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Salmonella pSLT-encoded effector SpvB promotes RIPK3-dependent necroptosis in intestinal epithelial cells

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Salmonella is one of the most important worldwide zoonotic pathogens. After invading a host orally, the bacteria break through the intestinal epithelial barrier for further invasion. Intestinal epithelial cells (IECs) play a crucial role in maintaining the integrity of the intestinal epithelial barrier. Necroptosis is considered one of the virulence strategies utilized by invasive *Salmonella*. Our previous work has shown that SpvB, an effector encoded by *S*. Typhimurium virulence plasmid (pSLT), promotes bacterial translocation *via* the paracellular route. However, it is still unknown whether SpvB could promote bacterial invasion through disrupting the integrity of IECs. Here, we demonstrated that SpvB promoted necroptosis of IECs and contributed to the destruction of the intestinal barrier during *Salmonella* infection. We found that SpvB enhanced the protein level of receptor-interacting protein kinase 3 (RIPK3) through inhibiting K48-linked poly-ubiquitylation of RIPK3 and the degradation of the protein in an autophagy-dependent manner. The abundant accumulation of RIPK3 upregulated the phosphorylation of MLKL, which contributed to necroptosis. The damage to IECs ultimately led to the disruption of the intestinal barrier and aggravated infection. In vivo, SpvB promoted the pathogenesis of *Salmonella*, favoring intestinal injury and colonic necroptosis. Our findings reveal a novel function of *Salmonella* effector SpvB, which could facilitate salmonellosis by promoting necroptosis, and broaden our understanding of the molecular mechanisms of bacterial invasion.

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INTRODUCTION

Salmonella is a common foodborne pathogen that poses an urgent health-safety problem. The species of *Salmonella* are highly diverse, and various serovars have different host specificity and clinical symptoms [1]. While most serovars cause mild gastroenteritis, some serovars in individuals with weakened immune systems can lead to severe and invasive infections, such as enteric fever and invasive nontyphoidal *Salmonella* disease, resulting in long-term health consequences and even death [2, 3]. *Salmonella enterica* serovar typhimurium (*S*. Typhimurium) is one of the most common isolates that can infect both humans and animals. A comprehensive understanding of the molecular mechanisms of *S*. Typhimurium is crucial for expanding therapeutic strategies against infectious diseases.

After breaking through the mucosal epithelial barrier, *S*. Typhimurium is mainly engulfed by immune cells such as macrophages. *S*. Typhimurium can replicate within phagocytic cells, facilitate further bacterial colonization, and cause systemic infection. A wide range of virulence determinants and effectors encoded by these genes are crucial for *S*. Typhimurium to invade the host and cause pathological changes [4]. Among them, *Salmonella* plasmid virulence (*spv*) is a highly conserved 8-kb-long region located on pSLT, which is crucial for intracellular survival and growth [5]. The *spv* gene consists of the positive regulatory *spvR* gene and the four structural *spvABCD* genes. Genetic analysis has demonstrated that the *spvB* gene contributes to the pathogenesis of *Salmonella* infection [6]. As shown in our previous work, *spvB*-encoded effector SpvB disrupts epithelial intercellular junctions, which is conducive to the paracellular translocation of bacteria across the intestinal epithelial barrier [7]. The intestinal epithelial barrier consists of IECs and junctional complexes [8]. In addition to the involvement of paracellular translocation, it remains to be clarified whether SpvB could promote *S*. Typhimurium invasion by disturbing the integrity of IECs.

S. Typhimurium infection generally causes obvious intestinal injury and cell death. Even though necrotic cell death was long defined as a form of unregulated and uncontrollable accidental cell death, recent studies have reported that a type of regulated cell death (RCD) shows morphological features similar to necrosis, termed necroptosis. Necroptosis has been implicated in many pathologies, such as inflammatory bowel disease. It is triggered by the dysregulation of either extracellular or intracellular homeostasis and requires the activity of mixed-lineage kinase domain-like protein (MLKL) and receptor-interacting protein kinase 3 (RIPK3). Phospho-MLKL (p-MLKL) mediates pore formation, which is a molecular basis for this lytic form of programmed necrosis [9].

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