterminal Z-DNA motif [135] of vaccinia E3L [136], and provide virulence characteristics [137], it remains uncertain whether this modulator interacts with or targets DAI. Thus, despite differences how necrosis is triggered, the programmed necrosis ‘trap door’ can open [27,32] (Figs. 1 and 3). In addition to these examples, the vFLIP proteins of *Molluscum contagiosum* virus, MC159, and equine herpesvirus 1, E8, inhibit TNF-induced necrosis when exogenously expressed [88], suggesting that further study of viral caspase inhibitors may add to the novel strategies for modulating necrosis.

Murine CMV-induced programmed necrosis occurs within 14 h in susceptible mouse or human cells following exposure to vIRA-deficient virus [89], acquiring morphological features of necrotic death, dying before viral DNA synthesis [89,138–140]. Moreover, this mutant virus is the most attenuated of any CMV cell death suppressor mutant [7–9,34,141], failing to disseminate from the inoculation site in immunocompetent or immunodeficient mice [89,142]. M45, which encodes vIRA, is located within in the cell death suppression locus (Fig. 4) along with Casp8 (vICA) and mitochondrial (vMIA and vIBO) inhibitors that are conserved in human CMV. The RHIM of vIRA is crucial (Fig. 3) and the phenotype of vIRA-deficient virus is normalized when either RIP3 or DAI is eliminated from the host [89,93]. The crucial role of the vIRA RHIM and the absence of a similar motif in human CMV raises questions of how suppression of programmed necrosis plays out in humans.

Regardless of whether RIP1-RIP3, DAI-RIP3 or TRIF-RIP3 starts the process, once triggered, RHIM-containing proteins (Fig. 3) may be recruited into oligomers independent of their functional contribution to death [75,94]. In fibroblasts, TLR3-induced necrosis is triggered by TRIF-RIP3 independent of DAI or RIP1 [94], although RIP1 is nevertheless recruited into complexes. Necroptosis mediated by RIP1-RIP3 complexes independent of TRIF and DAI nevertheless include DAI [75].

In addition to cell death, RHIM-dependent signal transduction leads to activation NF- κ B and other transcription factors, via RIP1 [80]. The pathways controlled by RIP1 have been characterized [80,143,144], although TRIF [140,145,146] and DAI [147,148] also activate NF- κ B as well as IRF3/IRF7 and IFN [41]. Activation of NF- κ B (Outcome I), which increases FLIP_L levels, prevents apoptosis (Outcome II), but not necrosis (Outcome III), whether induced by virus infection [93] or death receptor signaling [58,64]. This is likely due to the absence of any crosstalk between RIP3 and NF- κ B [149]. Even though RIP3 partners, RIP1, TRIF and DAI have well-established impact on NF- κ B, IRF3 and IFN, once RIP3 is activated, the value of increased FLIP_L levels becomes moot. Indeed, initial observations on DAI signaling showed it to be a DNA-specific pathogen sensor activating TBK and IRF3 [150] as well as NF- κ B in a RHIM-dependent manner [148]. Like the death pathway, DNA-induced DAI-dependent activation of NF- κ B and IRF3 is blocked by vIRA [147]. Curiously, a large component of the human CMV virion-induced IFN-like response occurs via DAI [151]. Thus, the potency of M45-encoded vIRA to suppress RHIM signaling was obvious before the pathway or steps in virus-induced programmed necrosis had been defined. Like other viral immunomodulators, vIRA prevents proinflammatory signaling independent of RHIM-signaling late during infection via the RR1 domain of M45 (Fig. 3) interacting with the adaptor IKK- γ /NEMO [53]. When necrosis is the outcome, it occurs within the first 14 h of infection and is independent of both NF- κ B activation and IFN signaling [93]. Thus, M45-encoded vIRA carries out additional RHIM-dependent and -independent functions during infection, however, it is the RHIM-dependent interference with RIP3-induced programmed necrosis that predominates in infection.

Conclusions and future directions

Evidence has accumulated that firmly establishes the evolutionary adaptation of cell death in host defense in animals and cell death suppressors that subvert host defense during infection by viruses. A new twist appears to be the way this evolution has created an opportunity for cell death pathways to become dysregulated and underlie developmental demise and inflammatory disease. As has repeatedly occurred in biology, model organisms, such as mice, and their natural pathogens have shown the path to discovery of pathways that are universal and will very likely continue to provide key insights into human disease.

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Highlights

1. Evolution of host cell death pathways was driven by virus-encoded cell death suppressors.
2. Caspase 8 regulates extrinsic apoptosis as well as programmed necrosis.
3. RIP3 (also called RIPK3) is the key mediator of programmed necrosis.
4. Programmed necrosis involves RIP3 RHIM-dependent interactions with RIP1, DAI or TRIF.
5. Programmed necrosis is suppressed by RHIM inhibitor vIRA encoded by MCMV.

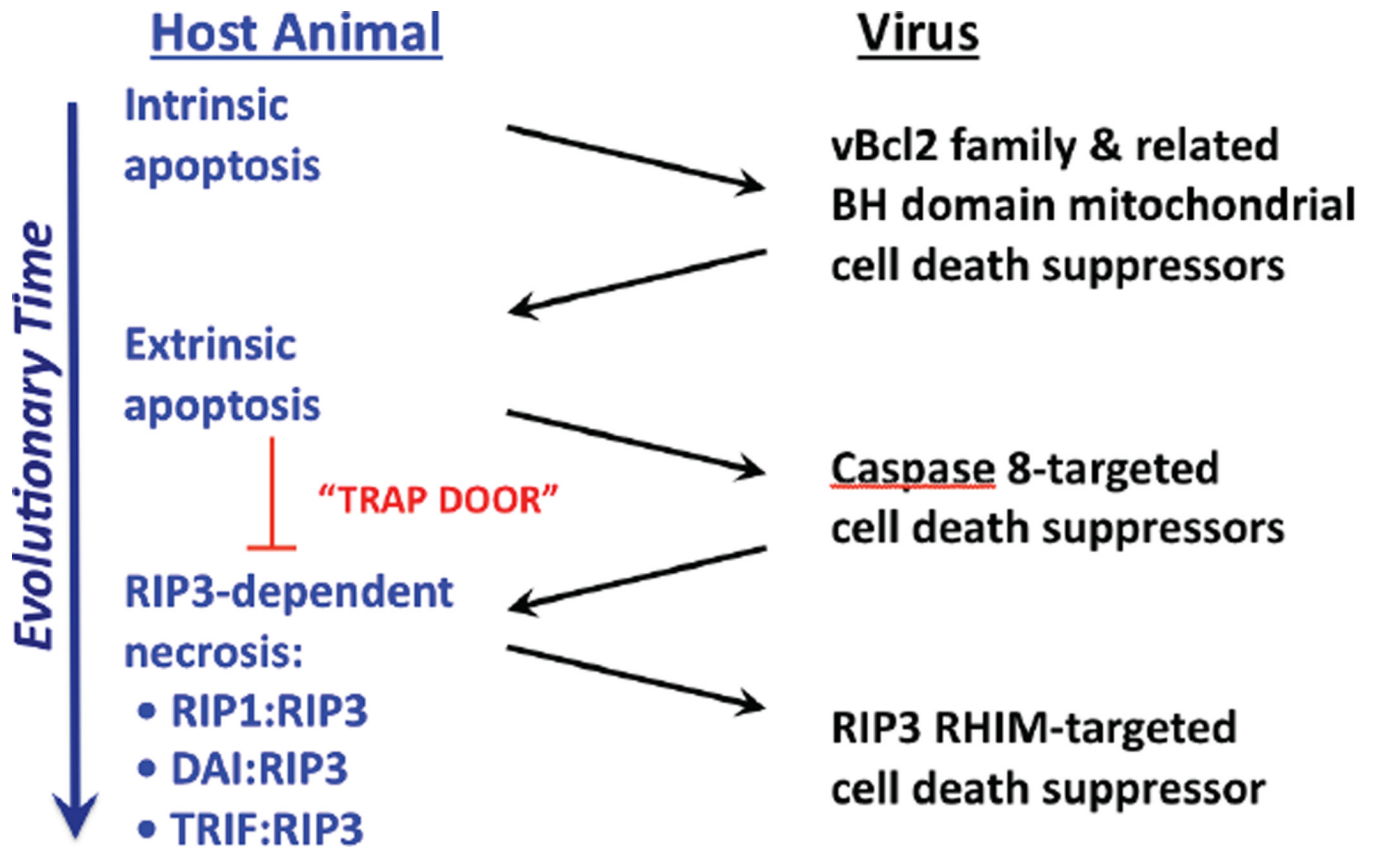


Figure 1. Evolution of cell death pathways in host defense

Diagram representing evolutionary time (large arrow) together with host death pathways targeted by known virus-encoded cell death suppressors. Intrinsic apoptosis is a primordial property of multicellular organisms to eliminate excess cells during development that contributes to host defense. Herpesviruses, poxviruses and adenoviruses encode mitochondrial cell death suppressors that limit the impact of pro-apoptotic BCL2 family member signaling in host defense [4–6,13]. Extrinsic apoptosis represents an adaptation to avoid virus-encoded inhibitors of mitochondrial apoptosis, by employing Casp8 as a self-activating protease that can directly target executioner caspases, Casp3 and Casp7. Herpesviruses, poxviruses and adenoviruses acquired suppressors of Casp8 that limit the contribution of extrinsic apoptosis to elimination of virus-infected cells. Programmed necrosis represents an adaptation to take advantage of Casp8 inhibition, by opening a trap door via any of three signaling platforms, RIP1-RIP3, DAI-RIP3 and TRIF-RIP3. Some viruses, such as vaccinia, remain susceptible to programmed necrosis; whereas, other viruses, such as murine CMV, encode suppressors that limit the impact of this pathway.

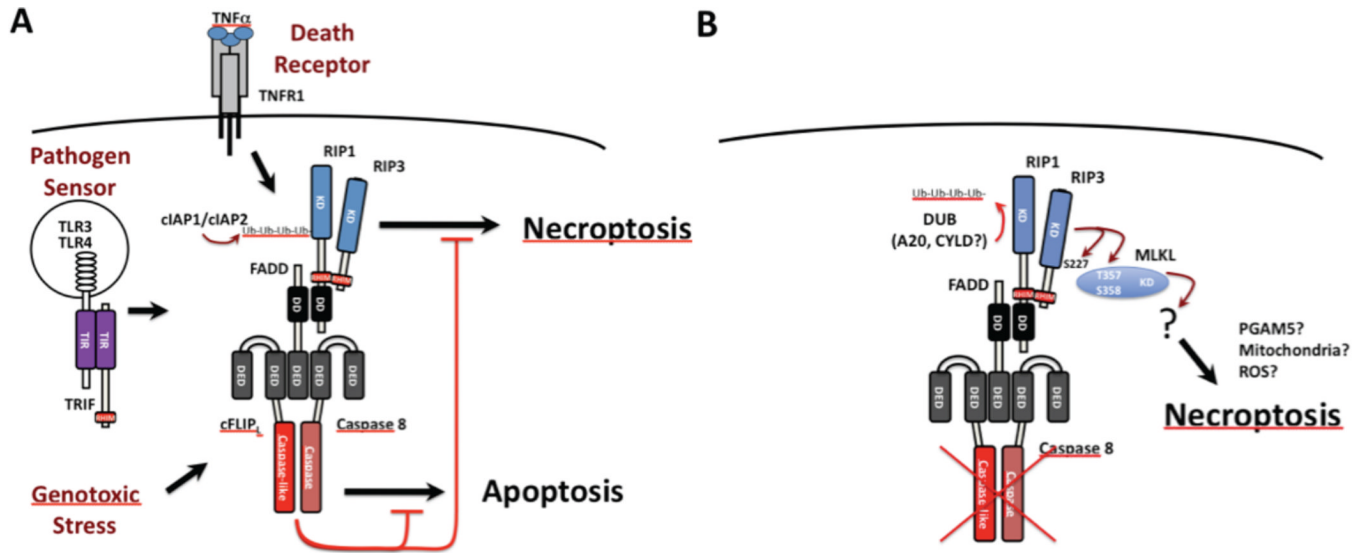


Figure 2. Caspase 8 regulation of extrinsic apoptosis and programmed necrosis

A. Diagrammatic summary of the cytoprotective FADD-Casp8-cFLIP_L-RIP1-RIP3 signaling complex (complex IIb or ripoptosome).

The cytosolic complex forms downstream of cell surface death receptor (e.g. TNFR1 signaling) [87,117,118], endosomal pathogen sensor (e.g. TLR3/TLR4 signaling) [60,152] or intracellular genotoxic stress [61]. The cFLIP_L-Casp8 heterodimer association with FADD prevents self-cleavage activation that is necessary to initiate apoptosis while maintaining sufficient basal activity to prevent unleashed RIP1-RIP3 necroptosis. E3 ubiquitin (Ub) ligases cIAP1 and cIAP2 also prevent RIP1-RIP3 necroptosis by adding K63 Ub chains to RIP1 and possibly other targets [61,62,69].

B. Diagrammatic summary of necroptosis unleashed by RIP3. In the presence of caspase compromise (red "X") or when cIAP1 and cIAP2 are compromised and de-ubiquitinases (DUBs) such as A20 and CYLD predominate and eliminate poly Ub chains, RIP3 kinase becomes activated and is essential for activating MLKL kinase activity [76], autophosphorylating at S277 and also phosphorylating T357 and S358 on MLKL [78] to promote the direct interaction between these two kinases, an essential step in necroptosis.

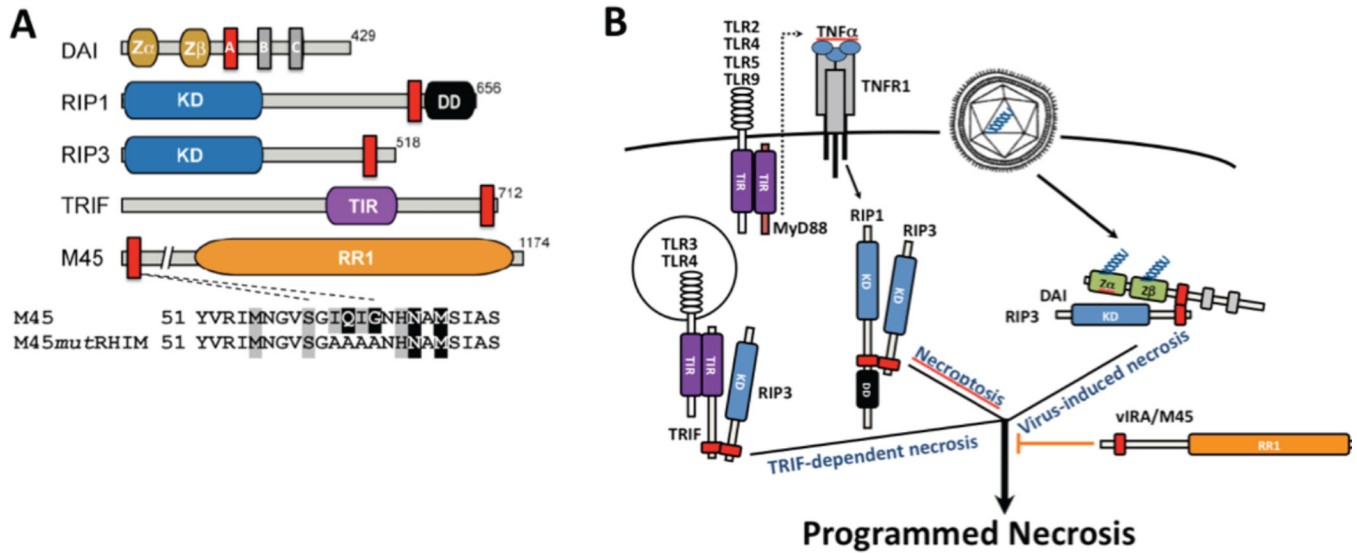


Figure 3. Signaling adaptors in control of RIP3 programmed necrosis

A. Diagrammatic depiction of host and viral RHIM-containing adaptors. Mouse DAI [148,150], RIP1 [153], RIP3 [154,155] and TRIF [145,146], together with murine CMV M45-encoded vIRA, an RR1 homolog [126]. The expanded regions depicts RHIM region from aa 51 and the tetra-alanine mutation employed to characterize M45 RHIM-specific inhibition [89,140]. Kinase domain (KD) of RIP1 and RIP3, death domain (DD) of RIP1, toll-IL-1 receptor (TIR) domain of TRIF and RIP homotypic interaction motif (RHIM; red boxes). DAI has three RHIM-like repeat (RLR) elements where RLR A is functional [148].

B. Summary diagram depicting three distinct triggers of RIP3 kinase-dependent programmed necrosis. TNFR1-induced RIP1-RIP3 necroptosis [87,117,118], DAI-RIP3 virus-induced necrosis [89,93] and TLR3 or TLR4 TRIF-dependent necrosis, as well as TLR2, TLR4, TLR5 and TLR9 MyD88-dependent activation of TNF and secondary induction of necroptosis [94]. These complexes depend on RHIM interactions and are blocked by murine CMV M45-encoded vIRA [89,93,94].

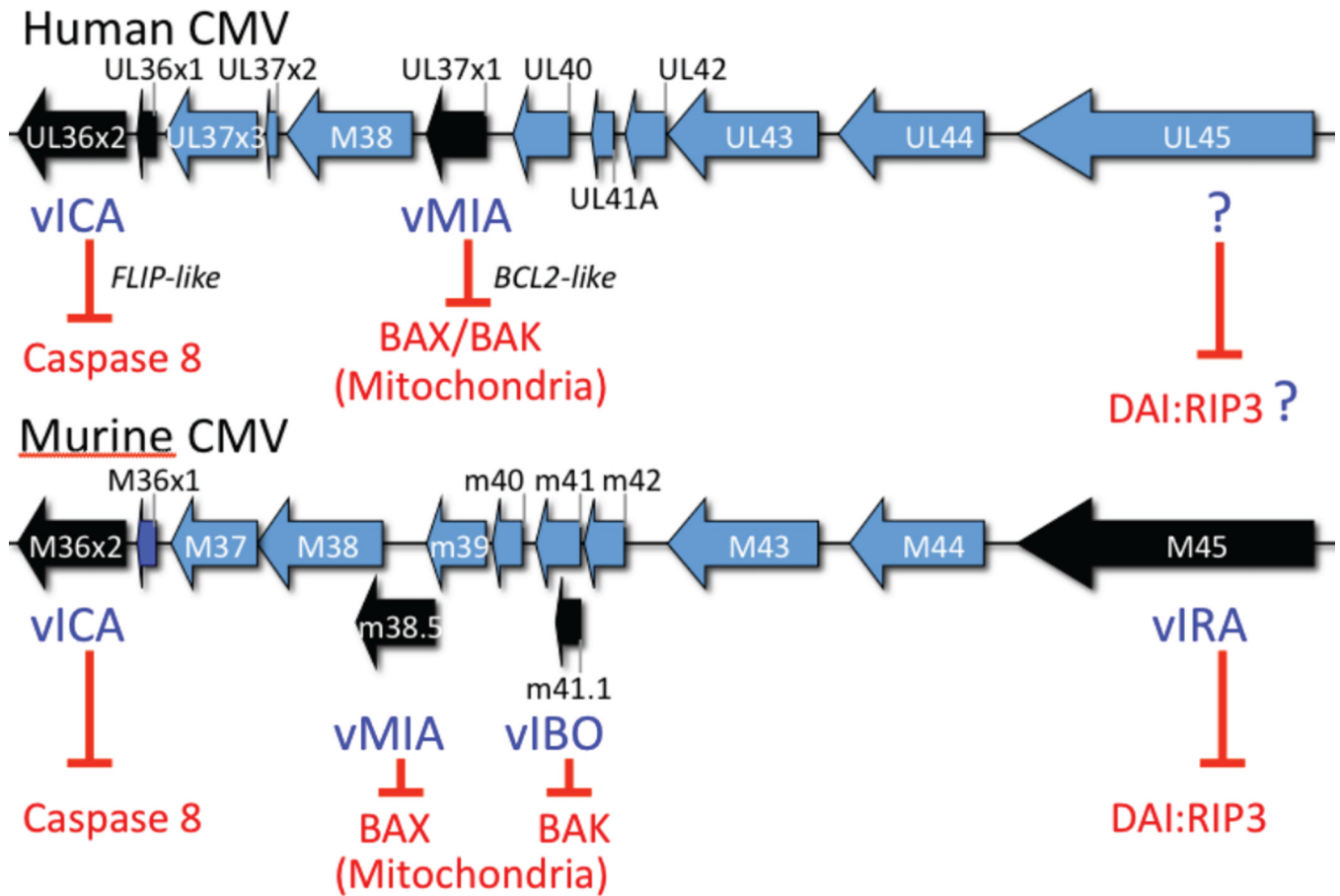


Figure 4. Cytomegalovirus cell death suppression locus

Colinear regions of the human and murine CMV genomes showing the location of ORFs that encode cell death suppressors. vICA is a sequence homolog lacking a DED that nevertheless functions in a FLIP-like manner, interacting with Casp8 to prevent self-activation [30,31]. Human CMV encoded vMIA inhibits BAX [156: Poncet, 2004 #5807] and modulates BAK [157], whereas murine CMV encodes vMIA to inhibit BAX and vIBO to inhibit BAK at mitochondria. Murine CMV M45-encoded vIRA suppresses apoptosis and necrosis [89,138–140], acting as a RHIM inhibitor to suppress DAI:RIP3 complex formation [93] (see Fig. 3).