Nature Reviews Immunology | AOP, published online 17 December 2010; doi:10.1038/nri2907

INNATE IMMUNITY

Calling on the neighbours

The enteric bacterium *Shigella flexneri* is known to produce multiple effector proteins that interfere with host cell inflammatory responses. But despite this, infection with this bacterium induces a dramatic inflammatory response, including the secretion of large amounts of CXC-chemokine ligand 8 (CXCL8; also known as IL-8). A recent study published in *Immunity* provides an explanation for this, by showing that infected intestinal epithelial cells communicate with uninfected neighbouring epithelial cells to promote inflammatory chemokine secretion that is unrestrained by bacterial effector proteins. A related, independent study in *PLoS Pathogens* describes another mechanism of intercellular communication that allows amplification of innate immune responses to *Listeria monocytogenes*.

In the first study, Kasper *et al.* noted, to their surprise, that when epithelial cells were exposed to low doses of *S. flexneri*, not only infected cells but also uninfected cells showed activation of the pro-inflammatory transcription factor nuclear factor-κB (NF-κB). The uninfected cells with active NF-κB seemed to neighbour infected cells, suggesting the occurrence of bystander activation. Such activation was not a result of bacterial spread to neighbouring cells, as it was not reduced following infection with a non-motile mutant of *S. flexneri*. Further analysis revealed that the uninfected bystander cells also showed activation of JUN N-terminal kinase (JNK), extracellular signal-regulated kinase (ERK) and the mitogen-activated protein kinase p38, all of which are involved in inflammatory responses to *S. flexneri*. In particular, p38 activation was much higher in these uninfected cells than in the infected cells, and this was shown to be a result of dephosphorylation of p38 in the infected cells by the bacterial effector protein OspF; accordingly, p38 activation was increased in cells that were infected with an OspF-deficient *S. flexneri* mutant. In keeping with the observed activation of pro-inflammatory signalling, uninfected epithelial cells produced much more CXCL8 than infected cells, as assessed on a single-cell level using immunofluorescence microscopy or mRNA hybridization.

So, how is the infection communicated to bystander cells? Treatment of the epithelial cells with brefeldin A, which blocks protein secretion, had no effect on bystander activation during *S. flexneri* infection, indicating that activation was not mediated by paracrine signalling involving secreted proteins. By contrast, pharmacological blockade of gap junctions, which allow the passage of small molecules between adjacent epithelial cells, did reduce CXCL8 secretion by bystander cells. Moreover, *S. flexneri* infection of an epithelial cell line that lacks expression of the gap junction protein connexin 43 (also known as GJA1) failed to induce activation of NF-κB, JNK, ERK and p38 in bystander cells. Finally, the finding that connexin 43 had to be expressed by both the infected and the uninfected bystander cells confirmed that epithelial cell inflammatory responses are propagated during bacterial infection through gap junction communication.

In the study by Dolowschiak *et al.*, communication between intestinal epithelial cells did not seem to depend on gap junctions but instead was shown to be mediated by reactive oxygen intermediates that were produced by NADPH oxidase in cells that were infected with the cytosolic bacterium *L. monocytogenes*. However, despite the differing mechanisms of intercellular communication that are described, these papers identify an important new way in which innate immune responses can be amplified at the early stages of bacterial infection.

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ORIGINAL RESEARCH PAPERS Kasper, C. A. *et al.* Cell-cell propagation of NF-κB transcription factor and MAP kinase activation amplifies innate immunity against bacterial infection. *Immunity* **33**, 804–816 (2010) | Dolowschiak, T. *et al.* Potentiation of epithelial innate host responses by intercellular communication. *PLoS Pathog.* **6**, e1001194 (2010)