Blood and CSF biomarkers for investigation of the immunopathogenesis of relapse in Multiple Sclerosis

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Based on the following papers

I

MALMESTROM, C., HAGHIGHI, S., ROSENGREN, L., ANDERSEN, O. & LYCKE, J. (2003) Neurofilament light protein and glial fibrillary acidic protein as biological markers in MS. *Neurology,* 61**,** 1720-5.

II

MALMESTROM, C., ANDERSSON, B. A., HAGHIGHI, S. & LYCKE, J. (2006) IL-6 and CCL2 levels in CSF are associated with the clinical course of MS: implications for their possible immunopathogenic roles. *J Neuroimmunol,* 175**,** 176-82.

III

MALMESTRÖM C, LYCKE. J., HAGHIGI S, ANDERSEN O, CARLSSON L, WADENVIK H, OLSSON B (2007) Relapses in Multiple Sclerosis are associated with increased $CD8^+$ T cell mediated cytotoxicity in CSF. Manuscript – Submitted

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Multiple sclerosis (MS) is an autoimmune disease of the central nervous system (CNS). MS usually starts with a relapsing-remitting course (RRMS) that later converts into a secondary progressive phase (SPMS). While inflammation is considered predominating in RRMS, neurodegenerative processes are probably more important in SPMS. The pathophysiology of MS includes autoreactive inflammatory cells that invade the CNS, causing demyelination, oligodendrocyte loss, axonal damage, astrocyte activation and subsequently gliotic scars. Over the last decade the importance of neuronal/axonal damage has been re-discovered. Although, most extensive during progressive MS, it is also an early phenomenon and can be noticed already soon after MS onset. It is the main cause of CNS atrophy and irreversible disability in MS. Several studies indicate that MS immunomodulatory therapy reduces this process, at least during RRMS. The most apparent clinical effect of MS therapy is relapse reduction.

The aim of this thesis was to investigate the relationship between inflammation, neuropathological processes and the clinical course of MS in order to identify biomarkers that could be useful for monitoring disease activity and therapy efficacy. The pathophysiological mechanisms behind clinical relapse were explored, including the possible role of T-cell mediated cytotoxicity.
Patients with RRMS or SPMS were included and healthy blood donors served as controls. Serum and cerebrospinal fluid (CSF) were obtained at relapse, remission or progression. A sub-group of RRMS patients with acute relapse were followed-up repeatedly 5 and 15 weeks after relapse onset. Pro- and antiinflammatory cytokines, neurofilament light chain protein (NFL), a marker of axonal damage and glial fibrillary acidic protein (GFAP), an astrogliosis marker were analysed in CSF. T-cell mediated cytotoxicity was investigated by analysing granzyme A and B in serum and CSF and by mRNA gene expression analysis of peripheral T-cells.

NFL in CSF was increased in all MS patients and showed a 10-fold increase during clinical relapse in relation to patients in remission and progression. The levels peaked after five weeks and were sustained for 15 weeks. CSF-levels of GFAP had a strong correlation to the EDSS in MS-patients, with the strongest correlation in the SPMS group. IL-6 in CSF was higher in RRMS patients than SPMS and controls. CCL2 in CSF was lower in RRMS patients with the lowest level at ongoing relapse compared with controls. Granzyme A and B were increased in CSF at acute relapse and increased level of granzyme A were sustained for up to 3 months.

Increased levels of NFL indicate a continuous axonal damage throughout the clinical course of MS with the most extensive damage during acute relapses. Increased levels of GFAP in MS, with a strong correlation to increasing disability, indicate that astrogliosis is more prominent during clinical progression. While demyelination is considered the pathophysiological hallmark of relapse development, our data suggests that axonal damage may be important in this process. Further, the change in the CSF levels of inflammatory markers (IL-6, CCL2) during relapses supports a relationship between inflammation and axonal damage. We also demonstrate that T-cell cytotoxicity within the CNS/CSF compartment might participate in the immunopathogenesis of clinical relapses. Prolonged increase of NFL and granzyme A levels in CSF after a relapse support an ongoing immunological attack even after apparent clinical remission suggesting a dissociation between immunological and clinical remission.

In conclusion, NFL appears to be a marker for relapse and GFAP for clinical progression. Although our data suggests that these markers might be useful in monitoring disease activity and MS therapies, further studies are needed. Intense treatment of relapse and reduction in relapse frequency during immunomodulatory treatments probably decrease axonal damage which should be beneficial for a positive long term outcome

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