



Mechanism of *Listeria monocytogenes* invasion in intestinal villi tips

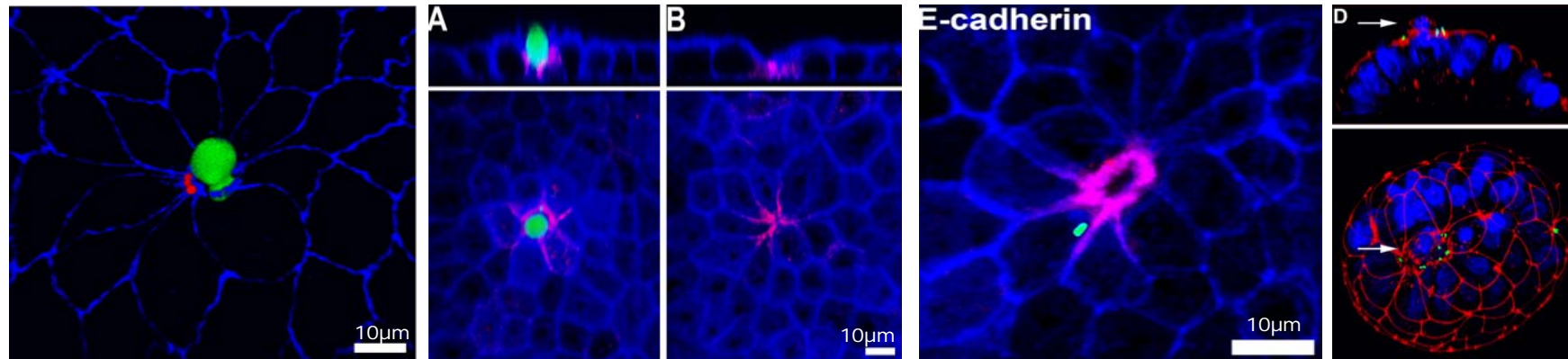


Fig 1

Fig 2

Fig 3

Fig 4

Dr Manuel Amieva from the Departments of Pediatrics and Microbiology & Immunology at Stanford University is researching the penetration and survival of pathogenic microorganisms in their chosen cellular environment. *Listeria monocytogenes* is a bacterial pathogen causing human illnesses ranging from gastroenteritis to invasive and disseminated infection. It is also known for its ability to infect the placenta in pregnant woman. *L. monocytogenes* from contaminated food enter the gastrointestinal track and reach the small intestine. For invasion to occur *Listeria* must interact with a host's cell-to-cell protein called E-cadherin. How it gains access to E-cadherin in vivo remained an important and unresolved issue, since E-cadherin is present primarily on the lateral membrane, but not on the accessible apical surface of intestinal epithelial cells.

In order to analyse the site of apical/basal invasion, Dr Amieva and his colleagues used confocal microscopy to image polarized MDCK cell monolayers infected with *Listeria*. At various time points after attachment and internalization of the bacteria, monolayers of MDCK cells were fixed and probed with anti-*L. monocytogenes* antibodies. The infected monolayers were also counterstained for the tight junctions. The images were then analysed in **Volocity**. The images above are taken from the Image view in **Volocity**. Fig 1 shows *L. monocytogenes* (red) adhering to the junctions (blue) surrounding an apoptotic cell (green) that is being extruded from the MDCK monolayer. This result suggested to the researchers that *L. monocytogenes* adhere to remodeling junctions surrounding sites of cell extrusion from the epithelium.

After showing that *L. monocytogenes* adhere to junctions at cell extrusion sites, the scientists hypothesised that this apical adherence may rely on an interaction with apically-exposed E-cadherin at sites of junction remodeling. To confirm that the receptor for the pathogen at multi-cellular junctions is E-cadherin, they stained the apical side of MDCK monolayers with antibodies to the extracellular domain of E-cadherin (red). Figure 2A shows that a cell in the process of extrusion (green) is surrounded by sites where E-cadherin (red) is exposed to the apical surface. In Fig 2B extrusion has completed, but apical E-cadherin (red) is still present. Finally, Fig 3 shows that *L. monocytogenes* (green) attach to sites where the E-cadherin receptor (red) is exposed at cell extrusion sites.

Fig 4 is a 3D image view of an intestinal villi tip (nuclei: blue; tight junctions: red). The sample was imaged using confocal microscopy and optical sections were taken at 0.5µm resolution through the intestinal villi. Z-stacks were rendered in 3D using **Volocity Visualization**. The 3D volume confirms that *L. monocytogenes* (green) associate with the junctions at cell extrusion sites in vivo at the intestinal villi tips.