Do Immunological Profiles of the Cerebrospinal Fluid Correlate with Disease Severity in Multiple Sclerosis?

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OBJECTIVE

To determine if there is an immunological difference between benign and aggressive courses of multiple sclerosis (MS) by studying the immune cell composition of the cerebrospinal fluid.

INTRODUCTION

Multiple sclerosis (MS) is thought to be an autoimmune disease mediated by a small fraction of our own immune cells. Demyelination of the Central Nervous System (CNS) caused by autoimmune inflammation is the hallmark of MS and results in various degrees of disease severity. We hypothesize that there may be immunological profiles that favor benign or aggressive MS courses. This would be important for our understanding of the pathogenesis of disease severity and optimizing therapeutic strategies.

DESIGN AND METHODS

Cell Separation and Culture: Cerebro-spinal fluid (CSF) and peripheral blood was obtained from MS patients with benign, stable disease or with aggressive, active disease (with or without treatment). Cells were separated from CSF by centrifugation and the supernatant discarded. Peripheral blood mononuclear cells (PBMCs) were isolated by density gradient centrifugation (BD vacutainer CPT tubes) and where necessary memory ($CD4^+CD45RO^+$) T cells purified by magnetic separation using the untouched memory T cell isolation kit (Stem Cell Technologies). All patient samples were obtained by informed consent and IRB approved.

For cell type studies CSF cells were re-suspended in 2% human serum in phosphate buffered saline, blocked with 1mg/ml mouse IgG, stained for cell surface markers (table 2) and the cells fixed in 1% paraformaldehyde. For cell subtype studies the cells were re-suspended in X-VIVO15 medium and stimulated by incubation for 5 hours at 37°C/5%CO_2 with PMA and ionomycin in the presence of brefeldin A. Cell surface staining was done as above except the 1% paraformaldehyde step and was instead followed by intracellular staining for cytokines and transcription factors by incubating in fix/perm buffer overnight at 4°C and following the FoxP3 kit (BioLegend) protocol for intracellular staining.

Table 1: Definitions of benign and aggressive disease types. Patients must have one or more of each definition to be considered for this study.

Benign	Aggressive
Low disability score (EDSS <2)	High disability score (EDSS >6)
No relapses within 5+ years	MRI enhancement
Active or "normal" lifestyle.	Severe recent or ongoing MS activity/symptoms
Little to no treatment required.	Poor response, or failure to respond, to treatment

RESULTS

Initial results showed no differences in the overall average number of cells in the CSF due to a significant amount of variation, Benign = 1385 cells/ml Aggressive = 1310 cells/ml, and no differences in the numbers of each cell type in the CSF. We tested for the cell types shown in table 2.

Table 2: Surface markers used to identify cell types in the CSF in this study.

Cell type	Surface markers
Naïve or memory T cells	CD4, CD25, CD45RA, CD45RO
Regulatory T cells	CD4, CD25, CD127, γ/δTCR
Differentiated T cells	CCR7, CD4, CD8, CD161
Natural Killer Cells	CD3, CD4, CD8, CD56
B cells & plasma cells	CD19, CD27, CD138, IgD
Antigen presenting cells	CD11b, CD11c, CD14, CD304

Since we could not discern any differences in cell numbers or types we decided to look at cell subtypes within the most numerous cell type in the CSF, the $CD4^+$ T cell. Several T cell subtypes may play a role in MS and we looked for these by doing intracellular staining for the following cytokines or transcription factors: IFNγ (T_H1), IL-4 (T_H2), IL-17 (T_H17), and FoxP3 (T_{reg}). We also compared PBMCs and memory T $CD4^+$ cells with the cells from CSF.

The only difference observed was that the percentage of $CD4^+$ IFNγ secreting T cells in the CSF is higher in patients not undergoing treatment who have aggressive disease than those with benign disease (Fig. 1. $p < 0.05$ by 2-tailed T-test assuming unequal variances).

We found no significant difference in our results when comparing IFNγ producing cells from patients on treatment. We also found no differences with cells producing IL-4 (T_H2), IL-17 (T_H17) or FoxP3 (T_{reg}).

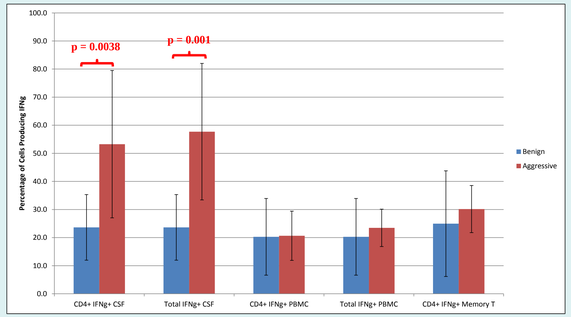

Figure 1: Percentage of cells producing IFNγ in the CSF, PBMC and memory T cell populations of MS patients with aggressive or benign disease who had not had drug therapy in greater than 1 year..

We measured the levels of the soluble factors present in concentrated (x4) CSF using 96-well plate ELISA or a 25-plex cytokine array (Life Technologies). IL-17, MIP-1β (CCL4) (Fig. 2) and osteopontin (Fig. 3) showed significantly increased levels in patients with aggressive disease as compared to those with benign disease.

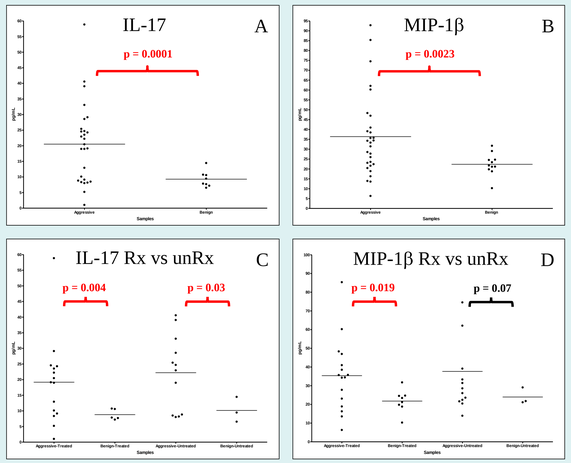

Figure 2: Amount (pg/ml) of IL-17 (A, C) and MIP-1β (B, D) in the CSF of all aggressive and benign MS samples (A, B) and those on treatment or not (C, D).

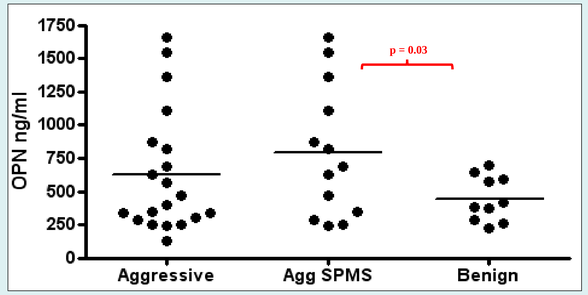

Figure 3: Amount of osteopontin (ng/ml by ELISA) in the CSF of all aggressive (PPMS and SPMS), SPMS with aggressive disease, and benign MS patients.

Molecules with levels below detection: Granulocyte-Macrophage-Colony Stimulating Factor (GM-CSF), Interferon-gamma (IFNγ), Interleukins (IL)-1β, -2, -10, -13, Matrix Metalloproteinase (MMP)-9, MIG (CXCL9), RANTES (CCL5); Detected but data extrapolated from below the linear part of the standard curve: Eotaxin (CCL11), IL-2R, -5, -7, and Tumor Necrosis Factor (TNF)α. Detected above threshold but with no significant differences: BCA-1 (CXCL13), Fetuin-A, IFNα, IL-1RA, -4, -6, -8, -12 (p40/p70), -15, IP-10 (CXCL10), MCP-1 (CCL2), MIP-1α (CCL3), Nitric Oxide, or Tau.

CONCLUSIONS

1. The pro-inflammatory cytokines IL-17, MIP-1β (CCL4), osteopontin are significantly higher in patients with aggressive disease..
2. T_H1 (IFNγ secreting) cells are more numerous in the CSF of patients with aggressive disease than those with benign disease in the subset not on treatment. However the levels of IFNγ in the CSF were below the limits of detection in the assays used.
3. These results suggest that pro-inflammatory responses play a role in disease activity and progression.

ONGOING WORK

1. We wish to determine whether these results hold true in longitudinal studies of patients.
2. We are also looking into whether there are markers of benign disease that indicate an immune balance in promoting or inhibiting disease.