TYPE IV SECRETION

A sprouting interest in *Bartonella*

Three *Bartonella* species, including *Bartonella henselae*, infect endothelial cells and can cause the formation of benign vasoproliferative tumours in humans in a manner that is reminiscent of tumour angiogenesis. Previously, our understanding of how *Bartonella* species trigger this complex process has been hindered by the fact that the existing animal and cell culture models do not directly recapitulate this feature of *Bartonella* infection.

Writing in *Cellular Microbiology*, Scheidegger and colleagues now report on the creation of a new *in vitro* model of *Bartonella*-induced angiogenesis.

Scheidegger and colleagues decided to adapt an existing three-dimensional cell culture model of angiogenesis. Human umbilical vein endothelial cells (HUVECs) were infected with *B. henselae* at a high multiplicity of infection for 24 hours. The infected cells were then grown overnight in a humidified atmosphere as hanging drops in endothelial growth medium containing methylcellulose. These growth conditions led to the formation of endothelial cell spheroids, which were then embedded within a collagen gel matrix. The effects of different factors on angiogenesis could then be quantified by assessing the formation of capillary-like projections, or sprouts, by the spheroids.

B. henselae uses a VirB/D4 type IV secretion system (T4SS) to transport *Bartonella* effector proteins (Beps) into host cells, and seven Beps (BepA–BepG) have been identified to date. Scheidegger *et al.* began work with their new *in vitro* model by pre-infecting the HUVECs with wild-type *B. henselae*, a *B. henselae* mutant strain that lacks the VirB/D4 T4SS and a Δ bepA–bepG mutant strain that lacks all known effectors. A comparison of

the cumulative length of the capillary sprouts formed revealed that although there is a basal level of *B. henselae*-induced angiogenesis that is independent of the VirB/D4 T4SS, there is also a strong VirB/D4- and Bep-dependent response. The authors went on to analyse the angiogenic response to isogenic strains of Δ bepA–G mutants that expressed the individual bep genes on a plasmid *in trans* and to isogenic mutants that carried in-frame deletions of the individual bep genes. Taken together, the results showed that two Beps, BepA and BepD, have pro-angiogenic activity and that one Bep, BepG, has anti-angiogenic activity.

This new *in vitro* model has enabled researchers to begin to make progress on understanding the molecular events that underlie *B. henselae*-triggered angiogenesis. Further work using this system will hopefully bring us closer to answering the key question of whether angiogenesis is a cause or consequence of *Bartonella* infection.

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Light microscopy images of spheroids infected either with a Δ bepA–G mutant or an isogenic strain that expresses BepA (Δ bepA–G + pbepA). The cells have been stained for nuclei (using DAPI (4',6-diamidino-2-phenylindole)) or entire cells (using CFSE (carboxyfluorescein succinimidyl ester)). Images courtesy of F. Scheidegger, University of Basel, Switzerland.

ORIGINAL RESEARCH PAPER Scheidegger, F. *et al.* Distinct activities of *Bartonella henselae* type IV secretion effector proteins modulate capillary-like sprout formation. *Cell. Microbiol.* 12 Mar 2009 (doi: 10.1111/j.1462-5822.2009.01313.x)

FURTHER READING Dehio, C. *Bartonella*–host cell interactions and vascular tumour formation. *Nature Rev. Microbiol.* 3, 621–631 (2005)