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Salmonella effector SpvB interferes with intracellular iron homeostasis *via* regulation of transcription factor NRF2

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ABSTRACT: Iron is a necessary nutrient for humans and nearly all bacterial species. During *Salmonella* infection, macrophages limit the availability of iron to intracellular pathogens in one of the central components of nutritional immunity. However, *Salmonella* also have mechanisms to interfere with the antimicrobial effect of host iron withdrawal and meet their own nutrient requirements by scavenging iron from the host. Here, we provide what is, to our knowledge, the first report that SpvB, a pSLT-encoded cytotoxic protein whose function is associated with the intracellular stage of salmonellosis, perturbs macrophage iron metabolism, thereby facilitating *Salmonella* survival and intracellular replication. In investigating the underlying mechanism, we observed that the *Salmonella* effector SpvB down-regulated nuclear factor erythroid-derived 2–related factor 2 (NRF2), and its C-terminal domain was necessary and sufficient for NRF2 degradation *via* the proteasome pathway. Decreased NRF2 expression in the nucleus resulted in a decrease in its transcriptional target ferroportin, encoding the sole macrophage iron exporter, thus ultimately decreasing iron efflux and increasing the intracellular iron content. Additionally, SpvB contributes to the pathogenesis of *Salmonella* including severe serum hypoferremia, increased splenic and hepatic bacterial burden, and inflammatory injury *in vivo*. Together, our observations uncovered a novel contribution of SpvB to *Salmonella* pathology *via* interference with host intracellular iron metabolism.—Yang, S., Deng, Q., Sun, L., Dong, K., Li, Y., Wu, S., Huang, R. *Salmonella* effector SpvB interferes with intracellular iron homeostasis *via* regulation of transcription factor NRF2. FASEB J. 33, 13450–13464 (2019). www.fasebj.org

KEY WORDS: salmonellosis · pSLT plasmid · macrophage · hypoferremia · ferroportin

Salmonellosis is a major public health concern worldwide, and there are ~180 million cases each year (1). Of the 2600 *Salmonella enterica* serovars associated with human infections, *S. enterica* serovar typhimurium (*S. typhimurium*) is one of the most common isolates; it causes gastroenteritis and systemic infection in humans and animals (2). A more comprehensive understanding of the ability of *S. typhimurium* to impair the host immune response may be helpful in expanding therapeutic strategies for the treatment or prevention of infectious diseases.

The host antiinfection immune response, which limits the nutrient metal requirement of invading bacterial pathogens and starves bacteria within cells, is termed nutritional immunity (3). In systemic infection, *Salmonella* compete with the host for essential transition metals to survive and replicate within phagocytic cells. Among these metals, iron is a necessary nutrient for humans and nearly all bacterial species, and it plays an integral role in a number of biologic processes (4). Humans maintain physiologic iron concentration and distribution because of the critical role of iron as an ideal redox catalyst for cellular and enzymatic processes, such as the host antimicrobial defense. Macrophages invaded by *Salmonella*, a facultative intracellular bacterium with an essential need for iron, show increased iron export, thereby decreasing the iron availability in intracellular bacteria (5, 6).

ABBREVIATIONS: AG, aminoguanidine; ARNT, aryl hydrocarbon receptor nuclear translocator; c-*spvB*, *SpvB* complemented strain; Cas9, CRISPR-associated protein 9; CFU, colony-forming unit; CRISPR, clustered regularly interspaced short palindromic repeats; DFP, deferiprone; DFX, deferasirox; DMT1, divalent metal transporter 1; FBS, fetal bovine serum; FPN, ferroportin; GAPDH, glyceraldehyde 3-phosphate dehydrogenase; HIF, hypoxia-inducible factor; LB, Luria-Bertani; MEF, mouse embryonic fibroblast; MOI, multiplicity of infection; MTF1, metal regulatory transcription factor 1; NAC, *N*-acetyl-L-cysteine; NOS2, inducible NO synthase; NRF2, nuclear factor erythroid-derived 2–related factor 2; RNS, reactive nitrogen species; ROS, reactive oxygen species; S-N-K, Student-Newman-Keuls; *S. typhimurium*, *Salmonella enterica* serovar typhimurium; sgRNA, single guide RNA; spv, *Salmonella* plasmid virulence; TBP, TATA-binding protein; TFR1, transferrin receptor 1; Ub, ubiquitin; WT, wild type; Δ*SpvB*, *SpvB* deletion mutant strain

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