BACTERIAL VIRULENCE

Control freak

As well as demonstrating enormous environmental versatility, *Pseudomonas aeruginosa* is an important source of Gram-negative nosocomial infections and a major cause of death and morbidity in cystic fibrosis patients. Key to the success of this microorganism is the complexity of regulation that controls crucial processes that contribute to its physiological adaptability. A recent report in *Microbiology* adds another level of understanding to the regulation of one such process — quorum sensing.

Evidence has amassed that quorum sensing, which allows bacteria to detect the density of their own species, is crucially important to the pathogenicity of *P. aeruginosa*. Two quorum-sensing systems, which rely on the *N*-acylhomoserine lactone (AHL) signal, have been identified in this microorganism: the *las* system and the *rhl* system. These two systems do not operate independently and are hierarchically arranged with the *las* system at the top of the signalling cascade. The quorum-sensing circuitry is subject to regulation by a number of different regulators, including Vfr, RsaL, GacA and DksA. Juhas and co-workers have now identified a new regulator, VqsR (virulence and quorum-sensing regulator) that has an important role in the quorum-sensing cascade of *P. aeruginosa*. When the *vqsR* gene was inactivated, the production and secretion of AHLs was abolished and the subsequent production of virulence factors was also severely compromised. To investigate the importance of VqsR to *P. aeruginosa* virulence, the mutant strain was tested in a nematode infection model system and was shown to be significantly attenuated. Oligonucleotide microarrays were then used to investigate the effect of VqsR on global changes in the gene-expression profile of the microorganism, both in the presence of human serum and under oxidative stress. Disruption of this regulator activated the expression of genes that are known to be repressed by quorum sensing and repressed the expression of genes previously shown to be promoted by this process. Furthermore, this analysis also demonstrated that VqsR is involved in the regulation of siderophores and membrane-bound elements of antibiotic resistance.

In conclusion, Juhas *et al.* have identified a new regulator of *P. aeruginosa* cell-to-cell communication and virulence, thereby adding a new layer to the regulatory complexity that underpins the extensive environmental adaptability of this unique organism.

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References and links

ORIGINAL RESEARCH PAPER Juhas, M. *et al.* Global regulation of quorum sensing and virulence by VqsR in *Pseudomonas aeruginosa*. *Microbiology* (April 2004) doi:10.1099/mic0.26906-0

INNATE IMMUNITY

PKR key to apoptosis

The molecular workings of a general strategy that bacterial pathogens use to thwart the innate immune response have been published in a recent issue of *Nature*.

Macrophages use cell-surface receptors known as Toll-like receptors (TLRs) to detect the presence of microbial pathogens by recognizing a variety of signals, known as pathogen-associated molecular patterns (PAMPs). The detection of a PAMP triggers the expression of intracellular effector molecules and signalling pathways. Some successful bacterial pathogens have evolved ways to hijack these pathways to facilitate their own survival and replication.

It was already known that the lethal toxin (LT) of *Bacillus anthracis* induces apoptosis of activated macrophages by preventing activation of the p38 signalling pathway, an important anti-apoptotic pathway. In this latest paper, Michael Karin's group follow-up on these findings by looking at the molecular mechanisms that are involved in apoptosis induction.

In the presence of a synthetic p38 inhibitor, the induction of apoptosis in wild-type macrophages by heat-inactivated *B. anthracis* was dependent on the presence of TLR4 and a TLR4 ligand. The importance of TLR4 was confirmed by the fact that bone-marrow-derived macrophages (BMDMs) in which TLR4 is inactivated were resistant to killing by heat-inactivated *B. anthracis*. Additionally, macrophages transfected with a vector expressing the TLR4 cytoplasmic domain underwent apoptosis in the presence of a p38 inhibitor. TLR4 therefore seems to be the key cell-surface receptor involved in *B. anthracis*-induced macrophage apoptosis.

Hsu *et al.* went on to look at the intracellular signalling pathways that are triggered by *B. anthracis*. They found that PKR, a dsRNA-responsive protein kinase, is the crucial factor in pathogen-mediated apoptosis through TLR4. How does the activation of PKR induce apoptosis? Activated PKR inhibits protein synthesis by phosphorylating the elongation initiation factor eIF2α. Hsu *et al.* obtained macrophages that express an eIF2α variant in which the key PKR phosphorylation site is mutated. BMDMs expressing this mutant initiation factor were less sensitive to apoptosis by LPS/p38 inhibitor incubation than cells expressing the wild-type factor.

Finally, the effects of infection with live *B. anthracis* and two other bacterial species, *Salmonella enterica* serovar Typhimurium and *Yersinia pseudotuberculosis*, were observed. In $PKR^{-/-}$ macrophages, the levels of apoptosis observed were reduced compared with the levels observed in $PKR^{+/+}$ macrophages. Although TLR4 recognizes a component of the bacterial cell wall, an additional bacterial virulence factor is also required to induce apoptosis — for *B. anthracis*, this was already known to be LT; the results from this study show that for *Y. pseudotuberculosis*, it is YopJ, and for *S. typhimurium*, it is encoded within the SPI2 pathogenicity island.

This work adds further weight to the idea that inducing apoptosis of macrophages, key components of the innate immune response, is a general strategy used by pathogenic bacteria to avoid detection by the host immune system.

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References and links

ORIGINAL RESEARCH PAPER Hsu, L.–C. *et al*. The protein kinase PKR is required for macrophage apoptosis after activation of Toll-like receptor 4. *Nature* **428**, 341–345 (2004)