Down the rabbit hole: Is necroptosis truly an innate response to infection?

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Abstract

Pathogenic microbes have evolved countless sophisticated mechanisms to subvert host immune responses and cause disease. Understanding evasion strategies employed by pathogens has led to numerous discoveries on specific host cell processes that are critical for controlling infection. Programmed cell death (PCD) is a key host defense to microbial infection, as well as being critical for organ development and cellular homeostasis in multicellular organisms. Much of our current understanding of PCD as a host response to infection has stemmed from the discovery and study of viral inhibitors of apoptosis, and more recently viral inhibition of the newly characterised from of PCD termed necroptosis, the mechanisms of which are still under intense investigation. Many bacterial pathogens also encode inhibitors of PCD, yet these discoveries are relatively more recent and thus the biological significance of such mechanisms are still under debate. In this viewpoint article, we will argue the concept that necroptosis is merely a 'back-up' mechanism in the event that

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apoptosis is inhibited, or whether it is a true host innate response to infection that has evolved in response to a growing arsenal of microbial evasion strategies.

Why would more than one programmed cell death pathway have evolved?

Aside from differing intrinsically in terms of the signalling componentry, the nature of the cell death modality provides important cues to the subject's immune system. Apoptosis, which is dependent on the activation and cleavage substrates by family of cysteine-aspartic proteases, termed caspases, which are serially activated in a caspase cascade, is largely considered immunologically-inert, as is necessary for a pathway which has such an important role in mammalian development. In contrast, necroptosis does not require caspase activity and is dispensable for normal mammalian development [1, 2], although dysregulated necroptosis is known to be deleterious to development [3-9]. As a lytic form of cell death, necroptosis is thought to release danger associated molecular patterns (DAMPs) to provoke an immune response that would benefit clearance of a pathogen.

What is necroptosis?

Our current understanding of necroptosis signaling is that activation and oligomerisation of receptor interacting serine/threonine kinase 3 (RIPK3) along with its substrate mixed lineage kinase-like (MLKL), is triggered downstream of the activation of pattern-recognition receptors (PRRs) or Toll-like receptors (TLRs) by pathogenic microbes, by members of the TNF receptor superfamily or by the interferon (IFN)-inducible innate sensor DAI (Figure 1). RIPK3 oligomerisation is driven by the RIP homotypic interaction motif (RHIM), C-terminal

to the kinase domain, which enables RIPK3 to assemble into higher order complexes within which trans-phosphorylation of kinase domains can occur to activate RIPK3 kinase activity [10] (Figure 1). Activated RIPK3 is then thought to recruit MLKL and phosphorylate its pseudokinase domain to induce MLKL's exposure of the N-terminal four-helix bundle ("executioner") domain, MLKL oligomerisation, membrane translocation and permeabilisation (Figure 1). While the mechanism of MLKL-mediated plasma membrane permeabilisation is still widely debated [11, 12], a key outcome is the release of DAMPs and other inflammatory signalling molecules, including cytokines [13, 14]. RHIM sequences from other cellular proteins [10, 15, 16], and TRIF [14, 17], are thought to act as glue to facilitate RIPK3 higher order complex assembly, and depending on the context, the RHIM of RIPK1 may promote or negate RIPK3 activation [18]. Viral proteins including M45 from mouse cytomegalovirus [19], ICP6 from Herpes simplex virus (HSV) 1 and ICP10 from HSV2 contain RHIM sequences that can compete with endogenous RHIM domains and thwart assembly of RIPK3 into higher order complexes [20], thereby preventing RIPK3 activation and the instigation of necroptosis (Figure 1). These observations provide strong circumstantial evidence for a role of necroptotic cell death in innate immunity, otherwise mechanisms to counteract necroptosis would not have been retained over millions of years of evolution.

The generally accepted dogma of necroptosis activation is that it only occurs upon inhibition of effector molecules that promote inflammatory signaling simultaneously with inhibition of caspase-8 mediated apoptosis. A number viral inhibitors of inflammation (e.g. NSP1 from

human Rotavirus, and A49 from VACV) [21, 22] and caspase-8 mediated apoptosis (e.g. CrmA from cowpox virus and v-FLIPs from several poxvirus) [23, 24] have been described (Figure 1). Coincidental perturbation of both cellular events downstream of transmembrane receptor activation leads to RIPK3 activation and subsequent necroptosis. Many studies utilise an artificial mechanism to activate necroptosis, which comprises a combination of TNF as a receptor stimulus, IAP antagonist compounds (Smac-mimetics) and caspase inhibitors (Z-VAD-FMK or Q-VD-OPh). This raises two key questions; 1. why have we evolved a cell death mechanism that is only activated if another is first inhibited? 2. Is necroptosis purely a back-up mechanism of immunity that has evolved in response to the numerous microbial effectors that inhibit apoptosis?

Is necroptosis simply a backup pathway to apoptosis?

Given apoptosis has been studied for decades longer than necroptosis, and is thus better understood, it is considered the archetypal programmed cell death pathway. However, two lines of thought suggest that apoptosis and necroptosis are parallel pathways, whose induction may be regulated by the relative abundance of the various signaling pathway effectors. For example, elevated c-FLIP levels negate caspase-8 activation and apoptosis [25] (Figure 1), depletion of cIAPs negates NF-κB-mediated inflammation [26] (Figure 1), and increased RIPK3 levels promote MLKL activation and necroptosis [27]. Firstly, mice genetically-deleted for RIPK1 – the key branch point protein in the apoptosis-necroptosis bifurcation – are embryonic lethal and exhibit systemic inflammation with elevated apoptosis in some tissues, and elevated necroptosis in others [5]. For instance, co-deletion of *Ripk1* and

Casp8 in the intestinal epithelium identified the intestine as a site that is predisposed to apoptosis, while necroptosis was most evident in the ileum [28]. Similarly, mice deficient in the TNFR-regulatory factor Sharpin, exhibit severe dermatitis and inflammation of liver and spleen [29]. Genetic deletion of the key necroptosis effector, MLKL, ameliorated liver and spleen inflammation in these mice, but did not significantly impact skin inflammation [29], consistent with the notion that apoptosis and necroptosis occur simultaneously within animals and there is some cellular predisposition to one pathway or the other. Secondly, as discussed further below, cytomegaloviruses and herpesviruses express protein inhibitors of both extrinsic apoptosis (targeting caspase-8) and necroptosis (targeting RHIM proteins, DAI/ZBP1 and RIPK3) [30]. While co-deletion of both viral suppressor proteins successfully blocks cell death, deletion or mutation of an individual suppressor protein does not [30]. However, it should be noted that, strictly speaking, these observations argue for the importance of RIPK3, rather than necroptosis as executed by MLKL *per se*, as an important target for viral inhibition. These findings, and the recent report that *Ripk3*, but not *Mlkl*, plays a key role in host defence and central nervous system chemokine production following West Nile Virus infection [31], may speak to RIPK3's broader functions in driving apoptosis and pro-inflammatory cytokine/chemokine synthesis as the basis for its viral targeting. We know that *Ripk3*$^{-/-}$ mice are more susceptible than wild-type counterparts to numerous viral infections [32], and that several pathogenic microbes have evolved mechanisms to specifically counteract necroptosis, suggesting that RIPK3 activation is important for pathogen control. Given this and the lack of developmental perturbation in *Ripk3*$^{-/-}$ or *Mlkl*$^{-/-}$

mice, there is a strong precedent that necroptosis has evolved as a purely innate response to infection.

Why do pathogens encode so many effectors to subvert immunity, especially when many appear to have redundant functions?

One possibility is that, as proposed for Influenza A viral infection, both apoptosis and necroptosis act in concert to destroy the infected cell and limit viral propagation [33], but it may also reflect different tributaries in pathways, such as necroptosis, that we are yet to fully understand. Viral inhibitors of inflammation and apoptosis have been fundamental in the discovery and characterisation of necroptosis, however, it is only more recently that specific bacterial inhibitors of caspases have been discovered. The bacterial gut pathogen, enteropathogenic *E. coli* (EPEC) utilises a type III secretion system (T3SS) to directly deliver virulence "effector" proteins called NleB1 and NleF into host cells to inhibit caspase activation and subsequent apoptosis during infection [34, 35] (Figure 1). EPEC also encodes numerous effectors that potently and irreversibly block TNF-induced inflammation (e.g. NleE and NleC) [36-40] (Figure 1), creating a strong precedent for necroptosis to occur during infection. Our recent study showed that EPEC can counteract necroptosis by injecting host cells with the effector, EspL, a novel cysteine protease that specifically targets RHIM-containing proteins for direct proteolytic cleavage within their RHIM domain (Figure 1) [41]. The cleavage consequently inhibits necroptosis via TNFR1 and TLR3 or TLR4 activation. Genetic deletion of *espL* renders host cells susceptible to necroptosis during infection *in vitro* [41], and deletion of *espL* results in attenuation of bacterial colonisation *in vivo* using the

established mouse-model of EPEC infection, *Citrobacter rodentium* [41], suggesting that the mechanism of EspL contributes to prolonging infection via protection of the bacterial niche (enterocytes) from necroptosis. Indeed, the three EPEC effectors that most potently inhibit inflammation, apoptosis and necroptosis, respectively (NleE, NleB1 and EspL) are all encoded on a pathogenicity island that is horizontally transferred as a single unit amongst certain pathogenic *E. coli* and is frequently found in strains that cause severe disease outbreaks [42]. It is therefore likely that if necroptosis evolved as a mechanism to control viral pathogens, it may also aid in clearance of bacterial gut pathogens which has in turn put selective pressure on EPEC to evolve a mechanism to also inhibit necroptosis. One might suggest that necroptosis would only be beneficial for clearance of bacterial pathogens that replicate intracellularly, like viruses, yet EPEC attach intimately to the apical surface of enterocytes where they orchestrate manipulation of host responses via the activity of the T3SS and its substrates to replicate and cause disease [43]. This raises the question of whether necroptosis is occurring predominantly within the enterocytes at the primary site of EPEC attachment or within immune cells that have engulfed bacteria around/within colonic crypts. This is yet to be investigated.

EPEC is not the only bacterial pathogen that delivers effector proteins to inhibit and stimulate immune processes such as inflammation and apoptosis in host cells. *Shigella* [44], *Yersinia* [45] and *Salmonella* spp. [46] all utilise T3SSs to deliver effectors that manipulate immune processes such as NF-κB and MAPK signaling and apoptosis to cause serious disease. The intracellular pathogen *Legionella pneumophila* translocates over 300 effector proteins into

the host cell cytosol during infection [47] whereas some pathogens such as *Pseudomonas* [48] and *Burkholderia* spp. [49] utilise multiple and diverse secretion systems to inject effectors during infection. Many of these bacterial pathogens have a diverse host range that require varied mechanisms to evade different immune systems, and with the vast number of uncharacterised bacterial effector proteins, we still have much to learn about innate responses to infection.

Why has evolution permitted necroptosis to be lost if it is integral to innate immunity?

An intriguing point of this discussion is the discrepancy of the presence/absence of RIPK3 and/or MLKL across species within the animal kingdom. For example, possums and Tasmanian devils lack RIPK3, MLKL, and DAI/ZBP1, and carnivores including cats, dogs and ferrets lack MLKL [18, 50]. The reason remains a great unknown and the basis for a good deal of speculation. One postulate is that the lifestyles of some animals, such as carnivores, would expose them to pathogens in scavenged carrion that might invoke death of digestive tract cells by necroptosis. Such an interaction would lead to inflammatory responses that would not be beneficial, and accordingly evolution may have disfavoured the preservation of this pathway in these animals. It will be important to test whether the lack of RIPK3 and/or MLKL in these species negates necroptosis when activated via the pathways that are known to induce necroptosis in humans. If death does still occur, it might infer that there are alternate mediators of necroptosis that exist. Currently our knowledge of the stimuli that induce necroptosis is quite limited, and most likely non-exhaustive, and some studies already suggest alternative mechanisms, independent of RIPK3 phosphorylation, exist to

regulate MLKL activation [51, 52]. Regardless, there are still many questions that remain to be answered to completely understand the role of necroptosis as an innate response to infection.

A final point to consider on the relevance of necroptosis as an innate response to infection is on the topic of bacterial and viral co-infection. Many studies have reported the co-incidence of viral and bacterial pathogens and have associated this with more severe disease phenotypes [53, 54]. Given that pathogens like EPEC can cleave all RHIM proteins, including TRIF, a key mediator of anti-viral inflammatory responses and necroptosis, could this then potentiate infection/disease caused by an enteric virus such as Rotavirus or Norovirus? This would further support a role for pathogens evolving mechanisms to overcome host responses such as necroptosis.

The fast-moving field of study on necroptosis is clearly still under intense debate, yet has opened-up a gold mine of research questions on immune regulation and homeostasis, host defence mechanisms and mechanisms of microbial pathogenesis. It appears the scientific community are making solid progress on understanding the mechanism of necroptosis itself and the fine details of how it is mediated in the host but our understanding of the physiological relevance of the response in *in vivo* models of disease or infection remains open. It will continue to be difficult to address this question when not all mechanistic aspects of necroptosis are comprehensively defined. After following our own set of arguments in this opinion piece, it seems that we have agreed that necroptosis is indeed a true innate response

to infection, but one that has evolved out of necessity upon the microbial evolution to inhibit inflammation and apoptosis. This means that we could still refer to necroptosis as a 'backup' mechanism to apoptosis, but this should not discount the increasing evidence that the key mediator of necroptosis, RIPK3, plays an important role in mediating inflammation and cell survival.

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References

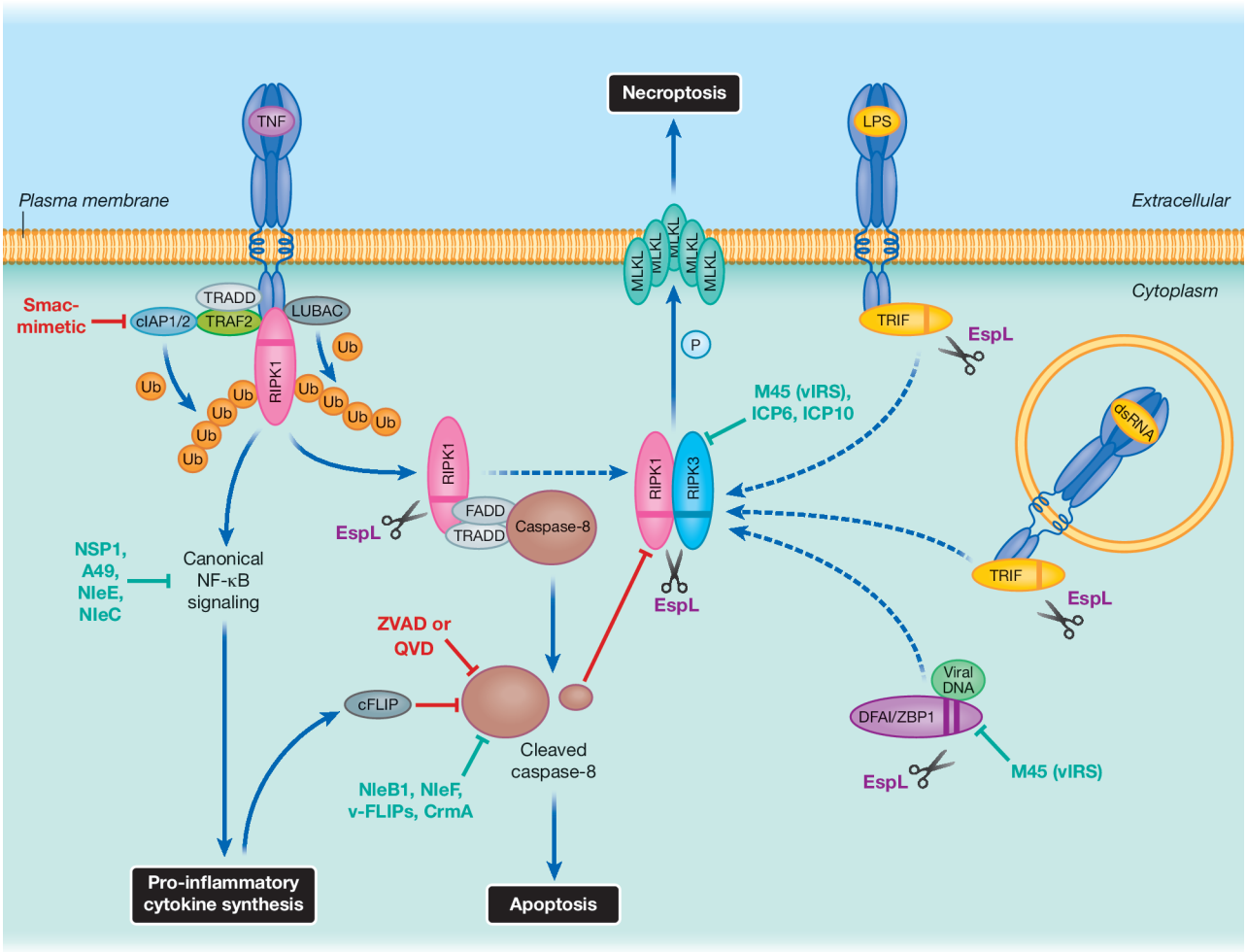
1. Newton, K, *et al.*, Kinase RIP3 is dispensable for normal NF-kappa Bs, signaling by the B-cell and T-cell receptors, tumor necrosis factor receptor 1, and Toll-like receptors 2 and 4. *Molecular and Cellular Biology*, 2004. 24(4): p. 1464-1469.
2. Murphy, J, *et al.*, The pseudokinase MLKL mediates necroptosis via a molecular switch mechanism. *Immunity*, 2013. 39(3): p. 443-453.
3. Dillon, C, *et al.*, RIPK1 blocks early postnatal lethality mediated by caspase-8 and RIPK3. *Cell*, 2014. 157(5): p. 1189-1202.
4. Kaiser, W, *et al.*, RIP1 suppresses innate immune necrotic as well as apoptotic cell death during mammalian parturition. *Proc Natl Acad Sci*, 2014. 111(21): p. 7753-7758.
5. Kelliher, M, *et al.*, The death domain kinase RIP mediates the TNF-induced NF-kappaB signal. *Immunity*, 1998. 8(3): p. 297-303.

6. Welz, P, *et al.*, FADD prevents RIP3-mediated epithelial cell necrosis and chronic intestinal inflammation. *Nature*, 2011. 477(7364): p. 330-334.
7. Kaiser, WJ, *et al.*, RIP3 mediates the embryonic lethality of caspase-8-deficient mice. *Nature*, 2011. 471(7338): p. 368-372.
8. Rickard, JA, *et al.*, RIPK1 Regulates RIPK3-MLKL-Driven Systemic Inflammation and Emergency Hematopoiesis. *Cell*, 2014. 157(5): p. 1175-88.
9. Zhang, J, *et al.*, Fas-mediated apoptosis and activation-induced T-cell proliferation are defective in mice lacking FADD/Mort1. *Nature*, 1998. 392(6673): p. 296-300.
10. Upton, JW, *et al.*, DAI/ZBP1/DLM-1 Complexes with RIP3 to Mediate Virus-Induced Programmed Necrosis that Is Targeted by Murine Cytomegalovirus vIRA. *Cell Host & Microbe*, 2012. 11(3): p. 290-297.
11. Petrie, EJ, *et al.*, Insane in the membrane: a structural perspective of MLKL function in necroptosis. *Immunol Cell Biol*, 2017. 95(2): p. 152-159.
12. Ros, U, *et al.*, Necroptosis Execution Is Mediated by Plasma Membrane Nanopores Independent of Calcium. *Cell Reports*, 2017. 19(1).
13. Li, J, *et al.*, The RIP1/RIP3 Necrosome Forms a Functional Amyloid Signaling Complex Required for Programmed Necrosis. *Cell*, 2012. 150(2): p. 339-350.
14. Pasparakis, M, *et al.*, Necroptosis and its role in inflammation. *Nature*, 2015. 517(7534): p. 311-320.
15. Newton, K, *et al.*, RIPK1 inhibits ZBP1-driven necroptosis during development. *Nature*, 2016. 540(7631): p. 129-133.
16. Lin, J, *et al.*, RIPK1 counteracts ZBP1-mediated necroptosis to inhibit inflammation. *Nature*, 2016. 540(7631): p. 124-128.
17. He, S, *et al.*, Toll-like receptors activate programmed necrosis in macrophages through a receptor-interacting kinase-3-mediated pathway. *Proc Natl Acad Sci*, 2011. 108(50): p. 20054-20059.
18. Newton, K, *et al.*, Necroptosis and inflammation. *Annual Review of Biochemistry*, 2016. 85: p. 743-763.
19. Upton, J, *et al.*, Cytomegalovirus M45 cell death suppression requires receptor-interacting protein (RIP) homotypic interaction motif (RHIM)-dependent interaction with RIP1. *Journal of Biological Chemistry*, 2008. 283(25): p. 16966-70.
20. Guo, H, *et al.*, Herpes simplex virus suppresses necroptosis in human cells. *Cell Host & Microbe*, 2015. 17(2): p. 243-251.
21. Holloway, G, *et al.*, Rotavirus Antagonizes Cellular Antiviral Responses by Inhibiting the Nuclear Accumulation of STAT1, STAT2, and NF-{kappa}B. *Journal of Virology*, 2009. 83(10): p. 4942-4951.
22. Mansur, D, *et al.*, Poxvirus targeting of E3 ligase β-TrCP by molecular mimicry: a mechanism to inhibit NF-κB activation and promote immune evasion and virulence. *PLoS Pathogens*, 2013. 9(2): p. e1003183.
23. Thome, M, *et al.*, Viral FLICE-inhibitory proteins (FLIPs) prevent apoptosis induced by death receptors. *Nature*, 1997. 386(6624): p. 517-521.
24. Zhou, Q, *et al.*, Target Protease Specificity of the Viral Serpin CrmA - ANALYSIS OF FIVE CASPASES. *The Journal of Biological Chemistry*, 1997. 272(12): p. 7797-7800.
25. Chang, D, *et al.*, c-FLIP(L) is a dual function regulator for caspase-8 activation and CD95-mediated apoptosis. *EMBO J*, 2002. 21(14): p. 3704-3714.

26. Damgaard, R, *et al.*, Inhibitor of apoptosis (IAP) proteins in regulation of inflammation and innate immunity. *Discovery Medicine*, 2011. 23(125): p. 221-231.
27. Moujalled, D, *et al.*, TNF can activate RIPK3 and cause programmed necrosis in the absence of RIPK1. *Cell Death and Disease*, 2013. 4: p. e465.
28. Takahashi N1, *et al.*, RIPK1 ensures intestinal homeostasis by protecting the epithelium against apoptosis. *Nature*, 2014. 513(7516): p. 95-99.
29. Rickard, J, *et al.*, TNFR1-dependent cell death drives inflammation in Sharpin-deficient mice. *eLIFE*, 2014. 3: p. e03464.
30. Upton, JW, *et al.*, Virus inhibition of RIP3-dependent necroptosis. *Cell Host & Microbe*, 2010. 7(4): p. 302-313.
31. Daniels, B, *et al.*, RIPK3 Restricts Viral Pathogenesis via Cell Death-Independent Neuroinflammation. *Cell*, 2017. 169(2): p. 301-313.
32. Cho, Y, *et al.*, Phosphorylation-driven assembly of the RIP1-RIP3 complex regulates programmed necrosis and virus-induced inflammation. *Cell*, 2009. 137(6): p. 1112-1123.
33. Nogusa, S, *et al.*, RIPK3 Activates Parallel Pathways of MLKL-Driven Necroptosis and FADD-Mediated Apoptosis to Protect against Influenza A Virus. *Cell Host & Microbe*, 2016. 20(1): p. 13-24.
34. Pearson, JS, *et al.*, A type III effector antagonizes death receptor signalling during bacterial gut infection. *Nature*, 2013. 501(7466): p. 247-251.
35. Pollock, G, *et al.*, Distinct Roles of the Antiapoptotic Effectors NleB and NleF from Enteropathogenic *Escherichia coli*. *Infection and Immunity*, 2017. 85(4): p. e01071-16
36. Mühlen, S, *et al.*, Proteasome-independent Degradation of Canonical NFκB Complex Components by the NleC Protein of Pathogenic *Escherichia coli*. *Journal of Biological Chemistry*, 2011. 286(7): p. 5100-5107.
37. Nadler, C, *et al.*, The type III secretion effector NleE inhibits NF-κB activation. *PLoS Pathogens*, 2010. 6(1): p. e1000743.
38. Newton, HJ, *et al.*, The Type III Effectors NleE and NleB from Enteropathogenic *E. coli* and OspZ from *Shigella* Block Nuclear Translocation of NF-κB p65. *PLoS Pathogens*, 2010. 6(5): p. e1000898.
39. Pearson, JS, *et al.*, A type III effector protease NleC from enteropathogenic *Escherichia coli* targets NF-κB for degradation. *Molecular Microbiology*, 2011. 80(1): p. 219-230.
40. Yen, H, *et al.*, NleC, a type III secretion protease, compromises NF-kappaB activation by targeting p65/RelA. *PLoS Pathogens*, 2010. 6(12): p. e1001231.
41. Pearson, JS, *et al.*, EspL is a bacterial cysteine protease effector that cleaves RHIM proteins to block necroptosis and inflammation. *Nature Microbiology*, 2017. 2: p. 16258.
42. Hazen, TH, *et al.*, Refining the pathovar paradigm via phylogenomics of the attaching and effacing *Escherichia coli*. *Proceedings of the National Academy of Sciences*, 2013. 110(31): p. 12810-12815.
43. Wong, ARC, *et al.*, Enteropathogenic and enterohaemorrhagic *Escherichia coli*: even more subversive elements. *Molecular Microbiology*, 2011. 80(6): p. 1420-1438.
44. Ashida, H, *et al.*, *Shigella* manipulates host immune responses by delivering effector proteins with specific roles. *Frontiers in Immunology*, 2015. 6(219).
45. Pha, K, *et al.*, *Yersinia* type III effectors perturb host innate immune responses. *World J Biol Chem*, 2016. 7(1): p. 1-13.

46. LaRock, DL, *et al.*, Salmonellae interactions with host processes. *Nature Reviews Microbiology*, 2015. 13(4): p. 191-205.
47. Ensminger, AW, *Legionella pneumophila*, armed to the hilt: justifying the largest arsenal of effectors in the bacterial world. *Current Opinion in Microbiology*, 2016. 29: p. 74-80.
48. Blevesa, S, *et al.*, Protein secretion systems in *Pseudomonas aeruginosa*: A wealth of pathogenic weapons. *International Journal of Medical Microbiology*, 2010. 300(8): p. 534-543.
49. Willcocks, SJ, *et al.*, Intracellular replication of the well-armed pathogen *Burkholderia pseudomallei*. *Current Opinion in Microbiology*, 2016. 29: p. 94-103.
50. Dondelinger, Y, *et al.*, An evolutionary perspective on the necroptotic pathway. *Trends in Cell Biology*, 2016. 26(10): p. 721-732.
51. Tanzer, M, *et al.*, Necroptosis signalling is tuned by phosphorylation of MLKL residues outside the pseudokinase domain activation loop. *Biochemical Journal*, 2015. 471(2): p. 255-265.
52. Günther, C, *et al.*, The pseudokinase MLKL mediates programmed hepatocellular necrosis independently of RIPK3 during hepatitis. *J Clin Invest*, 2016. 126(11): p. 4346-4360.
53. Bosch, AATM, *et al.*, Viral and Bacterial Interactions in the Upper Respiratory Tract. *PLoS Pathog*, 2013. 9(1): p. e1003057.
54. Almand, EA, *et al.*, Virus-Bacteria Interactions: An Emerging Topic in Human Infection. *Viruses*, 2017. 9(3): p. 58-68.

Figure 1. Schematic representation of necroptotic signaling pathways induced via the TNFR1, TLR3, TLR4 and cytosolic viral DNA. See in text for detailed description. Solid line arrows depict progression of pathways upon stimulation of receptor by ligand or pathogen-associated molecular pattern (PAMP), dotted line arrows depict pathway progression as a result of RHIM-mediated interactions. Solid colours lines depicted within RIPK1, RIPK3, DAI/ZBP1, TRIF proteins represent their respective RHIM domain/s. Bacterial (NleB1, NleC, NleE and NleF) and viral (M45, ICP6, ICP10, NSP1, A49, CrmA and v-FLIPs) inhibitors of pathways are coloured in aqua, chemical inhibitors of pathways (Z-VAD-FMK (ZVAD) and Q-VD-OPh (QVD)) are coloured in red.



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