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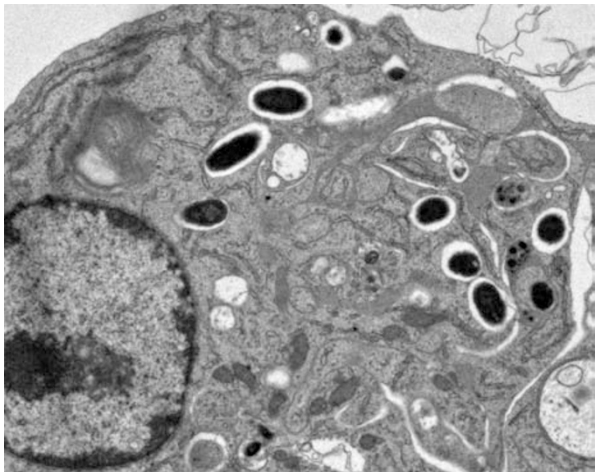
BACTERIAL VIRULENCE

With a little help from my enemies

> *S.* Typhimurium requires signals from the innate immune system to activate expression of … virulence genes

To optimize their survival in the host, pathogens such as *Salmonella enterica* subsp. *enterica* serovar Typhimurium need to recognize their surroundings and respond appropriately. In a recent article in *Cell*, Arpaia and colleagues show that *S.* Typhimurium requires signals from the innate immune system to activate expression of the virulence genes that are necessary for intracellular growth.

S. Typhimurium is a facultative intracellular pathogen that replicates within membrane-bound compartments (referred to as *Salmonella*-containing vacuoles (SCVs)) in host cells. However, host cells sense the presence of these invaders through various mechanisms, including the Toll-like receptors (TLRs), which play an essential part in the innate immune response by recognizing various microbial molecules and subsequently inducing an antimicrobial response. To study TLR-mediated recognition of *S.* Typhimurium, the authors generated mice with a genetic background that allows normal TLR signalling and lysosome function (hereafter referred to as 'wild type'), contrary to mouse strains typically used in the past. From these wild-type mice, the authors generated mice that lacked various TLRs and infected them with *S.* Typhimurium. Mice lacking a combination of two TLRs (either TLR2 and TLR4 or TLR4 and TLR9) were more susceptible to *S.* Typhimurium infection than wild-type animals. However, mice lacking three TLRs (TLR2, TLR4 and TLR9) were more resistant to infection and had fewer bacteria in their internal organs than mice lacking two TLRs, indicating that TLR signalling may be required for growth of *S.* Typhimurium. Similar experiments performed with the extracellular pathogen *Yersinia enterocolitica* revealed no significant difference in infection susceptibility between the double and the triple mutants, suggesting that the requirement for TLR signalling may be specific for *S.* Typhimurium.



Transmission electron microscopy image of *Salmonella enterica* subsp. *enterica* serovar Typhimurium growing within a macrophage lacking Toll-like receptor 2 (TLR2) and TLR4, 22 hours post-infection. Image courtesy of G. M. Barton, University of California, Berkeley, USA.

Growth of *S.* Typhimurium within bone marrow-derived macrophages (BMMs) followed the same patterns as that in mice: the bacteria replicated in BMMs lacking two TLRs but not in BMMs lacking the three TLRs, whereas *Listeria monocytogenes* and *Legionella pneumophila* could replicate in all TLR-deficient BMMs. Transmission electron microscopy of *S.* Typhimurium-infected BMMs indicated that the bacteria failed to transform the phagosomes into SCVs only in the triple-mutant macrophages. By comparing the gene expression patterns of *S.* Typhimurium isolated from infected BMMs, the authors found that 13 genes within the SPI-2 (*Salmonella* pathogenicity island 2) locus were upregulated in bacteria from wild-type BMMs and (to a lesser extent) in bacteria from double-mutant BMMs, but not in those from triple-mutant BMMs. Furthermore, bacteria that constitutively expressed the SPI-2 genes replicated similarly in double-mutant and triple-mutant BMMs.

These results suggest that TLR signalling is required for upregulation of SPI-2 genes and, hence, for intracellular bacterial growth. To find the part of the host response that induces this bacterial gene expression, the authors used several pharmacological inhibitors. Only inhibition of the vacuolar ATPase, which acidifies the phagosomes by transporting protons, blocked the production of PipB2 (an effector secreted by the SPI-2 locus) in both wild-type and double-mutant macrophages. Indeed, the authors found that *S.* Typhimurium-containing phagosomes in triple-mutant BMMs failed to acidify to the same extent and at the same pace as the phagosomes in double-mutant or wild-type macrophages.

Thus, *S.* Typhimurium uses TLR-dependent phagosome acidification as a cue to induce virulence genes and establish a successful infection. This allows the bacterium to time its secretion of virulence factors during infection of the host cell.

Cesar Sanchez

ORIGINAL RESEARCH PAPER Arpaia, N. *et al.* TLR signaling is required for *Salmonella typhimurium* virulence. *Cell* **144**, 675–688 (2011)
FURTHER READING Haraga, A., Ohlson, M. B. & Miller, S. I. Salmonellae interplay with host cells. *Nature Rev. Microbiol.* **6**, 53–66 (2008)