BACTERIAL PATHOGENESIS

Bartonella gets under the skin

> the cytoprotective effect of BepE results from the antagonism of BepC

Many bacteria secrete multiple effector proteins into host cells to modulate host cell signalling pathways and promote bacterial replication and transmission. The specific functions of many effectors have been investigated in depth but what is perhaps less well studied is the interactions that can occur between effectors within host cells. In a new paper in *PLoS Pathogens*, Okujava *et al.* report on an antagonistic interaction between two *Bartonella* effector proteins (Beps) that is essential for *Bartonella* bloodstream infection in a reservoir host.

Members of the genus *Bartonella* are facultative intracellular pathogens that establish a long-lasting intraerythrocytic infection in a variety of mammalian reservoir hosts. The bacteria are acquired from the bloodstream by arthropod vectors and transmitted intradermally through infected arthropod faeces. The exact nature of the primary niche for bacterial colonization outside of the bloodstream has been unclear but is thought to include dermal dendritic cells and vascular endothelial cells. *Bartonella* spp. use type IV secretion systems (T4SSs) to transfer effectors into host cells at different stages of the infection cycle. The VirB T4SS is used to inject Beps into nucleated host cells, and seven Beps (BepA–BepG) have been identified to date in the model species *Bartonella henselae*.

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Previous work had shown that three Beps (BepD, BepE and BepF) contain phosphotyrosine-rich amino-terminal motifs that could potentially be important in modulation of host cell signalling. Using an *in vitro* HUVEC (human umbilical vein endothelial cell) infection model, Okujuva *et al.* investigated the role of BepD, BepE and BepF and, using a combination of live cell imaging and confocal laser scanning microscopy, they found that a Δ*bepDEF* strain of *B. henselae* caused severe fragmentation of HUVECs. Each Bep was added back individually in complementation assays, which showed that the defect could be complemented only by the addition of BepE. The carboxy-terminal regions of Beps contain at least one *Bartonella* intracellular delivery (BID) domain, and one of the BID domains in BepE ($BID2.E_{)}$ was found to be sufficient to interfere with cell fragmentation. Immunocytochemistry confirmed that BepE translocation was dependent on the VirB T4SS.

Further confocal laser scanning microscopy to analyse the effects of different mutant combinations showed that the cell fragmentation defect conferred by the absence of BepE could be suppressed by the absence of BepC. Moreover, ectopic expression of BepC in HUVEC cells led to cell fragmentation, and this fragmentation was prevented by coexpression of BepE. Together, these data suggest that the cytoprotective effect of BepE results from antagonism of BepC.

The authors went on to investigate the localization of BepE within HUVECs and found that BepE was present at cell-cell contacts and near the rear edge of migrating HUVECs. The host protein RhoA is known to be involved in the release of the rear edges of migrating cells from the substratum. Treatment of HUVECs with a RhoA inhibitor disrupts stress fibres, causing the cells to round up and die, and this intoxication was prevented by ectopic expression of BepE, or the $BID2.E_{domain.}$ Finally, the authors moved to an *in vivo* model of intradermal infection with the related species *Bartonella tribocorum*, and showed that in rats infected with a Δ*bepDE* strain there was no noticeable bacteremia but bacteremia was restored following complementation with BepE or the BID domains. Additionally, an *in vitro* assay demonstrated that BepE had a cytoprotective effect on migrating dendritic cells.

The authors suggest that the cytoprotective effect of BepE may be important in the early stages following intradermal infection, protecting migrating dermal dendritic cells against the activity of BepC and thus allowing the bacteria to progress to the bloodstream. Further work is required to probe exactly how BepC induces cell fragmentation, and how BepE counteracts it.

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ORIGINAL RESEARCH PAPER Okujava, R. *et al.* A translocated effector required for *Bartonella* dissemination from derma to blood safeguards migratory host cells from damage by co-translocated effectors. *PLoS Pathogens* **e1004187** (2014)