two Kdo molecules — all of which indicates that toxin binding is inhibited by phosphorylation.

The authors propose that this structural difference in the Kdo LPS core of *V. cholerae* and ETEC is, at least in part, responsible for the differing toxicities of LT and CT — because by being confined to the ETEC outer membrane, delivery of LT in an infected host is restricted to delivery by outer membrane vesicles — and propose that transport by vesicles should be considered a specific secretion mechanism for virulence factors.

Jane Saunders

References and links

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WEB SITE

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IMMUNE EVASION

Proteasome power

A report in *Science* has revealed that the proteasome of *Mycobacterium tuberculosis* could have a role in immune evasion.

Worldwide, an estimated 2 billion people are infected with *M. tuberculosis*, the causative agent of tuberculosis (TB). *M. tuberculosis* is spread by aerosols — once inhaled, the pathogen travels to the lungs where it is engulfed by alveolar macrophages. The resulting host immune response includes the production of inducible nitric oxide synthase (iNOS) and reactive nitrogen intermediates (RNIs). However, in some infected individuals, the host response is sufficient to contain the infection but doesn't effect clearance — some mycobacteria evade the response, remain viable and can be reactivated and cause TB many years after the initial infection.

Darwin *et al.* were interested in this population of mycobacteria that cause persistent infection and, specifically, how they counter the effects of RNIs. Their starting point was a transposon mutagenesis strategy — a library of more than 10,000 transposon mutants was screened for increased sensitivity to a physiologically relevant nitrite. 12 mutants were isolated, each of which had a unique transposon insertion. Of these, five were found to have insertions in two genes associated with the mycobacterial proteasome, Rv2115c and Rv2097c. The predicted gene product of Rv2115c is an AAA family ATPase and Rv2097c is predicted to encode a proteasome accessory factor.

In vitro, the growth of all five mutants was impaired in macrophages from wild-type mice. However, growth was also impaired in macrophages isolated from iNOS^{-/-} mice, indicating that the Rv2115c and Rv2097c mutations cause *M. tuberculosis* to become sensitive to iNOS-independent stresses.

An aerosol infection protocol was used to infect both wild-type and iNOS^{-/-} mice with wild-type *M. tuberculosis* or *M. tuberculosis* bearing the proteasome-associated mutations. Several different assays showed that the virulence of the mutant bacteria was severely attenuated. Eight weeks after infection, the burden of infection in different organs was assessed. Between 1 and 4 log₁₀ fewer colony-forming units were recovered from the lungs, liver and spleen of mice infected with the Rv2115c or Rv2097c mutants compared with mice infected with wild-type bacteria. For Rv2115c, Darwin *et al.* went on to show that introducing a single chromosomal copy of the wild-type allele could reverse this effect. Inhibiting the activity of the mycobacterial proteasome with two different chemical inhibitors reproduced the RNI-sensitive phenotype associated with the Rv2115c and Rv2097c mutations, giving confidence to the conclusion that the protein products of Rv2115c and Rv2097c are indeed proteasome related. The authors propose Rv2115c should be renamed *mpa*, for mycobacterial proteasome, and Rv2097c renamed *paf*, for proteasome accessory factor.

From this work, it remains unclear whether the protective effects of the mycobacterial proteasome against RNIs are direct or indirect. Nevertheless, it provides us with a new insight into the function of the proteasome within *M. tuberculosis*, about which little knowledge has previously been available, and additionally has highlighted a potential new target for anti-TB drugs.

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