VLA-4 blockade promotes differential routes into human CNS involving PSGL-1 rolling of T cells and MCAM-adhesion of TH17 cells

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Research question and background

Immunohistological assessment of the expression levels of VCAM-1 and P-Selectin at possible central nervous system (CNS) entry sites in Multiple Sclerosis (MS) patients and control subjects. VCAM-1 is the receptor for VLA-4 (integrin $\alpha 4\beta 1$) and P-Selectin is the receptor for P-selectin glycoprotein ligand-1 (PSGL-1). VLA-4 and PSGL-1 are both adhesion molecules that are expressed on lymphocytes and together with their receptors are involved in tethering, rolling, and adhesion of T cells to endothelial barriers and are prerequisites for successful extravasation into the CNS.

T_H17 cells have been described to have a pathogenic role in MS. The melanoma cell adhesion molecule (MCAM; CD146) was recently been shown to be a specific marker for T_H17 cells. To investigate whether T_H17 cells might employ MCAM as adhesion molecule for trafficking into the CNS, the presence of MCAM+ cells within the CNS of patients with MS was assessed by immunofluorescence.

Methods and tissues used

Control tissues and tissues of Multiple Sclerosis patients were used. The regions of interest were the meninges, normal-appearing/lesion white matter and the choroid plexus. Formalin fixed paraffin embedded tissues sections were stained with antibodies targeting CD106 (VCAM1) and CD62P (P-Selectin). The secondary antibody was HRP conjugated and diaminobenzidine (DAKO) was used as a chromogenic substrate. Antigen retrieval was done at low pH (pH 6) and endogenous peroxidase activity was blocked with 3% hydrogen peroxide in methanol.

For immunofluorescence studies assessing the expression of MCAM on lymphocytes in MS lesions autopsies of gray and white matter of MS patients were stained with primary antibodies directed against CD146 (MCAM) and CD8. For staining MCAM, amplification with TSATM Plus Biotin kit was done according to the manufacturers instruction.

Results and conclusion

VCAM-1 expression was detected on the endothelium of vessels in white matter, on meningeal vessels and on choroid plexus vessels. Varying amounts of VCAM-1 were expressed on the endothelial barriers of control tissue and tissue of a patient with Multiple Sclerosis. The expression of P-Selectin on the other hand was much more restricted. There was no expression of P-Selectin on the endothelium of vessels in white matter lesions, very low expression on meningeal vessels, and pronounced expression on choroid plexus vessels. The P-Selectin expression on the choroid plexus endothelium was generally more pronounced in the tissue of MS patients as compared to control tissues. MCAM+ T cells could be detected in white matter lesions as well as in the gray matter of MS patients, suggesting a pathogenic role MCAM+ T_H17 cell in MS.