

B- and T-cell markers in opsoclonus–myoclonus syndrome

Immunophenotyping of CSF lymphocytes

M.R. Pranzatelli, MD; A.L. Travelstead, BS, MT (ASCP); E.D. Tate, FNP-C, MN; T.J. Allison, BS; E.J. Moticka, PhD; D.N. Franz, MD; M.A. Nigro, DO; J.T. Parke, MD; D.A. Stumpf, MD, PhD; and S.J. Verhulst, PhD

Abstract—Background: Although many lines of evidence suggest an autoimmune etiology, the pathophysiology of opsoclonus–myoclonus syndrome (OMS) remains poorly understood and no immunologic abnormalities have correlated with neurologic severity. Conventional immunotherapies often do not prevent relapse or permanent sequelae. **Objective:** To test the cellular immune hypothesis of OMS in a cross-sectional study and determine if CSF lymphocyte subset analysis provides biomarkers of disease activity. **Methods:** The expression of lymphocyte surface antigens was investigated in CSF and blood of 36 children with OMS and 18 control subjects, using a comprehensive panel of monoclonal antibodies to adhesion and activation proteins in combination with anti-CD3 and anti-CD45 antibodies in four-color fluorescence-activated cell sorting. **Results:** Although most children with OMS had normal CSF cell counts, they exhibited expansion of CD19⁺ B-cell (up to 29%) and $\gamma\delta$ T-cell (up to 26%) subsets and a lower percentage of CD4⁺ T-cells and CD4/CD8 ratio, which persisted even years after disease onset and conventional treatments. The percentage of activated CSF T-cells was also higher. Abnormalities correlated with neurologic severity, as scored blinded from videotapes using a 12-item motor scale, and disease duration. No significant differences were found between tumor and no-tumor groups. In children with neuroblastoma, tumor resection or cancer chemotherapy did not alter immunologic abnormalities. **Conclusions:** CSF B- and T-cell recruitment is linked to neurologic signs in pediatric OMS, which may relate to relapses and disease progression.

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Opsoclonus–myoclonus syndrome (OMS) is a neuropsychiatric disorder,¹ in which autoimmunity may play an important role, characterized by relapses² and often permanent sequelae.^{3,4} The brainstem and cerebellum are thought to give rise to the principal motor features.^{3,5–7} In children, neuroblastoma⁸ and viral infections^{9,10} are the most common causes.¹¹

The immunopathophysiology of childhood OMS has remained rather elusive. Basic information about the participant immune cell types in OMS is lacking, and there have been no convincing links between various autoantibodies^{6,12,13} and neurologic

abnormalities. Because autoantibodies are not found in all cases and they do not cause OMS in laboratory animals when passively transferred from humans, we hypothesized cellular immune co-involvement.¹⁴ The same immune cell types involved in host tumor defenses, such as tumor-infiltrating lymphocytes and lymphokine-activated killer cells, could participate in the paraneoplastic syndrome through blood–brain barrier permeation after activation by onconeural antigens.

Lymphocytes traffic from brain capillaries through the CNS as part of a physiologic process of immune surveillance.¹⁵ Under various pathologic conditions, CSF can host increased numbers of $\alpha\beta$ T-cells (helper/inducer; cytotoxic/suppressor), $\gamma\delta$ T-cells, B-cells, natural killer (NK) cells, as well as cell types not normally found in CSF,

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From the Departments of Neurology (Dr. Pranzatelli, E. Tate and T. Allison), Pediatrics (Dr. Pranzatelli), Medical Microbiology and Immunology (Dr. Moticka, A. Travelstead), and Statistics and Research Consulting (Dr. Verhulst), Southern Illinois University School of Medicine, Springfield, Departments of Pediatrics and Neurology (Dr. Franz), University of Cincinnati, OH, Michigan Institute of Neurological Disorders (Dr. Nigro), Farmington Hills, Department of Neurology (Dr. Parke), University of Oklahoma College of Medicine, Oklahoma City, and Department of Neurology (Dr. Stumpf), Northwestern University, Evanston, IL.

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Address correspondence and reprint requests to Dr. M.R. Pranzatelli, National Pediatric Myoclonus Center, SIU School of Medicine, PO Box 19658, Springfield, IL 62794-9658; e-mail: mpranzatelli@siu.edu

Table Clinical characteristics of children with OMS

Variable	All OMS*	Tumor found†	No tumor found‡
No. of cases (%)	36	16 (44)	20 (56)
Gender, no. (%)			
Boy	16 (44)	5	11
Girl	20 (56)	11	9
Mean ± SD age at testing, y	4.1 ± 0.6	3.2 ± 0.5	4.8 ± 0.9
Range, y	1.1–16.9	1.8–9.3	0.7–16.9
Infant <1.5 y, no. (%)	2 (6)	0	2
Toddler ≥1.5 < 3 y, no. (%)	15 (42)	8	7
Preschool ≥3 < 5 y, no. (%)	12 (33)	7	5
School age ≥5 y, no. (%)	7 (19)	1	6
Mean ± SD age at onset, y	1.9 ± 0.2	1.5 ± 0.2	2.2 ± 0.3
Range, y	0.5–5.6	0.5–3.6	0.7–5.6
Mean ± SD syndrome duration, y	2.2 ± 0.5	1.8 ± 0.5	2.5 ± 0.8
Range, y	0.1–14.4	0.3–7.7	0.1–14.4
Acute ≤0.3 y, no. (%)	5 (14)	2	3
Subacute ≥0.3 ≤ 1 y, no. (%)	13 (36)	7	6
Chronic >1 y, no. (%)	18 (50)	7	11
Neurologic severity‡	16.2 ± 1.9	15.7 ± 2.5	16.7 ± 2.8
Range	0–36	0–36	2–36
Mild, no. (%)	14 (39)	5	9
Moderate, no. (%)	17 (47)	10	7
Severe, no. (%)	5 (14)	1	4
Prior tumor chemotherapy,§ no. (%)			
No	27 (75)	7	20
Yes	9 (25)	9	0
Current immunotherapy, no. (%)			
None	10 (28)	4	6
Monotherapy	15 (42)	3	12
Two or more agents	11 (30)	9	2

* The χ^2 goodness-of-fit test of all OMS subjects showed significant differences in numbers of subjects by age category, syndrome duration category, neurologic severity category, and prior tumor chemotherapy, but not by current treatment category ($p < 0.05$).

† In statistical comparisons between the tumor and no-tumor groups, there were significant differences in numbers of individuals receiving prior chemotherapy ($p = 0.001$, χ^2) and current immunotherapy ($p = 0.007$, χ^2).

‡ Total score indicates the neurologic motor abnormality was mild if 0–12, moderate if 13–24, and severe if 25–36.

§ Chemotherapy was cyclophosphamide given either alone, which was most often the case, or in combination with other agents such as adriamycin or cisplatin, doxorubicin, and etoposide.

|| Treatments included adrenocorticotrophic hormone (H.P. Acthar gel; Questcor, Union City, CA), steroids, or IV immunoglobulins (infused monthly).

OMS = opsoclonus–myoclonus syndrome.

such as plasma cells and macrophages.^{16–20} Expanded lymphocyte subsets in CSF may reflect increased trafficking or proliferation within the CSF.²¹

Flow cytometry, the most reliable method for quantitative analysis of lymphocyte subsets, allows cells to be analyzed simultaneously for expression of multiple immunologic markers.²² We now report a cross-sectional study of CSF leukocyte phenotype, activation, and maturation status in a carefully defined cohort of children with OMS.

Methods. *Subjects.* Thirty-six children with OMS were recruited through the National Pediatric Myoclonus Center, and their parents signed consent for this institutional review board–approved study. The clinical characteristics are shown in the table. A thorough search was made for occult neuroblastoma using neuroimaging and blood and urine tumor markers. Those with a previously identified tumor, which was either stage I or II in all but one case, had undergone tumor resection.

Scoring of neurologic status. Each child was videotaped (see the supplementary material on the *Neurology* Web site; go to www.neurology.org). A trained observer blinded to treatment status rated motor impairment using the OMS Evaluation Scale, which we devised and validated.²³ Items of the 12-part scale were

rated from 0 to 3 as an index of increasing neurologic severity or impairment. Total score, which was calculated as the sum of subscores, tallied 36 in the most extreme cases.

Controls. Control subjects included 18 children with myoclonus, ataxia, idiopathic intracranial hypertension, chronic daily headache, or developmental delay, who were undergoing a diagnostic evaluation. Routine CSF studies such as cell count, protein, and glucose were normal. None of the children ever received immunotherapy. The mean age was 8.5 ± 1.2 years (range 1.3 to 16 years). Seven were boys.

Lumbar puncture. To prevent contamination of CSF with blood due to trauma, minimize sedation risks during lumbar puncture, standardize the degree of stress on immune function, and provide compassionate care, an anesthesiologist administered IV propofol after brief sevoflurane induction to insert the IV line. The lumbar puncture was performed in the lateral decubitus position, and the first 4 mL was sent for routine studies. An additional 8 to 10 mL, not to exceed 15% of the calculated total CSF volume, was collected for flow cytometry. Blood for parallel studies was also drawn.

CSF mononuclear cell count was 0 to 3 cells/mm³ in all children except three with OMS, who had pleocytosis with counts of 5 to 20 cells/mm³. The mean CSF white blood cell count of 1.3 ± 0.2 /mm³ in control subjects was not statistically different from 1.5 ± 0.2 /mm³ in OMS. The CSF red blood cell count was always ≤ 1 , and CSF protein and glucose levels were normal.

Flow cytometry. Cells were recovered from CSF through low-speed centrifugation (200 g, 7 minutes) and concentrated by resuspension in phosphate-buffered saline (PBS; pH 7.2) containing 0.5% bovine serum albumin (Sigma Chemicals, St. Louis, MO). CSF samples (100 μ L/tube) were stained with 15 to 20 μ L of monoclonal antibodies (mAb) directly conjugated with fluorescein isothiocyanate, phycoerythrin, allophycocyanin, or phycoerythrin-cyanine 5 (see table E-1 on the *Neurology* Web site), which were arranged as panels in several assay tubes (see table E-2 on the *Neurology* Web site).²⁴ Samples were incubated for 20 minutes at room temperature. CSF leukocytes were washed and resuspended in 200 μ L of PBS after final incubations.

Whole peripheral blood samples (100 μ L/tube) were stained with the same panel of directly conjugated mAb used for CSF cells. They were further incubated for 10 minutes with 100 μ L/tube Optilyse B (Immunotech, Marseille, France; Beckman-Coulter, Miami, FL). One milliliter of H₂O was subsequently added to each sample and incubated for a final 10 minutes at room temperature.

All samples were acquired and analyzed by flow cytometry on a FACSCalibur cytometer equipped with a 488-nm argon/633-nm HeNe laser (Becton-Dickinson, San Jose, CA). For CSF mononuclear cell count of >3 cells/mm³, 1,000 events were counted; otherwise, 500 were counted. Data acquisition and analysis were performed with CellQuest (Becton-Dickinson). Dependent on the surface marker fluorochrome, data were plotted as log vs log of fluorescence. The relative size of each lymphocyte subset (percentage of positive cells) was expressed as the percentage within the total lymphocyte population or "lympho/monocyte gate," as determined manually by forward and side scatter characteristics.

Quality control was maintained in several ways. The lymphocyte gate was checked by CD14/CD45 double staining and deemed pure if it included $\geq 95\%$ of all lymphocytes and $<5\%$ contamination with granulocytes, monocytes, or cell debris. Appropriately labeled isotopes (IgG₁) were used as internal controls in each analysis. Instrument calibration was monitored daily with Calibrite beads (Becton-Dickinson). A "lymphosum" was calculated as the sum of the percentage for T-cells, B-cells, and NK cells and found to equal 92 to 98%, as it should. For CD3, coefficients of variation were 2.0% (inratch) and 0.5% (interbatch), which fall within acceptable performance parameters.

Statistical analysis. Dependent variables (CSF and blood lymphocytes and the CSF/blood ratio) were analyzed as means by two-tailed *t*-tests or one-way analysis of variance (ANOVA) on the Statistical Analysis System (SAS, Cary, NC), a computer software program for statistical analysis,²⁵ using a significance level of $p < 0.05$. The Duncan Multiple Range Test was utilized for post-hoc subgroup comparisons of all statistically significant results from ANOVA to allow comparisons between OMS categories as well as with controls. The principal independent variables were diagnosis (OMS vs controls), etiology (tumor vs no tumor), neurologic sever-

ity (mild, moderate, severe), syndrome duration (acute, subacute, chronic), and treatment (current treatment vs no treatment, prior chemotherapy vs no chemotherapy). Pearson correlation coefficients were used for correlational analysis. Demographic data were analyzed by χ^2 and χ^2 goodness-of-fit tests. To evaluate unequal variances, we performed nonparametric tests, but as the results were substantially the same as the unequal-variance *t*-tests, only *t*-tests are reported here.

Because lymphocyte populations, in blood at least, may vary with age and there are no CSF data in healthy children, we looked for correlations between age and each type of lymphocyte in our control subjects. No significant correlations were found, so the data were pooled. This had the effect of increasing the mean age of our control subjects somewhat, but we felt it was more important, given the small sample size, to boost the statistical power. The same argument applies to blood, as the relative size of various lymphocyte populations in healthy children ages 2 to 5 years and 5 to 10 years differs by only 1 to 6%.²⁶ Published normative CSF data for healthy young adults²⁴ as well as children with various neurologic disorders¹⁹ were used to assess the comparability of our neurologic controls.

Results. Distribution of lymphocyte population in CSF. In control subjects, the prominent characteristic of normal CSF lymphocytes was the dominance of $\alpha\beta$ T-cells (CD3⁺) and almost total lack of B-cells. The rank order of CSF lymphocytes was helper/inducer T-cells (CD3⁺CD4⁺) > cytotoxic/suppressor T-cells (CD3⁺CD8⁺) > $\gamma\delta$ T-cells (T-cell receptor [TCR]- $\gamma\delta$ ⁺), NK cells (CD3⁻CD16/56⁺), NK-like T-cells (CD3⁺CD16/56⁺) >> B-cells (CD45⁺CD3⁻CD19⁺).

In OMS, abnormalities in the percentage of various cell types were found in all cases, despite the absence of CSF leukocytosis in most (figure 1). Percentages were lower for CD3⁺CD4⁺ cells (-19% ; $p = <0.0001$) and minimally higher for CD3⁺CD8⁺ cells ($+5\%$; $p = 0.047$). As a result, the mean ratio of CD4⁺ to CD8⁺ T-cells was less (-53% ; $p = 0.017$). Percentages were higher for $\gamma\delta$ T-cells (2.7-fold; $p = <0.0001$) and CD19⁺ B-cells (6.5-fold; $p = 0.0004$). There was a trend toward lower CD3⁺ cells ($p = 0.055$) but no intergroup difference in CD3⁻ or CD3⁺ NK cells. The immunophenotype was not changed by presence of tumor or treatment.

Certain cell types were found in neither controls nor OMS. Based on CD45 staining and light scatter characteristics, no plasma cells or macrophages were detected in CSF. Monocytes regularly constituted $<5\%$ within the lympho/monocyte gate (data not shown).

Expression of T-cell activation markers in CSF. We evaluated both human leukocyte antigen (HLA) DR and CD25 (interleukin-2 receptor) T-cell activation markers. HLA-DR⁺ T-cells accounted for approximately 12% of CD3⁺ cells in the CSF of control subjects, whereas few T-cells were CD25⁺ (figure 2). In OMS, the percentage of HLA-DR⁺ T-cells was 51% greater ($p = 0.0006$), as can be seen in representative flow cytometric displays (see figure E-1 on the *Neurology* Web site). The percentage of CD3⁺CD25⁺ cells was not significantly different in OMS. OMS etiology and treatment had no significant effect.

CSF T-cell markers of maturation. In a comparison of CD45R isoforms on CD3⁺ cells from control subjects, the percentage of "memory" (CD45RO⁺) T-cells was about two-fold higher than that of "naive" (CD45RA⁺) T-cells ($p = 0.0004$). However, there were no significant differences between controls and OMS for CD45RO⁺ (47.3 ± 4.4 and 43.5 ± 4.0 , respectively) or CD45RA⁺ (21.7 ± 4.8 and 18.5 ± 1.9) T-cells. OMS etiology and treatment also had no significant effects with the exception of slightly lower

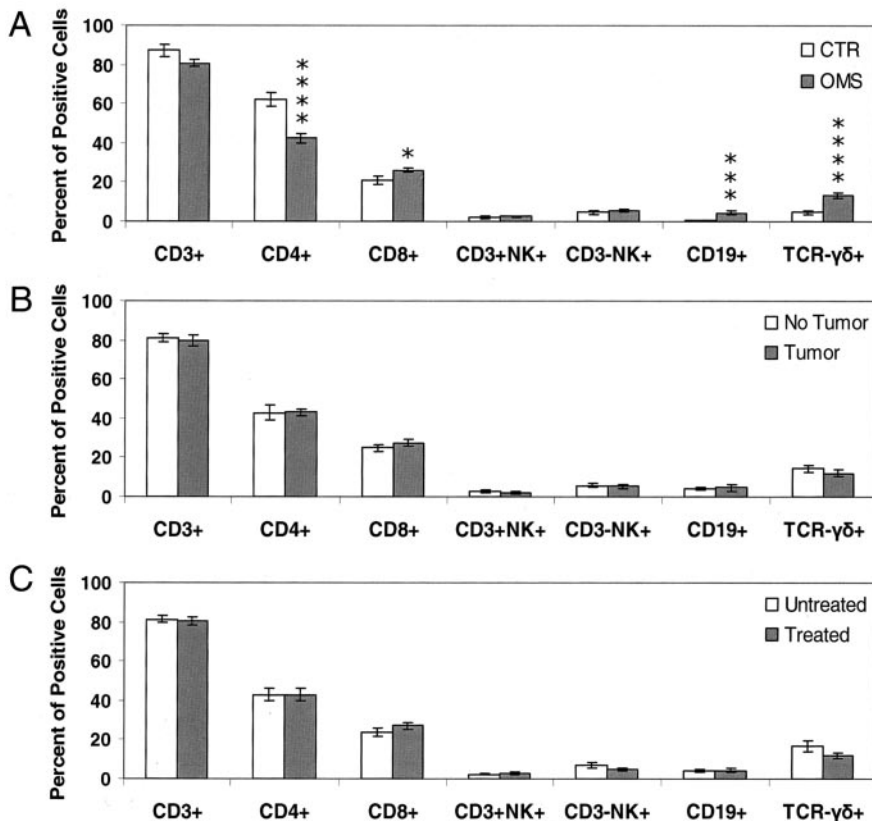


Figure 1. Distribution of CSF lymphocyte population. (A) In opsoclonus-myoclonus syndrome (OMS), the percentage of CD3⁺ cells and CD4⁺ T-cells was reduced compared with controls, but increases were found for CD19⁺ B-cells, $\gamma\delta$ T-cells, and CD8⁺ T-cells. There were no statistically significant differences between tumor and no-tumor groups (B) or untreated and treated groups (C). Data are means \pm SEM. The B-cell percentage for controls was $0.7 \pm 0.2\%$. Asterisks signify statistical significance by *t*-tests: * $0.01 \leq p < 0.05$, *** $0.0001 \leq p < 0.001$, **** $p < 0.0001$.

CD45RA⁺ cells in the no-tumor group (-9% ; $p = 0.019$) (data not shown).

Relation of neurologic severity of OMS to CSF cell types. The effect of neurologic severity on the percentage of CD19⁺ B-cells as well as CD4⁺ and TCR- $\gamma\delta$ ⁺ T-cells was statistically significant (figure 3). Reciprocal relations were seen between $\alpha\beta$ T-cells and $\gamma\delta$ T-cells. The two children with the highest percentage of CSF $\gamma\delta$ T-cells (26%) or B-cells (29%) were most severe (total scores 34 and 36, respectively). Severity correlated negatively with the percentage of CD3⁺ cells ($r = -0.56$, $p = 0.0012$) and CD3⁺CD4⁺ cells ($r = -0.38$, $p = 0.024$). A positive correlation was found with the percentage of CD19⁺ cells ($r = 0.37$, $p = 0.03$), TCR- $\gamma\delta$ ⁺ cells ($r = 0.54$, $p = 0.0019$), and CD3⁻ NK cells ($r = 0.38$, $p = 0.04$). There were no other significant correlations.

Relation of syndrome duration to CSF cell types. The duration of illness correlated with the CD4/CD8 ratio ($r = 0.40$, $p = 0.015$) and the percentage of CD4⁺ T-cells ($r = 0.44$, $p = 0.007$) and CD3⁺ cells ($r = 0.38$, $p = 0.039$). There was a negative correlation with TCR- $\gamma\delta$ ⁺ cells ($r = -0.37$, $p = 0.043$) and total score ($r = -0.45$, $p = 0.008$).

CSF/blood lymphocyte ratios. In control subjects, the percentage of all cells in CSF exceeded that in paired peripheral blood, except for B-cells and CD45RA⁺ T-cells. The magnitude of CSF/blood ratios differed significantly between cell types (figure 4). The percentage of CD3⁺HLA-DR⁺ cells was sevenfold higher in CSF than in blood, whereas the percentage of CSF CD19⁺ B-cells was only one-twentieth of that in blood. The means of other ratios were just above 1.0, with the exception of the CD3⁺ NK cell ratio and CD45RO⁺ cell ratio, which were about threefold higher.

In OMS, the CSF/blood ratio for CD19⁺ B-cells was

threefold higher than in control subjects ($p = 0.0005$). The $\gamma\delta$ T-cell ratio was 2.4-fold higher ($p = 0.0005$) and correlated with total score ($r = 0.61$, $p = 0.0004$), syndrome duration ($r = -0.42$, $p = 0.022$), and the CD4/CD8 ratio ($r = -0.49$, $p = 0.0075$). There was a 2.4-fold higher ratio for CD3⁺NK cells in OMS ($p = 0.04$). Presence of a tumor and treatment had no effect. There were no other significant differences in lymphocyte ratios, with the exception of a lower HLA-DR⁺ cell ratio in chemotherapy-treated children (-57% ; $p = 0.0019$).

The relative size of the peripheral blood mononuclear cell pool did not reflect the lymphocyte subset abnormalities found in CSF. There were no significant differences between control and OMS subjects. In the tumor group, approximately 1.5-fold higher percentages of CD19⁺ B-cells ($p = 0.038$) and HLA-DR⁺ T-cells ($p = 0.048$) were found (data not shown).

Comparability of pediatric neurologic control subjects and healthy young adults. No major discrepancies were found between our control subjects and data from healthy young adults²⁴ in the general distribution and relative proportions of CSF lymphocyte subsets. Our controls had a slightly lower percentage of CD3⁺ cells, CD4⁺ T-cells, and NK-like T-cells and a slightly higher percentage of NK cells and HLA-DR⁺ T-cells. Although we could find no study of CSF $\gamma\delta$ T-cells in normal individuals, the percentage of $\gamma\delta$ T-cells in our controls was comparable with that of a group of pediatric neurologic control subjects.¹⁹

Discussion. To our knowledge, this is the first immunophenotyping study of CSF lymphocytes in OMS. We found multiple immunologic abnormalities involving B-cells and T-cells, as is the case with mul-

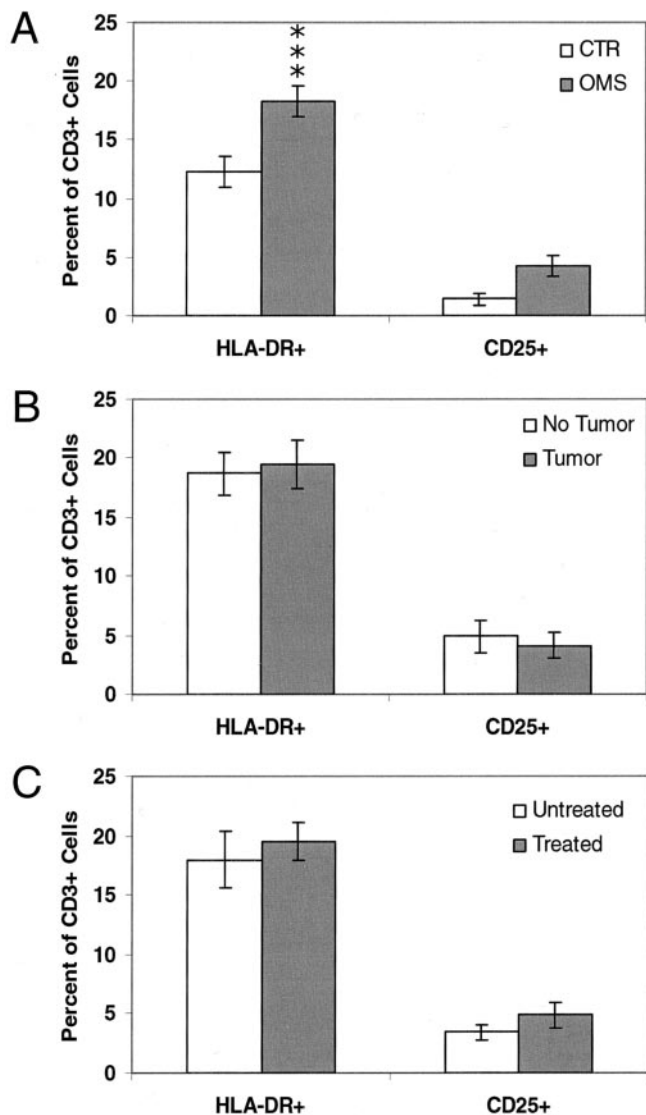


Figure 2. CSF CD3⁺ T-cell activation markers in control subjects vs opsoclonus–myoclonus syndrome (OMS) (A), tumor vs no tumor (B), and untreated vs treated OMS (C). Data are means \pm SEM. Asterisks signify statistical significance by t-tests: ***0.0001 \leq p < 0.001. In OMS, 21 of 36 children had values above the upper confidence limit of the control group for human leukocyte antigen DR⁺ T-cells (up to 34%) but only 11 children for CD3⁺CD25⁺ cells (up to 25%).

multiple sclerosis and a growing number of human autoimmune diseases. Both lymphocyte populations infiltrate neuroblastomas to a high degree²⁷ and may provide a link between peripheral indication of autoimmunity and CNS immunopathology. Because both B-cell and T-cell abnormalities were linked to neurologic dysfunction, they could account for relapses and disease progression. Although the pattern of abnormalities may suggest immune dysregulation, this is a descriptive observation; prospective longitudinal studies, which are in progress at our center, will be required. Long-term persistence of CSF abnormalities despite tumor resection, multiagent cancer che-

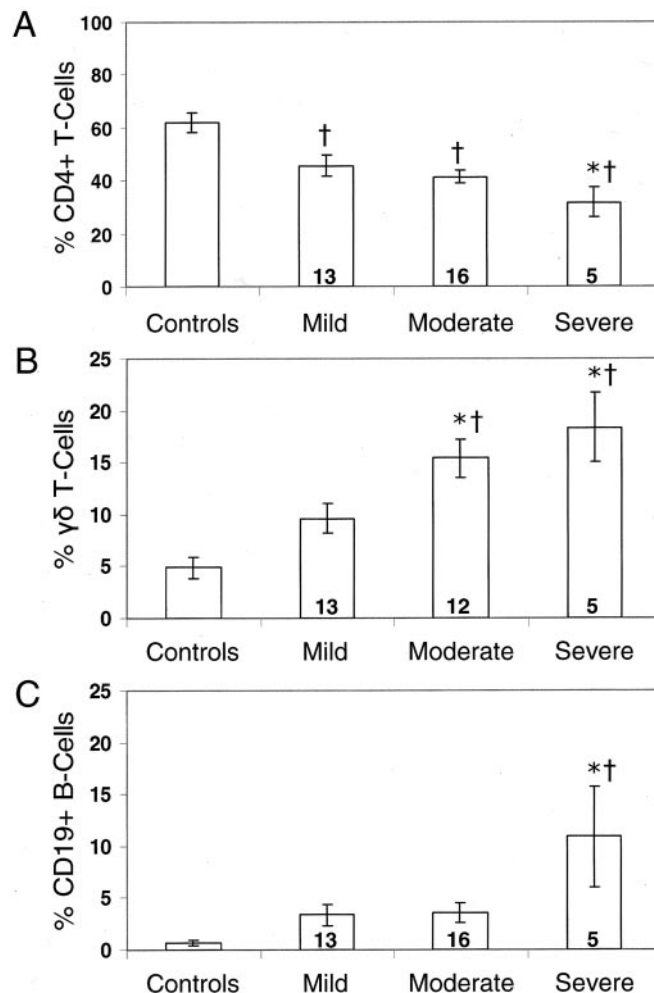


Figure 3. Relation between neurologic severity (total score) in opsoclonus–myoclonus syndrome (OMS) and percentage of CSF CD4⁺ T-cells (A), $\gamma\delta$ T-cells (B), and CD19⁺ B-cells (C). Sample size is shown at the base of each column. Data are means \pm SEM. Asterisks indicate statistically significant differences between OMS severity categories on Duncan test, p < 0.05. Dagger indicates significant differences between OMS and control subjects. Analysis of variance with linear trend analysis revealed that the more severely affected children (higher total score) had a lower percentage of CSF CD4⁺ T-cells (F = 20.6, p \leq 0.0001). In contrast, the percentage of CSF $\gamma\delta$ T-cells increased with severity (F = 25.6, p \leq 0.0001), being nearly double in the severe category, and the percentage of CSF CD19⁺ B-cells was also higher (F = 21.2, p \leq 0.0001).

motherapy, or prior treatment with conventional immunotherapies emphasizes the need for more effective therapies in OMS. The absence of a detectable tumor had little impact on the immunologic abnormalities in CSF, which, taken together with neuroblastoma's high incidence of spontaneous regression,²⁸ emphasizes the need for very careful tumor screening. However, some of our patients, even a substantial percentage, may not have had a tumor.

Our data suggest that one component of OMS may be B-cell mediated. The intrathecal expansion or in-

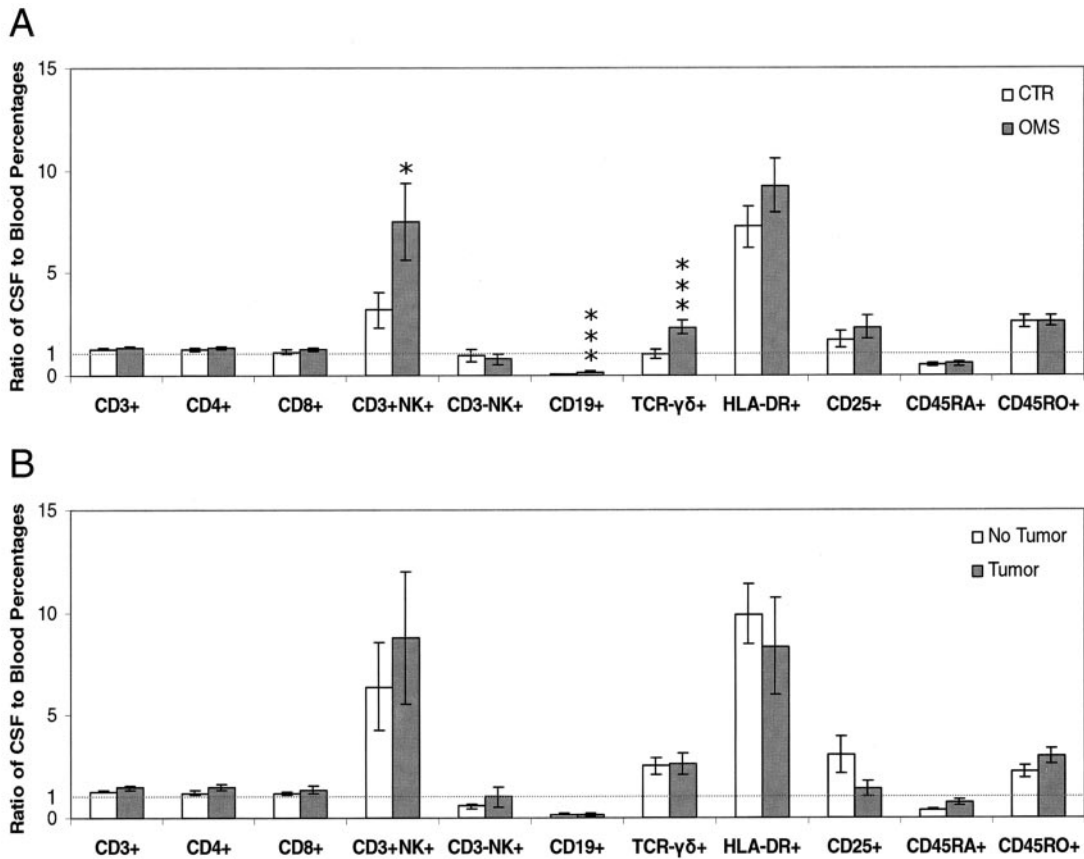


Figure 4. CSF/blood lymphocyte ratios. (A) In opsoclonus–myoclonus syndrome (OMS), the ratios for $\gamma\delta$ T-cells, CD19⁺ B-cells, and CD3⁺CD16/56⁺ cells were significantly increased. The B-cell ratios were 0.05 ± 0.01 for controls and 0.16 ± 0.03 for OMS. (B) There were no statistically significant differences between the tumor and no-tumor groups. Data are means \pm SEM. Asterisks signify statistical significance by t-tests: * $0.01 \leq p < 0.05$, *** $0.0001 \leq p < 0.001$.

crease in relative size of the B-cell pool is clinically important because B-cells usually are negligible in normal CSF and the high percentage of CSF B-cells in severe OMS indicates pronounced recruitment to the CNS. B-Cell expansion implies potential for autoantibody production, as B-cells are capable of synthesizing antibodies even if they are not plasma cells, and autoantibodies have been found in some children with OMS.^{6,12,13} However, autoreactive B-cells also may contribute to autoimmune disease merely by enhancing antigen presentation to T-cells. Studies of various inflammatory CNS disorders have revealed that CSF B-cells may be a particularly reactive population compared with peripheral blood,²⁹ preferentially compartmentalized to the CSF and lacking systemic feedback control.³⁰ The next step will be to determine if CSF B-cells in OMS are highly activated and lead to a humoral response that is pathogenic.

The $\gamma\delta$ T-cell is regarded as an “unconventional” T-cell, one with a unique role in immunologic disease. Also called “fetal-type lymphocytes,” $\gamma\delta$ T-cells are the first T-cells to develop and a possible third arm of immune response.³¹ Because they possess inherent autoreactivity, $\gamma\delta$ T-cells may recognize antigens directly in tissues rather than relying on the

professional antigen-presenting cells for antigen recognition required of conventional T-cells.³¹

CSF expansion of $\gamma\delta$ T-cells is abnormal; the TCR of most CSF T-cells in normal individuals expresses α - and β -chains, rather than γ - and δ -chains.³² However, our data do not allow us to determine if the expansion in OMS is primary or secondary or whether it is deleterious or helpful. The $\gamma\delta$ T-cells could be involved in the primary immune response against the tumor,³³ triggering a cascade of immunologic events that lead to inflammation once they have entered the brain. Alternatively, they may be drawn only secondarily to CNS inflammation already involving other T-cells and B-cells.³⁴ In contrast, $\gamma\delta$ T-cells could fulfill an anti-inflammatory role in OMS by down-regulating autoimmune disease,³⁵ because they normally do not function as helper cells³⁵ and they suppress B-cell expansion in vivo.³¹ Whatever role $\gamma\delta$ T-cells do have in OMS, it is likely to be important.

The principal abnormalities involving “conventional” T-cells in OMS were increased T-cell activation, greatly reduced helper/inducer T-cells, and slightly increased suppressor/cytotoxic T-cells. T-Cell activation, an important factor in the development of autoimmune disease, is required for optimal antigen

presentation by B-cells to T-cells. An increased percentage of activated T-cells in the CSF is evidence of significant T-cell recruitment in OMS.

In a study of three adults with paraneoplastic cerebellar degeneration (PCD), 20 to 40% of CSF cells were activated conventional T-cells,³⁶ which is above the normal range²⁴ and similar to what we found in OMS. A specific inhibitor of activated T-cells markedly reduced these cells in CSF.³⁶ In PCD, antigen-specific cytotoxic T-cells also have been found in peripheral blood.^{37,38} Although PCD and OMS are different disorders clinically, increased T-cell activation is a feature of both, and there may be other shared immunologic traits as well.

The explanation for the reduced percentage of CSF helper/inducer T-cells we found in OMS is unclear. Although circulating blood helper T-cells and the helper/suppressor T-cell ratio are reduced in epilepsy³⁹ and a subset of autism,⁴⁰ we are not aware of CSF helper T-cell reduction in other neurologic disorders. Because of the importance of helper T-cell subsets in autoimmune diseases, the next stage will be to evaluate Th1- and Th2-helper T-cell subsets in OMS.

We propose that CSF B-cell and T-cell abnormalities be factored into the design and monitoring of immunotherapy for OMS. We realize it is hazardous to draw conclusions about functional properties of immune cells from their phenotypic markers alone. However, given the cytotoxic capacity of these cells, their potential role in the pathophysiology of pediatric OMS warrants serious attention. Our study shows that some children who manifest neurologic abnormalities years after presentation appear to have an active autoimmune process and are potentially salvageable. CSF lymphocyte immunophenotyping allows this subgroup to be identified for further treatment.

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