**Mechanism of stimulation of host cell exocytosis**

**during entry of *Listeria monocytogenes***

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 **Project: KI-2**

**Description:**

The bacterial pathogen *Listeria monocytogenes* causes food-borne illnesses resulting in gastroenteritis, meningitis, or abortion (1). Critical for disease is the ability of *Listeria* to induce its internalization (entry) into cells of the intestinal epithelium. Entry of *Listeria* into these cells is mediated by interaction of the bacterial protein Internalin A (InlA) with its host receptor, E-cadherin. Recent results indicate that efficient InlA-dependent entry of *Listeria* requires the host membrane trafficking process of polarized exocytosis, which is mediated by a human octameric protein complex called the ’exocyst’ (2,3). How the exocyst is activated downstream of InlA/E-cadherin interaction is not understood.

We ***hypothesize*** that the stimulation of the exocyst during InlA-dependent entry is mediated by human proteins that interact with the cytoplasmic domain of E-cadherin to regulate the normal functions of this receptor in cell-cell adhesion (4). Such proteins include a-catenin, b-catenin, and type Ig phosphatidylinositol 4-phosphate 5-kinase (PI4P5K) (2). This hypothesis will be tested through three specific aims.

**Aims of the project:**

* Aim 1: Determine if a-catenin, b-catenin, and/or PI4P5K associate with E-cadherin at sites of InlA-mediated entry of *Listeria.*
* Aim 2: Assess if catenins and/or PI4P5K recruit the exocyst and promote exocytosis downstream of InlA/E-cadherin interaction.
* Aim 3:  Determine if catenins and/or PI4P5K are needed for efficient InlA-mediated entry of *Listeria*.

**Techniques to be used:**

* RNA interference (RNAi) to inhibit expression of catenins or PI4P5K.
* Western blotting to assess effects of RNAi on target protein expression.
* Laser scanning confocal microscopy to assess localization of catenins and PI4P5K, or to measure exocytosis during InlA-mediated entry.

**References:**

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4. Van Roy, F. *and Berx, B. 2008. The cell-cell adhesion molecule E-cadherin.* Cell. Mol. Life Sci. 65: 3756-3788.