



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.

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

The intrinsic apoptotic pathway contributes 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 *pI*, 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 phosphatidylinositol 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

(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 rapid 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-immunoprecipitate 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 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 apoptosis. 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-1 β 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- κ B 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

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- κ B, although RIPK1 is not absolutely required for NF- κ B activation in response to TNF α 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- κ B transcriptional target, caspase-8 activation does not occur. However, if cFLIP is not available for recruitment, or the ubiquitin platform to drive NF- κ B 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-1 α 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 TNF α 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 lethality 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 lethality 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

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 topic for discussion and further research.

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