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Review

Necroptotic signaling in adaptive and innate immunity

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ABSTRACT

The vertebrate immune system is highly dependent on cell death for efficient responsiveness to microbial pathogens and oncogenically transformed cells. Cell death pathways are vital to the function of many immune cell types during innate, humoral and cellular immune responses. In addition, cell death regulation is imperative for proper adaptive immune self-tolerance and homeostasis. While apoptosis has been found to be involved in several of these roles in immunity, recent data demonstrate that alternative cell death pathways are required. Here, we describe the involvement of a programmed form of cellular necrosis called "necroptosis" in immunity. We consider the signaling pathways that promote necroptosis downstream of death receptors, type I transmembrane proteins of the tumor necrosis factor (TNF) receptor family. The involvement of necroptotic signaling through a "RIPoptosome" assembled in response to innate immune stimuli or genotoxic stress is described. We also characterize the induction of necroptosis following antigenic stimulation in T cells lacking caspase-8 or FADD function. While necroptotic signaling remains poorly understood, it is clear that this pathway is an essential component to effective vertebrate immunity.

Contents

1. Introduction

Necrosis was initially defined as an accidental, uncontrolled type of cell death that is typically induced by energetic starvation or plasma membrane disruption [1]. Recent studies have revolutionized the definition of necrosis, and it is now known that certain forms are highly regulated processes activated by certain pathological or physiological stimuli [2]. Necroptosis is a form of programmed necrosis that occurs when caspases are inhibited, or otherwise fail to become activated [3]. Apoptosis is a caspase-dependent mode of cell death, leading to the orderly degradation of cellular components into "apoptotic bodies" that are engulfed

Abbreviations: TNF, tumor necrosis factor; RIP, receptor interacting protein; cIAP, cellular inhibitor of apoptosis protein; TRADD, TNF receptor associated death domain; FADD, Fas associated death domain; TAK1, transforming growth factor-β-activated kinase 1; NF-κB, nuclear factor kappa B; IKK, inhibitor of kappa B kinase; IKB, inhibitor of kappa B; CYLD, cylindromatosis.

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by surrounding cells via phagocytosis. With the efficient and rapid removal of dead cells, apoptosis has long been considered the immunologically quiescent form of cell death, whereas necrosis (and likely necroptosis due to its similarity to necrosis) is thought to provoke the immune system [4]. Given this paradigm, apoptosis is to be considered the preferred mode of cell death during the development of the immune system, particularly in regulating adaptive immune tolerance and the homeostasis of mature lymphocytes in the peripheral immune system. In contrast, necroptosis may be considered a "fail-safe" mechanism to prevent unrestrained growth of cells, particularly following infection by viruses that attempt to prevent apoptosis [5]. A key control point in the choice between necroptosis vs. apoptosis is mediated through the RIP kinase family [6]. The serine-threonine RIP kinases, RIPK1 and RIPK3, are critical mediators of necroptosis [7], while RIPK1 is also a key regulator of apoptosis [8]. The involvement of RIP kinases in the control of apoptotic vs. necroptotic cell death following death receptor stimulation is currently the most well-characterized. However, as described below, other roles for RIP kinase family members in death receptor independent forms of necroptosis are also under intense scrutiny.

2. Death-receptor signaling triggers cell death

TNFR1 stimulation can lead to diverse responses: anti-apoptosis, apoptosis, or necroptosis. The TNF signaling pathway is currently the most widely studied necroptotic signaling pathway. TNF binding to TNFR1 at the plasma membrane leads to the recruitment of TNFR1-associated death domain (TRADD), RIP1, cellular inhibitor of apoptosis protein 1 (cIAP1), cIAP2, TNF-receptor-associated factor 2 (TRAF2), and TRAF5 (Fig. 1). This assembly is called complex I and it is situated on the plasma membrane [8]. cIAP1 and cIAP2 polyubiquitinate RIP1 and induce NF-κB activation [9,10] while preventing apoptosis and necroptosis [11]. Transforming growth factor-β-activated kinase 1 (TAK1)-binding proteins TAB1 and TAB2 mediate the interaction between ubiquitinated RIP1 and TAK1. TAK1 in turn activates the inhibitor of NF-κB kinase (IKK) complex, and the IKK complex phosphorylates IκB. The phosphorylated IκB is polyubiquitinated and undergoes proteasomal degradation, which enables NF-κB translocation to the nucleus. The activation of the canonical NF-κB pathway results in the transcription of pro-survival and pro-inflammatory genes [12]. In the absence of cIAPs, the canonical NF-κB pathway is suppressed [10,13,14]. RIP1 is not polyubiquitinated, and complex I leads to the upregulation of NF-κB-inducing kinase (NIK) and the activation of the non-canonical NF-κB pathway [15–17]. Thus, the main function of TNF-induced complex I is likely to promote anti-apoptosis pathways.

Cylindromatosis (CYLD) is a RIP1 Lys63 deubiquitinating enzyme that prevents the pro-survival effect of RIP1 [18]. CYLD destabilizes complex I and allows RIP1 to dissociate from the plasma membrane. Subsequently, a TRADD-dependent complex (complex IIa) assembles in the cytoplasm: RIP1 associates with TRADD, FADD and pro-caspase 8 [19,20]. Pro-caspase 8 dimerizes [21] and terminates the necroptotic signal by cleaving RIP1 and RIP3 [22–24]. It has been suggested that the caspase 8-c-FLIP$_L$ heterodimer cleaves RIP3 [25] and CYLD [26] to promote apoptosis and prevent necroptosis. Caspase 8 activation leads to the activation of caspase 3 and caspase 7 to execute apoptosis. If cIAPs are depleted and RIP1 is not ubiquitylated, then a TRADD-independent complex forms. The assembly of RIP1, RIP3, FADD, and caspase 8 is called the RIP1-dependent complex IIb, or the ripoptosome [27]. Within this complex, caspase 8 cleaves RIP1 and RIP3, resulting in apoptosis and the prevention of further necroptotic signaling [28].

The inhibition of caspase 8 by caspase inhibitors or virally encoded proteins, such as cytokine response modifier protein A (CrmA), causes RIP1 and RIP3 to associate within necrosomes (complex III) [29]. RIP1 and RIP3 associate with one another through the RIP homotypic interaction motif (RHIM), leading to activation of downstream necroptotic signaling [30,31]. This occurs when mixed lineage kinase domain-like protein (MLKL) is phosphorylated by RIP3 and is recruited to the necrosome [32]. The intracellular location of the necrosome is not well understood, although it has been reported that RIPK3 does not co-localize with mitochondria, golgi, endoplasmic reticulum, peroxisomes, LAMP1-associated endosomes, nor RhoB-associated endosomes [33]. However, a recent study suggests that MLKL is required for the translocation of RIPK3-containing necrosomes to membrane-associated mitochondria (MAM) upon TNF-induced necroptosis [34].

RIP1 [35–37] and RIP3 [31,33,36,37] are key mediators of necroptosis induced by the DR ligands TNF, Fas ligand (FasL), and TNF-related apoptosis-inducing ligand (TRAIL). FasL binds to Fas, and in the absence of caspase 8 [38], this leads to the recruitment of FADD, RIP1 [35,39], and RIP3 [33,40], resulting in necroptosis. TRAIL binding to TRAIL-Rs can also result in RIP1/RIP3 necrosome formation [37]. Thus, while much remains to be determined, it is clear that ligation of death receptors of the TNF-R family can provoke apoptosis, necroptosis, or non cell-death associated events. The relative contribution of apoptosis vs. necrosis under physiological and pathological conditions *in vivo* remains to be fully elaborated.

3. Toll-like receptor signaling triggers cell death

The innate immune system is armed with pathogen recognition receptors that detect molecular components from a variety of pathogens including bacteria, viruses, yeast, and fungi. Although Toll-like receptors (TLRs) bind to foreign pathogen associated molecular patterns (PAMPs), they also recognize endogenous danger associated molecular patterns (DAMPs) such as self-nucleic acids released from dying cells [41]. TLR3 and TLR4 recognize double-stranded RNA [42] and lipopolysaccharide (LPS) [43], respectively. These receptors control cytokine induction, dendritic cell maturation, and antigen presentation to T cells [44]. TLR3 and TLR4 signal through the adaptor protein TRIF, but TLR4 signals through TRIF in cooperation with a TIR adaptor protein called TRAM. TLR4 is also able to signal through MyD88 [45]. RIP1 is also a critical mediator of the activation of the NF-κB pathway through TLR3 and TLR4 [46,47]. As described below, TLR3 and TLR4 signaling pathways also regulate necrosome formation and cell death.

Stimulation of TLR3 or TLR4 triggers the recruitment of TRIF, whose RHIM motif subsequently interacts with the RHIM motif of RIPK3 [48,49]. The deactivation of caspases is necessary for TNFα-induced RIP1/RIP3 necrosome formation [31,33]. Interestingly, the deactivation of caspases was not essential for the recruitment of RIP3 to TRIF in TLR3 and TLR4-mediated necroptosis in bone-marrow derived macrophages (BMDM)s [48]. It is important to note another study that showed caspase 8 inhibition in BMDMs was required for TLR4-induced necroptosis in BMDMs [50]. In addition, although TNFα is produced as a result of TLR3 or TLR4 signaling, autocrine TNFα contributes minimally to the necroptosis induced through TLR3 or TLR4 signaling [48].

4. Physiological importance of necroptosis to infection and tissue damage

Viruses and bacteria that can inhibit caspases could pose a threat if these organisms cannot be eliminated. Therefore, if caspase 8 is non-functional, then necroptosis may be an important way to