Title: CSF perturbation of human primary astrocytes to model the link between CSF pathogenicity and neurodegeneration in multiple sclerosis

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Background: The causal relationship between inflammatory and neurodegenerative paradigms of multiple sclerosis (MS) is poorly elucidated. Reactive astrocytes in MS lesions propagate an inflammatory cascade leading to immune cell influx and demyelination. Toxic soluble factors in cerebrospinal fluid (CSF) may contribute to neurodegeneration in MS.

Objectives: Our objectives are to (1) characterize which immune biomarkers in CSF correlate with neurodegeneration as assessed by MRI and (2) examine the cellular effects of exposing astrocytes to CSF and recombinant cytokines *in vitro*. We hypothesize that inflammatory factors in CSF will differentially induce astrocyte reactivity and toxicity, offering insight into neurodegenerative pathways in MS.

Methods: 120 subjects were selected from the UMass MS biobank based on available CSF and ≥1 MRI performed on a standardized platform (3T Pioneer scanner, GE). Extent of whole brain atrophy and pathological features of MS were assessed with two quantitative MRI metrics: brain parenchymal fraction (BPF, from CAT12 software) and normalized thalamic volume (nThal, from SIENAX). Age-adjusted Z-scores of BPF and nThal were created from all subjects. Four sub-groups were formed based on Z-scores and MS severity scores (MSSS), defined as follows: high neurodegeneration = Z-scores >1 SD below age-adjusted mean in BPF and nThal, and MSSS ≥3; low neurodegeneration = normal BPF/nThal with MSSS <3, "fragile" = normal BPF/nThal but MSSS ≥3, and non-MS neurological disease controls with normal BPF. CSF proteins were measured via Proximity Extension Assay (Olink) from matched subjects. Human primary astrocytes (ScienCell) were cultured and replated for CSF stimulation. Supernatant and lysed cells were collected for downstream ELISA and qPCR readouts of inflammation, demyelination, and toxicity.

Results: 20 age- and sex-matched subjects were selected for cell culture (N=5 per group). All subjects had a valid EDSS >1 year from symptom onset. Sub-groups significantly differed in terms of MSSS, nThal, and BPF scores (one-way ANOVA, $p<0.001$). Olink data showed CXCL-11 and FGF-21 significantly correlated with MSSS score ($p<0.05$), whereas 13 unique markers significantly correlated with MRI metrics ($p<0.05$). IL-8, FGF-5, and CSF-1 were also significantly lower in the high-ND group ($p<0.05$). CD40 was the only marker that correlated with MSSS and MRI metrics ($p<0.05$).

Conclusion: Unique CSF profiles are associated with neurodegeneration in MS. The correlation of CD40 with both MSSS and MRI supports the role of activated B-cells in neurodegeneration. Ongoing work includes stimulating astrocytes co-cultured with CSF and/or recombinant proteins to examine potential toxic effects of immune proteins in CSF.