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Deciphering the initial response of endothelial cells to Neisseria meningitidis adhesion

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Neisseria meningitidis is a commensal bacterium found in the nasopharyngeal mucosa. Upon crossing the epithelial barrier and disseminating into the bloodstream, the bacterium becomes a pathogen responsible for cerebrospinal meningitis and/or severe sepsis. *Neisseria meningitidis* ability to invade the bloodstream relies on a tight interaction between the bacterium type IV pilus (T4P) and endothelial cells. Meningococci first adheres to cells, presumably through the interaction of type IV pili with receptors CD147 and β2-Adrenergic Receptor (b2AR). This interaction results in the formation of plasma membrane microvilli-like protrusions, which protect the bacteria from being cleared by the bloodstream. The protrusions are highly enriched in receptors, cytoskeleton and scaffold proteins including ezrin (a member of the ERM family) and CD9, CD81, CD151 (several members of the tetraspanin family). Protrusions formation has been linked to T4P retraction forces and ezrin recruitment, presumably involved in actin polymerization and stability of actin fibers.

The process that leads from adhesion to plasma membrane protrusions is a multi-step process that still needs to be addressed. In this work, we focus on the initial steps and we aim to understand the mechanisms allowing for plasma membrane protrusions development and enrichment in proteins.

We followed ezrin, an organizer of membrane microvilli-like protrusions and the membrane-organizing factors tetraspanins CD9/81/151, using combination of immuno-staining and scanning electron microscopy approaches. We showed that the membrane-bound marker CD9 and cytoskeleton-bound marker ezrin are independently recruited at the site of bacterial adhesion. While T4P retraction is necessary to promote full protrusion growth, adhesion of a *pilT* mutant strain unable to retract T4P is still associated with immature protrusion enriched in CD9. The use of the ezrin phosphorylation inhibitor NSC668394 leads to the same phenotype as a *pilT* mutant strain, suggesting that T4P itself is not sufficient to stabilize membrane protrusion and that immature protrusion enriched in CD9 are independent of ezrin. Tetraspanins organise proteins at the plasma membrane and sense membrane curvature. As such, they can be passively recruited in villi-like structure along with interacting partners. Interestingly, BRET assays showed that CD9/81/151 are found in close proximity to GPCRs such as b2AR. Moreover, tetraspanin knock-out cell lines have a defect in bacterial adhesion.

We therefore propose a sequential model in which T4P-mediated adhesion promote recruitment of immature protrusion in which enrichment of tetraspanins is critical for accumulation of adhesion and signaling receptors. When present in enough quantity, their activation by T4P retraction promotes the elongation and stabilization of protrusions thanks to ezrin phosphorylation. The elongated protrusions are then further enriched in tetraspanins and adhesion/signaling receptors in an amplification loop.