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Molecular lipid raft composition of primary human brain and kidney endothelial cells emphasizing Shiga toxin glycosphingolipid receptors

Nadine Legros, Gottfried Pohlentz, Helge Karch and **Johannes Müthing**
University of Münster, Germany

Human brain and kidney endothelial cells are important targets for Shiga toxins (Stxs) produced by enterohemorrhagic *Escherichia coli* (EHEC) and play key roles in the pathogenesis of life-threatening extraintestinal complications. The clinically important Stx1a and Stx2a subtypes bind to glycosphingolipids (GSLs) of the globo-series. Primary endothelial cells are fastidious and sensible cells, which are eminently suitable as an optimal native cell type for analyzing Stx-mediated cellular injury. Lipid rafts represent supramolecular membrane microdomains that are enriched in certain types of lipids such as cholesterol, sphingomyelin and GSLs. Detergent-resistant membranes (DRMs) commonly used as lipid raft-analogous structures, represent the ruling method to assign association with lipid rafts. Here we present novel data on the composition of lipid rafts prepared from primary human brain microvascular endothelial cells (pHBMECs) and primary human renal glomerular endothelial cells (pHRGECs). Most prominent receptor lipoforms of Stx-receptor GSLs of both types of endothelial cells were globotriaosylceramide (Galα4Galβ4Glcβ1Cer, Gb3Cer) and globotetraosylceramide (GalNAcβ3Galα4Galβ4Glcβ1Cer, Gb4Cer) with Cer (d18:1, C16:0), Cer (d18:1, C22:0) and Cer (d18:1, C24:1/C24:0), determined by electrospray ionization mass spectrometry in combination with thin-layer chromatography (TLC) immunochemical detection. Gb3Cer and Gb4Cer were found to co-distribute with canonical lipid raft-markers cholesterol and sphingomyelin as well as flotillin-2 in DRMs, which represent the liquid-ordered membrane phase and indicate their association with lipid rafts. Figure-1 exemplarily shows the DRM and non-DRM distribution of Gb3Cer and Gb4Cer detected in sucrose density gradient fractions of pHBMECs using Stx1a- and Stx2a-TLC overlay assays. On the other hand, lysophosphatidylcholine was identified as a non-DRM marker phospholipid of the liquid-disordered membrane phase. Increasing knowledge on membranes of brain and kidney endothelial cells might help to develop new therapeutic strategies to fight EHEC infections.

Biography

Nadine Legros has studied Molecular Biotechnology at the University of Bielefeld in Germany. She is currently working on her PhD thesis in the group of Professor Doctor Johannes Müthing, Institute for Hygiene, Germany. Her project is part of the research consortium Infect Control 2020 (TFP, TV8, AS12) with the aim to develop new strategies for the early recognition, control and prevention of infectious diseases.

nadine.legros@ukmuenster.de

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