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RESEARCH HIGHLIGHT

Type I interferon: the mediator of bacterial infection-induced necroptosis

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COMMENTARY

Innate immune responses are essential for the host to fight against invading microbes. Innate immune receptors or pattern recognition receptors recognize conserved microbial molecules and trigger innate immune responses. Pattern recognition receptors such as Toll-like receptors primarily signal through the adaptor MyD88 to activate NF-κB, while other Toll-like receptors signal *via* TRIF to activate IRF-3 resulting in the expression of type I interferons (IFNs).[1] Type I IFNs play a crucial role in antiviral immune responses but are also important for the host response against bacterial infection.[2] Paradoxically, the production of type I IFNs leads to protection against some intracellular pathogens such as *Mycobacterium tuberculosis*,[3] while it enhances the susceptibility to other intracellular pathogens such as *Listeria monocytogens*.[4,5] The mechanisms whereby type I IFN signaling during intracellular bacterial pathogen infection causes differential outcomes remain to be established. In a recent study reported in *Nature Immunology*, Robinson *et al.* describe a pathway in which *Salmonella enterica* serovar Typhimurium (*S. Typhimurium*) exploits the host type I IFN signaling to kill macrophages through the induction of necroptosis, a specialized pathway of programmed necrosis.[6]

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To evaluate the role of type I IFNs in infection, Robinson *et al.* challenged mice deficient in the receptor for IFN-α and IFN-β (IFNAR-deficient ($Ifnar^{-/-}$)) with *S. Typhimurium*. Improved survival as well as lower bacterial burden in the spleen and liver was observed in $Ifnar^{-/-}$ mice relative to that of the wild-type mice. Furthermore, better protection against *S. Typhimurium* infection was also obtained *via* adoptive transfer of $Ifnar^{-/-}$ bone marrow into wild-type or $Ifnar^{-/-}$ hosts, compared with the bone marrow from wild-type mice, indicating that hematopoietic cell-derived type I IFNs play an prominent role in the susceptibility to infection in $Ifnar^{-/-}$ mice.

The improved early control of *S. Typhimurium* in $Ifnar^{-/-}$ mice was observed before T-cell activation, suggesting a dominant role of the modulation of the innate immune response. The researchers then assessed different innate immune cell types from the spleens of 5d-infected mice. Significantly, more macrophages were observed in $Ifnar^{-/-}$ mice than wild-type mice. To further determine whether these innate immune cells were sufficient for the enhanced protection against *S. Typhimurium* in $Ifnar^{-/-}$ mice, $Ifnar^{-/-}$ macrophages were transferred into naïve wild-type hosts. Indeed, improved protection was noted. However, there was no difference between bacterial burdens of *S. Typhimurium*-infected, bone marrow-derived macrophages from $Ifnar^{-/-}$ and wild-type mice, indicating that the improved control of *S. Typhimurium* was because of increased number of macrophages in $Ifnar^{-/-}$ mice. Considering that the induction of macrophage death is a key virulence strategy used by *S. Typhimurium*,[7] it was reasonable to rationalize that type I IFN signaling may be related to cell death of macrophages through infection. Indeed, macrophages from $Ifnar^{-/-}$ mice survived from *S. Typhimurium* infection, while massive death was observed in those form wild-type mice.

To discern if the death of macrophages was caused by apoptosis or necrosis, the workers examined the cleavage of poly(ADP-ribose) polymerase 1. Poly(ADP-ribose) polymerase 1 is cleaved to fragments of 89 and 24 kDa in size during apoptosis, while in necrosis, it is excised into fragments of 72 and 50 kDa, respectively.[8] The resultant data show a cleavage pattern consistent with necrotic death, demonstrating that *S. Typhimurium* infection exploited type I IFN signaling to induce macrophage death *via* necrosis.

The resistance of $Ifnar^{-/-}$ macrophages to death induction through *S. Typhimurium* infection could be *via* either cytokine signaling or inflammasome activation. However, little difference in cytokine expression was observed in $Ifnar^{-/-}$ macrophages *vs.* wild-type cells. Although the secretion of IL-1β in $Ifnar^{-/-}$ macrophages was elevated, there was no difference in caspase-1 activation between $Ifnar^{-/-}$ and wild-type macrophages. Furthermore, neutralization of IL-1β *in vivo* did not influence the bacterial burden in $Ifnar^{-/-}$ mice. These results indicate that there is no inflammasome activation or cytokine expression difference between $Ifnar^{-/-}$ and wild-type mice during *S. Typhimurium* infection.

Robinson *et al.* further assessed several mechanisms associated with cell death. TNF and nitrate ions are known to induce the death of macrophages. Neither genetic deficiency in TNF receptors nor neutralization of TNF-α could protect the death of macrophages during *S. Typhimurium* infection. Similar results were also retained when the production of nitrate ions was blocked. However, neutralization of IFN-β blocked the death of macrophages after infection. These results indicate that type I IFN signaling plays a commanding role in the

necrotic death induced by *S. Typhimurium* infection.

Apoptosis is considered a form of programmed cell death during development, homeostasis and disease, whereas necrosis is regarded as an unregulated and uncontrolled process. However, a recent study has revealed the existence of programmed necrosis termed necroptosis.[9] Necroptosis requires the kinase activity of receptor interacting protein 1 (RIP1)[10] and RIP3.[11–13] To determine whether the *S. Typhimurium* infection-induced macrophage death was *via* necroptosis, these researchers treated infected macrophages with necrostatin, which inhibit the activity of RIP1. As predicted, necrostatin protected macrophages from death induction. Furthermore, RIP3 deficiency also inhibited the death of infected macrophages. These data suggest that *S. Typhimurium* infection invokes the mechanism of necroptosis to abolish macrophages.

Since the RIP1 and RIP3 interaction protein complex is required for necroptosis, the authors hypothesized that infection of *S. Typhimurium* would manipulate the phosphorylation level of these two proteins. Compared with wild-type macrophages, *S. Typhimurium*-infected *Ifnar*$^{-/-}$ macrophages had less phosphorylated forms of RIP1 and RIP3, especially in the late infection period. When IC-21 macrophages were infected with *S. Typhimurium* or when macrophages were incubated with type I IFNs, phosphorylated RIP1 was induced. Furthermore, *S. Typhimurium* infection of macrophages induced the expression of RIP3, and immunoprecipitated RIP1 showed a stronger association of RIP3 with RIP1 in wild-type macrophages compared with the *Ifnar*$^{-/-}$ cells. These results indicate that *S. Typhimurium* infection activated RIP1 and RIP3 in a type I IFN signaling-dependent manner. Caspase-8 is an inhibitor of necroptosis, and blockade of caspase-8 induces necroptosis.[14] There was less caspase-8 in macrophages after infection with *S. Typhimurium*. Consistent with this result, blocking caspase-8 activation and the engagement of type I INF signaling resulted in necroptosis, resulting in macrophage death. To further confirm the role of necroptosis in *S. Typhimurium*-infected macrophage death, *in vivo* experiments were performed, which showed that there were less phosphorylated RIP1 in the *Ifnar*$^{-/-}$ splenic macrophages after *S. Typhimurium* infection compared with the wild-type. These outcomes raise the point that IFNAR-mediated RIP1 signaling may be a new pathway leading to macrophage death.

The direct evidence between type I IFN signaling and necroptosis comes from the observation of a physical interaction of RIP1 with the IFNAR. Robinson *et al.* infected wild-type macrophages with *S. Typhimurium* and found that RIP1 was co-immunoprecipitated with the IFNAR and the interaction was enhanced after infection. Furthermore, treating macrophages with type I IFN increased the association of RIP1 with IFNAR. Staining *S. Typhimurium*-infected macrophages also revealed colocalization of RIP1 and IFNAR. These results demonstrate that type I IFN signaling promotes the formation of a RIP1–RIP3 complex and leads to necroptosis.

To further confirm the role of necroptosis in *S. Typhimurium*-induced macrophage death, *Rip3*$^{-/-}$ macrophages were transferred into wild-type host, and enhanced control of *S. Typhimurium* infection was observed compared to the transfer from wild-type macrophages, in a manner similar to the adoptive transfer of *Ifnar*$^{-/-}$ macrophages. In the animal infection assay, mice deficient in Ciap1, which is known to inhibit necroptosis through inhibiting RIP1, showed a higher burden of *S. Typhimurium* during infection than that of the wild-type mice. These results indicate that necroptosis is dependent on type I IFN signaling during *S. Typhimurium*-induced macrophage death.

It is well known that *S. Typhimurium* induces the expression of type I IFNs by macrophages[15] and the induction of macrophage death is a crucial pathogenic strategy used by *S. Typhimurium*.[7] The Robinson study uncovers a novel mechanism by which *S. Typhimurium* exploits the host type I IFN signaling to destruct macrophages through the induction of RIP-dependent cell death, hereby boosts its own invasion and persistence (Figure 1). This mechanism is not only limited in *S. Typhimurium* infection since inhibition of necroptosis also protects macrophages from death in infection with another intracellular bacterium, *L. monocytogenes*.[16] Therefore, pathogens that manipulate host response to express type I IFNs and induce the death of macrophages may use this mechanism as a common strategy to facilitate their survival. Elucidation of this new pathway may lead to the development of innovative

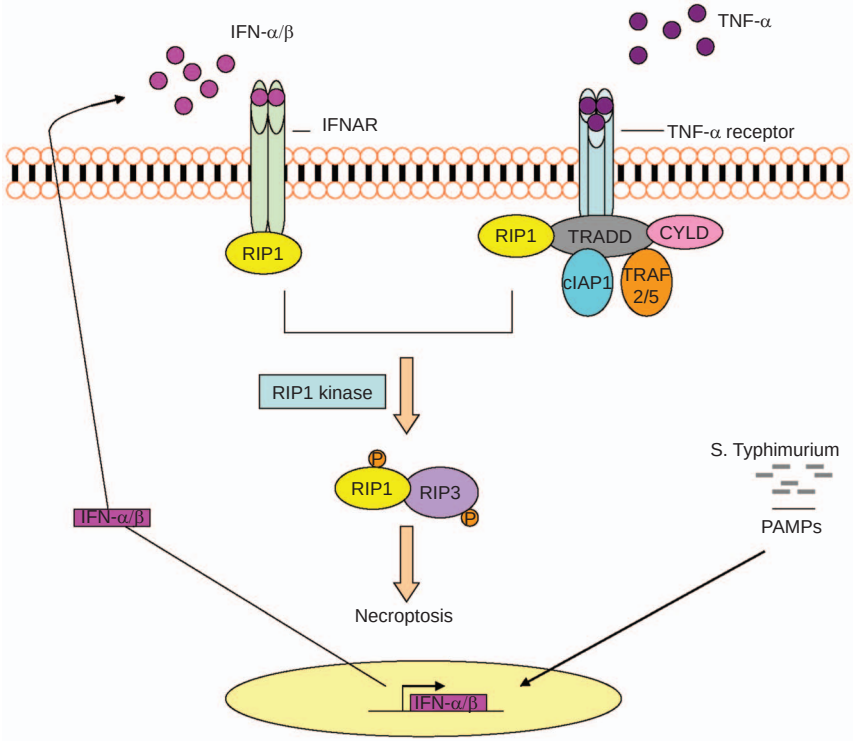

Figure 1 Type I IFN-mediated necroptosis in macrophages during infection with S. Typhimurimum. After S. Typhimurium infection, type I IFN signaling is activated through the production of type I IFNs. This signal induces the interaction of IFNAR and RIP1, promoting the formation of RIP1–RIP3 (also known as RIPK1–RIPK3) complex, leading to necroptosis of macrophages, invasion and persistence of the bacteria. CYLD, cylindromatosis; IAP, inhibitor of apoptosis protein; IFN, interferon; PAMP, pathogen-associated molecular pattern; RIP, receptor interacting protein; TRADD, TNF receptor-associated death domain; TRAF2/5, TNF receptor-associated factor 2/5.

strategies to fight against chronic infections by interfering with the induced necroptosis of macrophages.

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