Cell. Mol. Life Sci. (2010) 67:3241–3253
DOI 10.1007/s00018-010-0413-8

Cellular and Molecular Life Sciences

REVIEW

Going up in flames: necrotic cell injury and inflammatory diseases

Sreerupa Challa · Francis Ka-Ming Chan

Received: 30 March 2010 / Revised: 9 May 2010 / Accepted: 17 May 2010 / Published online: 8 June 2010

Abstract Recent evidence indicates that cell death can be induced through multiple mechanisms. Strikingly, the same death signal can often induce apoptotic as well as non-apoptotic cell death. For instance, inhibition of caspases often converts an apoptotic stimulus to one that causes necrosis. Because a dedicated molecular circuitry distinct from that controlling apoptosis is required for necrotic cell injury, terms such as "programmed necrosis" or "necroptosis" have been used to distinguish stimulus-dependent necrosis from those induced by non-specific traumas (e.g., heat shock) or secondary necrosis induced as a consequence of apoptosis. In several experimental models, programmed necrosis/necroptosis has been shown to be a crucial control point for pathogen- or injury-induced inflammation. In this review, we will discuss the molecular mechanisms that regulate programmed necrosis/necroptosis and its biological significance in pathogen infections, drug-induced cell injury, and trauma-induced tissue damage.

Keywords RIP1 · RIP3 · Programmed necrosis · Inflammation · TNF

S. Challa · F. K.-M. Chan
Department of Pathology, Immunology and Virology Program Diabetes and Endocrinology Center, University of Massachusetts Medical School, 55 Lake Avenue North, Worcester, MA 01655, USA

F. K.-M. Chan (✉)
Department of Pathology, University of Massachusetts Medical School, Room S2-125, Worcester, MA 01655, USA
e-mail: francis.chan@umassmed.edu

Non-apoptotic cell death comes in different flavors

Metazoan homeostasis is achieved through balancing cell proliferation and cell death. The critical role of apoptosis is demonstrated by the many experiments where genetic ablation of components of the apoptosis pathway leads to blunted embryonic development. Work in recent years shows that cell death in multi-cellular organisms comes in many flavors. For instance, paraptosis is a form of non-apoptotic cell death marked by mitochondrial/ER swelling and vacuolation. It was first described as a form of neuronal cell death [1, 2], but has recently been observed in certain tumors treated with the chemotherapeutic drug taxol [3]. Pyroptosis is characterized by potassium efflux and caspase-1-mediated activation of the inflammasome, and the rapid loss of plasma membrane integrity [4]. It is often activated during bacterial infections and thus is an important host defense against pathogens [5]. Autophagy, a cellular survival pathway in response to nutrient deprivation [6], has been shown to cause cell death in certain situations [7–9]. Although these non-apoptotic cell-death pathways often exhibit overlapping morphological features, it is unclear if they also utilize similar molecular machineries to execute the demise of the cell.

The term "necrosis" was once used to describe cell death from trauma such as that induced by heat shock or repeated cycles of freeze/thaw. In tissue culture, cells often undergo late-stage "secondary necrosis" in response to apoptotic stimuli. This observation further contributed to the notion that necrosis is a by-product of apoptosis. However, it is now clear that necrosis can be triggered as a direct result of "extrinsic" stimulation by death cytokines in the TNF superfamily (e.g., TNF, Fas ligand (FasL) and TRAIL (TNF-related apoptosis inducing factor) [10]) or by "intrinsic" signals such as DNA damage [11, 12]. Emerging

evidence indicates that specific signaling pathways are required to mediate necrosis induced by different death signals. Because specific triggers (e.g., death cytokines and their corresponding death receptors) and an elaborate biochemical signaling cascade are involved, terms such as "programmed necrosis" [13] or "necroptosis" [14] have been used to highlight the fact that necrotic cell injury is not merely an "accidental death", but rather a "programmed" form of cellular demise. In this review, we will review recent advances in the molecular regulation of programmed necrosis and discuss the physiological functions of this cell injury pathway in mediating the inflammatory responses against pathogens and other cellular trauma.

Morphological features of programmed necrosis

Programmed necrosis is marked by cell and organelle swelling, extensive formation of intracellular vacuoles, and rapid rupture of plasma membrane (Fig. 1a). The cell swelling observed in necrosis is reminiscent of a process referred to as oncosis [15]. These features are also found in other non-apoptotic forms of cell death. In addition to the rapid loss of plasma membrane integrity, necrosis induced by death cytokines in the TNF superfamily is also distinguished by the lack of classical apoptosis markers such as chromatin condensation or nucleosomal-sized DNA fragmentation (Fig. 1b, c). By flow cytometry, necrotic cells can be distinguished from apoptosis by the concomitant inclusion of fluorescent DNA dyes such as propidium iodide (PI) and positive Annexin V staining (Fig. 1d). DNA fluorescent dyes such as PI are impermeable to live cells and early apoptotic cells. Annexin V measures the exposure of phosphatidyl serine (PS) on the outer cell surface, an early event in apoptosis [16]. In necrotic cells, the positive Annexin V staining is likely due to a combination of PS exposure to the outer leaflet of the plasma membrane and staining of PS on the inner plasma membrane as the membrane becomes permeable.

The pleiotropic adaptor RIP1 mediates programmed necrosis

TNF was recognized as a host factor that could induce tumor regression in the form of necrosis in the 1970s [17]. Since that time, most research had focused on how TNF induces apoptosis. It took almost 30 years for TNF to regain its identity as a necrosis-inducing factor, when it was found that caspase inhibition enhanced cellular sensitivity to TNF-induced necrosis [18]. In 2000, Holler and colleagues reported that in Jurkat T cells that are deficient for FADD or caspase-8 or wild-type T cells treated with the pan-caspase inhibitor zVAD-fmk, the death cytokines TNF, FasL, and TRAIL induce necrosis through the serine/threonine kinase RIP1 [10]. These results were surprising because early experiments indicate that RIP1 is essential for TNF-induced NF-κB activation [19, 20], which confers pro-survival signals that counter the cytotoxic effects of TNF [21]. Subsequent investigations show that RIP1 was also required for NF-κB activation by Toll-like receptor (TLR)-3 and TLR-4 [22, 23].

How does RIP1 mediate pro-necrotic and pro-survival signals? Upon binding to TNF-R1, the E3 ligases cIAP-1 and cIAP-2 mediates K63 and non-K63-dependent RIP1 polyubiquitination at lysine 377 [24–26]. Polyubiquitinated RIP1 binding to NEMO, the regulatory subunit of the IKK complex, is a critical event for NF-κB activation [27, 28]. It is noteworthy that mouse embryonic fibroblasts (MEFs) isolated from RIP1$^{-/-}$ mice were recently shown to exhibit normal TNF-induced NF-κB activation [29]. The basis for these discrepant results is unclear at the moment, but they do raise the possibility that NF-κB activation could occur in a RIP1-independent manner. In addition to RIP1, the linear ubiquitin chain assembly complex (LUBAC) comprised of the RING finger ligases HOIL-1 and HOIP promote linear ubiquitination of NEMO and NF-κB activation [30, 31]. HOIL-1$^{-/-}$ cells fail to activate NF-κB in response to TNF [31]. Interestingly, recruitment of HOIL-1 to the TNF-R1 signaling complex is independent of RIP1 [30, 31]. It remains to be tested whether LUBAC might cooperate with RIP1 to promote TNF-induced NF-κB induction in certain cell types. Nonetheless, these results indicate that RIP1-driven NF-κB activation plays an important role in cell survival in most situations.

In contrast to NF-κB activation, which does not require RIP1 kinase activity, programmed necrosis is completely dependent upon RIP1 kinase function. For example, expression of a kinase inactive RIP1 dominantly inhibits TNF-induced programmed necrosis [32]. Reconstitution of RIP1$^{-/-}$ cells with a kinase inactive RIP1 mutant fails to restore TNF-induced programmed necrosis [10, 32]. Recently, the kinase activity of RIP1 has also been shown to be required for apoptosis induced through an alternative caspase-8 activating pathway [33]. The apoptosis promoting effect of RIP1 is reminiscent of the early observation that RIP1 over-expression causes spontaneous apoptosis [34]. Collectively, these results show that RIP1 is a pleiotropic adaptor that utilizes distinct domains to promote cell survival and cell death by apoptosis or programmed necrosis (Fig. 2).

RIP3: a critical determinant for programmed necrosis

The pleiotropic nature of RIP1 suggests that additional signal adaptors are required to activate or to mediate its

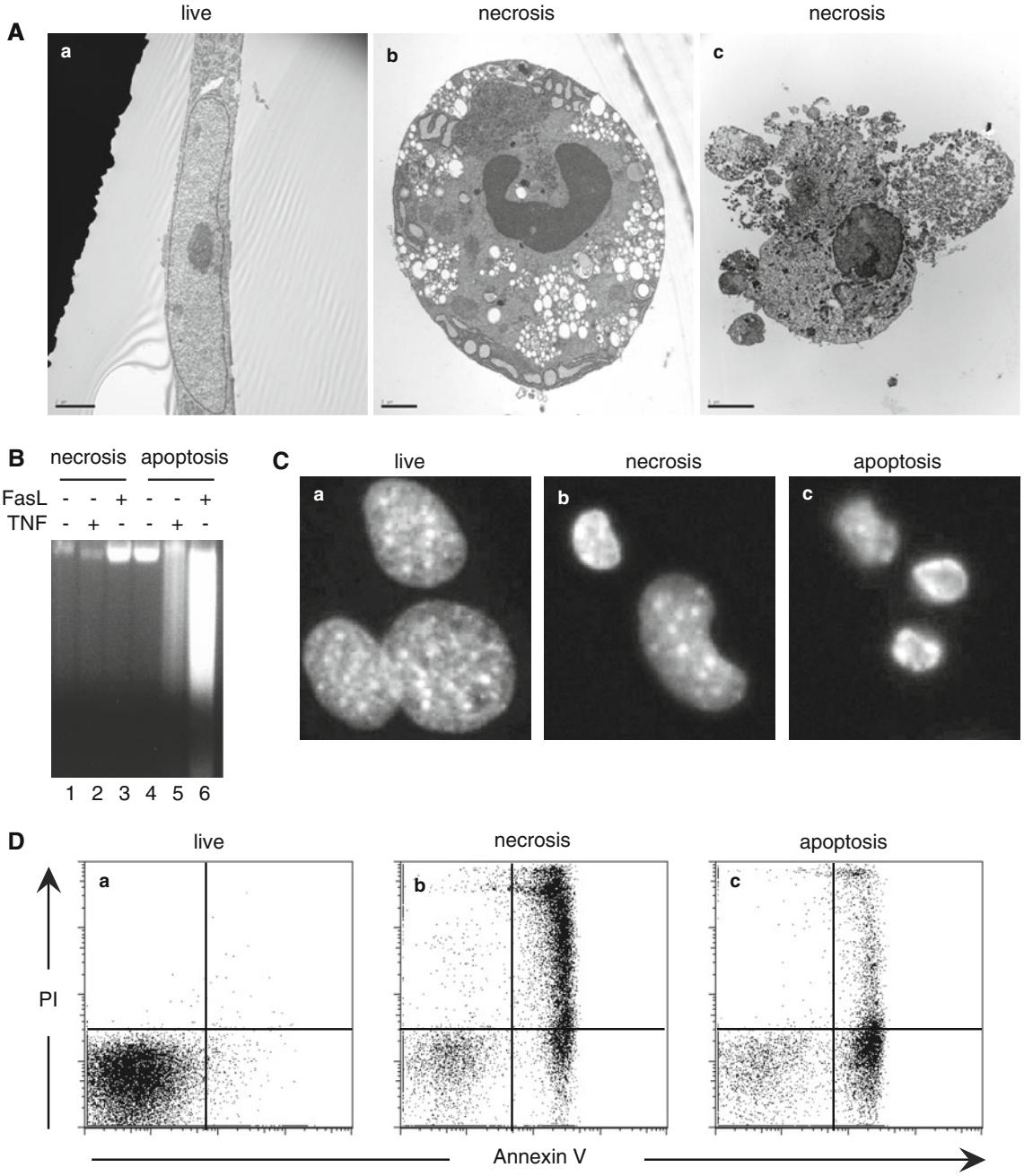

Fig. 1 Morphological distinctions of programmed necrosis. **a** Extensive intracellular vacuolation and rupture of plasma membrane in cells undergoing programmed necrosis. Mouse embryonic fibroblasts (MEFs) were infected with vaccinia virus and treated with TNF. Electron micrograph shows that the MEFs underwent necrosis marked by extensive formation of intracellular vacuoles (*panel b*) and dissolution of plasma membrane integrity (*panel c*). In *panel a*, an uninfected MEF was included for comparison. *Scale bar* 1 μm. **b** TNF-induced programmed necrosis does not result in chromosomal DNA fragmentation. Caspase-8 deficient (*lanes 1–3*) or wild-type (*lanes 4–6*) Jurkat cells were treated with FasL or TNF for 5 h as indicated. Chromosomal DNA was extracted from the cells and resolved by electrophoresis. The results show that DNA fragmentation is only observed in wild-type cells undergoing apoptosis, but is absent in caspase-8 deficient cells undergoing programmed necrosis. **c** Lack of nuclear condensation in programmed necrosis. Untreated wild-type Jurkat cells (*panel a*), TNF-treated caspase-8 deficient Jurkat cells (*panel b*), and FasL-treated wild-type Jurkat cells (*panel c*) were stained with Hoechst 33342. Nuclear morphology was imaged by epi-fluorescence microscopy. The results show that crescent-shaped nuclear condensation was only observed in apoptotic cells, but not necrotic cells. **d** Necrotic cells exhibit concomitant positive staining for Annexin V and PI. Wild-type untreated Jurkat cells (*panel a*), TNF-treated caspase-8 deficient Jurkat cells (*panel b*) or FasL-treated wild-type Jurkat cells were stained with Annexin V and PI and analyzed by flow cytometry. The majority of necrotic cells are positive for both markers, whereas the majority of apoptotic cells exhibit only Annexin V staining

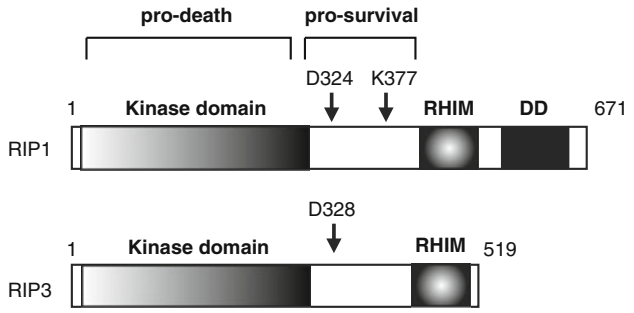

Fig. 2 Domain structures of RIP1 and RIP3, the two crucial kinases for programmed necrosis. The kinase and RHIM domains of RIP1 and RIP3 are required for death cytokine (TNF, FasL, and TRAIL)-induced programmed necrosis. Evidence indicates that the kinase activity of RIP1 is also required for assembly of an alternative caspase-8 activating complex in response to apoptosis induced by TNF and IAP antagonist [33]. In contrast, cleavage by caspase-8 at D324 (for RIP1) [45] and D328 (for RIP3) [46] releases the kinase domains from the RIP kinases and likely prevents the phosphorylation and activation of downstream substrates. Polyubiquitination of RIP1 at K377 inhibits TNF-induced apoptosis, possibly by blocking the transition of the receptor-associated complex to the cytoplasmic death signaling complexes [41]. *DD* death domain

pro-necrotic activity. Recently, three independent groups identified another RIP family kinase, RIP3, as an essential component of death ligand-induced programmed necrosis [35–37]. Like RIP1, RIP3 contains an amino terminal serine/threonine kinase domain (Fig. 2). In addition, RIP3 contains a RIP homotypic interaction motif (RHIM) that is also found in RIP1 and several other signal adaptors involved in inflammatory signaling (Fig. 2). During programmed necrosis, RIP1 and RIP3 form a cytoplasmic "pro-necrotic complex" via the RHIM (Fig. 3). The stable assembly of this RIP1-RIP3 complex further requires the phosphorylation of both kinases, an event that occurs specifically during programmed necrosis, but not in apoptosis or NF-κB activation [35, 36]. Although the precise hierarchy of RIP1 and RIP3 activation during programmed necrosis has yet to be determined, the fact that RIP1, but not RIP3, is recruited to the receptor signaling complex suggests that RIP1 is the upstream activating kinase (Fig. 3). Interestingly, cells that lack RIP3 expression are resistant to programmed necrosis despite normal RIP1 expression [36]. Hence, RIP3 relays the pro-necrotic signal from RIP1 and determines cellular sensitivity to programmed necrosis.

Protein ubiquitination and deubiquitination in programmed necrosis

RIP1 recruited to TNF-R1 is heavily modified by ubiquitination. By contrast, RIP1 found in the cytoplasmic RIP1-RIP3 complex was largely unmodified by ubiquitination [35, 36]. The differential ubiquitination pattern implies that RIP1 might undergo deubiquitination as it transitions from the membrane receptor-signaling complex to the cytoplasmic signaling complex. The deubiquitinating enzymes A20 and cylindromatosis (CYLD) have been shown to facilitate hydrolysis of the polyubiquitin chain on RIP1 [38, 39]. Interestingly, CYLD was recently identified in a genome-wide RNAi screen as a positive regulator for programmed necrosis [40]. CYLD was also found to promote the assembly of an alternative caspase-8 activating complex containing RIP1 and FADD [33]. Although the biochemical mechanism is still vague at present, it is tempting to speculate that CYLD or A20 might facilitate programmed necrosis by removing the polyubiquitin chain from RIP1 (Fig. 3). According to this model, polyubiquitination of RIP1 might interfere with programmed necrosis by sterically hindering the recruitment of RIP3 or other components of the pro-necrotic signaling complex. In fact, such a mechanism appears to be important for the anti-apoptotic function of RIP1. Ting and colleagues showed that RIP1 confers protection against apoptosis in cells that stably express a dominant negative mutant of IκBα, especially early during TNF stimulation [41]. Strikingly, mutation at the RIP1 polyubiquitination site, K377, abolished the early protective effect of RIP1 [41]. Polyubiquitinated RIP1 binds to NEMO, which prevents the interaction between RIP1 and caspase-8 and blocks the activation of caspase-8 by RIP1 [41, 42]. Although it is unclear if deubiquitinases like A20 or CYLD are also involved in controlling this anti-apoptotic function of RIP1, these results are consistent with the notion that ubiquitination and deubiquitination of RIP1 plays a critical role in controlling its pro-survival and pro-death cell death activities (Fig. 3).

Caspase-8 inhibits programmed necrosis by cleavage of RIP1 and RIP3

As we have discussed above, one of the early clues indicating a dedicated molecular circuitry for programmed necrosis came from the observation that caspase inhibition often sensitize cells to programmed necrosis. However, although caspase inhibition exacerbates the necrotic response, it is not an obligate requirement for programmed necrosis. For example, in Jurkat T cells expressing TNFR-1 and TNFR-2 [43], TNF stimulation causes both apoptosis and programmed necrosis [44]. In fact, induction of necrosis-specific RIP1-RIP3 kinase activity could be detected in these cells [35]. Caspase inhibition blocks apoptosis and renders programmed necrosis the predominant form of cell death [32, 44]. The sensitizing effect of caspase inhibition on programmed necrosis is at least in part due to the fact that RIP1 and RIP3 are substrates for

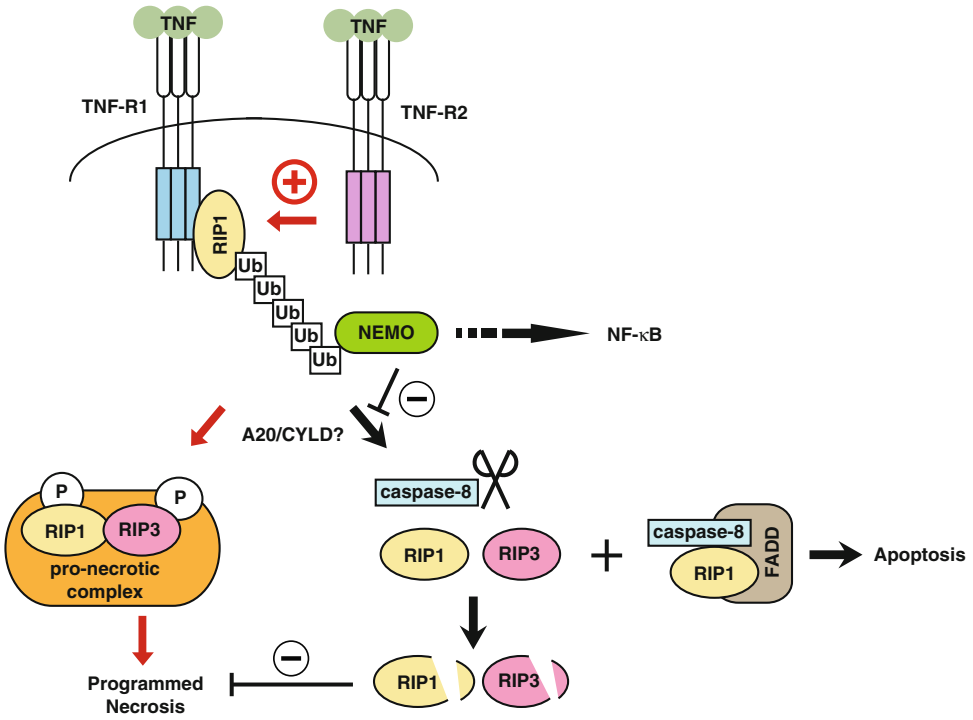

Fig. 3 Regulation of programmed necrosis by ubiquitination, phosphorylation, and caspase cleavage. TNF-induced programmed necrosis is regulated at multiple steps involving positive (indicated by *red arrows*) and negative (indicated by *black arrows*) mechanisms. TNF-R2 signaling enhances TNF-R1 mediated programmed necrosis through a poorly defined mechanism [32, 43]. Upon binding to TNF-R1, RIP1 becomes modified by polyubiquitination at K377. Polyubiquitinated RIP1 binds to NEMO, the regulatory subunit of NF-κB, to promote NF-κB activation. NF-κB activation counters the death signals by inducing pro-survival genes. The plasma membrane-associated receptor signaling complex containing polyubiquitinated RIP1 migrates to the cytoplasm where the receptor falls off the complex and RIP1 becomes deubiquitinated. The deubiquitinating enzymes A20 or CYLD may facilitate this reaction. In the presence of caspase-8 inhibition, RIP1 and RIP3 interact with each other via the RHIM to form the pro-necrotic signaling complex. This interaction is further stabilized by phosphorylation of both kinases. However, active caspase-8 cleaves RIP1 and possibly RIP3 to blunt the pro-necrotic complex. The active caspase-8 complex can go onto cleave additional substrates, culminating in cell death by apoptosis

caspase-8 [45, 46]. In fact, RIP1 cleavage could be detected within the caspase-8-associated complex in apoptotic cells, but not in cells undergoing programmed necrosis [35]. The caspase cleavage site for RIP1 has been mapped to the boundary between the kinase domain and the carboxyl end of the protein at D324 (Fig. 2) [45]. Thus, caspase-8-mediated cleavage of RIP1 will result in the release of the kinase domain from the protein–protein interaction domains of RIP1. In this scenario, access and phosphorylation of downstream substrates of RIP1 will likely be blocked (Fig. 3).

Compared to the results on RIP1 cleavage, the evidence supporting RIP3 cleavage and inactivation by caspase-8 is weaker. Although RIP3 cleavage at D328 has been observed in transfection studies [46], it has not been detected in endogenous signaling complexes. Nonetheless, a non-cleavable mutant of RIP3 is sufficient to abrogate the necrosis-enhancing effect of the pan-caspase inhibitor zVAD-fmk [37]. Taken together, these results strongly support the model that caspase inhibitors such as zVAD-fmk facilitate programmed necrosis in part by preserving the integrity of RIP1 and RIP3 (Fig. 3).

Reactive oxygen species: critical effectors for programmed necrosis

The molecular events that drive the morphological changes associated with programmed necrosis, namely intracellular vacuoles, organelle and cell swelling, and eventually rupture of the plasma membrane, are poorly defined. A widely accepted view is that reactive oxygen species (ROS) play crucial roles in the execution of programmed necrosis [47]. For example, high levels of ROS could be detected in necrotic cell death. ROS scavengers such as butylated hydroxyanisole (BHA) are effective inhibitors of programmed necrosis in many cell types [48]. At present, it is not clear how ROS cause the cellular damages observed in necrosis. High levels of ROS could induce lipid membrane peroxidation and plasma membrane leakage [49].

However, since ROS also regulate other biological processes including apoptotic death [50, 51], additional factors are clearly required to synergize with ROS to promote necrosis.

ROS are produced as by-products of mitochondrial oxidative phosphorylation. Inhibitors that block mitochondrial Complex I, a major site of ROS production, was effective against TNF-induced programmed necrosis in L929 cells [48, 52]. Several reports indicate that the necrosis-specific kinases RIP1 and RIP3 act upstream to regulate ROS production [35, 53]. Although the results are somewhat controversial, RIP1 and RIP3 have both been shown to co-localize with the mitochondria [53, 54]. Interestingly, several mitochondrial metabolic enzymes were reported to be possible substrates for RIP3 [37]. Thus, these results support a role for the cytoplasmic RIP1-RIP3 complex in ROS production, possibly by acting directly on the mitochondrial machinery that regulates oxidative phosphorylation. In contrast to these results, several recent reports show that plasma membrane-associated complex containing TNFR-1, riboflavin kinase (RFK) and NADPH oxidase 1 (Nox1) is essential for ROS production in response to TNF-induced programmed necrosis [55, 56]. These discrepant results might be reconciled by different mechanisms of ROS generation in different cell types. Alternatively, membrane-induced ROS could synergize with mitochondrial-produced ROS to drive programmed necrosis. Clearly, more work is needed to clarify the mechanism that generates ROS during necrotic cell injury.

Programmed necrosis: a driver of inflammation

The identification of a molecular pathway that regulates programmed necrosis raises questions about its physiological functions. From an immunological perspective, apoptosis is a non-inflammatory form of cell death because apoptotic cells expose "eat-me" signals such as PS on the cell surface early during the cell death process. This ensures the rapid clearance of apoptotic cells by phagocytes before they progress to secondary necrosis and prevents the release of cellular adjuvants that can stimulate inflammation [57]. The importance of timely removal of apoptotic cells is demonstrated by the induction of autoimmune inflammatory diseases when phagocytosis of apoptotic cells is inhibited [58]. By contrast, cells undergoing necrosis rapidly lose plasma membrane integrity, which leads to the release of immuno-stimulatory adjuvants such as monosodium urate crystals and the DNA-binding factor HMGB1 [59–62]. Interestingly, specific receptors in the C-type lectin family have recently been implicated to facilitate necrotic cell detection and inflammation signaling [63, 64]. All in all, these results suggest a unique role for programmed necrosis in inflammatory processes. In the next few sections, we will provide several examples in which programmed necrosis plays crucial roles in various physiological and pathological responses.

Programmed necrosis in T-cell expansion

Immune homeostasis is maintained through the delicate balance between cell proliferation and cell death. Surprisingly, many components of the machinery that control lymphocyte cell death also possess the dual function of regulating lymphocyte proliferation [65, 66]. In particular, the essential adaptors for death cytokine-induced apoptosis, FADD and caspase-8, are required for $CD4^+$ T-cell proliferation in response to antigen receptor activation [67, 68]. Lymphocytes from mice that lack FADD or caspase-8 or those that express a dominant negative mutant FADD (FADD-dn) exhibit defective proliferation and undergo death in response to antigen receptor stimulation [67, 69–71]. These results were corroborated by the immuno-deficiencies observed in human patients with caspase-8 mutations [68, 72]. Initial reports suggest that the defective activation in response to antigen receptor stimulation in caspase-8-deficient T-cells was due to the failure to activate NF-κB via the Bcl10-CARMA1-MALT1 complex [71, 72]. However, recent results indicate that NF-κB activation in caspase-$8^{-/-}$ $CD4^+$ T cells is normal [73]. Instead, antigen receptor-induced cell death and expansion of caspase-$8^{-/-}$ and FADD-dn T-cells were rescued by the RIP1-specific inhibitor necrostatin-1 [73, 74]. Necrostatin-1 specifically inhibits the pro-necrotic RIP1 kinase activity, but not the kinase activities of other kinases including RIP2 and RIP3 [75, 76]. These results imply that FADD and caspase-8 actively suppress RIP1-dependent programmed necrosis to ensure antigen-specific lymphocyte expansion. FADD and caspase-8 are also required for TLRs-induced B-cell proliferation [77, 78]. However, it is unclear if the defective proliferation in $FADD^{-/-}$ and caspase-$8^{-/-}$ B-cells was due to RIP1-dependent programmed necrosis. Interestingly, $RIP3^{-/-}$ T-cells are resistant to activation-induced cell death in the presence of caspase inhibitors [35]. It will be interesting to determine whether RIP3 might similarly control antigen-induced lymphocyte expansion.

Programmed necrosis in poxvirus infection

One of the hallmarks of programmed necrosis is the rapid loss of plasma membrane integrity. For cytolytic viruses that require host-cell lysis to release new viral progenies,

necrotic cell death might facilitate viral dissemination within the host. Many viral inhibitors potently inhibit the host apoptosis machinery [79]. When apoptosis is inhibited, the host might employ programmed necrosis as a backup or alternative mechanism to eliminate the infected cells. Moreover, the release of endogenous adjuvants from the necrotic cells might facilitate innate inflammatory responses against the virus. This appears to be the case for certain poxviruses. Poxviruses encoded serpins such as CrmA that inhibit caspase-1, -6, and -8 by complexing with the p20 subunit of the caspases via thioester bonds [80]. Despite inhibition of caspases and apoptosis, cells infected with the poxvirus vaccinia virus remain sensitive to the cytotoxic effects of TNF with classical necrotic morphologies [81]. Deletion of RIP1 or RIP3 rescues vaccinia virus infected cells from TNF-induced programmed necrosis [32, 35]. Thus, programmed necrosis circumvents viral inhibition of host apoptosis to eliminate vaccinia virus-infected cells.

Could programmed necrosis be more than a backup cell death response to apoptosis? Poxvirus infection elicits strong inflammatory responses. Recent evidence indicates that TNF-induced programmed necrosis might play a crucial role in eliciting the early inflammatory response in vaccinia virus infections. In mice, vaccinia virus infection induces TNF expression as early as 24 h [35]. Mice deficient in TNFR-1 or TNFR-2 exhibited defective viral clearance [32, 82]. Vaccinia virus causes lethal infection in nude mice. However, nude mice were protected from death when recombinant vaccinia virus encoding mouse TNF was used [83]. These results highlight a crucial role for TNF in the early innate immune control of poxvirus infections.

The RIP1–RIP3 pro-necrotic complex was detected in the liver of vaccinia virus infected mice 24–48 h post-infection [35]. Moreover, extensive necrotic cell injury was detected in the liver and the visceral fat pads of infected animals. Interestingly, inflammatory cells were concentrated in these regions of extensive necrotic cell death [35], an observation that is consistent with the notion that necrosis drives the anti-viral inflammatory response. Strikingly, necrotic cell injury and inflammation were conspicuously absent in similarly infected RIP3$^{-/-}$ mice. The failure to mount an innate inflammatory response led to drastically increased viral replication and mortality in the RIP3$^{-/-}$ mice [35]. Furthermore, TNF-R2$^{-/-}$ mice also exhibited increased viral replication, lack of necrotic cell injury, or inflammation [32, 35]. Thus, TNF-R2$^{-/-}$ mice were phenocopy of the RIP3$^{-/-}$ mice. Taken together, these results indicate that TNF- and RIP3-induced programmed necrosis is an important anti-viral response against poxvirus infections (Fig. 4a).

Programmed necrosis in MCMV infection

Cytomegaloviruses (CMVs) are betaherpesviruses that employ multiple strategies to subvert the host apoptosis machinery [84]. Recent evidence indicates that productive infection with murine cytomegalovirus (MCMV) requires inhibition of RIP3-dependent programmed necrosis by the MCMV inhibitor M45 [85]. M45 was originally identified as an apoptosis inhibitor in MCMV-infected endothelial cells [86]. More recently, M45 was shown to also inhibit programmed necrosis through binding to RIP1 and RIP3 via the RHIM-like motif at its amino terminus [87, 88]. M45 also shares homology with mammalian ribonucleotide reductase (RNR) at the carboxyl terminus but exhibits no enzymatic activity [89]. Thus, the carboxyl terminal RNR domain is unlikely to contribute to its anti-death activity.

The significance of inhibition of programmed necrosis in MCMV infection was beautifully demonstrated recently using mutant MCMV infection in RIP3$^{-/-}$ mice [85]. An MCMV virus expressing a M45 mutant with tetra-alanine substitutions in the RHIM failed to establish productive infection in tissue culture as well as in mice due to extensive programmed necrosis (Fig. 4b). Strikingly, infection of the M45 mutant virus into RIP3$^{-/-}$ cells or RIP3$^{-/-}$ mice restored productive infection [85]. Thus, productive infection by MCMV requires M45-mediated inhibition of RIP3-dependent programmed necrosis. Surprisingly, siRNA-mediated silencing of RIP1 or the RIP1-specific inhibitor necrostatin-1 did not protect mutant MCMV-infected cells from programmed necrosis [85]. This last result is significant because it implies that RIP1 is dispensable for some types of programmed necrosis.

Although the evidence strongly favors M45 inhibition of RIP3-dependent programmed necrosis as an essential step in establishing productive MCMV infection, it is noteworthy that M45 could modulate other cellular processes to ensure productive infection. As we have discussed above, M45 was first identified as an apoptosis inhibitor in MCMV-infected endothelial cells [86]. Inhibition of apoptosis could serve similar function to that of programmed necrosis inhibition to prevent premature death of infected cells. In addition, M45 was shown to inhibit NF-κB signaling by the DNA-dependent activator of interferon regulatory factor or Z-DNA binding protein 1 (DAI/ZBP1) [90]. DAI mediates NF-κB activation through interaction with RIP1 and RIP3 via the RHIM [91]. Inhibition of NF-κB by M45 might enhance viral replication by suppressing the innate inflammatory responses against the virus. However, it does not explain why the M45 mutant virus also failed to establish productive infection in tissue culture [85].

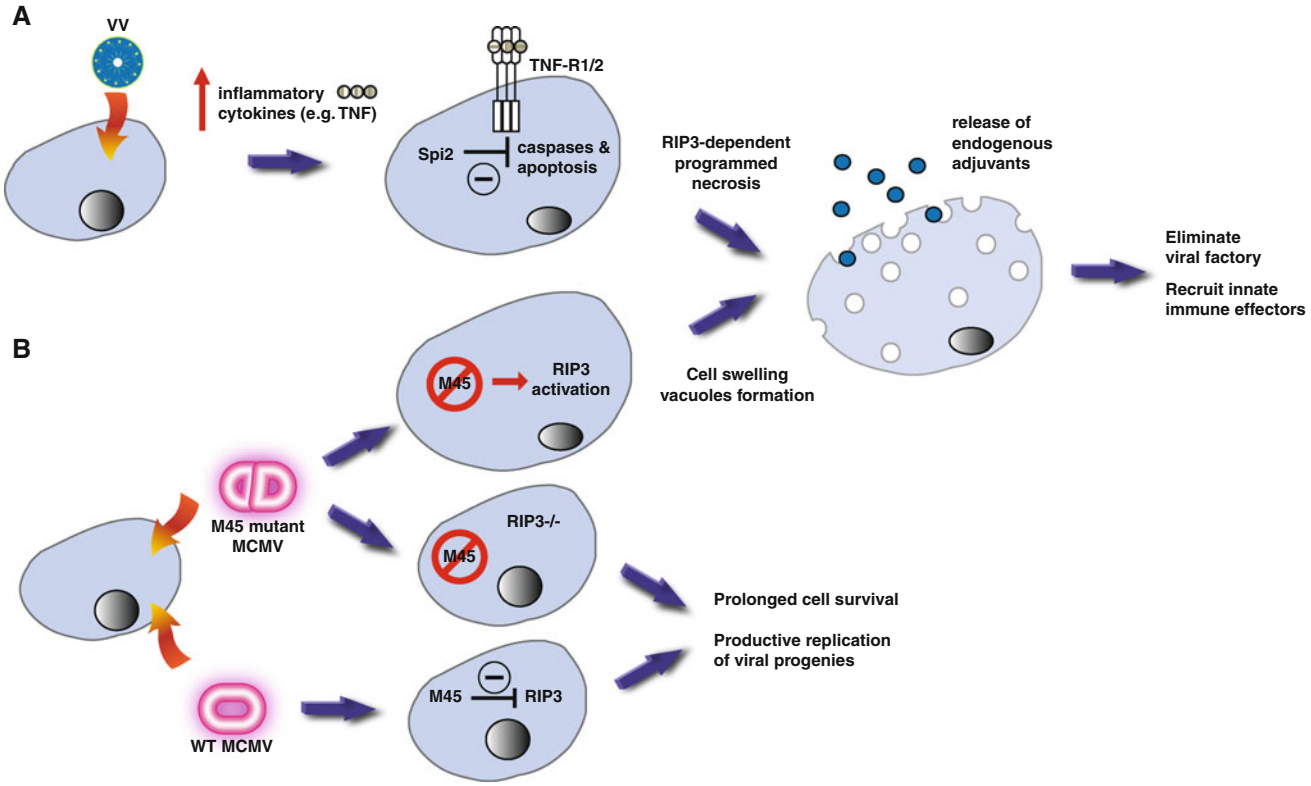

Fig. 4 RIP3-dependent programmed necrosis controls the outcome of vaccinia virus and MCMV infections. **a** TNF-induced, RIP3-dependent programmed necrosis is an important innate inflammatory response against vaccinia virus infection. TNF and other inflammatory cytokines are rapidly induced early during vaccinia virus infection. TNF-induced apoptosis is inhibited by the virally encoded caspase inhibitor Spi2, which skews the response towards RIP3-dependent programmed necrosis. The death of the infected cells limits the viral factory and contributes to the clearance of the virus. In addition, programmed necrosis might facilitate cross-priming of dendritic cells as the plasma membrane ruptures. This might result in further production of inflammatory cytokines and the recruitment of innate immune effector cells to the site of infection. **b** Inhibition of RIP3-dependent programmed necrosis is crucial for productive infection by MCMV. Infection with MCMV containing a mutant M45 leads to RIP3-dependent programmed necrosis, premature termination of the viral replication cycle and failure for the virus to establish productive infection. Inactivation of RIP3 by siRNA or infection in RIP3$^{-/-}$ cells restored productive infection by the mutant virus similar to that observed with wild-type MCMV

Viral inhibitors of programmed necrosis

The presence of a programmed necrosis inhibitor in MCMV strongly implicates that inhibition of programmed necrosis is an important viral immune evasion strategy. Indeed, this notion is further bolstered by the discovery of certain viral FLIPs (FLICE/caspase-8 inhibitory protein) that inhibit programmed necrosis [32]. Viral FLIPs were first identified as inhibitors against death cytokine-induced apoptosis [92, 93]. They contain two tandem death effector domains (DEDs) that interact with the DEDs of caspase-8 and FADD [94]. Interestingly, a subset of vFLIPs, including MC159 encoded by the poxvirus *Molluscum contagiosum* and E8 encoded by the *Equine herpesvirus-2* (EHV-2), also potently inhibit TNF-induced programmed necrosis [32]. Thus, the RHIM-containing M45 and the vFLIPs represent two distinct classes of viral inhibitors of programmed necrosis.

Structural studies indicate that the vFLIP MC159 binds to FADD to prevent FADD oligomerization [95]. However, binding to FADD and caspase-8 is not sufficient for apoptosis inhibition, since this interaction was preserved in some MC159 mutants that fail to prevent apoptosis [96]. This surprising finding might be reconciled by the apoptosis inhibitory function of a carboxyl terminal TRAF3 binding site in MC159 [97]. By contrast, the mechanism by which vFLIPs inhibit programmed necrosis is less defined. MC159 has been shown to interact with many signal adaptors that regulate NF-κB activation including TRAF2 and RIP1 [98]. As such, it is possible that MC159 and E8 inhibit programmed necrosis by targeting RIP1. Interestingly, we found that MC159 also enhances NF-κB induction (unpublished observation). Consequently, mice expressing a MC159 transgene under the ubiquitously expressed H2-K^{b} promoter [99] exhibited enhanced clearance of vaccinia virus infection (unpublished observation). These results are in contrast to an early report that shows an inhibitory effect of MC159 on NF-κB activation [100]. These disparate results could be reconciled by the

observation that MC159 modulates NF-κB activation in a dose-dependent manner. At low concentrations, MC159 enhances NF-κB activation, whereas at high expression level, it suppresses NF-κB activation (unpublished observation). Thus, vFLIPs such as MC159 might inhibit programmed necrosis through direct interference of the necrotic signaling pathway or by enhancing the NF-κB driven pro-survival signals. Regardless of the mechanisms, the discovery of viral inhibitors against programmed necrosis highlights the importance of this response in antiviral immunity. Necrotic cell injury has also been reported in certain bacterial infections including Clostridium [101] Yersinia [102], and Pseudomonas [103]. However, the role of RIP1 and RIP3 in bacteria-induced necrosis has yet to be tested.

Necrosis in chemical- and trauma-induced cell injury

Besides regulating pathogen-induced inflammation, the RIP1-RIP3 axis has also been implicated to regulate sterile inflammation. Cerulein causes injury to the pancreatic acinar cells and inflammation [104]. Cerulein-induced pancreatitis might involve TNF signaling, since neutralization of TNF with the TNF blocker Etanercept attenuates cerulein-induced pancreas necrosis [105]. Moreover, the induction of inflammatory effectors such as IL-6 and iNOS is reduced in cerulein-treated TNF$^{-/-}$ mice [106]. Importantly, RIP3$^{-/-}$ mice were also resistant to cerulein-induced pancreatitis [36, 37]. However, at 10 h post-treatment, RIP3$^{-/-}$ mice exhibit similar tissue injury in the pancreas compared with their wild-type counterparts. The differential protection against pancreatic acinar cell necrosis was only apparent after 24 h of cerulein treatment in the RIP3$^{-/-}$ mice [36]. Hence, RIP3-dependent necrosis appears to exacerbate rather than initiate cerulein-induced cell injury in the pancreas.

In addition to drug-induced cell injury, RIP1/3-dependent programmed necrosis also appears to be involved in trauma-induced tissue damage. Ischemia reperfusion induced injury of the brain and myocardial tissues are accompanied by an increase in inflammatory cytokine production including TNF [107, 108]. In mouse models of brain damage induced by ischemia reperfusion or controlled cortical impact, the RIP1-specific kinase inhibitor necrostatin-1 was effective in ameliorating the injury [76, 109]. Similarly, necrostatin-1 reduced the infarct size of the heart in a mouse model of ischemia–reperfusion induced myocardial injury [110]. Although it is not clear if TNF-like cytokines are the triggers for the tissue injury, these results do support a role for RIP1-dependent necrosis in tissue necrosis induced by trauma. However, since chemical inhibitors often have off-target effects, further experiments such as those comparing the responses of RIP3$^{-/-}$ mice will be useful in validating the requirement for programmed necrosis in these trauma-induced disease models. Nonetheless, these results raise the interesting possibility that RIP1 and RIP3 might function as an "alarm" for multi-cellular organisms in response to injury induced by pathogens, drugs, or physical trauma (Fig. 5).

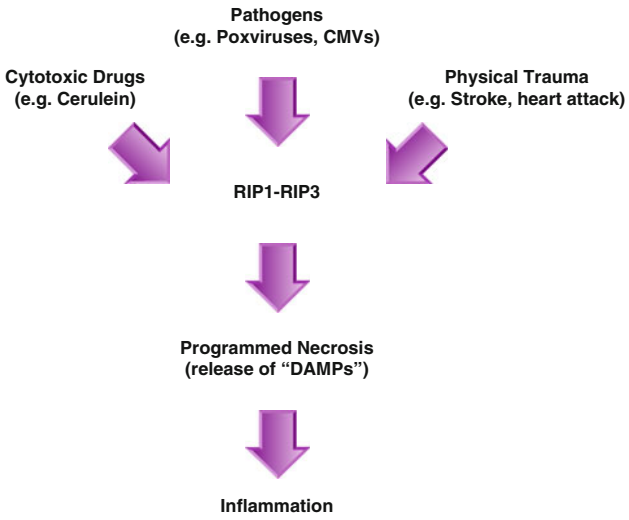

Fig. 5 Hypothetical model of programmed necrosis as a sensor for different forms of cellular insults. Programmed necrosis can be induced by diverse insults from pathogens, cytotoxic drugs, and physical trauma. As RIP1/RIP3-dependent programmed necrosis occurs, cellular "danger-associated molecular patterns (DAMPs)" are released into the tissue milieu. The released DAMPs can cause inflammation through activation of various DAMP receptors such as Toll-like receptors (TLRs) or the inflammasomes [57]

Concluding remarks

From a molecular perspective, we are only beginning to understand the biochemical pathway that controls programmed necrosis. Future work will no doubt focus on the identification of additional components that regulate this pathway. However, from the limited information we have at present, it is clear that programmed necrosis is not merely a backup form of cell suicide. Rather, programmed necrosis is linked with many disease pathologies, especially those that are associated with inflammation. We have described several examples in which inflammation induced by pathogens, cytotoxic drugs, and trauma requires an intact programmed necrotic response pathway. These studies raise the tantalizing possibility that programmed necrosis might have broader roles in controlling other inflammatory conditions such as autoimmune diseases or even cancers. Inhibitors that target components of the necrosis pathway, such as necrostatin-1, might have therapeutic value in treating these diseases.

Acknowledgments This work is supported by NIH grants AI088502 and AI083497 (to F.K.C.). F.K.C. is a member of the UMass DERC (DK32520). We thank J. Upton, W. Kaiser and E. Mocarski for sharing their results ahead of print publication.

References

1. Schneider D, Gerhardt E, Bock J, Muller MM, Wolburg H, Lang F, Schulz JB (2004) Intracellular acidification by inhibition of the Na+/H+-exchanger leads to caspase-independent death of cerebellar granule neurons resembling paraptosis. Cell Death Differ 11:760–770
2. Jadus MR, Chen Y, Boldaji MT, Delgado C, Sanchez R, Douglass T, Al-Atar U, Schulz W, Lloyd C, Wepsic HT (2003) Human U251MG glioma cells expressing the membrane form of macrophage colony-stimulating factor (mM-CSF) are killed by human monocytes in vitro and are rejected within immunodeficient mice via paraptosis that is associated with increased expression of three different heat shock proteins. Cancer Gene Ther 10:411–420
3. Chen TS, Wang XP, Sun L, Wang LX, Xing D, Mok M (2008) Taxol induces caspase-independent cytoplasmic vacuolization and cell death through endoplasmic reticulum (ER) swelling in ASTC-a-1 cells. Cancer Lett 270:164–172
4. Fernandes-Alnemri T, Wu J, Yu JW, Datta P, Miller B, Jankowski W, Rosenberg S, Zhang J, Alnemri ES (2007) The pyroptosome: a supramolecular assembly of ASC dimers mediating inflammatory cell death via caspase-1 activation. Cell Death Differ 14:1590–1604
5. Bergsbaken T, Fink SL, Cookson BT (2009) Pyroptosis: host cell death and inflammation. Nat Rev Microbiol 7:99–109
6. Nakatogawa H, Suzuki K, Kamada Y, Ohsumi Y (2009) Dynamics and diversity in autophagy mechanisms: lessons from yeast. Nat Rev Mol Cell Biol 10:458–467
7. Berry DL, Baehrecke EH (2007) Growth arrest and autophagy are required for salivary gland cell degradation in Drosophila. Cell 131:1137–1148
8. Samara C, Syntichaki P, Tavernarakis N (2008) Autophagy is required for necrotic cell death in *Caenorhabditis elegans*. Cell Death Differ 15:105–112
9. Koike M, Shibata M, Tadakoshi M, Gotoh K, Komatsu M, Waguri S, Kawahara N, Kuida K, Nagata S, Kominami E, Tanaka K, Uchiyama Y (2008) Inhibition of autophagy prevents hippocampal pyramidal neuron death after hypoxic-ischemic injury. Am J Pathol 172:454–469
10. Holler N, Zaru R, Micheau O, Thome M, Attinger A, Valitutti S, Bodmer JL, Schneider P, Seed B, Tschopp J (2000) Fas triggers an alternative, caspase-8-independent cell death pathway using the kinase RIP as effector molecule. Nat Immunol 1:489–495
11. Xu Y, Huang S, Liu ZG, Han J (2006) Poly(ADP-ribose) polymerase-1 signaling to mitochondria in necrotic cell death requires RIP1/TRAF2-mediated JNK1 activation. J Biol Chem 281:8788–8795
12. Zong WX, Ditsworth D, Bauer DE, Wang ZQ, Thompson CB (2004) Alkylating DNA damage stimulates a regulated form of necrotic cell death. Genes Dev 18:1272–1282
13. Declercq W, Vanden Berghe T, Vandenabeele P (2009) RIP kinases at the crossroads of cell death and survival. Cell 138:229–232
14. Christofferson DE, Yuan J (2010) Necroptosis as an alternative form of programmed cell death. Curr Opin Cell Biol 22:263–268
15. Majno G, Joris I (1995) Apoptosis, oncosis, and necrosis. An overview of cell death. Am J Pathol 146:3–15
16. Krysko DV, Vanden Berghe T, D'Herde K, Vandenabeele P (2008) Apoptosis and necrosis: detection, discrimination and phagocytosis. Methods 44:205–221
17. Carswell EA, Old LJ, Kassel RL, Green S, Fiore N, Williamson B (1975) An endotoxin-induced serum factor that causes necrosis of tumors. Proc Natl Acad Sci USA 72:3666–3670
18. Vercammen D, Beyaert R, Denecker G, Goossens V, Van Loo G, Declercq W, Grooten J, Fiers W, Vandenabeele P (1998) Inhibition of caspases increases the sensitivity of L929 cells to necrosis mediated by tumor necrosis factor. J Exp Med 187:1477–1485
19. Hsu H, Huang J, Shu HB, Baichwal V, Goeddel DV (1996) TNF-dependent recruitment of the protein kinase RIP to the TNF receptor- 1 signaling complex. Immunity 4:387–396
20. Ting AT, Pimentel-Muinos FX, Seed B (1996) RIP mediates tumor necrosis factor receptor 1 activation of NF-kappaB but not Fas/APO-1-initiated apoptosis. EMBO J 15:6189–6196
21. Karin M, Lin A (2002) NF-kappaB at the crossroads of life and death. Nat Immunol 3:221–227
22. Meylan E, Burns K, Hofmann K, Blancheteau V, Martinon F, Kelliher M, Tschopp J (2004) RIP1 is an essential mediator of Toll-like receptor 3-induced NF-kappa B activation. Nat Immunol 5:503–507
23. Cusson-Hermance N, Khurana S, Lee TH, Fitzgerald KA, Kelliher MA (2005) Rip1 mediates the Trif-dependent toll-like receptor 3- and 4-induced NF-{kappa}B activation but does not contribute to interferon regulatory factor 3 activation. J Biol Chem 280:36560–36566
24. Varfolomeev E, Goncharov T, Fedorova AV, Dynek JN, Zobel K, Deshayes K, Fairbrother WJ, Vucic D (2008) c-IAP1 and c-IAP2 are critical mediators of tumor necrosis factor alpha (TNFalpha)-induced NF-kappaB activation. J Biol Chem 283:24295–24299
25. Bertrand MJ, Milutinovic S, Dickson KM, Ho WC, Boudreault A, Durkin J, Gillard JW, Jaquith JB, Morris SJ, Barker PA (2008) cIAP1 and cIAP2 facilitate cancer cell survival by functioning as E3 ligases that promote RIP1 ubiquitination. Mol Cell 30:689–700
26. Mahoney DJ, Cheung HH, Mrad RL, Plenchette S, Simard C, Enwere E, Arora V, Mak TW, Lacasse EC, Waring J, Korneluk RG (2008) Both cIAP1 and cIAP2 regulate TNFalpha-mediated NF-kappaB activation. Proc Natl Acad Sci USA 105:11778–11783
27. Ea CK, Deng L, Xia ZP, Pineda G, Chen ZJ (2006) Activation of IKK by TNFalpha requires site-specific ubiquitination of RIP1 and polyubiquitin binding by NEMO. Mol Cell 22:245–257
28. Wu CJ, Conze DB, Li T, Srinivasula SM, Ashwell JD (2006) Sensing of Lys 63-linked polyubiquitination by NEMO is a key event in NF-kappaB activation [corrected]. Nat Cell Biol 8:398–406
29. Wong WW, Gentle IE, Nachbur U, Anderton H, Vaux DL, Silke J (2010) RIPK1 is not essential for TNFR1-induced activation of NF-kappaB. Cell Death Differ 17:482–487
30. Haas TL, Emmerich CH, Gerlach B, Schmukle AC, Cordier SM, Rieser E, Feltham R, Vince J, Warnken U, Wenger T, Koschny R, Komander D, Silke J, Walczak H (2009) Recruitment of the linear ubiquitin chain assembly complex stabilizes the TNF-R1 signaling complex and is required for TNF-mediated gene induction. Mol Cell 36:831–844
31. Tokunaga F, Sakata S, Saeki Y, Satomi Y, Kirisako T, Kamei K, Nakagawa T, Kato M, Murata S, Yamaoka S, Yamamoto M, Akira S, Takao T, Tanaka K, Iwai K (2009) Involvement of linear polyubiquitylation of NEMO in NF-kappaB activation. Nat Cell Biol 11:123–132

32. Chan FK, Shisler J, Bixby JG, Felices M, Zheng L, Appel M, Orenstein J, Moss B, Lenardo MJ (2003) A role for tumor necrosis factor receptor-2 and receptor-interacting protein in programmed necrosis and antiviral responses. J Biol Chem 278:51613–51621
33. Wang L, Du F, Wang X (2008) TNF-alpha induces two distinct caspase-8 activation pathways. Cell 133:693–703
34. Stanger BZ, Leder P, Lee TH, Kim E, Seed B (1995) RIP: a novel protein containing a death domain that interacts with Fas/APO-1 (CD95) in yeast and causes cell death. Cell 81:513–523
35. Cho YS, Challa S, Moquin D, Genga R, Ray TD, Guildford M, Chan FK (2009) Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. Cell 137:1112–1123
36. He S, Wang L, Miao L, Du F, Zhao L, Wang X (2009) Receptor interacting protein kinase-3 determines cellular necrotic response to TNF-α. Cell 137:1100–1111
37. Zhang DW, Shao J, Lin J, Zhang N, Lu BJ, Lin SC, Dong MQ, Han J (2009) RIP3, an energy metabolism regulator that switches TNF-induced cell death from apoptosis to necrosis. Science 325:332–336
38. Wertz IE, O'Rourke KM, Zhou H, Eby M, Aravind L, Seshagiri S, Wu P, Wiesmann C, Baker R, Boone DL, Ma A, Koonin EV, Dixit VM (2004) De-ubiquitination and ubiquitin ligase domains of A20 downregulate NF-kappaB signalling. Nature 430:694–699
39. Wright A, Reiley WW, Chang M, Jin W, Lee AJ, Zhang M, Sun SC (2007) Regulation of early wave of germ cell apoptosis and spermatogenesis by deubiquitinating enzyme CYLD. Dev Cell 13:705–716
40. Hitomi J, Christofferson DE, Ng A, Yao J, Degterev A, Xavier RJ, Yuan J (2008) Identification of a molecular signaling network that regulates a cellular necrotic cell death pathway. Cell 135:1311–1323
41. O'Donnell MA, Legarda-Addison D, Skountzos P, Yeh WC, Ting AT (2007) Ubiquitination of RIP1 regulates an NF-kappaB-independent cell-death switch in TNF signaling. Curr Biol 17:418–424
42. Legarda-Addison D, Hase H, O'Donnell MA, Ting AT (2009) NEMO/IKKgamma regulates an early NF-kappaB-independent cell-death checkpoint during TNF signaling. Cell Death Differ 16:1279–1288
43. Chan FK, Lenardo MJ (2000) A crucial role for p80 TNF-R2 in amplifying p60 TNF-R1 apoptosis signals in T lymphocytes. Eur J Immunol 30:652–660
44. Zheng L, Bidere N, Staudt D, Cubre A, Orenstein J, Chan FK, Lenardo M (2006) Competitive control of independent programs of tumor necrosis factor receptor-induced cell death by TRADD and RIP1. Mol Cell Biol 26:3505–3513
45. Lin Y, Devin A, Rodriguez Y, Liu ZG (1999) Cleavage of the death domain kinase RIP by caspase-8 prompts TNF-induced apoptosis. Genes Dev 13:2514–2526
46. Feng S, Yang Y, Mei Y, Ma L, Zhu DE, Hoti N, Castanares M, Wu M (2007) Cleavage of RIP3 inactivates its caspase-independent apoptosis pathway by removal of kinase domain. Cell Signal 19:2056–2067
47. Moquin D, Chan FK (2010) The molecular regulation of programmed necrotic cell injury. Trends Biochem Sci. doi: 10.1016/j.tibs.2010.03.001
48. Festjens N, Kalai M, Smet J, Meeus A, Van Coster R, Saelens X, Vandenabeele P (2006) Butylated hydroxyanisole is more than a reactive oxygen species scavenger. Cell Death Differ 13:166–169
49. Pamplona R (2008) Membrane phospholipids, lipoxidative damage and molecular integrity: a causal role in aging and longevity. Biochim Biophys Acta 1777:1249–1262
50. Hildeman DA, Mitchell T, Teague TK, Henson P, Day BJ, Kappler J, Marrack PC (1999) Reactive oxygen species regulate activation-induced T cell apoptosis. Immunity 10:735–744
51. Sidoti-de Fraisse C, Rincheval V, Risler Y, Mignotte B, Vayssiere JL (1998) TNF-alpha activates at least two apoptotic signaling cascades. Oncogene 17:1639–1651
52. Schulze-Osthoff K, Bakker AC, Vanhaesebroeck B, Beyaert R, Jacob WA, Fiers W (1992) Cytotoxic activity of tumor necrosis factor is mediated by early damage of mitochondrial functions. Evidence for the involvement of mitochondrial radical generation. J Biol Chem 267:5317–5323
53. Temkin V, Huang Q, Liu H, Osada H, Pope RM (2006) Inhibition of ADP/ATP exchange in receptor-interacting protein-mediated necrosis. Mol Cell Biol 26:2215–2225
54. Kasof GM, Prosser JC, Liu D, Lorenzi MV, Gomes BC (2000) The RIP-like kinase, RIP3, induces apoptosis and NF-kappaB nuclear translocation and localizes to mitochondria. FEBS Lett 473:285–291
55. Kim YS, Morgan MJ, Choksi S, Liu ZG (2007) TNF-induced activation of the Nox1 NADPH oxidase and its role in the induction of necrotic cell death. Mol Cell 26:675–687
56. Yazdanpanah B, Wiegmann K, Tchikov V, Krut O, Pongratz C, Schramm M, Kleinridders A, Wunderlich T, Kashkar H, Utermohlen O, Bruning JC, Schutze S, Kronke M (2009) Riboflavin kinase couples TNF receptor 1 to NADPH oxidase. Nature 460:1159–1163
57. Rock KL, Latz E, Ontiveros F, Kono H (2010) The sterile inflammatory response. Annu Rev Immunol 28:321–342
58. Nagata S, Hanayama R, Kawane K (2010) Autoimmunity and the clearance of dead cells. Cell 140:619–630
59. Martinon F, Petrilli V, Mayor A, Tardivel A, Tschopp J (2006) Gout-associated uric acid crystals activate the NALP3 inflammasome. Nature 440:237–241
60. Chen CJ, Shi Y, Hearn A, Fitzgerald K, Golenbock D, Reed G, Akira S, Rock KL (2006) MyD88-dependent IL-1 receptor signaling is essential for gouty inflammation stimulated by monosodium urate crystals. J Clin Invest 116:2262–2271
61. Park JS, Svetkauskaite D, He Q, Kim JY, Strassheim D, Ishizaka A, Abraham E (2004) Involvement of Toll-like receptors 2 and 4 in cellular activation by high mobility group box 1 protein. J Biol Chem 279:7370–7377
62. Kokkola R, Andersson A, Mullins G, Ostberg T, Treutiger CJ, Arnold B, Nawroth P, Andersson U, Harris RA, Harris HE (2005) RAGE is the major receptor for the proinflammatory activity of HMGB1 in rodent macrophages. Scand J Immunol 61:1–9
63. Yamasaki S, Ishikawa E, Sakuma M, Hara H, Ogata K, Saito T (2008) Mincle is an ITAM-coupled activating receptor that senses damaged cells. Nat Immunol 9:1179–1188
64. Sancho D, Joffre OP, Keller AM, Rogers NC, Martinez D, Hernanz-Falcon P, Rosewell I, Reis e Sousa C (2009) Identification of a dendritic cell receptor that couples sensing of necrosis to immunity. Nature 458:899–903
65. Krammer PH, Arnold R, Lavrik IN (2007) Life and death in peripheral T cells. Nat Rev Immunol 7:532–542
66. Siegel RM (2006) Caspases at the crossroads of immune-cell life and death. Nat Rev Immunol 6:308–317
67. Zhang J, Cado D, Chen A, Kabra NH, Winoto A (1998) Fas-mediated apoptosis and activation-induced T-cell proliferation are defective in mice lacking FADD/Mort1. Nature 392:296–300
68. Chun HJ, Zheng L, Ahmad M, Wang J, Speirs CK, Siegel RM, Dale JK, Puck J, Davis J, Hall CG, Skoda-Smith S, Atkinson TP, Straus SE, Lenardo MJ (2002) Pleiotropic defects in lymphocyte activation caused by caspase-8 mutations lead to human immunodeficiency. Nature 419:395–399

69. Newton K, Harris AW, Bath ML, Smith KG, Strasser A (1998) A dominant interfering mutant of FADD/MORT1 enhances deletion of autoreactive thymocytes and inhibits proliferation of mature T lymphocytes. EMBO J 17:706–718
70. Walsh CM, Wen BG, Chinnaiyan AM, O'Rourke K, Dixit VM, Hedrick SM (1998) A role for FADD in T cell activation and development. Immunity 8:439–449
71. Salmena L, Hakem R (2005) Caspase-8 deficiency in T cells leads to a lethal lymphoinfiltrative immune disorder. J Exp Med 202:727–732
72. Su H, Bidere N, Zheng L, Cubre A, Sakai K, Dale J, Salmena L, Hakem R, Straus S, Lenardo M (2005) Requirement for caspase-8 in NF-kappaB activation by antigen receptor. Science 307:1465–1468
73. Ch'en IL, Beisner DR, Degterev A, Lynch C, Yuan J, Hoffmann A, Hedrick SM (2008) Antigen-mediated T cell expansion regulated by parallel pathways of death. Proc Natl Acad Sci USA 105:17463–17468
74. Bell BD, Leverrier S, Weist BM, Newton RH, Arechiga AF, Luhrs KA, Morrissette NS, Walsh CM (2008) FADD and caspase-8 control the outcome of autophagic signaling in proliferating T cells. Proc Natl Acad Sci USA 105:16677–16682
75. Degterev A, Hitomi J, Germscheid M, Ch'en IL, Korkina O, Teng X, Abbott D, Cuny GD, Yuan C, Wagner G, Hedrick SM, Gerber SA, Lugovskoy A, Yuan J (2008) Identification of RIP1 kinase as a specific cellular target of necrostatins. Nat Chem Biol 4:313–321
76. Degterev A, Huang Z, Boyce M, Li Y, Jagtap P, Mizushima N, Cuny GD, Mitchison TJ, Moskowitz MA, Yuan J (2005) Chemical inhibitor of nonapoptotic cell death with therapeutic potential for ischemic brain injury. Nat Chem Biol 1:112–119
77. Imtiyaz HZ, Zhang Y, Zhang J (2005) Structural requirements for signal-induced target binding of FADD determined by functional reconstitution of FADD deficiency. J Biol Chem 280:31360–31367
78. Beisner DR, Ch'en IL, Kolla RV, Hoffmann A, Hedrick SM (2005) Cutting edge: innate immunity conferred by B cells is regulated by caspase-8. J Immunol 175:3469–3473
79. Benedict CA, Norris PS, Ware CF (2002) To kill or be killed: viral evasion of apoptosis. Nat Immunol 3:1013–1018
80. Dobo J, Swanson R, Salvesen GS, Olson ST, Gettins PG (2006) Cytokine response modifier a inhibition of initiator caspases results in covalent complex formation and dissociation of the caspase tetramer. J Biol Chem 281:38781–38790
81. Li M, Beg AA (2000) Induction of necrotic-like cell death by tumor necrosis factor alpha and caspase inhibitors: novel mechanism for killing virus-infected cells. J Virol 74:7470–7477
82. Ruby J, Bluethmann H, Peschon JJ (1997) Antiviral activity of tumor necrosis factor (TNF) is mediated via p55 and p75 TNF receptors. J Exp Med 186:1591–1596
83. Sambhi SK, Kohonen-Corish MR, Ramshaw IA (1991) Local production of tumor necrosis factor encoded by recombinant vaccinia virus is effective in controlling viral replication in vivo. Proc Natl Acad Sci USA 88:4025–4029
84. Goldmacher VS (2005) Cell death suppression by cytomegaloviruses. Apoptosis 10:251–265
85. Upton JW, Kaiser WJ, Mocarski ES (2010) Virus inhibition of RIP3-dependent necrosis. Cell Host Microbe 7:302–313
86. Brune W, Menard C, Heesemann J, Koszinowski UH (2001) A ribonucleotide reductase homolog of cytomegalovirus and endothelial cell tropism. Science 291:303–305
87. Mack C, Sickmann A, Lembo D, Brune W (2008) Inhibition of proinflammatory and innate immune signaling pathways by a cytomegalovirus RIP1-interacting protein. Proc Natl Acad Sci USA 105:3094–3099
88. Upton JW, Kaiser WJ, Mocarski ES (2008) Cytomegalovirus M45 cell death suppression requires receptor-interacting protein (RIP) homotypic interaction motif (RHIM)-dependent interaction with RIP1. J Biol Chem 283:16966–16970
89. Lembo D, Donalisio M, Hofer A, Cornaglia M, Brune W, Koszinowski U, Thelander L, Landolfo S (2004) The ribonucleotide reductase R1 homolog of murine cytomegalovirus is not a functional enzyme subunit but is required for pathogenesis. J Virol 78:4278–4288
90. Rebsamen M, Heinz LX, Meylan E, Michallet MC, Schroder K, Hofmann K, Vazquez J, Benedict CA, Tschopp J (2009) DAI/ZBP1 recruits RIP1 and RIP3 through RIP homotypic interaction motifs to activate NF-kappaB. EMBO Rep 10:916–922
91. Kaiser WJ, Upton JW, Mocarski ES (2008) Receptor-interacting protein homotypic interaction motif-dependent control of NF-kappa B activation via the DNA-dependent activator of IFN regulatory factors. J Immunol 181:6427–6434
92. Thome M, Schneider P, Hofmann K, Fickenscher H, Meinl E, Neipel F, Mattmann C, Burns K, Bodmer JL, Schroter M, Scaffidi C, Krammer PH, Peter ME, Tschopp J (1997) Viral FLICE-inhibitory proteins (FLIPs) prevent apoptosis induced by death receptors. Nature 386:517–521
93. Bertin J, Armstrong RC, Ottilie S, Martin DA, Wang Y, Banks S, Wang GH, Senkevich TG, Alnemri ES, Moss B, Lenardo MJ, Tomaselli KJ, Cohen JI (1997) Death effector domain-containing herpesvirus and poxvirus proteins inhibit both Fas- and TNFR1-induced apoptosis. Proc Natl Acad Sci USA 94:1172–1176
94. Shisler JL, Moss B (2001) Immunology 102 at poxvirus U: avoiding apoptosis. Semin Immunol 13:67–72
95. Yang JK, Wang L, Zheng L, Wan F, Ahmed M, Lenardo MJ, Wu H (2005) Crystal structure of MC159 reveals molecular mechanism of DISC assembly and FLIP inhibition. Mol Cell 20:939–949
96. Garvey TL, Bertin J, Siegel RM, Wang GH, Lenardo MJ, Cohen JI (2002) Binding of FADD and caspase-8 to molluscum contagiosum virus MC159 v-FLIP is not sufficient for its antiapoptotic function. J Virol 76:697–706
97. Thurau M, Everett H, Tapernoux M, Tschopp J, Thome M (2006) The TRAF3-binding site of human molluscipoxvirus FLIP molecule MC159 is critical for its capacity to inhibit Fas-induced apoptosis. Cell Death Differ 13:1577–1585
98. Chaudhary PM, Jasmin A, Eby MT, Hood L (1999) Modulation of the NF-kappa B pathway by virally encoded death effector domains-containing proteins. Oncogene 18:5738–5746
99. Woelfel M, Bixby J, Brehm MA, Chan FK (2006) Transgenic expression of the viral FLIP MC159 causes lpr/gld-like lymphoproliferation and autoimmunity. J Immunol 177:3814–3820
100. Murao LE, Shisler JL (2005) The MCV MC159 protein inhibits late, but not early, events of TNF-alpha-induced NF-kappaB activation. Virology 340:255–264
101. Kennedy CL, Smith DJ, Lyras D, Chakravorty A, Rood JI (2009) Programmed cellular necrosis mediated by the pore-forming alpha-toxin from *Clostridium septicum*. PLoS Pathog 5:e1000516
102. Grobner S, Autenrieth SE, Soldanova I, Gunst DS, Schaller M, Bohn E, Muller S, Leverkus M, Wesselborg S, Autenrieth IB, Borgmann S (2006) Yersinia YopP-induced apoptotic cell death in murine dendritic cells is partially independent from action of caspases and exhibits necrosis-like features. Apoptosis 11:1959–1968
103. Kirschnek S, Scheffel J, Heinzmann U, Hacker G (2004) Necrosis-like cell death induced by bacteria in mouse macrophages. Eur J Immunol 34:1461–1471
104. Mareninova OA, Sung KF, Hong P, Lugea A, Pandol SJ, Gukovsky I, Gukovskaya AS (2006) Cell death in pancreatitis:

caspases protect from necrotizing pancreatitis. J Biol Chem 281:3370–3381
105. Malleo G, Mazzon E, Genovese T, Di Paola R, Muia C, Centorrino T, Siriwardena AK, Cuzzocrea S (2007) Etanercept attenuates the development of cerulein-induced acute pancreatitis in mice: a comparison with TNF-alpha genetic deletion. Shock 27:542–551
106. Sandoval J, Pereda J, Rodriguez JL, Escobar J, Hidalgo J, Joosten LA, Franco L, Sastre J, Lopez-Rodas G (2010) Ordered transcriptional factor recruitment and epigenetic regulation of TNF-alpha in necrotizing acute pancreatitis. Cell Mol Life Sci 67:1687–1697
107. Zhang M, Chen L (2008) Status of cytokines in ischemia reperfusion induced heart injury. Cardiovasc Hematol Disord Drug Targets 8:161–172
108. Stoll G, Jander S, Schroeter M (2002) Detrimental and beneficial effects of injury-induced inflammation and cytokine expression in the nervous system. Adv Exp Med Biol 513:87–113
109. You Z, Savitz SI, Yang J, Degterev A, Yuan J, Cuny GD, Moskowitz MA, Whalen MJ (2008) Necrostatin-1 reduces histopathology and improves functional outcome after controlled cortical impact in mice. J Cereb Blood Flow Metab 28:1564–1573
110. Smith CC, Davidson SM, Lim SY, Simpkin JC, Hothersall JS, Yellon DM (2007) Necrostatin: a potentially novel cardioprotective agent? Cardiovasc Drugs Ther 21:227–233