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Deciphering the initial response of endothelial cells to Neisseria meningitidis adhesion

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Background

Neisseria meningitidis ability to invade the bloodstream relies on a tight interaction between their type IV pilus (T4P) and endothelial cells. 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 enriched in adhesion and signaling receptors, cytoskeleton and scaffold proteins including ezrin and CD9, CD81, CD151 (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.

Aim/Methods

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.

Results

We followed the membrane-bound marker Tetraspanin CD9 and cytoskeleton-bound marker ezrin, using combination of immuno-staining and scanning electron microscopy approaches. We showed that the CD9 and 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 ezrin phosphorylation inhibitor 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 sense membrane curvature. As such, they can be passively recruited in villi-like structure along with interacting partners. Interestingly, BRET assays showed that CD9 is found in close proximity to GPCRs such as b2AR. Moreover, a CD9 knock-out cell line does not support bacterial adhesion anymore.

Conclusions

We propose a sequential model in which T4P-mediated adhesion promote recruitment of immature protrusion in which enrichment of CD9 is critical for accumulation of adhesion/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 receptors in an amplification loop.