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Pathogenic bacteria attack RHIM

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Abstract

Attaching and effacing enteropathogenic *Escherichia coli* causes gastrointestinal inflammation and diarrhoea. In this issue of *Nature Microbiology*, Pearson and colleagues find that this pathology involves bacterial cleavage of a class of host cell death signal adaptors that encode a unique protein interaction motif called the RHIM.

Evasion of host cell death is crucial for the successful survival, propagation and dissemination of many intracellular pathogens. Necroptosis is a recently described form of non-apoptotic cell death with distinct morphological features, such as cell and organelle swelling and rapid disintegration of cellular membranes. Upon rupture of the plasma membrane, certain cellular factors, which are collectively referred to as damage-associated molecular patterns (DAMPs), are leaked out from the necroptotic cells. Recent studies show that DAMPs can potentially stimulate inflammation to promote robust immune response against invading microorganisms. These characteristics suggest that necroptosis may be a key host-defence mechanism against pathogens¹.

The key players that mediate necroptosis are receptor-interacting protein kinase 1 (RIPK1) and RIPK3, two serine/threonine kinases that form a tight complex with one another. This binding reaction requires the receptor-interacting protein homotypic interaction motif (RHIM), present in both RIPK1 and RIPK3. Other RHIM-containing mammalian proteins include the innate immune signal adaptors TIR-domain-containing adapter-inducing interferon- β (TRIF) and Z-DNA-binding protein 1/DNA-dependent activator of IFN-regulatory factors (ZBP1/DAI); all four mammalian RHIM-containing adaptors can stimulate necroptosis (Fig. 1a). Besides promoting cell death, they are also potent inducers of inflammatory cytokine expression, and this cell-death-independent activity is mostly mediated by activating the transcription factor NF- κ B (nuclear factor κ -light-chain-enhancer of activated B cells). Moreover, RIPK1 and RIPK3 also facilitate cleavage of pro-interleukin-1 β (IL-1 β) into the mature cytokine IL-1 β . The cytokine-inducing capacity of the RIP kinases can further promote antimicrobial inflammation beyond that achieved by cell death alone¹ (Fig. 1a).

Recent studies seem to suggest that bacterial pathogens stimulate RIPK1- and RIPK3-dependent cell death. For example, *Yersinia pestis*, the etiological agent for pandemic Black

Competing interests

The authors declare no competing financial interests.

Death in medieval Europe, causes RIPK1 caspase-8-dependent apoptosis in macrophages³, while *Salmonella enterica* serovar Typhimurium promotes RIPK3-dependent necroptosis in macrophages⁴. In contrast, large DNA viruses are adept at countering this cell death machinery to limit antiviral inflammation, with several RHIM-containing inhibitors identified in the herpesvirus family². Interestingly, these inhibitors bind to the mammalian adaptors through the RHIM to sequester them from the necroptosis machinery. Thus, when it comes to dealing with the threat of host cell death, there appears to be a dichotomy between viral and bacterial pathogens.

However, in this issue of *Nature Microbiology*, Pearson and colleagues present exciting data that suggest certain attaching and effacing enteropathogenic *Escherichia coli* (EPEC) encode inhibitors that can also counteract bactericidal signalling using the RHIM-containing adaptors⁵. The authors found that EPEC infection led to a reduction in RIPK1 protein levels. Using mutant bacteria that lack different genes in the type III secretion system (T3SS), the authors traced the infection-induced disappearance of RIPK1 to the effector EspL. Structural modelling suggested that EspL encodes a cysteine protease, and ectopic EspL expression induced RIPK1 cleavage in host cells. However, EspL does not exhibit homology to other known cysteine proteases, and broad-spectrum cysteine protease inhibitors did not restore degradation of RIPK1. Hence, EspL appears to be an atypical cysteine protease.

Infection with wild-type EPEC, but not EspL-deficient EPEC, conferred resistance to tumour necrosis factor (TNF)-induced necroptosis. Complementation of EspL-deficient EPEC with wild-type EspL, but not an EspL mutated in the putative catalytic triad, restored sensitivity to TNF-induced necroptosis. In addition to RIPK1, EspL also cleaved the other mammalian RHIMs — RIPK3, TRIF and ZBP1 (Fig. 1b). Thus, TRIF-mediated type I interferon (IFN) expression in response to Toll-like receptor 3 (TLR3) and TLR4 stimulation was also inhibited by EspL. These EspL-mediated effects are important for pathogenesis, as *Citrobacter rodentium* that lacked EspL was cleared much more rapidly than wild-type bacteria in the intestines of infected mice. These results therefore provide compelling evidence that pathogenic bacteria can also target the host RHIM-containing adaptors to maximize survival and persistence within the host.

How common is bacterial inhibition of RHIM and necroptosis? Bioinformatics analysis suggests that other pathogenic bacteria, such as *Shigella*, *Yersinia* and *Salmonella*, all encode an EspL-like factor. However, previous studies show that both *Yersinia* and *Salmonella* activate RIPK1 and RIPK3 rather than inhibit them^{3,4}, suggesting that these bacteria do not encode functional EspL that can cleave RHIM adaptors. Given that EspL does not resemble other known cysteine proteases, comparison of EspL from different bacteria may reveal further insights into their mechanism of inhibition of necroptosis. Another interesting feature of EspL is that it cleaves only the inactive monomeric RHIM, and not the active amyloid form of the RHIM complex⁶. While we cannot rule out the possibility that EspL also targets other cellular proteins, this observation does suggest that EspL is most effective at curbing the host response at the onset of infection. In this light, it is noteworthy that EPEC encodes a second T3SS effector, NleB1, that targets the apoptosis signal adaptor fas-associated death domain (FADD) protein, as well as RIPK1⁷. NleB1 inhibits FADD signalling through its N-acetyl glucosamine transferase activity, but it is

unclear if NleB1 is equally active against the active amyloid form of RIPK1. In this case, EspL and NleB1 may act in synergy to maximize inhibition of the host cell death machinery.

EspL now joins a growing list of inhibitors that target host cell necroptosis. This represents quite a paradigm shift from the early days when pathogen infection was largely thought to sensitize cells to necroptosis. Although these viral and bacterial necroptosis inhibitors share the same cellular targets, they do so in a different manner. The convergent attack on RHIM-containing adaptors raises the tantalizing possibility that they are key players in shaping the evolution of diverse pathogenic microorganisms.

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Figure 1.

RHIM protein cleavage in EPEC infection. **a**, In uninfected enterocytes, RHIM domain (green boxes)-containing proteins can promote inflammatory cytokine production and necroptosis. RIPK1 and RIPK3 assemble into a higher-order complex when activated. **b**, EPEC injects the cysteine protease EspL effector upon host cell infection, which leads to cleavage of RHIM domain-containing proteins. RIPK1 and RIPK3 degradation is thought to prevent their assembly and thus block necroptosis, while TRIF and DAI cleavage may also downregulate IFN signalling. EspL is required to combat host clearance as infection with an inactive EspL strain reduces *C. rodentium* intestinal persistence.