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Review

The molecular relationships between apoptosis, autophagy and necroptosis

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ABSTRACT

Cells are constantly subjected to a vast range of potentially lethal insults, which may activate specific molecular pathways that have evolved to kill the cell. Cell death pathways are defined partly by their morphology, and more specifically by the molecules that regulate and enact them. As these pathways become more thoroughly characterized, interesting molecular links between them have emerged, some still controversial and others hinting at the physiological and pathophysiological roles these death pathways play. We describe specific molecular programs controlling cell death, with a focus on some of the distinct features of the pathways and the molecular links between them.

Contents

1. "Intrinsic" or "Bcl-2 regulatable" apoptosis

The intrinsic apoptotic pathways contribute to normal embryonic development and the homeostasis of cell number in all multicellular organisms. When the intrinsic cell death pathway is inactivated, for example in gene-deletion mouse models, abnormally increased cell numbers accumulate in various organs. This has been observed in the developing nervous system when two critical genes called BAX and BAK are deleted and in the hematopoietic compartment when another gene, BIM, is inactivated [1,2]. The intrinsic cell death pathway is activated by a variety of cellular stresses, which include DNA damage, growth factor deprivation, oxidative stress, heat shock and endoplasmic reticulum stress. While there are diverse molecular elements involved in activating the intrinsic apoptotic pathway, they ultimately all converge on a core molecular pathway.

Mutagenesis studies in *C.elegans* defined the genes regulating a cell death pathway that is conserved in all other multicellular organisms. This pathway regulates the activity of cell death proteases called caspases, whose aspartate protease functions are the ultimate cause of cell death. In the worm, the cell death genes *ced-4* and *ced-3* are required for developmental apoptosis. *ced-4* encodes a protein that functions as an adaptor molecule to activate the caspase encoded by *ced-3*. The protein encoded by *ced-9* that in turn

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could be repressed by *egl-1* suppressed the apoptotic functions of CED-4 and CED-3. CED-9 and EGL-1 are members of the BCL-2 family of proteins, which are the principal regulators of this apoptosis pathway [3,4].

B cell lymphoma 2 (BCL-2) is the founding member of the BCL-2 family of proteins in mammals and was originally discovered at the breakpoint region of a chromosomal translocation t(14;18)(q32;q21) in patients with follicular lymphoma [5]. Further studies demonstrated that enforced expression of BCL-2 in hematopoietic cells rendered cells resistant to cytokine deprivation [6]. From these original discoveries the number of identified BCL-2 family members has expanded with all proteins containing at least one of the four regions of homology called BCL-2 homology (BH) domains. Indeed, there may also be proteins that contain BH-like domains that function in other non-apoptotic pathways, such as Beclin 1, but more of that later. This BCL-2 family of proteins is functionally sub-divided into proteins that promote cell survival, homologues of CED-9, and proteins that promote apoptosis. There are a variety of pro-survival BCL-2 proteins such as BCL-2, MCL-1, BCL-X_L, BCL-W, BCL-B and A1, the latter known as BFL1 in humans. There are also two distinct groups of pro-apoptotic proteins with BAK, BAX and BOK containing four BH domains and another group known as the BH3-only proteins (BIM, PUMA, BID, BAD, NOXA, BIK, HRK, BMF) that contain only the BH3 domain [7] and which are functional and sequence homologues of EGL-1.

The apical molecules in the intrinsic apoptosis pathways are the BH3-only proteins, which may be activated by transcription or post-translational modification in response to an apoptotic stimulus. Some members of the BH3-only proteins function by antagonizing the pro-survival BCL-2 proteins, whilst other BH3-only proteins also directly interact with BAX and BAK. Both these mechanisms of action ultimately result in conformational change, multimerisation and activation of BAX and BAK [8]. The exact mechanism by which this occurs is still debated. Some of the mystery has been revealed by recent structures of dimeric forms of these proteins, but the nature of the higher order structures of BAX and BAK remain to be determined [9,10]. However, what is clear is that activation of BAX and BAK is the point of no return, the molecular step at which cells commit to apoptosis. The generation of homo-oligomers of BAX or BAK forms structures that permeabilize the outer mitochondrial membrane leading to a number of irreversible outcomes for the cell [11]. Mitochondrial transmembrane potential is lost and a number of proteins are released from the intermembrane space including cytochrome *c* (CYTC) [12], Second Mitochondria-derived Activator of Caspases (SMAC, also known as direct IAP-binding protein with low p*I*, DIABLO) [13,14], Apoptosis-Inducing Factor (AIF) [15], and High Temperature Requirement protein A2 (HTRA2) [16–19]. CYTC joins with an adapter protein APAF1, pro-caspase 9 and dATP to form a protein complex known as the apoptosome. This complex then activates the effector caspase-9, which in turn cleaves and activates the effector caspases-3 and -7 leading to proteolysis of numerous cellular components [20,21]. SMAC/DIABLO and HTRA2 are also responsible for disrupting Inhibitor of APoptosis (IAP) proteins, in particular XIAP, which leads to further caspase activity [19,22–24]. Caspases cleave their substrates, one of which is the Inhibitor of Caspase Activated DNase (ICAD) which leads to the activation of Caspase-Activated DNase (CAD) [25] and results in the characteristic DNA fragmentation of apoptotic cells (DNA laddering) as well as the other morphological changes typical of apoptotic cell death that are the basis of the experimental detection of intrinsic apoptosis.

Cytotoxic lymphocyte mediated cell killing is mediated by a family of serine proteases called Granzymes. These are introduced into target cells through a pore for which the protein Perforin is an essential requirement [26]. In humans there are 5 Granzyme-encoding genes. Broadly speaking, Granzymes may induce cell death in target cells through mechanisms that engage with components of the BCL-2 regulatable pathway, or by mechanisms that are independent of this apoptosis pathway. Granzyme B is the archetypal example of cytotoxic lymphocyte mediated cell death that engages with the apoptotic machinery. Granzyme B can cleave and activate the BH3-only protein BID, which results in BAX and BAK activation [27]. Furthermore, Granzyme B can directly cleave and activate apoptotic caspases (interestingly caspases involved in inflammation), so initiating the apoptotic destruction of a cell, but bypassing the regulation of caspase activation by BCL-2 family members [28]. Granzyme A, in contrast, induces cytotoxicity independently of the BCL-2 regulated pathway, or other caspase-dependent pathways, and instead induces cell death as a result of direct cleavage of a range of important cellular substrates. The mechanisms by which cytotoxic lymphocytes kill target cells is of more than academic interest, since mutations in this pathway are associated with Hemophagocytic lymphohistiocytosis (HLH) (reviewed in [29]). There is also evidence to suggest that a relatively common hypomorphic Perforin polymorphism, may increase susceptibility to lymphocytic malignancies (reviewed in [30]). Most disease-causing mutations occur in Perforin itself, or other proteins involved in the mechanisms by which Perforin induces pore-formation and the introduction of Granzymes into target cells. Mutations in Granzymes or apoptosis genes that engage with Granzymes have not been described as phenocopying Perforin-related diseases. This suggests that, from a pathological perspective at least, the mechanisms by which Granzymes induce cytotoxicity are less critical than the mechanisms by which Granzymes are introduced and trafficked into target cells.

2. Autophagy

More recently, there has been interest in the role autophagy may play in cell death, and how this may intersect with the intrinsic cell death pathway. Autophagy is a cellular mechanism of "self-eating", in which proteins and organelles are encased in specialized intracellular vesicles and are then broken down by lysosomal proteases for recycling. A basal or low level of autophagy occurs in most cells at resting state. During times of stress, such as nutrient starvation or treatment with certain toxic agents such as etoposide, the rate of autophagy increases. Autophagosomic vesicles envelope the contents of the cytoplasm, either seemingly non-specifically (macroautophagy) or in a specific manner, such as targeting damaged mitochondria (mitophagy).

Autophagosome formation is instigated by the ULK protein complex, which is negatively regulated by the mechanistic Target Of Rapamycin (mTOR) pathway, a major nutrient sensor. Withdrawal of growth factors, amino acid deprivation, and the chemical inhibitor rapamycin all induce autophagy by targeting this pathway. The ULK complex initiates the nucleation of the autophagosome membrane and activates the phosphatylinositol 3-kinase (PI3K) complex III, which includes Beclin 1 and the PI3K Vps34. PI3K inhibitors such as 3-methyladenine (3-MA), wortmannin, and LY294002 inhibit autophagy at this early stage.

Microtubule-associated protein Light Chain 3 (LC3) is a cytoplasmic protein that is quickly cleaved to form LC3-I. It is "activated" by lipidation with phosphatidylethanolamine to form LC3-II by a ubiquitin-like ligation pathway that include Autophagy protein 7 (ATG7) and ATG5, both of which are required for this modification to take place [31]. LC3-II is incorporated into the vesicle membrane and promotes membrane elongation. To degrade their contents, autophagosomes fuse with lysosomes, in which the proteases digest the cytoplasmic material under acidic conditions. Chemical inhibitors such as chloroquine and bafilomycin A1 inhibit both autolysosome and lysosomal function. While an increase in LC3-II

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(and corresponding decrease in LC3-I) typically corresponds with an increase in the rate of autophagy, there are important exceptions. For instance, LC3-II may not be incorporated into vesicles and immunoblotting cell lysate does not establish LC3-II localization. Indeed, LC3 lipidation has been reported to occur even if there are blocks to autophagosome formation [32]. Further, LC3-II is degraded in the autolysosome, so if late stage autophagy is inhibited, LC3-II will accumulate. Therefore, an increase in LC3-II can actually be an indication of both stimulation or inhibition of autophagy [33].

3. Autophagy and cell death

Sometimes referred to as Type II cell death, autophagic cell death has had a confusing history. In some instances, autophagic cell death has been identified principally by the presence of autophagosomes or increased rates of autophagy only, while others have a stricter definition and include a functional outcome – cell death must be directly due to autophagy. The latter seems most informative and the general trend in the field seems to be shifting in this direction. The Nomenclature Committee on Cell Death has recommended "the use of the expression 'autophagic cell death' . . . to indicate a cell death subroutine that is limited or delayed by the pharmacologic or genetic inhibition of the autophagic machinery" [34].

Stimulation of autophagy (or the presence of autophagosomes) often accompanies cell death. This is unsurprising since both autophagy and cell death may be activated in response to similar stresses. Indeed, many cytotoxic compounds induce autophagy [35]. Therefore, it is important to determine if autophagy can be induced independently of cell death. For instance, while the BH3 mimetic ABT-737 has been shown to induce autophagy [36] this only occurred in cells which expressed BAX and BAK, indicating that autophagy in this response was dependent on the activation of the intrinsic apoptosis pathway [37].

The pro-survival BCL-2 family members, which are the targets of the BH3-mimetic compound ABT-737, have been reported to directly inhibit autophagy by binding to a BH3-like domain of the autophagy protein Beclin 1 (BECN1/ATG6/VPS30) [38]. This idea was supported by the observation that BCL-2 can co-immunopreciptate with Beclin 1 when over-expressed. However, recent evidence indicates that inhibition of autophagy by the pro-survival BCL-2 family is dependent on the presence of an intact intrinsic apoptosis pathway. When the intrinsic pathway is completely disabled by the deletion of both BAX and BAK, over-expression of anti-apoptotic BCL-2 family members cannot block the activation of autophagy by a variety of stimuli [37]. Thus it would seem that the regulation of autophagy by BCL-2 family members or BH3-mimetics is indirect and due to activation of cell death via BAX and BAK as opposed to an independent regulation of autophagy.

There are, however, examples of cell death stimuli that do activate autophagy independently of cell death. The DNA intercalator etoposide induces autophagy even in cells devoid of BAX and BAK [37,39], however, whether the drug can cause cell death due to autophagy is controversial. Deletion of key autophagy genes (e.g. ATG5 or ATG7) is the clearest way to establish the role of autophagy as a type of cell death. For example, one could genetically separate autophagic and apoptotic cell death responses by comparing $Bax^{-/-}Bak^{-/-}$ and $ATG5^{-/-}$ cells with cells deficient in all three genes. Although this direct experiment has not yet been performed (to our knowledge), a screen of a large number of cytotoxic compounds concluded that even when compounds do specifically induce autophagy, inhibition of autophagy by knockdown of ATG7 expression could not inhibit cell death [35]. There are relatively few examples of autophagic cell death based on these criteria.

One of the most convincing instances of autophagic cell death occurs in Drosophila. The removal of larval midgut structures during development can still take place, even in the absence of caspases. Silencing of autophagy genes under these experimental conditions provided convincing evidence that autophagic pathways were primarily responsible for removing these cells, independent of caspase activity [40]. Other experimental work suggests that autophagy pathways, generally considered a mechanism by which cells conserve and recycle resources, can tip cell fate in favor of cell death. For instance, Beclin 1, ATG12, ATG5, and ATG7 have been implicated in modulating apoptosis and caspase-dependent cell death [41–43]. In each of these instances, caspase activation appeared to be the final mediator of cell death. Other data point to components of the extrinsic cell death pathways having a role in the regulation of autophagy [44,45]. Thus there may be many levels at which cell death pathways and autophagy pathways interact. The challenge continues to be establishing how critical this cross talk is to determining cell fate in response to various apoptotic and autophagic stimuli, by establishing the independent roles of these pathways.

4. Extrinsic cell death pathways

In many immune cells, and particularly in lymphocytes, cell death can be triggered by the specific engagement of a subset of the TNF Receptor family by their ligands. The most well characterized ligands of these receptors to date are FasL and TNF-α. This form of cell death plays a critical role in the deletion of autoreactive immune cells to limit inflammatory responses. When this pathway is inactivated by naturally occurring mutations or by gene deletion, autoimmune disease is a consequence [46,47]. Extrinsic cell death pathways have been extensively reviewed so here we provide a brief description since it is the connections between extrinsic apoptosis and another cell death pathway necroptosis that are of interest.

The subgroup of TNF Receptors that can signal to activate cell death contain a cytoplasmic domain known as a "death domain". The archetypal example is the TNF Receptor family member FAS/CD95 that is the receptor for FAS Ligand. When the receptor is activated, the death inducing signaling complex (DISC) complex is formed at the membrane, which functions primarily to activate caspase-8 and enforce the cell death outcome. In the context of FAS receptor, the binding of its ligand induced the recruitment of Fas Associated Death Domain (FADD), an adaptor molecule that associates with procaspase-8 via dimerization of their death effector domains. Within the DISC, oligomerisation of the procaspase-8 results in its autocatalytic activation to form caspase-8. cFLIP (FADD like Interleukin-1ß Converting Enzyme (FLICE) Inhibitory Protein) is a homologue of caspase-8, containing a DED domain and an inactive catalytic domain. cFLIP can inhibit apoptosis by binding to FADD and caspase 8. [48–50]. Several isoforms, produced by alternative splicing, are present as proteins within cells. These include a long isoform, $cFLIP_L$, and short form, $cFLIP_S$ in humans. The morphological features of extrinsic apoptosis pathway are the same as those of the intrinsic cell death pathway. Although there are established molecular links between the extrinsic and intrinsic pathways, BAX and BAK are not required for extrinsic cell death.

Amongst the TNF family of cytokine receptors, cell death is only one of a number of outcomes from receptor signaling which also include NF-κB activation, cell proliferation and active repression of caspase-dependent cell death. TNF receptor 1 (TNFR1) is perhaps the best-studied example. These competing outcomes (life or death) result from the assembly of a signaling complex related to the DISC that represses caspase-8 activity (complex I) or which promote caspase-8 activation (complex II) [51]. Complex I includes

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the adaptor molecules TNFR1-Associated Death Domain (TRADD), Receptor Interacting Protein Kinase I (RIPK1), TNF Receptor Associated Factor proteins (TRAFs) and cIAP1&2 and cFLIP (reviewed in [52]). Complex I functions to repress caspase-8 activation through the recruitment of cFLIP. RIPK1 functions as a scaffold for the assembly of ubiquitin chains that are a platform that ultimately results in the activation of NF-κB, although RIPK1 is not absolutely required for NF-κB activation in response to TNFα signaling through TNFR1 [53]. This ubiquitin platform relies crucially on the E3 ligase activity of cIAP1 and cIAP2 [54]. It is likely that complex II also forms when TNFR1 is engaged by TNF, but whilst sufficient cFLIP is available, itself a NF-κB transcriptional target, caspase-8 activation does not occur. However, if cFLIP is not available for recruitment, or the ubiquitin platform to drive NF-κB activation does not form, then conditions favor the persistence of complex II and caspase-8 activation and so the extrinsic apoptosis pathway proceeds.

Experimentally, whether apoptosis pathways are triggered in response to TNFR1 signaling is dependent on the recruitment of the cIAPs to the signaling complex and their function as ubiquitin ligases [54]. When cIAPs are degraded by the addition of specific inhibitor drugs, or removed by gene depletion, the caspase-8 activating complex is favored [55–57]. Further, cFLIP, which has a caspase-8 cleavage site, may be further cleaved by caspase-8 activity. Several groups studying this pathway took the next step and asked what would happen if caspase-8 activity was blocked, reasoning that this should prevent cell death in response to TNFR1 signaling. What became evident was that under these circumstances, the combined degradation of IAPs and the inhibition of caspases, cells had another route to death that was triggered by TNFR1. This pathway, necroptosis, is morphologically distinct from apoptosis and does not require caspase activity, but much of the molecular machinery that can drive this specific cell death pathway is familiar.

5. Necroptosis: a programmed necrosis

Necrotic death is generally a result of an over-whelming cytotoxic insult, and requires no specific molecular events in order for it to occur. However, in the last decade, a regulated form of necrotic cell death has been characterized and termed necroptosis, because it shares features of apoptosis and necrosis. Like apoptosis, a defined molecular cascade controls necroptosis. Like necrosis, necroptosis is characterized by swelling of the cell and its organelles leading to cell rupture. Rupture results in the release of cellular contents, a number of which serve as damage-associated molecular patterns (DAMPs) such as mtDNA, HMGB1, IL-33, IL-1α or S100a9, which can potentiate inflammation [58]. Proinflammatory cytokines and chemokines also play an important role in this inflammatory response. Consistent with this idea, necroptosis is associated with a number of inflammatory pathologies such as pancreatitis [59] inflammatory bowel diseases [60] and infection diseases [61–64].

The first indication of a necroptotic type of death was observed in murine L929 fibroblast cells treated with TNFα or FasL and caspase inhibitors ([65]; [66]). Later studies revealed that inhibition of caspase-8 in particular induces necroptosis in the presence of a diverse range of stimuli including signaling through TNF Receptors [67,68], T-Cell Receptor stimuli [69], interferons [70], some anticancer drugs [71–73], pathogen-associated molecular patterns activating RIG-I-like or Toll-like receptors [74–76], genotoxic or oxidative stresses [55,56,77], and virus-mediated activation of DNA-dependent activator of IFN-regulatory factors (DAI) [78]. Necroptosis requires the serine/threonine Receptor Interacting Protein (RIP) kinases, RIPK1 and RIPK3, and Mixed Lineage Kinase Like (MLKL). Currently, necroptosis is defined by a caspase-independent cell death mediated by RIP kinases and by MLKL. Experimentally, this commonly involves the use of caspase inhibitors, and the inhibition of RIPK1 by necrostatin, or by the deletion or silencing of RIPK3 or MLKL.

Most of the reported necroptotic stimuli result in TNF production and signaling through TNFR1. In experimental settings, inhibition of caspase-8 and cIAPs during TNFR1 signaling promotes necroptosis. Binding of TNF to TNFR1 leads to polyubiquitination of RIPK1 by cIAPs, which sterically restricts RIPK1 from forming cell death complexes [54,79]. When cIAPs are absent or have been degraded by treatment with IAP antagonists, a cell death-signaling complex, called RIPoptosome, is formed consisting of deubiquitinated RIPK1, FADD, caspase-8 and cFLIP [55,56]. Within this dynamic platform the cellular fate appears to be critically dependent on caspase-8 activity. Both RIPK1 and RIPK3 are substrates of caspase-8 and their cleavage leads to apoptosis and simultaneously prevents extensive necrosis during embryonic development and inflammation [74,80,81].

In the absence of caspase-8 activation, RIPK1 and RIPK3 are no longer cleaved and can interact through their RHIM domains to function as kinases that drive necroptosis [82]. RIPK1 and RIPK3 together with MLKL form the necrosome [83]. In this complex, RIPK1 phosphorylates RIPK3, which in turn phosphorylates MLKL that directly causes cell death. Once phosphorylated, the pseudokinase MLKL undergoes a conformational change that results in the insertion of a unique structural domain, a 4 helical bundle, into plasma membranes that disrupts membrane integrity [84–89]. Interestingly, other molecules with this 4 helical bundle are recognized, including in plants, where they contribute to the response to pathogens. This suggests that necroptosis may be a more ancient cell death response than apoptosis pathways.

It is worth noting that the unconditional requirement of RIPK1 in necroptosis has been recently questioned. Recently, two separate groups have provided evidence suggesting that RIPK1 suppresses spontaneous RIPK3 activation by controlling RIPK3 oligomerisation. [90,91]. Unexpectedly, cells lacking RIPK1 increased spontaneous RIPK3-dependent necroptosis, while cells treated with necrostatin remained protected from necroptosis [90,91]. Consistent with this, the perinatal lethally of *Ripk1*-deficient mice is ameliorated by loss of *Ripk3* or *Mlkl*, suggesting the existence of a RIPK1-independent (RIPK3/MLKL-dependent) necroptosis. This idea is supported by the fact that deletion of *Ripk1* does not rescue the lethally of *Caspase-8*-deficient mice like loss of *Ripk3* does [92,93]. Further studies have showed that necroptosis induced by TLR3/4 or by MCMV is independent of RIPK1 [74,78,94].

There are other levels of cross regulation of caspase-dependent and–independent death responses by components of the RIPoptosome, many of which are incompletely understood. For example, the $cFLIP_L$ isoform forms a catalytically active heterodimer with caspase-8 that can alter the spectrum of caspase-8 substrate specificity [95,96]. This heterodimer of $cFLIP_L$ and caspase-8 functions to block necroptosis, by inhibiting RIPK3 [97]. In contrast, the short isoform of cFLIP inhibits caspase-8 activity and can potentiate the formation of the necroptotic complex in the absence of cIAPs [55,56]. More recent studies of conditional cFLIP knockout mice describe a role for cFLIP in regulating both apoptosis and necroptosis pathways, since the lethal phenotype of cFLIP knockout mice is rescued by co-deletion of RIPK3 and FADD, but not by either alone [98]. Another example of molecular co-regulation of apoptosis and necroptosis is Cylindromatosis (CYLD). This gene encodes a cytoplasmic protein that functions as a deubiquitinase and was identified in a genome-wide screen as a necrosis mediator [99]. CYLD promotes necroptosis by facilitating RIPK1 deubiquitination at the TNFR1 membrane receptor complex [100]. Caspase-8 suppresses necroptosis by cleaving CYLD [101]. Together, these examples serve to highlight the fact that the necroptosis and

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extrinsic apoptosis pathways are tightly integrated and regulated in complex and intersecting ways. Doubtless, other examples of co-regulation will be described. The challenge is to untangle the distinct roles of each cell death pathway from these complex interactions.

6. Autophagy and necroptosis together?

Currently, it is less clear how potential interactions between autophagy and necroptosis pathways fit into the increasingly complex network of the different ways in which cells can die. Nevertheless, there are data that point to such interactions. In studies of murine fibroblast L929 cells, cell death was initiated solely by the treatment with a pan-caspase inhibitor zVAD [45]. This death response was in part characterized by the development of autophagosomic vacuoles. Because reducing RIPK1, ATG7 or Beclin-1 expression inhibited the death response, this data suggested on the one hand that these autophagic genes contribute to the necroptotic cell death, and that caspase activation (presumably caspase-8) can repress autophagic cell death. Studies of T-cells from genetically modified mice suggest that a DISC-like complex, composed of FADD caspase-8 and RIPK1 is assembled on autophagosomic membranes in response to T-cell activation [102]. A protein-protein interaction between ATG5 and FADD is key to the assembly of this complex. At a functional level, this model suggests that some level of autophagy is required for a normal proliferative T-cell response. FADD, caspase-8 and RIPK1 all regulate the magnitude of the response, since deletion of FADD and caspase-8 enhances autophagy, blunts proliferation, whilst inhibition of RIPK1 by necrostatin can restore the proliferative response in T-cell lacking normal FADD function. More recently it was shown that deletion of RIPK3 also restores cell proliferation [103]. This level of regulation may check the possibility of an unregulated autophagy causing T-cell death during clonal expansion. Moreover, these data suggest that the regulation of caspase-8 activity has an important role in the regulation of T-cell proliferative responses. There is clearly more to be learnt about the specific physiological contexts in which autophagic and necroptotic pathways cross-regulate one another in other receptor signaling systems.

7. Conclusion

The same deleterious insult can engage different, molecularly distinct routes to cell death. There are also several points at which these pathways converge on shared molecules and, in many cases, these are regulatory points at which cells may “decide” the manner of their death. Whilst it is evident that certain cellular stresses always engage a particular cell death pathways such as the BCL-2 regulatable pathway, it is also clear that other cytotoxic responses, such as cytotoxic lymphocyte killing, have evolved to have multiple mechanisms by which cell death can be caused, presumably because the consequences of a failure in the death response may be severe. The intersections between receptor-mediated apoptosis pathways and necroptosis are an intriguing and evolving area of study. The level of expression or activity of critical components of the molecular machinery that regulates the activity of caspase-8, for example, will determine whether cells will engage apoptotic or necroptotic pathways. Whilst the physiological consequence is cell death, the “downstream” consequences of which cell death pathway is activated fundamentally influences whether an inflammatory response is promoted. Whether autophagy and intrinsic apoptosis responses to similar stimuli have distinct physiological consequences even if the outcome of both processes results in the demise of the cell remains to be established and is a lively tropic for discussion and further research.

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References

[1] Bouillet P, Metcalf D, Huang DC, Tarlinton DM, Kay TW, Kontgen F, et al. Proapoptotic Bcl-2 relative Bim required for certain apoptotic responses, leukocyte homeostasis, and to preclude autoimmunity. Science 1999;286:1735–8.
[2] Lindsten T, Thompson CB. Cell death in the absence of Bax and Bak. Cell Death Differ 2006;13:1272–6.
[3] Conradt B, Horvitz HR. The *C. elegans* protein egl-1 is required for programmed cell death and interacts with the bcl-2-like protein ced-9. Cell 1998;93:519–29.
[4] Ellis HM, Horvitz HR. Genetic control of programmed cell death in the nematode *C. elegans*. Cell 1986;44:817–29.
[5] Tsujimoto Y, Cossman J, Jaffe E, Croce CM. Involvement of the bcl-2 gene in human follicular lymphoma. Science 1985;228:1440–3.
[6] Vaux DL, Cory S, Adams JM. Bcl-2 gene promotes haemopoietic cell survival and cooperates with c-myc to immortalize pre-B cells. Nature 1988;335:440–2.
[7] Strasser A. The role of BH3-only proteins in the immune system. Nat Rev Immunol 2005;5:189–200.
[8] Westphal D, Dewson G, Czabotar PE, Kluck RM. Molecular biology of Bax and Bak activation and action. Biochim Biophys Acta 2011;1813:521–31.
[9] Czabotar PE, Westphal D, Dewson G, Ma S, Hockings C, Fairlie WD, et al. Bax crystal structures reveal how BH3 domains activate Bax and nucleate its oligomerization to induce apoptosis. Cell 2013;152:519–31.
[10] Westphal D, Dewson G, Menard M, Frederick P, Iyer S, Bartolo R, et al. Apoptotic pore formation is associated with in-plane insertion of Bak or Bax central helices into the mitochondrial outer membrane. Proc Natl Acad Sci U S A 2014;111:E4076–85.
[11] Czabotar PE, Lessene G, Strasser A, Adams JM. Control of apoptosis by the BCL-2 protein family: implications for physiology and therapy. Nat Rev Mol Cell Biol 2014;15:49–63.
[12] Kluck RM, Bossywetzel E, Green DR, Newmeyer DD. The release of cytochrome *c* from mitochondria – a primary site for bcl-2 regulation of apoptosis. Science 1997;275:1132–6.
[13] Du C, Fang M, Li Y, Li L, Wang X. Smac, a mitochondrial protein that promotes cytochrome *c*-dependent caspase activation by eliminating IAP inhibition. Cell 2000;102:33–42.
[14] Verhagen A, Ekert PG, Silke J, Connolly LM, Reid GE, Moritz RL, et al. Identification of DIABLO, a mammalian protein that promotes apoptosis by binding to and antagonizing IAP proteins. Cell 2000;102:43–53.
[15] Susin SA, Lorenzo HK, Zamzami N, Marzo I, Snow BE, Brothers GM, et al. Molecular characterization of mitochondrial apoptosis-inducing factor. Nature 1999;397:441–6.
[16] Hegde R, Srinivasula SM, Zhang Z, Wassell R, Mukattash R, Cilenti L, et al. Identification of Omi/HtrA2 as a mitochondrial apoptotic serine protease that disrupts inhibitor of apoptosis protein–caspase interaction. J Biol Chem 2001;277:432–8.
[17] Martins LM, Iaccarino I, Tenev T, Gschmeissner S, Totty NF, Lemoine NR, et al. The serine protease Omi/HtrA2 regulates apoptosis by binding XIAP through a Reaper-like motif. J Biol Chem 2001. M109784200.
[18] Suzuki Y, Imai Y, Nakayama H, Takahashi K, Takio K, Takahashi R. A serine protease, HtrA2, is released from the mitochondria and interacts with XIAP, inducing cell death. Mol Cell 2001;8:613–21.
[19] Verhagen AM, Silke J, Ekert PG, Pakusch M, Kaufmann H, Connolly LM, et al. HtrA2 promotes cell death through its serine protease activity and its ability to antagonize inhibitor of apoptosis proteins. J Biol Chem 2001;277:445–54.
[20] Li P, Nijhawan D, Budihardjo I, Srinivasula SM, Ahmad M, Alnemri ES, et al. Cytochrome *c* and datp-dependent formation of apaf-1/caspase-9 complex initiates an apoptotic protease cascade. Cell 1997;91:479–89.
[21] Zou H, Henzel WJ, Liu XS, Lutschg A, Wang XD. Apaf-1, a human protein homologous to *C. elegans* ced-4, participates in cytochrome *c*-dependent activation of caspase-3. Cell 1997;90:405–13.
[22] Chai J, Du C, Wu JW, Kyin S, Wang X, Shi Y. Structural and biochemical basis of apoptotic activation by Smac/DIABLO. Nature 2000;406:855–62.
[23] van Loo G, van Gurp M, Depuydt B, Srinivasula SM, Rodriguez I, Alnemri ES, et al. The serine protease Omi/HtrA2 is released from mitochondria during apoptosis. Omi interacts with caspase-inhibitor XIAP and induces enhanced caspase activity. Cell Death Differ 2002;9:20–6.
[24] Yang QH, Church-Hajduk R, Ren J, Newton ML, Du C. Omi/HtrA2 catalytic cleavage of inhibitor of apoptosis (IAP) irreversibly inactivates IAPs and facilitates caspase activity in apoptosis. Genes Dev 2003;17:1487–96.
[25] Nagata S. Apoptotic DNA fragmentation. Exp Cell Res 2000;256:12–8.
[26] Hoves S, Trapani JA, Voskoboinik I. The battlefield of perforin/granzyme cell death pathways. J Leukoc Biol 2010;87:237–43.

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[27] Waterhouse NJ, Sedelies KA, Browne KA, Wowk ME, Newbold A, Sutton VR, et al. A central role for Bid in granzyme B-induced apoptosis. J Biol Chem 2005;280:4476–82.
[28] Adrain C, Murphy BM, Martin SJ. Molecular ordering of the caspase activation cascade initiated by the cytotoxic T lymphocyte/natural killer (CTL/NK) protease granzyme B. J Biol Chem 2005;280:4663–73.
[29] Weitzman S. Approach to hemophagocytic syndromes. Hematology: Am Soc Hematol Educ Program 2011;2011:178–83.
[30] Brennan AJ, Chia J, Trapani JA, Voskoboinik I. Perforin deficiency and susceptibility to cancer. Cell Death Differ 2010;17:607–15.
[31] Mizushima N, Yoshimori T, Levine B. Methods in mammalian autophagy research. Cell 2010;140:313–26.
[32] Matsui Y, Takagi H, Qu X, Abdellatif M, Sakoda H, Asano T, et al. Distinct roles of autophagy in the heart during ischemia and reperfusion: roles of AMP-activated protein kinase and Beclin 1 in mediating autophagy. Circ Res 2007;100:914–22.
[33] Mizushima N, Yoshimori T. How to interpret LC3 immunoblotting. Autophagy 2007;3:542–5.
[34] Galluzzi L, Bravo-San Pedro JM, Vitale I, Aaronson SA, Abrams JM, Adam D, et al. Essential versus accessory aspects of cell death: recommendations of the NCCD 2015. Cell Death Differ 2014;22:58–73.
[35] Shen S, Kepp O, Michaud M, Martins I, Minoux H, Metivier D, et al. Association and dissociation of autophagy, apoptosis and necrosis by systematic chemical study. Oncogene 2011;30:4544–56.
[36] Maiuri MC, Criollo A, Tasdemir E, Vicencio JM, Tajeddine N, Hickman JA, et al. BH3-only proteins and BH3 mimetics induce autophagy by competitively disrupting the interaction between Beclin 1 and Bcl-2/Bcl-X(L). Autophagy 2007;3:374–6.
[37] Lindqvist LM, Heinlein M, Huang DC, Vaux DL. Prosurvival Bcl-2 family members affect autophagy only indirectly, by inhibiting Bax and Bak. Proc Natl Acad Sci U S A 2014;111:8512–7.
[38] Pattingre S, Tassa A, Qu X, Garuti R, Liang XH, Mizushima N, et al. Bcl-2 antiapoptotic proteins inhibit Beclin 1-dependent autophagy. Cell 2005;122:927–39.
[39] Shimizu S, Kanaseki T, Mizushima N, Mizuta T, Arakawa-Kobayashi S, Thompson CB, et al. Role of Bcl-2 family proteins in a non-apoptotic programmed cell death dependent on autophagy genes. Nat Cell Biol 2004;6:1221–8.
[40] Denton D, Shravage B, Simin R, Mills K, Berry DL, Baehrecke EH, et al. Autophagy, not apoptosis, is essential for midgut cell death in Drosophila. Curr Biol 2009;19:1741–6.
[41] Altman BJ, Wofford JA, Zhao Y, Coloff JL, Ferguson EC, Wieman HL, et al. Autophagy provides nutrients but can lead to Chop-dependent induction of Bim to sensitize growth factor-deprived cells to apoptosis. Mol Biol Cell 2009;20:1180–91.
[42] Gonzalez P, Mader I, Tchoghandjian A, Enzenmuller S, Cristofanon S, Basit F, et al. Impairment of lysosomal integrity by B10, a glycosylated derivative of betulinic acid, leads to lysosomal cell death and converts autophagy into a detrimental process. Cell Death Differ 2012;19:1337–46.
[43] Rubinstein AD, Eisenstein M, Ber Y, Bialik S, Kimchi A. The autophagy protein Atg12 associates with antiapoptotic Bcl-2 family members to promote mitochondrial apoptosis. Mol Cell 2011;44:698–709.
[44] Mills KR, Reginato M, Debnath J, Queenan B, Brugge JS. Tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) is required for induction of autophagy during lumen formation in vitro. Proc Natl Acad Sci U S A 2004;101:3438–43.
[45] Yu L, Alva A, Su H, Dutt P, Freundt E, Welsh S, et al. Regulation of an ATG7-Beclin 1 program of autophagic cell death by caspase-8. Science 2004;304:1500–2.
[46] Drappa J, Vaishnaw AK, Sullivan KE, Chu JL, Elkon KB. Fas gene mutations in the canale-smith syndrome, an inherited lymphoproliferative disorder associated with autoimmunity. N Engl J Med 1996;335:1643–9.
[47] Watanabe FR, Brannan CI, Copeland NG, Jenkins NA, Nagata S. Lymphoproliferation disorder in mice explained by defects in Fas antigen that mediates apoptosis. Nature 1992;356:314–7.
[48] Kischkel FC, Hellbardt S, Behrmann I, Germer M, Pawlita M, Krammer PH, et al. Cytotoxicity-dependent APO-1 (Fas/CD95)-associated proteins form a death-inducing signaling complex (DISC) with the receptor. EMBO J 1995;14:5579–88.
[49] Medema JP, Scaffidi C, Kischkel FC, Shevchenko A, Mann M, Krammer PH, et al. FLICE is activated by association with the CD95 death-inducing signaling complex (DISC). EMBO J 1997;16:2794–804.
[50] Muzio M, Chinnaiyan AM, Kischkel FC, O'Rourke K, Shevchenko A, Ni J, et al. FLICE, a novel FADD-homologous ICE/CED-3-like protease, is recruited to the CD95 (Fas/APO-1) death-inducing signaling complex. Cell 1996;85:817–27.
[51] Micheau O, Tschopp J. Induction of TNF receptor I-mediated apoptosis via two sequential signaling complexes. Cell 2003;114:181–90.
[52] Silke J. The regulation of TNF signalling: what a tangled web we weave. Curr Opin Immunol 2011;23:620–6.
[53] Wong WW, Gentle IE, Nachbur U, Anderton H, Vaux DL, Silke J. RIPK1 is not essential for TNFR1-induced activation of NF-kappaB. Cell Death Differ 2010;17:482–7.
[54] Bertrand MJM, Milutinovic S, Dickson KM, Ho WC, Boudreault A, Durkin J, et al. cIAP1 and cIAP2 facilitate cancer cell survival by functioning as E3 ligases that promote RIP1 ubiquitination. Mol Cell 2008:p689–700.
[55] Feoktistova M, Geserick P, Kellert B, Dimitrova DP, Langlais C, Hupe M, et al. cIAPs block Ripoptosome formation, a RIP1/caspase-8 containing intracellular cell death complex differentially regulated by cFLIP isoforms. Mol Cell 2011;43:449–63.
[56] Tenev T, Bianchi K, Darding M, Broemer M, Langlais C, Wallberg F, et al. The Ripoptosome, a signaling platform that assembles in response to genotoxic stress and loss of IAPs. Mol Cell 2011;43:432–48.
[57] Vince JE, Wong WW, Khan N, Feltham R, Chau D, Ahmed AU, et al. IAP antagonists target cIAP1 to induce TNFalpha-dependent apoptosis. Cell 2007;131:682–93.
[58] Kaczmarek A, Vandenabeele P, Krysko DV. Necroptosis: the release of damage-associated molecular patterns and its physiological relevance. Immunity 2013;38:209–23.
[59] He S, Wang L, Miao L, Wang T, Du F, Zhao L, et al. Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-alpha. Cell 2009;137:1100–11.
[60] Gunther C, Martini E, Wittkopf N, Amann K, Weigmann B, Neumann H, et al. Caspase-8 regulates TNF-alpha-induced epithelial necroptosis and terminal ileitis. Nature 2011;477:335–9.
[61] Cho YS, Challa S, Moquin D, Genga R, Ray TD, Guildford M, et al. Phosphorylation-driven assembly of the RIP1–RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell 2009;137:1112–23.
[62] Pan T, Wu S, He X, Luo H, Zhang Y, Fan M, et al. Necroptosis takes place in human immunodeficiency virus type-1 (HIV-1)-infected CD4+ T lymphocytes. PLOS ONE 2014;9:e93944.
[63] Robinson N, McComb S, Mulligan R, Dudani R, Krishnan L, Sad S. Type I interferon induces necroptosis in macrophages during infection with *Salmonella enterica serovar* Typhimurium. Nat Immunol 2012;13:954–62.
[64] Wang X, Li Y, Liu S, Yu X, Li L, Shi C, et al. Direct activation of RIP3/MLKL-dependent necrosis by herpes simplex virus 1 (HSV-1) protein ICP6 triggers host antiviral defense. Proc Natl Acad Sci U S A 2014;111:15438–43.
[65] Vercammen D, Beyaert R, Denecker G, Goossens V, Van Loo G, Declercq W, et al. Inhibition of caspases increases the sensitivity of L929 cells to necrosis mediated by tumor necrosis factor. J Exp Med 1998;187:1477–85.
[66] Vercammen D, Brouckaert G, Denecker G, Van de Craen M, Declercq W, Fiers W, et al. Dual signaling of the Fas receptor: initiation of both apoptotic and necrotic cell death pathways. J Exp Med 1998;188:919–30.
[67] Holler N, Zaru R, Micheau O, Thome M, Attinger A, Valitutti S, et al. Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. Nat Immunol 2000;1:489–95.
[68] Wilson CA, Browning JL. Death of HT29 adenocarcinoma cells induced by TNF family receptor activation is caspase-independent and displays features of both apoptosis and necrosis. Cell Death Differ 2002;9:1321–33.
[69] Ch'en IL, Beisner DR, Degterev A, Lynch C, Yuan J, Hoffmann A, et al. Antigen-mediated T cell expansion regulated by parallel pathways of death. Proc Natl Acad Sci U S A 2008;105:17463–8.
[70] Thapa RJ, Nogusa S, Chen P, Maki JL, Lerro A, Andrake M, et al. Interferon-induced RIP1/RIP3-mediated necrosis requires PKR and is licensed by FADD and caspases. Proc Natl Acad Sci U S A 2013;110:E3109–18.
[71] Basit F, Cristofanon S, Fulda S. Obatoclax (GX15-070) triggers necroptosis by promoting the assembly of the necrosome on autophagosomal membranes. Cell Death Differ 2013;20:1161–73.
[72] Chromik J, Safferthal C, Serve H, Fulda S. Smac mimetic primes apoptosis-resistant acute myeloid leukaemia cells for cytarabine-induced cell death by triggering necroptosis. Cancer Lett 2014;344:101–9.
[73] Han W, Li L, Qiu S, Lu Q, Pan Q, Gu Y, et al. Shikonin circumvents cancer drug resistance by induction of a necroptotic death. Mol Cancer Ther 2007;6:1641–9.
[74] Kaiser WJ, Sridharan H, Huang C, Mandal P, Upton JW, Gough PJ, et al. Toll-like receptor 3-mediated necrosis via TRIF, RIP3, and MLKL. J Biol Chem 2013;288:31268–79.
[75] Kalai M, Van Loo G, Vanden Berghe T, Meeus A, Burm W, Saelens X, et al. Tipping the balance between necrosis and apoptosis in human and murine cells treated with interferon and dsRNA. Cell Death Differ 2002;9:981–94.
[76] Ma Y, Temkin V, Liu H, Pope RM. NF-kappaB protects macrophages from lipopolysaccharide-induced cell death: the role of caspase 8 and receptor-interacting protein. J Biol Chem 2005;280:41827–34.
[77] Davis CW, Hawkins BJ, Ramasamy S, Irrinki KM, Cameron BA, Islam K, et al. Nitration of the mitochondrial complex I subunit NDUFB8 elicits RIP1- and RIP3-mediated necrosis. Free Radic Biol Med 2010;48:306–17.
[78] Upton JW, Kaiser WJ, Mocarski ES. DAI/ZBP1/DLM-1 complexes with RIP3 to mediate virus-induced programmed necrosis that is targeted by murine cytomegalovirus vIRA. Cell Host Microbe 2012;11:290–7.
[79] O'Donnell MA, Legarda-Addison D, Skountzos P, Yeh WC, Ting AT. Ubiquitination of RIP1 regulates an NF-kappaB-independent cell-death switch in TNF signaling. Curr Biol 2007;17:418–24.
[80] Bonnet MC, Preukschat D, Welz PS, van Loo G, Ermolaeva MA, Bloch W, et al. The adaptor protein FADD protects epidermal keratinocytes from necroptosis in vivo and prevents skin inflammation. Immunity 2011;35:572–82.
[81] Zhang J, Zhang H, Li J, Rosenberg S, Zhang EC, Zhou X, et al. RIP1-mediated regulation of lymphocyte survival and death responses. Immunol Res 2011;51:227–36.
[82] Li J, McQuade T, Siemer AB, Napetschnig J, Moriwaki K, Hsiao YS, et al. The RIP1/RIP3 necrosome forms a functional amyloid signaling complex required for programmed necrosis. Cell 2012;150:339–50.
[83] Sun L, Wang H, Wang Z, He S, Chen S, Liao D, et al. Mixed lineage kinase domain-like protein mediates necrosis signaling downstream of RIP3 kinase. Cell 2012;148:213–27.

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[84] Cai Z, Jitkaew S, Zhao J, Chiang HC, Choksi S, Liu J, et al. Plasma membrane translocation of trimerized MLKL protein is required for TNF-induced necroptosis. Nat Cell Biol 2014;16:55–65.
[85] Chen X, Li W, Ren J, Huang D, He WT, Song Y, et al. Translocation of mixed lineage kinase domain-like protein to plasma membrane leads to necrotic cell death. Cell Res 2014;24:105–21.
[86] Dondelinger Y, Declercq W, Montessuit S, Roelandt R, Goncalves A, Bruggeman I, et al. MLKL compromises plasma membrane integrity by binding to phosphatidylinositol phosphates. Cell Rep 2014;7:971–81.
[87] Hildebrand JM, Tanzer MC, Lucet IS, Young SN, Spall SK, Sharma P, et al. Activation of the pseudokinase MLKL unleashes the four-helix bundle domain to induce membrane localization and necroptotic cell death. Proc Natl Acad Sci U S A 2014;111:15072–7.
[88] Murphy JM, Czabotar PE, Hildebrand JM, Lucet IS, Zhang JG, Alvarez-Diaz S, et al. The pseudokinase MLKL mediates necroptosis via a molecular switch mechanism. Immunity 2013;39:443–53.
[89] Wang H, Sun L, Su L, Rizo J, Liu L, Wang LF, et al. Mixed lineage kinase domain-like protein MLKL causes necrotic membrane disruption upon phosphorylation by RIP3. Mol Cell 2014;54:133–46.
[90] Kearney CJ, Cullen SP, Clancy D, Martin SJ. RIPK1 can function as an inhibitor rather than an initiator of RIPK3-dependent necroptosis. FEBS J 2014;281:4921–34.
[91] Orozco S, Yatim N, Werner MR, Tran H, Gunja SY, Tait SW, et al. RIPK1 both positively and negatively regulates RIPK3 oligomerization and necroptosis. Cell Death Differ 2014;21:1511–21.
[92] Kaiser WJ, Upton JW, Long AB, Livingston-Rosanoff D, Daley-Bauer LP, Hakem R, et al. RIP3 mediates the embryonic lethality of caspase-8-deficient mice. Nature 2011;471:368–72.
[93] Rickard JA, O'Donnell JA, Evans JM, Lalaoui N, Poh AR, Rogers T, et al. RIPK1 regulates RIPK3-MLKL-driven systemic inflammation and emergency hematopoiesis. Cell 2014;157:1175–88.
[94] Upton JW, Kaiser WJ, Mocarski ES. Virus inhibition of RIP3-dependent necrosis. Cell Host Microbe 2010;7:302–13.
[95] Chang DW, Xing Z, Pan Y, Algeciras-Schimnich A, Barnhart BC, Yaish-Ohad S, et al. c-FLIP(L) is a dual function regulator for caspase-8 activation and CD95-mediated apoptosis. EMBO J 2002;21:3704–14.
[96] Pop C, Oberst A, Drag M, Van Raam BJ, Riedl SJ, Green DR, et al. FLIP(L) induces caspase 8 activity in the absence of interdomain caspase 8 cleavage and alters substrate specificity. Biochem J 2011;433:447–57.
[97] Oberst A, Dillon CP, Weinlich R, McCormick LL, Fitzgerald P, Pop C, et al. Catalytic activity of the caspase-8-FLIP(L) complex inhibits RIPK3-dependent necrosis. Nature 2011;471:363–7.
[98] Dillon CP, Oberst A, Weinlich R, Janke LJ, Kang TB, Ben-Moshe T, et al. Survival function of the FADD-CASPASE-8-cFLIP(L) complex. Cell Rep 2012;1:401–7.
[99] Hitomi J, Christofferson DE, Ng A, Yao J, Degterev A, Xavier RJ, et al. Identification of a molecular signaling network that regulates a cellular necrotic cell death pathway. Cell 2008;135:1311–23.
[100] Moquin DM, McQuade T, Chan FK. CYLD deubiquitinates RIP1 in the TNFalpha-induced necrosome to facilitate kinase activation and programmed necrosis. PLOS ONE 2013;8:e76841.
[101] O'Donnell MA, Perez-Jimenez E, Oberst A, Ng A, Massoumi R, Xavier R, et al. Caspase 8 inhibits programmed necrosis by processing CYLD. Nat Cell Biol 2011;13:1437–42.
[102] Bell BD, Leverrier S, Weist BM, Newton RH, Arechiga AF, Luhrs KA, et al. FADD and caspase-8 control the outcome of autophagic signaling in proliferating T cells. Proc Natl Acad Sci U S A 2008;105:16677–82.
[103] Lu JV, Weist BM, van Raam BJ, Marro BS, Nguyen LV, Srinivas P, et al. Complementary roles of Fas-associated death domain (FADD) and receptor interacting protein kinase-3 (RIPK3) in T-cell homeostasis and antiviral immunity. Proc Natl Acad Sci U S A 2011;108:15312–7.

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