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Neisseria meningitidis exploits SphK-S1P-S1P2 axis for EGFR activation and invasion in brain endothelial cells

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Background

Neisseria meningitidis (Nm) is able to modulate sphingolipid content on brain endothelial cells (BECs) and uses ceramide-enriched membrane platforms (CRPs) as an entry portal into these cells. Formation of CRPs is the result of transient activation of the enzyme acid sphingomyelinase, followed by the release of ceramide on BECs. Ceramide can further be metabolized to sphingosine-1-phosphate (S1P), a bioactive lipid mediator that regulates the integrity of the blood-cerebrospinal fluid barrier by modulating cytoskeleton structures and tight junction assembly via S1P receptors.

Aim/Methods

hCMEC/D3s were used as a cell culture model and infected with Nm serogroup B strain MC58. LC-MS/MS was used to quantify sphingolipid metabolites in BECs. Expression and activity of S1P- synthesising enzymes (sphingosine kinase 1/2 (Sphk1/2), S1P phosphatase 1/2 (SGPP1/2), S1P lyase (SGPL1)) or S1P receptors (S1P1-3) was measured using commercial available assays or by qPCR. Gentamicin protection assays were conducted to determine levels of Nm adherence and invasion. Western Blotting was performed to determine the levels of total EGFR and pEGFR.

Results

LC-MS/MS revealed a significant time-dependent increase in S1P levels in MC58-infected BECs. Elevated S1P could be attributed to a transient increase in the expression and activity of Sphk1, while the expression of Sphk2, SGPP1/2 or S1P lyase was not altered. Treatment of cells with pilus-enriched supernatants resulted in a significant increase of SphK activity, demonstrating that the type IV pilus of Nm contributes to SphK activity. We found that infection with Nm also increases the expression of S1P2, whereas expression of S1P1 or S1P3 is not affected. We have previously shown that Nm causes epidermal growth factor receptor (EGFR) activation in BECs to induce cytoskeletal rearrangements necessary for uptake. Interestingly, EGFR phosphorylation could by significantly inhibited by the S1P2 receptor antagonist JTE-013. In parallel, infection in the presence of JTE-013 also blocked bacterial uptake by BECs, demonstrating that Nm exploits the S1P-S1P2 axis for EGFR activation and thus invasion.

Conclusions

We showed that Nm exploits the S1P-S1P2 axis for EGFR activation and thus invasion. The SphK-S1PR axis may therefore prove to be a promising target for pharmacological intervention in meningococcal meningitis.

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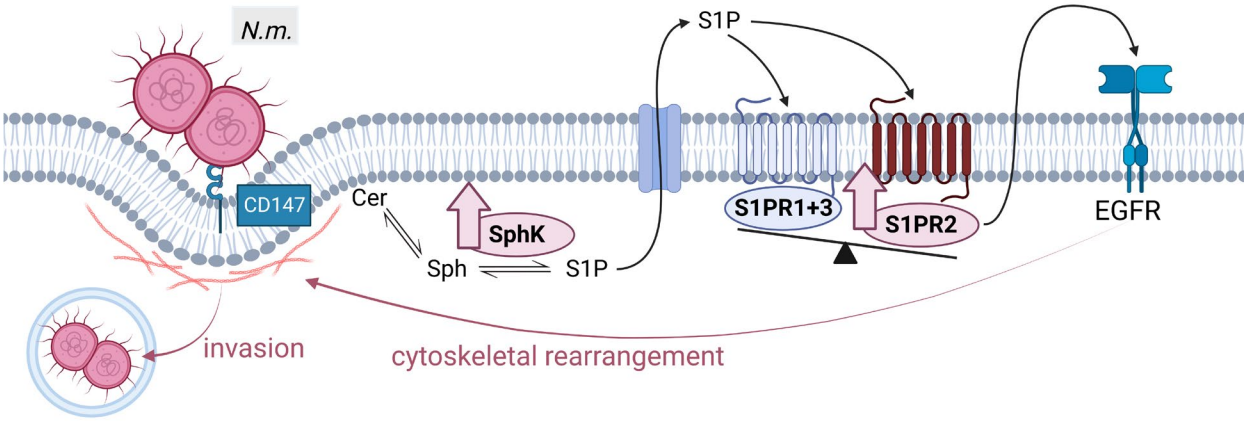
Graphical Abstract: Proposed mechanism of EGFR activation through the SphK-S1P-S1PR2 axis


N.m.
CD147
Cer
SphK
Sph
S1P
S1P
S1PR1+3
S1PR2
EGFR
invasion
cytoskeletal rearrangement