

## CSF B-Cell Expansion in Opsoclonus–Myoclonus Syndrome: A Biomarker of Disease Activity

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**Abstract:** Lack of a biomarker of disease activity has hindered the therapy of childhood opsoclonus-myoclonus syndrome (OMS), which is purported to be mediated humorally. To determine if the cerebrospinal fluid (CSF) B lymphocyte, which may traffic into the central nervous system (CNS) to produce antibody locally, is one such biomarker, B lymphocytes were immunophenotyped in the CSF and blood of 56 children with OMS and 26 pediatric controls by dual-laser flow cytometry. Neurological severity was rated blindly from videotapes using a validated 12-item motor evaluation scale. Children with OMS manifested a 4- to 7-fold higher percentage of total B-cells in CSF ( $P < 0.0001$ ), including CD5<sup>+</sup> ( $P = 0.001$ ) and CD5<sup>-</sup> ( $P = 0.0004$ ) B-cell subsets, compared with controls, in whom the percentages were negligible and unchanging with age. CSF expansion of both B-cell subsets increased with disease severity and decreased

with disease duration ( $P \leq 0.0001$ , ANOVA). Previous treatment with conventional immunotherapies, chemotherapy, or tumor resection had not normalized B-cell percentages in those with lingering symptoms. These studies reveal that CSF B-cell expansion in OMS is characteristic and often persistent. Presence of the autoreactive CD5<sup>+</sup> B-cell subset and correlations with neurological severity and disease duration suggest CSF B-cell expansion is a biomarker of disease activity and possible target for B-cell-specific therapy. Immunophenotyping of CSF lymphocytes by flow cytometry yields valuable clinical information missed by routine studies and allows crucial treatment decisions to be made rapidly. © 2004 Movement Disorder Society

**Key words:** paraneoplastic syndrome; Kinsbourne syndrome; B lymphocytes; B-cell trafficking; neuroblastoma; myoclonic disorders

Opsoclonus-myoclonus syndrome (OMS) is a striking and unique movement disorder that appears across the age spectrum.<sup>1</sup> In childhood OMS, also known as Kinsbourne syndrome,<sup>2</sup> an occult neuroblastoma, the only commonly associated tumor,<sup>3</sup> can be found in up to one-half of the cases.<sup>1</sup> Although tumor survival is improved greatly compared with neuroblastoma without OMS, the paraneoplastic syndrome causes significant neurological and developmental morbidity, such as

ataxia, mental retardation, and behavioral problems.<sup>1</sup> Evidence suggests an autoimmune basis for OMS, but much information about disease pathophysiology is lacking, and treatment has been hampered by lack of a biomarker of disease activity.

According to the humoral hypothesis, onconeural antigens shared by the brain and embryologically similar neural crest-derived tumors trigger autoantibody production.<sup>4</sup> Antibodies to unidentified antigens are found on Western blots<sup>5</sup>; however, commercially available testing for known autoantibodies in pediatric OMS is seldom positive.<sup>6</sup> No unique autoantibody (one not found in unrelated disorders) has been identified as a marker,<sup>7</sup> and there is no animal model to establish a causal relation between a putative candidate antibody and neurological dysfunction. Even if the autoantibody were known, con-

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ventional immunotherapies and plasmapheresis have limited capacity to remove antibodies in the central nervous system (CNS),<sup>4</sup> and plasmapheresis is risky in toddlers, the age group predisposed to develop OMS.<sup>1</sup>

For all of these reasons, we decided to shift focus from the antibody to the “smoking gun” and source of antibody production, the B lymphocyte, looking for direct evidence of intrathecal B-cell expansion. Under normal circumstances, cerebrospinal fluid (CSF) is nearly devoid of B-cells in adults.<sup>8</sup> B-cell trafficking<sup>9</sup> and proliferation within the CSF compartment<sup>10</sup> have been found in some inflammatory CNS diseases, such as multiple sclerosis,<sup>11</sup> but have not been reported previously in OMS. Finding B-cell expansion and linking it to neurological severity would help substantiate the autoimmune basis of the disorder and provide a possible therapeutic target for innovative treatment.

We report on the phenotyping of B lymphocyte subsets in CSF and blood from 56 children with OMS using flow cytometry, a rapid and reliable method for quantitative analysis of lymphocyte subsets.<sup>12</sup> We evaluated two different B-cell markers: CD19, a pan-B-cell marker, and CD5, for immature B cells or the fetal subset.<sup>13</sup> CD19 is a pivotal B-cell receptor signaling effector<sup>14</sup> and CD5 is associated with the B-cell antigen receptor complex.<sup>15</sup> A new clinical tool for assessment of disease severity in OMS was used to correlate immunologic and neurological parameters.<sup>16</sup>

## SUBJECTS AND METHODS

### Subjects

In total, 56 children with OMS (23 boys, 33 girls; mean age  $\pm$  SD,  $4.0 \pm 3.4$  years; age range, 0.92–17 years) were recruited through the National Pediatric Myoclonus Center and their parents signed consent for this Institutional Review Board-approved study. There was no significant age difference between tumor or treatment subgroups. Besides a complete neurological assessment, a thorough search was made for occult neuroblastoma using neuroimaging as well as blood and urine tumor markers. Each of 25 children with a previously identified tumor had undergone tumor resection, and 14 children, one without a tumor, had received chemotherapy. Forty-one children were already receiving one or more immunotherapies, including ACTH (corticotropin), intravenous immunoglobulins (IVIG), or steroids (prednisone, methylprednisolone, dexamethasone). Fifteen were untreated.

Controls were 26 children (11 boys, 15 girls; mean age  $\pm$  SD,  $7.8 \pm 4.5$  years; age range, 1.3–16 years) with chronic daily headaches, seizures, ataxia, or myoclonus,

who were not known to have an autoimmune or neoplastic disorder and who had never received immunotherapy or chemotherapy.

### Lumbar Puncture

Lumbar puncture was carried out in the lateral decubitus position. 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 to provide compassionate care, an anesthesiologist administered intravenous propofol after brief sevoflurane anesthesia to insert the IV line.<sup>17</sup> The first 4 ml were sent for routine studies, which were typically normal. The mean white blood cell (WBC) count with SD was  $1.0 \pm 1.1$  cells/mm<sup>3</sup> for controls, and  $2.3 \pm 4.6$  for OMS (tallying a cell count of 0 to 1 as 1). There also was no significant difference in the percentage of lymphocytes or monocytes in OMS compared with controls. In 5 OMS patients, CSF WBC count was  $>4$  (range, 5–26). The subsequent 8 to 10 ml were collected for flow cytometry and blood was drawn for parallel studies.

### Scoring of Neurological Status

Each child was videotaped. A trained observer, who was blinded to treatment status, rated motor impairment from videotapes using the Opsoclonus-Myoclonus Evaluation Scale (Table 1), which we devised and validated.<sup>16</sup> Items of the 12-item scale were rated from 0 to 3 as an index of increasing neurological severity or dysfunction. Total score was calculated as the sum of subscores, with a score of 36 indicating maximum abnormality.

**TABLE 1.** *Opsoclonus-myoclonus evaluation scale of motor performance*

Item no.	Description
1	Walking: side-to-side imbalance
2	Walking: front-to-back imbalance
3	Walking: wide base
4	Instability while standing (feet apart)
5	Difficulty achieving standing position
6	Truncal instability while sitting
7	Targeting difficulty
8	Difficulty grasping with one hand
9	Difficulty with pincer grasp
10	Abnormal eye movements while tracking (fixation)
11	Abnormal eye movements while resting
12	Speech abnormality (dysarthria)

Videotapes were made to include a segment of each item needed for scoring. Each item is scored from 0 to 3 as follows: 0, normal; 1, mild; 2, moderate; 3, severe.

### Flow Cytometry

Cells were recovered from CSF through low-speed centrifugation (200g, 7 minutes) and resuspended in phosphate-buffered saline (PBS; pH 7.2) containing 0.5% bovine serum albumin (BSA; Sigma, St. Louis, MO). CSF samples (100  $\mu$ l/tube) were stained with directly conjugated antibodies (monoclonal antibodies [mAbs]),<sup>8</sup> including anti-CD5 (Immunotech, Marseille, France), anti-CD19 (Beckman-Coulter, Miami, FL), anti-CD14 (Beckman-Coulter), anti-CD45 (Beckman-Coulter), and anti-IgG<sub>1</sub> isotypes, which were labeled with fluorescein isothiocyanate (FITC), phycoerythrin (PE), allophycocyanin (APC), or PC5 (Beckman-Coulter). The tube configuration was 5/14/19/45. Samples were incubated for 20 minutes at room temperature, and cells were washed and resuspended in 200  $\mu$ l PBS after final incubations.

Whole peripheral blood samples (100  $\mu$ l/tube) were stained with the same panel of directly conjugated mAbs and incubated for 10 minutes with 100  $\mu$ l/tube Optilyse B (Immunotech). A 1-ml aliquot of H<sub>2</sub>O was added to each sample, which was incubated for 10 minutes.

Double staining with CD45, a pan-leukocyte marker, and CD14, a monocyte marker, was used to check the lympho-monocyte gate for purity. The anti-CD5 marker was added to the antibody panel partway through the study and included in the analysis of all subsequent samples.

All samples were acquired and analyzed by flow cytometry on a fluorescence-activated cell sorter (FACS)/Calibur cytometer equipped with a 488-nm argon/633-nm HeNe laser (Becton-Dickinson, San Jose, CA). For the 5 children with CSF mononuclear cell count of >4 cells/mm<sup>3</sup>, 1,000 events were counted; otherwise, 500 were counted. Data were acquired and analyzed with CellQuest (Becton-Dickinson) and plotted as log versus log fluorescence (Fig. 1). Quality was controlled by daily standardization with Becton-Dickinson Calibrite 4 color

beads and checking linearity with Sperotech rainbow particles (Sperotech Inc., Libertyville, IL). As to the performance characteristics for the CSF FACS, the intrabatch coefficient of variation (CV) for CD19 was 0.8% and the interbatch CV was 1.2%, both well within acceptable parameters.

The percentage of lymphocytes refers to the percentage of cells positive within the gate defined for that specific combination of antibodies. The percentage of CSF B-cells that co-express CD5 was calculated using the following equation:

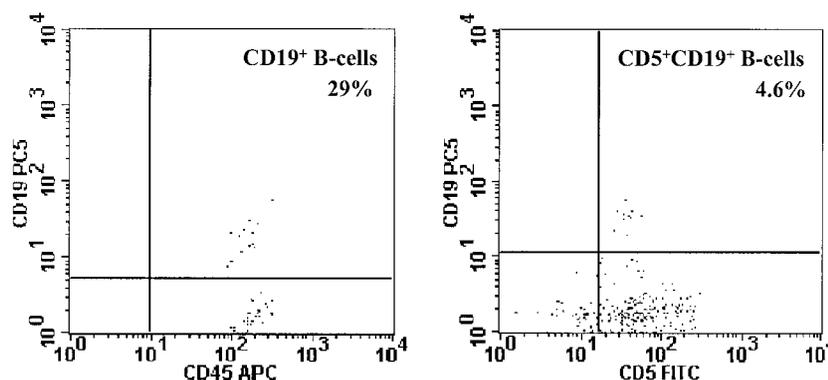
CD5<sup>+</sup> B-cell % of total B-cells

$$= \frac{(\% \text{ CD5}^+ \text{CD19}^+ \text{ cells of all lymphocytes})}{(\% \text{ CD5}^+ \text{CD19}^+ \text{ cells} + \% \text{ CD5}^- \text{CD19}^+ \text{ cells of all lymphocytes})}$$

### Statistical Analysis

Because our controls were older than were the children with OMS, we needed to ascertain whether the percentage of CSF B-cells normally changes with age. Controls were divided at the median age (7.2 years) into two groups: a younger group (n = 13), which was age-equivalent with the OMS group and not statistically different, and an older group (n = 13). In the younger group, the mean age with SD was 3.9  $\pm$  1.8 years (age range, 1.3–7.2), and in the older group it was 11.7  $\pm$  2.6 years (age range 7.3–16). The mean percentage of CD19<sup>+</sup> B-cells was 1.0  $\pm$  1.1 in the younger group and 0.60  $\pm$  0.91 in the older group. For CD5<sup>+</sup> B-cells, the percentages were 0.44  $\pm$  0.76 and 0.06  $\pm$  0.18, respectively. Because there was no statistically significant difference between groups, the controls were pooled.

In contrast, blood lymphocyte populations do change throughout childhood.<sup>18</sup> The percentage of blood CD19<sup>+</sup> B-cells is higher in children aged 1 to 6 years (mean 24%) than 7 to 17 years (mean 16%).<sup>18</sup> We therefore



**FIG. 1.** Flow cytometric displays for CSF B-cells in OMS from children with the greatest B-cell expansion. The dot-plot data represent two-color immunofluorescence analysis of CD19 (left) and CD5 (right) expression on B lymphocytes. CD19<sup>+</sup>CD45<sup>+</sup> and CD5<sup>+</sup>CD19<sup>+</sup> cells appear in the right upper quadrant of their respective figures. CD5<sup>-</sup>CD19<sup>+</sup> cells would be found in the left upper quadrant of the right figure but are not present in that patient.

used these age groupings in analyzing our own blood lymphocyte data.

Percentages were analyzed statistically by *t* tests using the statistical analysis system (SAS; SAS Institute, Cary, NC), a computer software program for statistical analysis.<sup>19</sup> Analysis of variance (ANOVA) with trend analysis was used to test for a statistical effect of multiple levels of neurological severity and syndrome duration on the percentage of B-cell subsets, and post-hoc comparisons of means were made by Duncan's Multiple Range Test. In other analyses, numbers of subjects were compared by  $\chi^2$  or Fisher's exact test. Pearson correlation coefficients were used for statistical correlations. The data are reported as means  $\pm$  SD.

**RESULTS**

**Total CSF CD19<sup>+</sup> B-Cells**

The mean percentage of B-cells positive for CD19 was 5.1-fold higher in OMS than in controls (*P* < 0.0001). It did not differ statistically between any subgroups of OMS, such as those created by etiology, chemotherapy, or treatment with conventional immunotherapies (data not shown). Significantly more children with OMS than controls exhibited a CSF B-cell percentage 2 standard deviations above the control mean (Table 2).

The relation between neurological severity and CSF B-cell expansion was statistically significant and showed a linear trend (*F*<sub>3,78</sub> = 33.0; *P* < 0.0001, ANOVA). In the severe subgroup of OMS (*n* = 10), the percentage of CSF CD19<sup>+</sup> B-cells was highest, 11-fold higher than that in controls. This relation was also seen in statistical correlations (Table 3).

There was also a significant relation between disease duration and CSF B-cell expansion, including a quadratic

**TABLE 2.** Numbers of subjects with elevated CSF B-cell percentages

Cell type	Positive/total subjects	Percent of subjects (%)	<i>P</i>
CD19 <sup>+</sup> B-cells			
Controls	1/26	4	<0.0001 <sup>a</sup>
OMS	27/56	48	
CD5 <sup>+</sup> B-cell subset			
Controls	1/18	5	0.056
OMS	10/36	28	
CD5 <sup>-</sup> B-cell subset			
Controls	1/18	5	0.016 <sup>a</sup>
OMS	13/36	36	

Two standard deviations above the control mean were used for comparisons: 2.84% for CD19<sup>+</sup> B-cells, 1.30% for CD5<sup>+</sup> B-cells, and 1.89% for CD5<sup>-</sup> B-cells.

<sup>a</sup>Statistically significant by  $\chi^2$  test. Results of Fisher's exact test were similar. CSF, cerebrospinal fluid; OMS, opsoclonus-myoclonus.

**TABLE 3.** Statistical correlations of percent CSF CD19<sup>+</sup> B-cells with clinical variables in OMS

Variable	<i>r</i>	<i>P</i>
Total score (severity)	0.36	0.0069 <sup>a</sup>
Onset age	0.42	0.0011 <sup>a</sup>
Syndrome duration category	-0.32	0.017
Gender	0.15	0.26
Syndrome cause	-0.04	0.79
CSF WBC count	0.07	0.65
Current treatment	0.06	0.66
Prior chemotherapy	-0.12	0.38

Correlation coefficient *r* indicates Pearson's correlation.

<sup>a</sup>Correlations with *P* values < 0.05 are statistically significant.

CSF, cerebrospinal fluid; OMS, opsoclonus-myoclonus; WBC, white blood cell.

trend (*F*<sub>3,78</sub> = 18.5; *P* ≤ 0.0001, ANOVA). The percentage of CSF CD19<sup>+</sup> B-cells was highest during the acute phase of OMS (*n* = 13), 7.9-fold higher than that in controls. By the chronic phase (*n* = 26), it was not significantly different from control values.

**CSF CD5<sup>+</sup> B-Cell Subset**

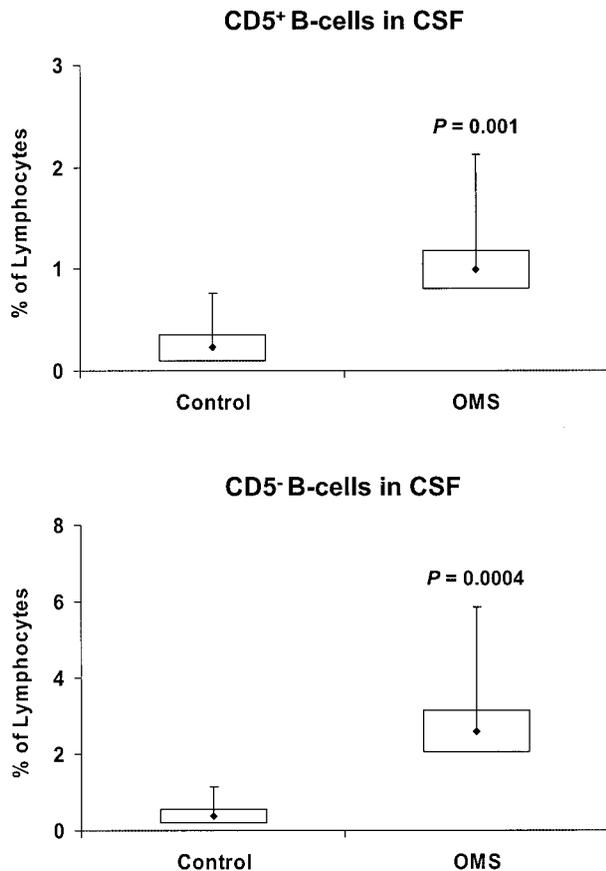
CD19<sup>+</sup>CD5<sup>+</sup> cells, or CD5<sup>+</sup> B-cells, represented a 4.3-fold higher percentage of all CSF lymphocytes in OMS (Fig. 2), reaching 4.6% of phenotyped cells (*P* = 0.0014). Prior neuroblastoma, cancer chemotherapy, or treatment with ACTH, steroids, or IVIG had no significant effect (data not shown). In OMS, 28.0  $\pm$  31.2% of all B-cells were CD5<sup>+</sup>, twofold higher than that in controls, but the difference was not statistically significant. CD5<sup>+</sup> B-cells did not correlate with CD19<sup>+</sup> B-cells. In a few children with OMS, all B-cells were CD5<sup>+</sup>, which was also the case in a few controls.

The relation between CD5<sup>+</sup> B-cell expansion and neurological severity (Fig. 3) was statistically significant and showed a linear trend (*F*<sub>3,50</sub> = 14.7; *P* = 0.0004, ANOVA). The highest percentage was in the severe subgroup, which was eightfold above control values. There was also a significant relation with disease duration, including a quadratic trend (*F*<sub>3,50</sub> = 21.0; *P* ≤ 0.0001) (Fig. 4). The peak expansion (7.3-fold) occurred during the acute phase of illness.

Unlike in controls, the effect of age on the percentage of CD5<sup>+</sup> B-cells in CSF was significant in OMS (*F*<sub>4,49</sub> = 4.31; *P* = 0.004, ANOVA), with toddlers having the highest values. This was due to the fact that total score also was significantly higher at this same time (data not shown), which is the typical age of onset.

**CSF CD5<sup>-</sup> B-Cell Subset**

Most CD19<sup>+</sup> B-cells in controls and in OMS were CD5<sup>-</sup>. CD5<sup>-</sup> B-cells constituted a 6.6-fold higher per-



**FIG. 2.** Box-and-whisker graphs of the percentage of CD5<sup>+</sup> (upper figure) and CD5<sup>-</sup> (lower figure) B-cell subsets in CSF. The mean is shown as the central dot, the standard error as the box, and the standard deviation above the mean as the bar. Asterisk indicates statistical significance by *t* tests. B-cell expansion in OMS was statistically significant compared with controls.

centage of all CSF lymphocytes in OMS than in controls ( $P = 0.0004$ ). Significantly more children with OMS had a percentage of CSF CD5<sup>-</sup> B-cells  $>2$  SD above the control mean (Table 2). CD5<sup>-</sup> B-cells were  $66.4 \pm 34.5\%$  of all B-cells, which was not significantly different from that in controls. There were no significant tumor or treatment effects (data not shown). CD5<sup>-</sup> B-cells correlated with CD19<sup>+</sup> B-cells ( $r = 0.71$ ,  $P < 0.0001$ ).

There was a significant relation between CD5<sup>-</sup> B-cell expansion and neurological severity (Fig. 3), which showed a linear trend ( $F_{3,50} = 14.2$ ,  $P = 0.0004$ , ANOVA). The severe group manifested the highest percentage, which was 13.5-fold above controls. The relation to disease duration (Fig. 4) was also significant and quadratic ( $F_{3,50} = 12.96$ ,  $P = 0.0007$ ).

#### CSF/Blood B-Cell Ratios

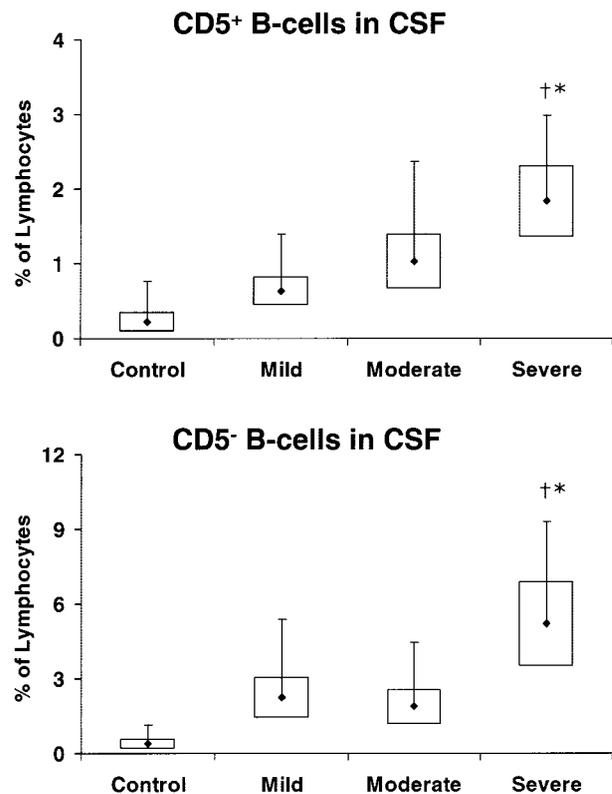
The CSF/blood ratios for CD19<sup>+</sup> B-cells and both B-cell subsets were significantly higher in OMS than

controls (Table 4). There were no significant differences in ratios between no-tumor and tumor groups (data not shown).

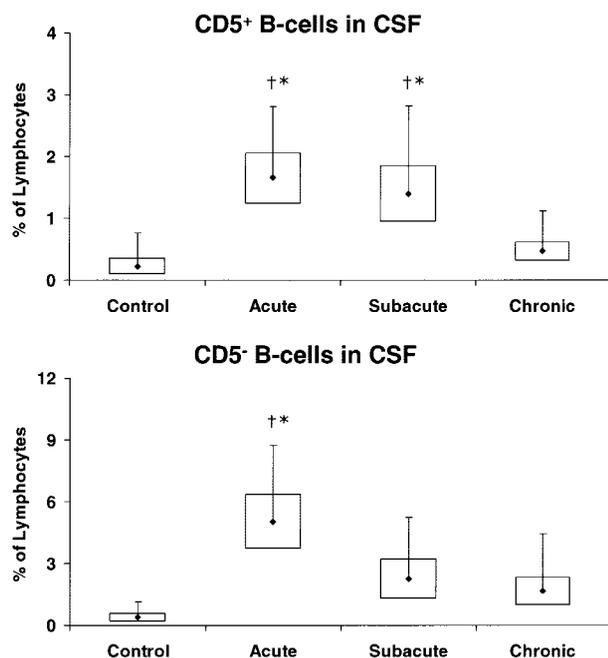
Disease severity had a significant effect on the CSF/blood ratio of CD19<sup>+</sup> B-cells ( $F_{3,73} = 7.7$ ;  $P = 0.0001$ ). The duration of illness, too, increased the ratios for CD19<sup>+</sup> B-cells ( $F_{3,73} = 5.5$ ;  $P = 0.0018$ ) and CD5<sup>+</sup> B-cells ( $F_{3,35} = 3.2$ ;  $P = 0.036$ ) ( $P < 0.05$ , Duncan's test). For CD19<sup>+</sup> B-cells, the ratio was highest in the severe group (5.7-fold) and in the acute group (4.9-fold) ( $P < 0.05$ , Duncan's test). The graphed data closely resembled those for CSF B-cell percentages and are not shown.

#### Peripheral Blood B-Cells

The percentage of blood lymphocytes comprised by CD19<sup>+</sup> B-cells and CD5<sup>+</sup> or CD5<sup>-</sup> B-cells was not different in OMS and controls (data not shown). CD5<sup>+</sup> B-cells constituted  $12.6 \pm 23.4\%$  of all peripheral blood



**FIG. 3.** Relation of neurological severity to percentage of CD5<sup>+</sup> (upper figure) and CD5<sup>-</sup> (lower figure) B-cell subsets in CSF. As an aid to clinical interpretation, neurological severity was defined as mild if the videotape total score was  $\leq 12$ , moderate if 13–24, and severe if 25–36. Controls are shown for comparison. Asterisk indicates significant difference among severity categories,  $P < 0.05$  by Duncan test. Cross signifies significant difference compared with controls. From left to right,  $n = 18, 16, 14,$  and  $6$ .



**FIG. 4.** Relation of syndrome duration to the percentage of CSF B-cell subsets. The syndrome duration category was defined as acute if  $\leq 3$  months, subacute if 3–12 months, and chronic if  $> 1$  year. Controls are shown for comparison. Asterisk indicates significant difference among duration categories,  $P < 0.05$  by Duncan test. Cross signifies significant difference compared with controls. From left to right,  $n = 18, 8, 10,$  and  $18$ . As a point of comparison, neurological severity (total score) also varied with the duration of the illness ( $F_{2,51} = 4.6; P = 0.014$ , ANOVA). Scores in the chronic phase ( $11.8 \pm 8.8$ ) were about one-half of those in the acute and subacute phases ( $P < 0.05$ , Duncan's test).

B-cells in OMS, which was not significantly different from that in controls.

**DISCUSSION**

**CSF B-Cells in OMS**

Our study implicates B-cell involvement in the pathophysiology of pediatric OMS. CSF expansion of B-cells, which are usually sparse in the CSF of healthy young individuals,<sup>8</sup> indicates significant B-cell recruitment to the CNS and the potential for autoantibody production.<sup>20,21</sup> Although B-cell abnormalities are not the only CSF lymphocyte derangements in OMS,<sup>22</sup> as is often the case in human autoimmune disorders,<sup>23</sup> the positive correlation with neurological severity supports their pathogenicity. The negative correlation we found between B-cell percentage and disease duration, which also has been found in multiple sclerosis,<sup>24</sup> suggests an early role for B-cells in the autoimmune process. Lack of change in relative size of the peripheral blood B-cell pool emphasizes the importance of CSF studies.

These findings contribute to growing evidence that pediatric OMS is associated with long-term immunological abnormalities. B-cell expansion was found in some children who had received multiple chemotherapeutic agents for their tumors and treatment with steroids, ACTH, or IVIG. The persistence of CSF B-cell expansion in children who did not achieve a neurological remission and had lingering neurological symptoms suggests a role for humoral immunity in the mechanism of relapse and neurodevelopmental morbidity. It also supports our hypothesis that failure to restore the normal distribution of the CSF lymphocyte population early in the course of the illness may result in neurological sequelae. Using CSF lymphocyte immunophenotyping, this hypothesis can be tested.

**CD5+ and CD5- B-Cells in Autoimmune Disease**

In humans, B-cells comprise at least two main subpopulations with different origins and anatomic distributions: B-1 and B-2. B-1 cells encompass the CD5+ subset, whereas B-2 cells constitute the "conventional" B-cell population.<sup>25</sup> B-1 cells are mainly T-cell independent, although they are influenced by T-cell-derived cytokines.<sup>26</sup> B-2 cells, which emanate from the bone marrow, migrate to secondary lymphoid organs and react to T-cell-dependent foreign antigens.

Two CD5+ B-cell subgroups are purported to exist, one with native expression of CD5 (B-1a or fetal B cells), the other induced in disease states to express CD5 (B-1b or B-2 cells). CD5+ B lymphocytes account for the majority of B-cells in the perinatal period, but 70 to 90% are replaced gradually by conventional (CD5-) B-cells by adulthood.<sup>25</sup> Highly relevant to neuroblastoma, which is usually thoracic or abdominal, is the observation that most B-cells in the human pleural and peritoneal cavity are CD5+, having a greater distribution there than in the circulation.<sup>27,28</sup>

Although CD5 is a pan-T-cell marker, its expression on B-cells is associated commonly with production of

**TABLE 4.** CSF/blood B lymphocyte ratios

Cell type	Group	n	Mean ratio ± SD	P
CD19+ B-cells	Controls	21	0.04 ± 0.06	<0.0001 <sup>a</sup>
	OMS	56	0.15 ± 0.14	
CD5+ B-cell subset	Controls	12	0.14 ± 0.29	0.004 <sup>a</sup>
	OMS	27	1.3 ± 1.8	
CD5- B-cell subset	Controls	12	0.02 ± 0.04	0.014 <sup>a</sup>
	OMS	27	0.09 ± 0.13	
% CD5+ of total B-cells	Controls	12	1.5 ± 2.7	0.006 <sup>a</sup>
	OMS	27	9.4 ± 13.4	

<sup>a</sup>Statistically significant by *t* test.  
CSF, cerebrospinal fluid; OMS, opsoclonus-myooclonus.

autoreactive antibodies.<sup>29</sup> Disease-associated CD5<sup>+</sup> B-cells interact with T-cells.<sup>25</sup> Their immunoglobulin repertoire is dominated by antibodies that cross-react with self-antigens and are increased in autoimmune diseases.<sup>14</sup>

OMS joins the list of other autoimmune disorders, such as rheumatoid arthritis, Sjögren's syndrome, myasthenia gravis, and hyperthyroid Grave's disease, in which increased percentages of CD5<sup>+</sup> B-cells have been reported.<sup>30–32</sup> In multiple sclerosis, one study found about 7% CD5<sup>+</sup> B-cells, ranging up to 40 to 50%,<sup>33</sup> which is higher than we found in OMS. Of 17 neurological adult controls, 9 lacked CD5<sup>+</sup> B-cells in CSF, which is fewer than we found in our pediatric controls.

Recently, the role of the CD5<sup>-</sup> B-cell subset in autoimmune disorders has also drawn attention. CD5 expression has been implicated in B-cell tolerance, and in some disorders, CD5<sup>-</sup> B-cells may be associated with a higher incidence of autoantibody production.<sup>34</sup> In our patients, CD5<sup>-</sup> B-cells were increased compared with that in controls, especially in the acute and severe subgroups, suggesting a possible role in the pathophysiology of OMS.

#### CSF B-Cells in the Pediatric Population

Our study provides pediatric control data for other investigators and new information on B-cell regulation in the CSF compartment of children. Although the controls were neurological, not completely healthy children, the proportion of B-cells did not change with age. Taken together with similar data from young, healthy adults,<sup>8</sup> we conclude that B-cells are regulated tightly and are unchanging in the CSF compartment across that age spectrum. Lack of an age influence on CSF B-cells in controls contrasts with normative data on B-cells in peripheral blood, which do change significantly over time.<sup>18</sup>

#### CSF Lymphocyte Immunophenotyping as a Clinical Tool

Immunophenotyping of CSF lymphocytes in autoimmune movement disorders represents a paradigm shift, one that opens new diagnostic and therapeutic avenues. Although the quest for the underlying autoantigen in OMS continues, we believe it is an important clinical tool. Normal routine CSF studies, which are typical in OMS, may lead to the false conclusion that there is no autoimmune process in the CNS. Our study shows that even in this situation, immunophenotyping of CSF lymphocytes provides valuable and otherwise unrecognized information.

Flow cytometry is available at most large hospitals. The same-day results it affords allow treatment decisions

to be made promptly. Most technologists, however, have more experience dealing with lymphocytes from blood than from CSF. Unlike blood samples, which can be processed up to 24 hours after collection, CSF samples must be obtained fresh to yield valid data. The CSF lymphocytes must be concentrated, as a straight CSF sample does not have sufficient cell density for flow cytometry in the absence of substantial CSF leukocytosis. Also, traumatic spinal taps and hence bloody CSF may cause artifact and should be avoided. Using our protocol, however, an experienced flow cytometrist can learn to immunophenotype CSF lymphocytes reliably and retrieve clinically relevant information.<sup>35</sup>

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