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RESEARCH HIGHLIGHT

Critical role of type I interferon-induced macrophage necroptosis during infection with *Salmonella enterica* serovar Typhimurium

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Salmonella enteric strains are facultative intracellular pathogens that can produce both localized enteritis and disseminated systemic disease in humans and a variety of other vertebrates.[1] Extensive evidence obtained from genetic and cell biology studies indicates that Salmonella has evolved specific virulence mechanisms to evade innate immune responses.[1] However, the underlying molecular and cellular mechanisms have not been understood very well. A recent paper published by Robinson *et al.* in *Nature Immunology*[2] reported that type I interferon (IFN)-induced macrophage necroptosis plays a critical role in evading host innate immune responses during infection with *Salmonella enterica* serovar Typhimurium (*S.* Typhimurium). This finding helps us to further understand the pathogenesis of Salmonella enteric infection.

The macrophage, an important component of innate immune cells, has long been thought to be central to the dissemination of *S.* Typhimurium.[3] Macrophages are among the earliest activated lymphocytes controlling the infectious pathogens before adaptive immune responses are developed. Macrophages provide an effective mechanism in rapid control of intracellular bacteria such as *S.* Typhimurium in the early phase of infection.[4,5] During the host bacteria interaction, virulent *S.* Typhimurium develops several mechanisms to avoid killing by host's macrophages.[6] The bacterium controls maturation of the intracellular vacuole where it shields itself from cytosol macrophage degradative pathway. In addition, *S.* Typhimurium has the ability to avoid killing by free radical-dependent macrophage antimicrobial mechanisms.[6] Further, the intracellular bacteria such as *S.* Typhimurium induce the macrophage death, which is a key virulence strategy used by those bacterial to evading host innate immune responses.[5] However, the underlying mechanism has not been elucidated very well. Regarding the processes of the pathogen-induced macrophage death including the well-known apoptosis and necrosis, the cells can also die through the inflammasome-mediated pyrosis, which involves in inflammatory process of caspase-1-dependent programmed cell death and proinflammatory cytokine release during cell lysis. Recently, a novel 'prototypic' apoptotic pathway termed as necropotosis has been defined. The necropotosis is normally triggered by Fas and TNFR family of death receptors and this programmed cell necrosis is competitive with apoptosis.[7,8] The necroptosis pathway depends on formation of death domain-containing kinase RIP1 and RIP3 complex and subsequent recruitment of downstream adaptor proteins, such as Fas-associated death domain protein, and upstream caspases, such as caspase-8.[7,8] The new findings reported by Robinson *et al.*[2] has demonstrated that *S.* Typhimurium exploites type I IFN to induce necroptosis of macrophages. This sheds light on the importance of *S.* Typhimurium-induced macrophage necroptosis in the pathogenesis of the infection.

Inflammatory cytokines such as type I IFN have long been recognized as another crucial component of innate immune defense against viral and bacterial infections. Interestingly, the impact of type I IFNs on nonviral pathogen infection is variable. This family of cytokines could be neural or protective, but is often detrimental to the host especially in the intracellular bacteria infection status usually by inducing the host cell death.[9,10] Accumulating data showed that expression of type I IFNs are very important in protecting host from extracellular bacteria infection[11,12] but lead to high burden with intracellular bacteria, e.g. *L. monocytogenes* in mice.[13,14] Intracellular bacteria, like *L. monocytogenes* and *M. tuberculosis*, are intracytosolic pathogens that are routinely cleared through phagocytosis by macrophages. However, macrophages also act as the primary host cell for many intracellular pathogens especially when a chronic infection has been

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established. Once infected, intracytosolic pathogens induce type I IFNs expression in macrophages; interestingly, however, type I IFN enhances the susceptibility of mice to the bacteria infection. Further, the mice lacking the type I IFN receptor are shown to be resistant to intracellular bacteria burden and be survived from high burden of infection.[13] Together, these data strongly suggested type I IFNs signaling activation may be involved in the sustained lethal intracellular bacteria infection. However, whether type I IFNs signaling activation is indeed associated in the infection with *S.* Typhimurium, one of intracellular bacteria, has not been investigated before. Further, whether this signaling activation is related to the *S.* Typhimurium-induced macrophage death remains unclear.

Robinson *et al.*[2] used the mice deficient in the receptor for IFN-α and IFN-β (*Ifnar1*$^{-/-}$) together with other multiple approaches to address these questions. They found that the mice deficient in the receptor for IFN-α and IFN-β but in both TNFR1 and TNFR2, or $iNOS_2$, IFN-γ alone or IL-6 had a dramatically improved survival and a much lower S. Typhimurium burden compared to wild-type mice. They also demonstrated that the enhanced control of *S.* Typhimurium in *Ifnar1*$^{-/-}$ mice was related specifically to the greater number of the macrophages but not an intrinsic property of the *Ifnar1*$^{-/-}$ macrophages themselves. The *Ifnar1*$^{-/-}$ macrophages were much more resistant to *S.* Typhimurium-induced death than the wild-type macrophages. In contrast, there was no significant difference between the sensitivities to *S.* Typhimurium-induced death for other innate immune cells, such as natural killer (NK) cells, natural killer T (NKT) cells, dendritic cells (DCs) or neutrophils from *Ifnar1*$^{-/-}$ and wild-type mice. This result clearly indicates that type I IFNs downstream signaling activation may participate in the underlying mechanism of *S.* Typhimurium-inducted macrophage death. Moreover, they found that *S.* Typhimurium-infected macrophages had a cleavage pattern consistent with necrotic death but not apoptosis. Supportively, *Ifnar1*$^{-/-}$ macrophages had much necrotic death than wild-type ones. Together, these results indicate that *S.* Typhimurium-induced macrophage death via necrosis in a type I IFN-dependent manner.[2]

Further, they explored the underlying molecular mechanisms.[2] They documented that: (1) type I IFN induced a RIP1-Ifnar association and RIP1-dependent macrophage death; (2) down-regulation of the expression of RIP1 or RIP3 complex prevented against the death of wild-type macrophages; (3) there were more activation of RIP1 and RIP3 during *S.* Typhimurium infection; (4) RIP3-deficient macrophages enhanced control of *S.* Typhimurium *in vivo* although they had similarly less death; and (5) the mice deficient in cIAP proteins, a known molecular to limit necroptosis by inhibiting RIP1, had more impaired control of *S.* Typhimurium than wild-type mice.[2] In contrast, the macrophages death under *S.* Typhimurium infection does not correlate with the inflammasome or caspase-1 activation or IL-1β expression. There was no significant difference between type I IFN, IL-12 and IL-6 secretion, cytokines between *Ifnar1*$^{-/-}$ and wild-type mice. Although proinflammatory cytokine IL-1β has proved to be key factors triggered by type I IFNs and high level of the cytokine leads to host cell death during pathogen infection, it did not correlate the inflammasome or caspase-1 activation or IL-1β expression with the resistance to *S.* Typhimurium infection in the *Ifnar1*$^{-/-}$ mice. Taken together, these data suggest that *S.* Typhimurium induces the type I IFN signaling activation, which drives necroptosis of macrophages and allows them to evade the immune response.

Although these findings highlight the critical new mechanism of the type I IFN-induced macrophage necroptosis in evading host innate immune responses during infection with *S.* Typhimurium, the following questions still need to be investigated in future: (1) whether the type 1 IFN-medicated necroptosis of macrophages in *S.* Typhimurium is cytokine dependent;[2] (2) whether its mechanism can be used to dissect the pathogenesis of other intracellular bacteria infection; (3) whether this happens under some extracellular bacteria infection as well; and (4) what are the key moleculars derived from *S.* Typhimurium to drive type I IFN-induced macrophage necroptosis?

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