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Caspase crosstalk: integration of apoptotic and innate immune signalling pathways

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The caspase family of cysteine proteases has been functionally divided into two groups: those involved in apoptosis and those involved in innate immune signalling. Recent findings have identified 'apoptotic' caspases within inflammasome complexes and revealed that 'inflammatory' caspases are capable of inducing cell death, suggesting that the earlier view of caspase function may have been overly simplistic. Here, I review evidence attributing nonclassical functions to many caspases and propose that caspases serve as critical mediators in the integration of apoptotic and inflammatory pathways, thereby forming an integrated signalling system that regulates cell death and innate immune responses during development, infection, and homeostasis.

Apoptosis and inflammation: interlinked mechanisms on a common axis

Apoptosis, or programmed cell death, is an essential process that occurs in all tissues during development, homeostasis, and disease [1]. In contrast to necrosis (see Glossary) and other forms of cell death where membrane disruption occurs, such as secondary necrosis, pyroptosis, and necroptosis, apoptosis is an immunologically silent form of cell death, whereby cells are rapidly phagocytosed and cleared without the initiation of an inflammatory response. By contrast, the inflammatory response is triggered by innate immune sensors following cellular damage, infection, or stress, and serves to clear the harmful stimulus and initiate healing.

Caspases are a family of proteases that have been subdivided functionally into those involved in either apoptosis or inflammation. However, as research into both of these essential processes progresses, it has become evident that apoptosis and inflammation are inextricably linked in both lower and higher organisms (Box 1). Caspases are central to coordinating and integrating signals that result in not only apoptosis and inflammation but also other forms of programmed death, including pyroptosis and necroptosis. This view is supported by observations that proteins involved in apoptosis and inflammation contain common conserved protein domains, including caspase-associated recruitment domains (CARDs) and death effector domains (DEDs), which are also present in caspases. Recent findings have revealed that classically 'apoptotic' caspases, particularly caspase-8, have essential roles in initiating inflammation, both directly and via inflammatory cell death pathways. Conversely, classically 'inflammatory' caspases are also emerging as essential drivers of cell death processes. Here, I review these recent findings and, based on this evaluation, propose that cell death and inflammation are outcomes of an integrated signalling system which is governed by caspases.

Caspase activation mechanisms

Caspases are cysteine proteases with primary specificity for aspartic acid (Asp) residues, cleaving their substrates after tetrapeptide sequences containing Asp in the P1 position. All caspases are synthesised as inactive single chain zymogens (procaspases), and they are all obligate heterodimers in their active forms; additional signals are required to facilitate procaspase modification and initiation of caspase activation pathways. Caspase zymogens consist of an N-terminal prodomain and a C-terminal protease domain, which has a large and a small subunit that contains the catalytic cysteine residue [1]. Initiator

Glossary

Apoptosome: a wheel-shaped heptameric protein complex, consisting of cytochrome *c*, Apaf-1, and caspase-9, which is formed during the intrinsic apoptosis pathway. The net result of apoptosome formation is the activation of caspase-9.

Death-induced signalling complex (DISC): a multiprotein complex, which forms following the engagement of death receptors with their cognate ligands, recruiting adaptor proteins and caspases-8 or -10 into the complex. The net result of DISC formation is the activation of caspases-8 or -10.

Extrinsic apoptosis: the pathway of DISC-mediated apoptotic cell death, activated following the engagement of death receptors of the TNF receptor family.

Inflammasome: a multiprotein complex, which forms following the detection of pathogen or danger signals in the cellular cytosol. The net result of inflammasome formation is the activation of caspase-1.

Intrinsic apoptosis: the pathway of mitochondrial-mediated apoptotic cell death, activated in response to cellular stresses, such as UV irradiation and chemotherapeutic agents.

Necroptosis: a proinflammatory form of cell death, which is dependent on receptor-interacting protein kinases 1 and 3 (RIPK1 and RIPK3), and is inhibited by caspase-8.

Necrosis: a passive, uncontrolled form of cell death characterised by cellular swelling, membrane lysis, and release of intracellular contents.

Pyroptosis: an inflammatory form of programmed cell death, activated following cellular insults such as bacterial infection or exposure to toxins, and mediated by caspase-1 and/or caspase-11.

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Box 1. Cell death and immunity in the fly

Drosophila melanogaster studies have greatly contributed to the functional understanding of caspases to date, and also serve to illustrate how cell death and immune processes are also regulated by caspases in lower organisms. There are seven *Drosophila* caspases, three of which (DRONC, DREDD, and STRICA) structurally resemble initiator caspases. The remaining four (DrICE, DCP-1, DAMM, and DECAY) have short prodomains and are classed as executioner caspases (reviewed in [86]).

DRONC, the *Drosophila* orthologue of caspase-9, assembles into an apoptosome structure with the Apaf-1-related protein, DARK, resulting in the activation of executioner caspase, DrICE [87]. Apoptosome-mediated DRONC activation drives stress-induced apoptosis and much of the developmentally related cell death that occurs in flies [88,89]. IAPs have a prominent role in *Drosophila* caspase regulation, as DIAP1 directly inhibits DRONC [90]. Apoptosis cannot proceed until a proapoptotic member of the RHG (Reaper, Hid, Grim) family, Sickle, or Jafrac-2 disrupts DIAP1-mediated caspase inhibition [91–93]. In addition to its apoptotic role, DRONC can also mediate processes, such as border cell migration, spermatid individualisation, and compensatory proliferation [94].

DREDD is the *Drosophila* orthologue of caspase-8. It contains two DED domains within its prodomain, through which it interacts with the fly homologue, dFADD, to mediate immune responses. The humoral immune response in the fly is composed of two pathways: Toll and immune deficiency (IMD), both of which regulate alternative NFκB transcription factors. The IMD pathway, similar to the mammalian TNFR pathway, induces activation of Relish/NFκB transcription and the expression of anti-Gram-negative microbial peptides. Activation and cleavage of Relish (and its upstream adaptor protein, IMD) is mediated by dFADD and DREDD [95,96]. The IAP, DIAP2, is also essential for Relish activation [97]. DIAP2 promotes the proteolytic activity of DREDD towards Relish, revealing a novel, contrasting role for IAPs in the promotion of caspase activity [98].

An apoptotic role was originally predicted for DREDD following its characterisation, as Reaper- and Grim-induced killing was suppressed by heterozygosity at the *dredd* locus [99]. Overexpression of the *Drosophila* RING1 and YY-binding protein (dRYBP) induces RHD-mediated apoptosis which requires dFADD and DREDD [100], although the physiological setting for this pathway has yet to be identified. However, a requirement for DREDD and Relish for neuronal cell death has been reported [101], suggesting that, similar to caspase-8, DREDD has the ability to mediate both immune and cell death pathways.

caspases have long prodomains that consist of protein interaction domains: CARDs in caspases-1, -2, -4, -5, -9, -11, and -12, and DEDs in caspases-8 and -10. Caspase-10 was lost in rodents during evolution and- thus in mice, caspase-8 is the sole initiator caspase of the extrinsic death pathway [2]. These protein interaction domains facilitate the recruitment of initiator caspases into multiprotein activation complexes, such as the apoptosome, death-induced signalling complex (DISC), and inflammasomes (discussed further later). These multiprotein complexes serve to promote initiator caspase dimerisation and activation via the induced proximity mechanism [1,3]. By contrast, effector caspases (caspases-3, -6, and -7) have minimal prodomains and, once synthesised, exist as inactive homodimers until they are subsequently cleaved and activated by specific initiator/upstream caspases in a cascade-like manner. Effector caspase activation results in the cleavage of over 600 cellular substrates, some of which are involved in the apoptotic programme, while the consequences of the cleavage of other targets are still being examined [4].

Caspases during apoptosis

Apoptosis, first described in 1972 by Kerr, Wyllie, and Currie [5], is activated via two distinct pathways, commonly referred to as the intrinsic and the extrinsic apoptotic pathways [3]. The intrinsic pathway is mediated by a component of the mitochondrial electron transport chain, cytochrome *c*, upon its release from the mitochondrial intermembrane space in response to cell stress. Once detected in the cytosol, cytochrome *c* triggers the rapid oligomerisation of apoptotic protease-activating factor-1 (Apaf-1), which recruits caspase-9 (via CARD/CARD interactions) into the apoptosome [6] (Figure 1). Active caspase-9 then directly cleaves and activates downstream effector caspases, resulting in apoptosis. The extrinsic pathway results in initiator caspase-8 and caspase-10 activation within the DISC, via their DED-mediated interactions (Figure 1), which ultimately results in the activation of effector caspases and apoptosis.

It is important to note that the engagement of death receptors and the recruitment of alternative adaptors [e.g., tumour necrosis factor receptor type 1-associated DEATH domain–receptor-interacting protein kinase (TRADD) and receptor-interacting serine/threonine protein kinase 1 (RIPK1) or Fas-associated death domain (FADD) and RIPK1] also signals for survival, differentiation, or other immune stimulatory activities, such as inflammation, via nuclear factor κB (NFκB), mitogen-activated protein kinase (MAPK), and c-Jun N-terminal kinase (JNK) signalling pathways [7]. Thus, differential regulation of the caspase-activating DISC complex can govern the fate of the cell. Although apoptosis is classically described as an immunologically silent mode of cell death, a study has shown that both transformed and primary cells, stimulated to undergo Fas-mediated apoptosis, produce moderate amounts of monocyte chemoattractant protein-1 (MCP-1) chemokine and IL-6 and IL-8 cytokines [8]. Fas stimulation was also shown to induce phagocyte migration *in vivo*, suggesting that it activates both apoptotic and proinflammatory pathways to facilitate the swift removal of dying cells. Apoptotic lymphocytes have also been shown to attenuate proinflammatory cytokine secretion from lipopolysaccharide (LPS)-stimulated peripheral blood mononuclear cells (PBMCs) [9], and it has been proposed that apoptotic caspases serve to dampen inflammation by cleaving and inactivating otherwise proinflammatory cellular signals during apoptosis [10]. Therefore, certain apoptotic processes actively engage with the immune system to coordinate their efficient clearance from tissues, while minimising inflammation and its consequences on surrounding tissues.

Caspases during inflammation

Caspase-1: the prototypical inflammatory caspase

IL-1β and IL-18 are potent proinflammatory cytokines that induce fever and interferon γ (IFNγ) secretion, respectively. Their production is under tight regulation: firstly, NFκB activation is required for the transcriptional upregulation