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Brain Endothelial Cells Segregate Lymphatic-Venous Biomarkers into Microparticles Following Inflammatory Cytokine Stimulation

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Abstract

Background

Elevated inflammatory cytokines seen in multiple sclerosis (MS) promote cerebrovascular stress and provoke endothelial disturbances. Despite the high metabolic rate of neurons and their sensitivity to the local environmental milieu, the brain lacks a 'conventional' lymphatic system, which in peripheral tissues aids in clearance of metabolic wastes and immune cells. Recently changes in lymphatic-venous biomarkers have been observed in human brain tissue and serum in MS. Whether and how these molecular features might contribute to clearance of fluid and debris remains unclear, but the transfer of lymphatic venous proteins from brain endothelial cells ('BEC') to MPs during neuroinflammation could represent a valuable marker and mediator of neurovascular stress.

Objective

We determined the individual and combined effects of pro-inflammatory cytokines tumor necrosis factor-α (TNF-α) and interferon-γ (IFN-γ) on the expression levels of lymphatic-venous biomarkers including lymphatic vessel endothelial hyaluronan receptor (LYVE-1), Prospero Homeobox-1 (Prox-1), forkhead box C2 (FoxC2), vascular endothelial growth factor receptor-3 (VEGFR-3) and podoplanin in murine BEC as well as in microparticles (MPs) derived from these cells.

Methods

Murine BEC, bEnd.3, were treated with TNF-α (20 ng/ml) and/or IFN-γ (1000 U/ml) for 48 hours. The number of MPs shed from cells under different treatment conditions were analyzed using flow cytometry. Levels of lymphatic-venous biomarker expressions were determined in both cells and MPs using western blotting. Expression patterns of these proteins were examined using immunocytochemistry. Fluorescently labeled BODIPY-cholesterol was used to observe cholesterol partitioning of cellular membranes into MPs.

Results

Several lymphatic-venous biomarkers were basally expressed by bEnd.3 cells and transferred into endothelial MPs following TNF-α/IFN-γ stimulation. IFN-γ treatment caused a significant decrease of LYVE-1 (p<0.01), VEGFR-3 (p<0.01) as well as Prox-1 (p<0.01). TNF-α significantly increased podoplanin (p<0.05) and VEGFR-3 (p<0.05) levels in the cells. Following combined treatment MP LYVE-1 increased significantly (p<0.01) but decreased in cells (p<0.05). Combined treatment also significantly decreased cellular Prox-1 levels (p<0.001). Combined stimulation also potently induced transfer of caveolin-1 into MPs, measured as a significant decrease in cells (p<0.05) and increase in MPs (p<0.05). Similarly, TNF-α/IFN-γ stimulated bEnd.3 cells to shed membrane cholesterol, (measured by BODIPY-cholesterol) consistent with endothelial MP identity as caveolae released by cytokine-activated BEC.

Conclusion

These findings show that pro-inflammatory cytokines alter lymphatic-venous proteins as well as caveolar partitioning from BEC into MPs; such events may represent important indices of neurovascular/neurodegenerative disease.

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