Title: Intrathecal Opsonins and Spinal Cord Axonal Loss in Multiple Sclerosis

Short Title: CSF Opsonins and MS Spinal Cord Axonal Loss

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Background:
Multiple sclerosis (MS) is an inflammatory, demyelinating, and neurodegenerative disease afflicting approximately 2.8 million people worldwide. Pathological studies have demonstrated that axonal loss is an important substrate for disease progression, though biomarkers and mediators of this process have remained elusive. Work from our group and others has implicated disruption of the blood-brain and blood-cerebrospinal fluid (CSF) barriers as an important step involved in MS-related neurodegeneration. Therefore, the CSF may provide a biologically-relevant repository to identify molecules that 1) correlate with clinical measures of MS-related disability and 2) are involved in the pathogenic cascade of axonal loss.

Objectives:
1. Identify molecular signatures in the CSF that segregate with the extent of axonal loss in the post-mortem MS spinal cord

Methods:
A post-mortem cohort (n=40) of MS cases with indexed CSF derived from the UK MS Tissue Bank was available for study. We devised a novel approach to integrate post-mortem quantitative neuropathology with CSF proteomics. Immunohistochemical analysis and a robust automated MATLAB analysis pipeline was used to quantify the extent of axonal loss (palmgren silver) within functionally relevant white matter tracts (dorsal columns, anterior corticospinal tracts, and lateral corticospinal tracts) of cervical, thoracic, and lumbar spinal cord tissue from each case. Unbiased principal component analysis (PCA) models were used to derive individual case-level

scores of axonal loss. In parallel, shotgun proteomics of CSF was performed using an Orbitrap Fusion Lumos mass spectrometer. MaxQuant was used for for label-free quantitation with peptides identified using Mascot (Matrix Sciences). Protein-level data was pre-processed through novel integration of the following R packages: pMartR, NormalyzerDE, DEP, and WGCNA. Subsequent over-representation of analysis was used to understand gene ontology enrichment within protein modules that correlated with traits of interest. P-values were adjusted using the Benjamini-Hochberg procedure.

Results:

Weighted protein correlation network analysis identified one module (salmon) that was positively associated with axonal loss ($r=0.41$, $p=0.007$). Gene ontology enrichment analysis of this module highlighted a role for immunoglobulins and complement in driving this relationship. The top enriched terms included GO:0006958 complement activation, classical pathway ($p = 5.39 \times 10^{-23}$) and GO:0002455 humoral immune response initated by circulting immunolglobulin ($p=9.82 \times 10^{-23}$).

Conclusions:

By employing an innovative approach linking post-mortem quantitative neuropathology and CSF proteomics, we have implicated opsonins in the CSF as potential biomarkers and/or molecular mediators of axonal loss in the MS spinal cord.