Published in final edited form as:

J Neuroimmunol. 2012 August 15; 249(1-2): 86–92. doi:10.1016/j.jneuroim.2012.04.017.

Expression of CCR7 and CD45RA in CD4⁺ and CD8⁺ subsets in cerebrospinal fluid of 134 patients with inflammatory and non-inflammatory neurological diseases

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Abstract

We investigated CD45RA and CCR7 expression in CD4⁺ and CD8⁺ subsets of cerebrospinal fluid (CSF) lymphocytes, both immediately ex vivo and after stimulation, from 134 patients with a variety of inflammatory and non-inflammatory neurological diseases. Most inflammatory diseases had a higher CD4⁺: CD8⁺ ratio and higher percentage of effector memory T cells (T_{EM}) than non-inflammatory controls, excluding active infection. Moreover, we found that patients with highly elevated cell counts in the CSF tended to have a lower percentage of central memory T cells (T_{CM}) than patients with low or absent pleiocytosis, with a concomitant increase in T_{EM}. We also found that samples with elevated IgG index or presence of oligoclonal bands had a significantly higher CD4⁺:CD8⁺ ratio than normal samples, consistent with increased CD4⁺ help for intrathecal IgG synthesis by B cells.

Keywords

CSF; T lymphocytes; effector memory T cells; immunoglobulin; multiple sclerosis

1. Introduction

Inflammation in the central nervous system (CNS), whether of infectious or autoimmune etiology, is associated with infiltration of immune cells into the cerebrospinal fluid (CSF) and brain parenchyma. This infiltration has been described to occur via three distinct routes of entry: 1. from the blood to CSF across the choroid plexus, 2. from blood to the subarachnoid space, and 3. from blood to parenchymal perivascular space (Ransohoff et al., 2003). Harvesting the CSF by lumbar puncture is valuable both for refining a differential diagnosis and for investigating the etiology of various diseases. The chemokine receptor profile and activation kinetics of immune cells infiltrating the subarachnoid space may offer

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a window into the pathogenic effects of these cells by highlighting their migratory and effector functions.

During inflammation, activated cells are known to preferentially enter the CNS via a process involving selectins, chemokines, chemokine receptors, and cell adhesion molecules (Hickey, 1991; Hickey et al., 1991; Springer, 1994; Wekerle, 1986). These cells are not necessarily antigen-specific, although antigen-specific cells have been located in the CNS (Hafler and Weiner, 1987; Olsson et al., 1990). It is not known, however, whether the cells in the CSF of a patient with an autoimmune disease such as multiple sclerosis (MS) represent a select subset of effector cells already primed to cause damage to the CNS, or if local conditions within the perivascular space or brain parenchyma in MS induce reactivation of a subset of circulating cells that secondarily become pathogenic in the CNS. It has been shown recently that infiltrating CD8⁺ T cells found in the CSF of MS patients are mostly of the effector-memory phenotype (Iferrgan, 2011). Herein we examined whether CSF lymphocytes are primarily effector-memory cells (T_{EM}), or primed, but not chronically activated, central memory cells (T_{CM}) in a variety of inflammatory and non-inflammatory CNS conditions seen at our center. Characterization of the CSF surface receptor profile may provide insight into the timing and location (peripheral versus central) of activation of cells recruited across the blood-CSF barrier into the subarachnoid space.

Expression of CCR7, a chemokine receptor which recruits cells to the lymph nodes, in combination with the naïve cell marker CD45RA, has been shown to discriminate naïve (CD45RA⁺CCR7⁺) and T_{CM} (CD45RA⁺CCR7⁺) from T_{EM} (CD45RA⁺CCR7⁺) and T_{EM}-RA⁺ (CD45RA⁺CCR7⁺, found mainly in CD8⁺ cells) subsets (Sallusto et al., 1999a) (Sallusto et al., 1999b). CCR7 has been shown to be highly expressed on CSF cells, while cells within MS brain lesions have a CCR7⁺ effector phenotype (Giunti et al., 2003; Kivisakk et al., 2004; Kivisakk et al., 2003a; Kivisakk et al., 2003b; Rus et al., 2005). Previous studies have used such markers as CD25, CD26, CD29, and chemokine receptors such as CXCR3 to differentiate between active and quiescent stages of MS (Kraus et al., 2000a; Kraus et al., 2000b; Matsui et al., 2004; Okuda et al., 2005; Sintern et al., 2002).

Herein, we demonstrate that CSF cells from a variety of inflammatory and non-inflammatory diseases are more likely to be T_{EM} than peripheral blood cells, both immediately ex vivo and after T cell stimulation with anti-CD3 and anti-CD28 antibodies. CSF cells from inflammatory disease processes tend to have a higher percentage of T_{EM} cells and fewer T_{CM} cells as compared to non-inflammatory controls. Further, the CD4⁺:CD8⁺ ratio in CSF was markedly increased in most inflammatory diseases, particularly RRMS, compared to non-inflammatory diseases. Exceptions included PPMS and active infection. We also found that samples with elevated IgG index or presence of oligoclonal bands (OCB) had a significantly higher CD4⁺:CD8⁺ ratio than normal samples, consistent with increased CD4⁺ help for intrathecal IgG synthesis by B cells.

2. Materials and Methods

2.1 CSF collection and ethics statement

CSF was collected at the Johns Hopkins Lumbar Puncture Clinic or the Johns Hopkins Hospital from 210 patients referred for diagnostic lumbar puncture. All patients gave consent according to a JHMI-IRB approved protocol. Patient demographics are presented in Table 1. The majority of MS patients included in the study were not receiving any treatment. Only three of the patients with relapsing remitting MS were being treated with Avonex or Copaxone. The primary progressive patients were not on any therapy and had not received steroids within the 3 months prior to the lumbar puncture. All patients receiving steroids recently were excluded from the study. CSF was centrifuged at 1000 rpm for 10 min to

pellet cells. Twenty-six samples with contaminating red blood cell content were excluded, assumed to be the result of a traumatic tap and unrepresentative of the lymphocyte population specifically recruited into the CSF. Further, only samples with CD4⁺ or CD8⁺ lymphocyte subsets numbering greater than 250 cells (acquired by flow cytometry) were included, in order to base our analysis on adequate representative populations. Our final number of analyzed patient samples, therefore, was 134.

2.2 Flow cytometric assay

After centrifugation, supernatant was removed and cells were reconstituted in 500uL of medium (IMDM supplemented with pen-strep, L-glutamine, gentamicin, and 5% human serum) and counted. For flow cytometry, we stained with FITC CD45RA, PE CCR7, PerCP CD8, APC CD4 (all from BD Pharmingen except CCR7 antibody, from R&D) and fixed in 1% paraformaldehyde. Samples were acquired on a BD FACSCalibur machine within one week of staining and analyzed using Cellquest software. If cells numbered $>2 \times 10^4$, half of sample was stained for flow cytometry and half was cultured in a round-bottom 96 well plate in 250uL media and stimulated with 0.125uL anti-CD3/anti-CD28 beads (T cell expander, Dynal Biotech, Invitrogen). At day 7, cells were restimulated with anti-CD3/anti-CD28 beads, and at days 7 and 14 of this culture, samples were harvested for flow cytometry.

2.3 Periperal blood collection and culture

Peripheral blood was obtained by venipuncture from 8 controls. Peripheral blood mononuclear cells (PBMC) were isolated by density gradient centrifugation with Ficoll/LSM, then stained and cultured according to CSF protocol.

2.4 Data analysis

Patients were divided into groups according to clinical presentation and laboratory results by a blinded observer prior to analyzing flow cytometric profiles. Groups were: non-inflammatory neurological disease; MS, divided by McDonald criteria into clinically definite primary progressive (PP) and relapsing-remitting subtypes or possible MS; other inflammatory neurological disease, including subgroups with active infection (including TB meningitis, viral meningitis, or neurosyphilis, within two weeks of onset of active disease), recovering infection (2 to 3 month followup after viral encephalitis or meningitis), and neuroimmunological diseases such as neuromyelitis optica, transverse myelitis, acute disseminating encephalomyelitis, and neurosarcoidosis. Other non-inflammatory neurological disease (OND) controls included patients with over 20 different diagnoses most commonly including normal pressure hydrocephalus, headache, peripheral neuropathy, and neurodegeneration. Demographics are included in Table 1.

2.5 Statistics

Mann-Whitney (non-parametric) t-tests were used to compare groups.

3. Results

3.1 CSF cells from a variety of inflammatory and non-inflammatory diseases are more likely to be T_{EM} than peripheral blood cells

We first analyzed the memory phenotype and CD4⁺:CD8⁺ ratio of T cells from CSF of all patients. Patient demographics are presented in Table 1. We also stained cells after 7 or 14 days of stimulation with anti-CD3/CD28 beads to compare the activation kinetics of CSF cells to PBMCs. For this analysis, CSF samples with too few cells to culture were excluded from the group at day 0.

Representative FACS gates for CSF cells and PBMC are shown in Figure 1A–B. The relative percentages of naïve, T_{CM}, and T_{EM} on CD4⁺ or CD8⁺ T cells at days 0, 7, and 14 as well as the CD4/CD8 cell ratio for CSF cells (Figure 1C–E) and PBMC (Figure 1F–H) are compared. Fresh CD4⁺ cells from the peripheral blood had an average of 53.3% naïve, 35.4% T_{CM}, and 11.2% T_{EM} subsets, while CD8⁺ T cells had more effector than central memory cells, with 62.5% naïve, 9.4% T_{CM}, and 28.1% T_{EM}. In contrast, T cells from the CSF were more heavily weighted towards activated central and effector memory phenotypes, consistent with previous reports (Kivisakk et al., 2003a). Within the CD4⁺ subset, we saw an average of 13.7% naïve, 41.7% T_{CM}, and 44.6% T_{EM}, while the CD8⁺ subset showed a strong majority of effector cells, with 75.5% T_{EM}, and only 15.8% naïve and 8.7% T_{CM}. All differences in memory phenotype between CSF and PBMC subsets were significant ($p < 0.05$), with disparities in naïve and T_{EM} subsets particularly strong ($p < 0.001$). The average CD4⁺:CD8⁺ ratio, on the other hand, was higher in CSF than PBMC populations, at 2.5 in PBMCs and 3.5 in CSF cells.

In both PBMC and CSF populations, the strong stimulus of TCR crosslinking by anti-CD3 antibody coated beads in conjunction with costimulation by anti-CD28 antibody coated beads induced a prominent shift from naïve to T_{CM} and T_{EM} subsets. The shift towards effector memory phenotype in CSF cells leveled off between d7 and d14, unlike PBMCs, which again shifted substantially after stimulation between days 7 and 14. By day 14, therefore, PBMC and CSF populations were much closer in memory phenotype than at the outset.

The CD4⁺:CD8⁺ ratio, meanwhile, did not significantly change in PBMCs over the time course, while the average CSF ratio significantly decreased from 3.5 on day 0 to 1.9 on day 7 ($p = 0.0009$) and trended further downwards to 1.3 on day 14 ($p = 0.0698$). This may indicate rapid death of activated CD4⁺ T cells in CSF, or conversely preferential survival of activated/terminally differentiated CD8⁺ cells in CSF.

3.2 CSF cells from inflammatory disease processes tend to have a higher percentage of T_{EM} cells and fewer T_{CM} cells as compared to non-inflammatory controls

Since CSF cells were derived from patients with a variety of diseases, including a spectrum of inflammatory disorders ranging from MS to infection, we proceeded to investigate correlations between disease type and CSF phenotype. As would be expected with human disease, some variability was found to exist among individuals and between groups of patients with differing diagnoses (Supplemental Fig. 1). We compared CSF samples from inflammatory and non-inflammatory disorders. Inflammatory diseases overall had fewer central memory CD4⁺ cells in the CSF than non-inflammatory diseases (51.6% vs. 43.1%, $p = 0.0303$), with a coinciding increase in T_{EM} CD4⁺ cells (total T_{EM}, 38.0% vs. 45.4%, $p = 0.0159$) (Figure 2A). There were no significant differences among the groups in the CD8⁺ profile (Figure 2B). Most significantly, CD4⁺:CD8⁺ ratios in CSF were higher in subjects with inflammatory than non-inflammatory diseases (3.7 vs. 2.1, $p = 0.0026$) (Figure 2C).

We then further subcategorized our group of heterogeneous inflammatory diseases to differentiate between different subtypes of MS (PPMS, RRMS, and possible MS) and several different inflammatory disorders (acute infection, recovering infection, neuromyelitis optica/Devic's, idiopathic transverse myelitis, and "others") (Figure 2D–F). Neuroinflammatory disorders not directly linked to infection tended to have a higher average CD4⁺:CD8⁺ ratio in CSF than non-inflammatory controls, except for patients with PPMS, who were indistinguishable from non-inflammatory controls (2.3 vs. 2.1). The average ratio in PPMS, indeed, was significantly lower than in RRMS ($p = 0.0029$). The elevation of CD4⁺:CD8⁺ ratio in CSF of RRMS patients (4.6) and possible MS patients (4.8) were particularly high compared to non-inflammatory controls ($p = 0.0001$, and $p = 0.0190$,

respectively). We did not find significant variations in memory profile between MS subtypes and other diseases; the MS groups' averages tended to be intermediate between non-inflammatory and other inflammatory groups, possibly due to the heterogeneity of MS disease courses.

The percentage of CD4⁺ naïve cells in the CSF in acute infection (16.9%) was significantly greater than the percentage of naïve cells in cases of recovering infection (9.0%, $p=0.0091$) or that in other inflammatory conditions (6.7%, $p=0.0303$) (Figure 2G–I). There was a similar, non-significant trend in CD8⁺ subsets. This is consistent with a non-specific and massive influx of cells. Cases of acute infection also had a significantly lower ratio of CD4⁺ to CD8⁺ cells in the CSF than other inflammatory disorders ($p=0.013$), consistent with high CD8⁺ cell influx to combat infection. CSF obtained from patients recovering from infection; however, reverted to an elevated CD4⁺:CD8⁺ ratio similar to other inflammatory diseases. Further, compared to non-inflammatory controls, the percentage of T_{CM} in CSF in cases of recovering infection was significantly decreased (38.44% vs. 51.6%, $p=0.0324$), and the percentage of T_{EM} increased (52.0% vs. 38.0%, $p=0.0188$).

3.3 Elevated IgG index or presence of oligoclonal bands (OCB) correlates with a higher CD4⁺:CD8⁺ ratio, consistent with increased CD4⁺ help for intrathecal IgG synthesis by B cells

When we grouped the patients not by diagnosis but solely on presence or absence of laboratory signs of inflammation, we found that patients with highly elevated cell counts in the CSF (>15 cells/mm³) tended to have a lower percentage of T_{CM} ($p=0.0487$) than patients with low or absent pleiocytosis, with a concomitant increase in T_{EM}. The memory profile in the CSF did not differ significantly between patients with and without other signs of inflammation (IgG index >0.8 , protein >45 , OCB >0). However, patients with elevated IgG index or positive oligoclonal bands did have a significantly higher ratio of CD4⁺:CD8⁺ cells in the CSF ($p=0.0011$ and $p=0.0468$ respectively), suggesting that intrathecal IgG synthesis is supported by the presence of CD4⁺ T helper cells (Figure 3A–D).

4. Discussion

Herein, we studied the memory profile and CD4⁺:CD8⁺ ratio of CSF cells in a large heterogeneous group of 134 patient samples with both inflammatory and non-inflammatory diseases.

Immediately ex vivo, CSF cells clearly have a higher percentage of memory cells in both CD4 and CD8 subsets than peripheral blood cells. This supports previous reports that activated cells preferentially enter the CSF. In addition, we found that the profile of fresh CSF cells resembles that of PBMCs after stimulation in vitro. Both CSF and PBMC cultures convert from naïve and central memory to effector memory phenotypes upon strong stimulation by anti-CD3 and anti-CD28 beads. The CD4⁺:CD8⁺ ratio remains constant in PBMCs over the timecourse, while the ratio in CSF cells decreases. This may indicate that terminally differentiated CD4⁺ effector memory cells from activated CSF die preferentially after stimulation.

CD4⁺:CD8⁺ ratio in the CSF was elevated in all inflammatory diseases except acute infection and PPMS. The ratio in RRMS was particularly high, consistent with increased influx of activated CD4⁺ T cells. The increased CD4⁺:CD8⁺ ratio in the CSF in inflammatory diseases, particularly in RRMS, has been reported previously (Kivisakk et al., 2004; Kolmel and Sudau, 1988; Matsui et al., 1988; Oreja-Guevara et al., 1998). Indeed, CD4⁺ T cells have been reported to preferentially migrate into the CSF (Hafler and Weiner, 1987). Moreover, we report that elevated OCB and IgG index significantly correlated with a

greater CD4+:CD8+ ratio in the CSF, consistent with intrathecal CD4+ T-cell help for B-cell cytokine production.

We found fewer TCM and more TEM in CSF of inflammatory than non-inflammatory diseases, consistent with a more activated population of cells. In acute infection specifically, there were more naïve cells, indicating non-specific influx; however, in patients recovering from infection, there was an elevated percentage of TEM cells in the CSF, consistent with an adaptive memory response to infection. As a group, patients with high CSF cell counts had a significantly lower percentage of TCM and higher percentage of TEM than patients with low number of cells, correlating the degree of cellular infiltration with an activated phenotype. This observation suggests a functional role for effector memory T cells within the CSF (possibly contributing to disease pathogenesis) in addition to providing immune surveillance. A previous study by Kivisakk et al. reported that greater than 90% of memory cells found in the CSF of a small number of MS patients and patients with non-inflammatory neurological disease had a CCR7+ central memory phenotype. However, the patients enrolled in this study were confirmed to be MS patients in remission. We postulate that the increase in effector memory CCR7- T cells found in the CSF in our study correlates more closely with inflammation and may vary depending upon the clinical state of the patients studied.

Limitations in our study should be acknowledged, particularly the large degree of overlap in phenotype of different diseases. Contributing factors may include differences in localization of inflammation within the CNS and in the timing of lumbar puncture within the disease course, particularly in diseases like MS with highly variable progression rates. Our capacity to compare different disease subtypes was further limited due to the relatively small number of patients within any one disease category. Finally, it is difficult to extrapolate from CSF findings to local conditions in the brain parenchyma, due to the different mechanisms of entry into the CSF and brain (Ransohoff et al., 2003).

Nevertheless, we found significant differences in the average memory profile and CD4+:CD8+ ratio in the CSF between inflammatory and non-inflammatory disease groups, in addition to correlating phenotypes with IgG index, oligoclonal bands, and cell count, which are parameters important in clinical diagnosis of CNS inflammatory disease. Moreover, these findings suggest that chronically activated effector memory T cells present within the CSF could have a functional role and may have differentiated from CCR7+ central memory T cells following reactivation locally in the subarachnoid space in the context of inflammation. These cells may then be retained in the CSF or migrate to the brain parenchyma where they could contribute to the pathogenesis of diseases such as MS. These data provide new information regarding the phenotype of CSF cells involved in the pathogenesis of CNS inflammatory diseases.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

This study was supported in part by NIH R01 NS41435 (PAC)

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Figure 1. CSF cells from a variety of inflammatory and non-inflammatory diseases are more likely to be T_{EM} than peripheral blood cells

The expression of CD45RA and CCR7 were analyzed within CD4⁺ and CD8⁺ subsets of CSF (A) and peripheral blood (B) by flow cytometry. CD4⁺ cells from the CSF (C) had an average of 13.7% naïve, 41.7% T_{CM}, and 44.6% T_{EM} ex vivo. Seven days after stimulation, the percentage of naïve had declined to 3.6% ($p < 0.0001$) and T_{CM} to 29.8% ($p = 0.0003$) while T_{EM} increased to 66.6% ($p < 0.0001$). These percentages did not significantly change between day 7 and d14. The CD8⁺ subset (D) showed 75.5% T_{EM}, and only 15.8% naïve and 8.7% T_{CM} ex vivo. Seven days after stimulation, the proportion of naïve CD8⁺ had declined to 4.9% ($p < 0.0001$) while T_{EM} increased to 87.0% ($p = 0.0003$). Between day 7 and 14, naïve and T_{CM} populations slightly declined ($p = 0.0482$ and $p = 0.0003$ respectively), while T_{EM} continued to increase ($p = 0.0005$). In the peripheral blood ex vivo, CD4⁺ cells (F) had an average of 53.3% naïve, 35.4% T_{CM}, and 11.2% T_{EM} subsets, while CD8⁺ cells (G) had 62.5% naïve, 9.4% T_{CM}, and 28.1% T_{EM}. In CD4⁺ PBMCs, as with CSF cells, stimulation induces a significant decrease in naïve and T_{CM} percentages (to 42.0%, $p = 0.0156$, and 22.6%, $p = 0.0078$, respectively) and an increase in T_{EM} (to 35.4%, $p = 0.0078$) by day 7. Between day 7 and day 14, unlike CSF, the percentage of naïve again significantly declines (to 22.3%, $p = 0.0078$), while T_{EM} significantly increases (to 52.1%, $p = 0.0078$) and T_{CM} remains constant. CD8⁺ PBMCs have the same pattern in naïve and

TEM subsets from day 0 to day 7 (naïve to 54.3 %, $p=0.0234$, and TEM to 42.3%, $p=0.0078$), and day 7 to day 14 (naïve to 37.3%, $p=0.0078$, and TEM to 56.0% $p=0.0078$), while the TCM subset declines between d0 and d7 (to 3.3%, $p=0.0078$) but slightly rebounds by d14 (to 6.7%, $p=0.0156$). The average CD4+: CD8+ ratio ex vivo was 2.5 in PBMCs (H) and 3.5 in CSF cells (E). In PBMCs, the CD4+: CD8+ ratio does not significantly change over the timecourse (H), while the CSF average ratio (E) significantly decreases from 3.5 on day 0 to 1.9 on day 7 ($p=0.0009$) and trends further downwards to 1.3 on day 14 ($p=0.0698$).

Figure 2. CSF cells from inflammatory disease processes tend to have a higher percentage of T_{EM} cells and fewer T_{CM} cells as compared to non-inflammatory controls

When samples were subgated according to diagnosis, inflammatory diseases overall had fewer central memory CD4+ cells in the CSF than non-inflammatory diseases (A: 51.6% vs. 43.1%, $p=0.0303$), but a greater percentage of TEM CD4+ cells (A: 38.0% vs. 45.4%, $p=0.0159$). There were no significant differences among the groups in the CD8+ profile (B). CD4+: CD8+ ratios were higher in subjects with inflammatory than non-inflammatory diseases (C: 3.7 vs. 2.1, $p=0.0026$). The CD4+: CD8+ ratio in patients with PPMS was indistinguishable from non-inflammatory controls (F: 2.3 vs. 2.1), and was significantly lower than in RRMS ($p=0.0029$). The CD4+: CD8+ ratio in RRMS (4.6) and possible MS (4.8) were significantly elevated compared to non-inflammatory controls (F: $p=0.0001$, and $p=0.0190$, respectively). The MS groups' memory profiles tended to be intermediate between non-inflammatory and other inflammatory groups, but did not significantly differ in

either CD4+ or CD8+ subsets (D, E). The percentage of CD4+ naïve cells in acute infection (G: 16.9%) was significantly greater than the percentage of naïve cells in cases of recovering infection (9.0%, $p=0.0091$) or that in other inflammatory conditions (6.7%, $p=0.0303$). There was a similar, non-significant trend in CD8+ subsets (H). Cases of acute infection also had a significantly lower ratio of CD4+ to CD8+ cells than other inflammatory disorders (I: $p=0.013$). CSF obtained from patients recovering from infection, however, revert to an elevated CD4+:CD8+ ratio similar to other inflammatory diseases (I). Further, compared to non-inflammatory controls, the percentage of CD4+ TCM in cases of recovering infection is significantly decreased (G: 38.44% vs. 51.6%, $p=0.0324$), and the percentage of TEM increased (52.0% vs. 38.0%, $p=0.0188$).

Figure 3. Elevated IgG index or presence of oligoclonal bands (OCB) correlated with a higher CD4+:CD8+ ratio, consistent with increased CD4+ help for intrathecal IgG synthesis by B cells
 The CD4+:CD8+ ratio was significantly higher in CSF with abnormal (>0.8) IgG index than in normal (IgG index<0.8) (A; 5.0 vs. 3.0, $p=0.0011$). The CD4+:CD8+ ratio was also elevated in CSF with oligoclonal bands (B; 3.9 with OCB vs. 3.1 without OCB, $p=0.0468$). Further, within the CD4+ subset, CSF with high cell counts (>15 cells/uL) had a lower percentage of TCM than CSF with cell counts <15 (C; 41.3 vs. 48.6, $p=0.0487$), with a coinciding but non-significant increase in the percentage of TEM (C). There was no difference in the CD8+ subset between samples with high and low cell count (D).

Table 1

Diagnosis	Number	Age (Average)	Age (Range)	% Female
RRMS	28	36.3	6–57	78.6
PPMS	9	51.7	39–61	44.4
Possible MS	11	39.9	25–62	54.5
OIND	51	43.7	18–73	52.9
Acute Infection	3	34.9	24–48	33.3
Recovering Infection	9	38.2	21–52	55.6
Sarcoidosis	5	44.3	23–67	40.0
ADEM	2	46.2	45–47	50.0
TM/NMO	16	46.5	18–69	75.0
Other	8	46.6	26–73	75.0
OND	43	53.5	1–85	58.1