

## **BBB-endothelial cell response to cerebral cortex demyelination in a mouse model of chronic EAE**

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Changes in blood-brain barrier (BBB) function have been implicated in demyelinating diseases. This study aimed to investigate the response of cerebral cortex microvessels to nerve fibre demyelination in a chronic model of murine experimental autoimmune encephalomyelitis (EAE) characterized by areas of extensive subpial demyelination along with well-demarcated lesions extended to deeper cortex layers. These cortices showed activation of microglia and astrogliosis with absence of typical perivascular inflammatory infiltrates.

On the basis of these data, we have analyzed the expression of two integral proteins of endothelial tight junctions, claudin-5 and occludin, a structural protein of caveolae, caveolin-1, as well as the BBB-specific endothelial transporter, Glut1 in the cerebral cortex of EAE-affected mice by immunofluorescence confocal microscopy.

Microvascular endothelial cells showed an increased expression of caveolin-1 and a coincident decrease of both claudin-5 and occludin junctional staining pattern. At a very early disease stage, claudin-5 molecules formed aggregates and vacuoles that also stained for Glut 1, whereas occludin pattern became diffusely cytoplasmic at advanced stages of the disease. Internalization/dismantling and loss of tight junction proteins and impairment of BBB function were confirmed by coexpression of claudin-5 with the autophagosomal marker MAP1LC3A and by FITC-dextran experiments that showed leakage of the tracer into the perivascular neuropil.

Overall, these observations indicate that in the cerebral cortex of EAE mice, during demyelination and independently from the inflammatory involvement of the cortex, a 'microvascular disease' characterized by a differential involvement of claudin-5 and occludin occurs, thereby possibly contributing to demyelinating disease progression.

**Keywords:** Blood-brain barrier, cerebral cortex demyelination, Claudin-5, experimental autoimmune encephalomyelitis, inflammation, occludin, oligodendrocyte precursor cell.