Rituximab (anti-CD20) Adjunctive Therapy for Opsoclonus-Myoclonus Syndrome

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Purpose: To determine if rituximab, an anti-CD20 monoclonal antibody, reduces cerebrospinal fluid (CSF) B-cell expansion in opsoclonus-myoclonus syndrome (OMS) and results in clinical improvement.

Methods: Sixteen children with OMS and increased % $CD20^+$ B-cells in CSF received 4 rituximab infusions (375 mg/m^2 IV) as add-on therapy to corticotropin (ACTH), intravenous immunoglobulins, or both, and were reevaluated 6 months later. Outcome measures were clinical (motor function, behavior, sleep) and immunologic (CSF and blood immunophenotype and Ig levels). Controls were 16 age-matched and sex-matched children, who did not have OMS.

Results: After rituximab, 81% of OMS had a lower motor severity score, and 44% improved one severity category. Mean total score decreased by 44% ($P = 0.0005$). Rituximab reduced rage score, nighttime awakenings, and the number of children with opsoclonus, action myoclonus, drooling, gait ataxia, and rage. Despite a 51% reduction in ACTH dose, 9 of 11 children on ACTH did not relapse. The percentage of CSF $CD19^+$ (and $CD20^+$) B-cells was lowered in all children (undetectable in 6), with a 90% reduction in the group mean ($P = 0.00003$). CSF B-cells were no longer expanded compared with controls. In blood, $CD19^+$ B-cells decreased (-90%, $P = 0.0003$), as did the CSF:blood $CD19^+$ B-cell ratio ($P = 0.00003$). Serum IgM fell by 69% (below reference range), with no statistically significant change in IgG or IgA.

Conclusions: Rituximab seems efficacious and safe as adjunctive therapy for OMS. Selective targeting of CSF B lymphocytes represents a novel and valuable paradigm shift in the therapy for centrally mediated paraneoplastic disorders.

Key Words: B lymphocytes, B-cell trafficking, corticotropin, CSF immunophenotyping, dancing eyes, IVIg, Kinsbourne syndrome, neuroblastoma, paraneoplastic syndrome

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Opsoclonus-myoclonus syndrome (OMS) is a serious neurologic complication of neuroblastoma, characterized by motor, behavioral, and sleep disturbances.[1] Cerebrospinal fluid (CSF) B-cell over-representation or "expansion" is a biomarker of disease activity in OMS, persisting in children with lingering symptoms, correlating with clinical severity, and resisting conventional treatment.[2] Although small numbers of B-cells are found in the CSF of healthy individuals,[3,4] expansion of CSF B-cells indicates B-cell recruitment to the CNS,[5] with possible proliferation, inflammation,[6] and production of potentially pathogenic antineural autoantibodies,[7] such as those reported in OMS.[8–11] We hypothesized that an agent targeting peripheral blood B-cells also would destroy B-cells in CSF, and that normalization of CSF B-cell percentages would reduce neurologic abnormalities.

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Rituximab, a chimeric $IgG_{1\kappa}$ monoclonal antibody directed against B-cells, seems ideal for testing these hypotheses. It binds to the CD20 antigen on the surface of mature B-cells,[12] which it targets for apoptosis and immune-mediated destruction, but not to stem cells or pro-B-cells.[13] Rituximab depletes circulating B-cells for 6 to 9 months, presumably eliminating B-cell clones,[14] and binds to lymphoid cells in the spleen, thymus, and lymph nodes.[15] Although rituximab was approved by the US Food and Drug Administration in 1997 to treat B-cell non-Hodgkin lymphomas,[16] successful off-label treatment of several autoimmune disorders has been reported.[17–36] To date, more than 300,000 patients, mostly adults, have been treated with rituximab worldwide.[14]

We now report the open-label, add-on use of rituximab in children with OMS who had CSF B-cell expansion despite ongoing treatment with gold standards of therapy corticotropin (ACTH) and intravenous immunoglobulin (IVIg).[37] Children with and without an identified neuroblastoma were included, because the lack of differences in the CSF immunophenotype,[2] age of onset,[37] or clinical phenotype[37] between groups, and the propensity for neuroblastoma to spontaneously regress,[38] suggest that both are the same entity.[2] This is a short-term proof of concept study.

PATIENTS AND METHODS

Patients

Sixteen children with OMS were recruited through the National Pediatric Myoclonus Center and their parents signed consent for this institutional review board-approved study (age 3.2 ± 0.5 y; OMS onset age 1.7 ± 0.2 y; OMS duration 1.6 ± 0.4 y; 44% boys; 2 severe, 5 moderate, 9 mild in severity). After a thorough search had been made for occult neuroblastoma, using body imaging and blood and urine tumor markers, a tumor was found in 50% (4 thoracic, 4 abdominal; 7 neuroblastoma, 1 ganglioneuroblastoma; 7 International Neuroblastoma Staging System stage I, 1 stage IIA), and tumors were resected. All children still had static motor, behavioral, or sleep abnormalities of OMS despite ACTH (n = 11) and IVIg (n = 13) immunotherapy, which had been given usually by referring physicians for 1.0 ± 0.3 years, and some had failed weaning. Nine were on both treatments at the time of evaluation. No children had been treated with chemotherapy or rituximab. None were immunized for at least 3 months before, during, or after rituximab therapy, both to avoid immune cell activation[39] and for possible effects of rituximab on immune responsiveness.[40] Each was evaluated clinically, videotaped, and underwent lumbar puncture and blood drawing before and 6 months (range 5 to 6.5) after completion of rituximab therapy.

Controls for immunologic studies were 16 immunotherapy-naive, age-matched and sex-matched children (age 4.2 ± 0.5 y; 38% boys), whose lumbar puncture was part of a diagnostic evaluation for a variety of other neurologic disorders, such as myoclonus and ataxia. Their routine CSF studies were normal.

Motor Function Assessment

Children were videotaped using a standardized format. A trained observer blinded to treatment status rated motor impairment using the OMS Evaluation Scale, which we devised and validated.[41] Each of the 12 scale items was rated from 0 to 3, as an index of increasing neurologic severity or motor impairment. Total score was calculated as the sum of subscores, a score of 36 indicating maximum abnormality. The neurologic examination was videotaped.

From the videotape, the number of 1″ smooth wooden blocks (out of 8 offered) that a child stacked into a tower was recorded. Children were allowed more than one try and their best effort was used. Because some children showed no dominance for handedness, the block counts for both hands were averaged for all.

Subjective Sleep Assessment

Parents were interviewed as to whether their child regularly had trouble sleeping. They were asked how long it took for the child to fall asleep, the total number of hours usually slept each night, and the number of nightly awakenings.[42]

Behavioral Assessment

Parents were questioned about rage attacks, which were described to them as extremely exaggerated temper tantrums lasting > 5 minutes.[42] Rage was semiquantitated on a published Likert scale from 0 to 5 as follows: 0 = none, 1 = 1 to 2 episodes/wk, 2 = 3 episodes/wk, 3 = 1 episode/d, 4 = 2 episodes/d, 5 = multiple episodes/d.[42]

Lumbar Puncture

Lumbar puncture was performed in the left lateral decubitus position under intravenous propofol anesthesia 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.[43] One milliliter of CSF for quantitative immunoglobulins (Ig) and an additional 10 mL for flow cytometry were collected. Blood for parallel studies also was drawn.

Treatment

Rituximab (Rituxan, Genentech/IDEC, South San Francisco, CA/San Diego, CA) was given by intravenous infusion once weekly for 4 consecutive weeks at a dose of 375 mg/m^2 of body surface area, the Food and Drug Administration-approved dosing regimen.[16] It was diluted to a concentration of 1 mg/mL. Patients were pretreated with 15 mg/kg acetaminophen and 1 to 2.5 mg/kg diphenhydramine[27,31]; most also received 0.05 to 0.08 mg/kg dexamethasone.[24] If there was no hypersensitivity reaction at the initial 10 cm^3/h rate of infusion, the rate was increased stepwise to infuse over 4 to 5 hours.

Children were at different stages in the course of ACTH treatment at the start of the study, but none was

on high-dose or daily dosing by the second lumbar puncture. H.P. ACTHAR GEL (80 IU/cm^3, Questcor, Union City, CA) was injected intramuscularly, except in a few cases subcutaneously. Our 40 week protocol had initiated at 75 IU/m^2 twice a day for week, daily for 1 week, subsequently switching to alternate days for 2 weeks, then gradually dropping to 40 IU/m^2 over 2 months, when the rate of taper decelerates to 5 IU/m^2 every month until a final dose of 5 IU/m^2 is reached.[44] The taper was halted at any signs of backsliding, and the previous dose that controlled symptoms was resumed. In this study, the average dose before rituximab treatment was just over 20 IU/m^2. ACTH-treated children received intermittent antimicrobial prophylaxis with trimethoprim/sulfamethoxazole oral suspension at standard doses. They were also on ranitidine HCl, calcium with vitamin D supplementation, and a low sodium diet. Children on IVIg, who had been induced at 2 g/kg, received 1 g/kg as maintenance about once a month with acetaminophen and diphenhydramine pretreatments,[39] but not concurrently with rituximab infusions. Most evaluations were performed approximately 4 weeks after the last IVIg dose.

Flow Cytometry

CSF was processed within 30 to 60 minutes of collection, as described previously.[2] Leukocyte counts ranged from 1 to 3 cells/mm^3, with a mean of 1.1 ± 0.1, or about 1100 cells/10 mL sample. Briefly, cells recovered from CSF through low-speed centrifugation (2500 rpm, 4 min, Damon/IEC clinical centrifuge) were concentrated by resuspension in phosphate-buffered saline (pH 7.2) containing 0.5% bovine serum albumin. CSF samples (100 µL/tube) were stained with directly conjugated anti-CD19 and anti-CD20 antibodies, in combination with anti-CD3, anti-CD45, and anti-CD14, which were labeled with 15 to 20 µL fluorescein isothiocyanate, phycoerythrin, allophycocyanin, or phycoerythrin-cyanine. All supplies were obtained from commercial sources.[2] Samples were incubated for 20 minutes at room temperature. CSF leukocytes were washed and resuspended in 200 µL of phosphate-buffered saline after final incubations.

Whole peripheral blood samples (100 µL/tube) were stained with the same panel of directly conjugated monoclonal antibodies used for CSF cells. After a 10-minute incubation with 100 µL/tube Optilyse B, 1 mL H_2O was subsequently added to each sample, which was then incubated for 10 minutes.

Flow cytometry was performed on a Becton-Dickinson FACS/Calibur cytometer equipped with a 488-mm argon/633-mm HeNe laser. Data acquisition and analysis used CellQuest, and data were plotted as log versus log of fluorescence. Performance characteristics were kept within acceptable performance parameters through multiple quality control measures.[2]

For peripheral blood, we computed both the relative and absolute size of the B-cell pool. Lymphocytes were counted in a separate blood sample on a Sysmex 9500 automated counter in the clinical laboratory. Data from our controls compares favorably with the literature.[45]

Quantitative Ig

Serum Ig were quantitated using Tina-quant assay, an antigen-antibody turbidity test, in the clinical laboratory. CSF Ig samples were sent to Specialty Labs (Santa Monica, CA), where they were assayed by Nephelometry. In most cases, Ig levels were measured 3 to 4 weeks after IVIg treatments.

Statistical Analysis

Statistical analyses used Excel, SPSS, and SAS. Comparisons between OMS and controls were made by the 2-tailed t test. For rituximab treatment, each subject served as his/her own control, having both a pretreatment and posttreatment assessment, and differences were analyzed statistically by the paired samples t test, using percentage of CD19$^+$ B-cells (to avoid competition between any residual rituximab in blood and the anti-CD20 monoclonal antibody used in flow cytometry) and total score as the principal outcome measures. Any missing values required dropping of the paired sample for that variable. Analyses of number of subjects responding were done by paired χ^2 test (McNemar's) or binomial distribution (with test proportion set at 0.5) to determine the statistical significance of individual proportions. Frequency comparisons with controls were made by independent χ^2 tests. Pearson correlations were used for correlational analyses. For all tests, the level of significance was set at $P < 0.05$.

RESULTS

Clinical Response to Rituximab

Mean neurologic severity (total score on OMS Evaluation Scale) decreased significantly by 44% from 13.1 ± 2.1 to 7.4 ± 1.4 ($P = 0.00053$). It dropped in 81% of the subjects ($P = 0.00052$), and in 44%, there was improvement by at least one severity category, such as from severe-to-moderate, or moderate-to-mild (Fig. 1). Before rituximab treatment, there were 2 severe, 5 moderate, and 9 mild cases; after rituximab, there were 0 severe, 2 moderate, and 14 mild cases. The number of children in the combined moderate and severe categories became significantly lower than in the mild category ($P = 0.025$, paired χ^2 test). Children with minimal motor impairment (total scores ≤ 6 or about 0.5 out of 3 per item) showed no significant change in motor scores after rituximab. Total score did not differ significantly between the tumor and no-tumor-found groups.

Six of the 12 subscores on the OMS Evaluation Scale dropped significantly by 39% to 88% after rituximab therapy; for the rest, most of which were already low at the start, the decrease was not statistically significant (Table 1). The largest improvements in motor function were in the 3 gait subscores (1 to 3) and both opsoclonus subscores (10 and 11). Speech deficits, which

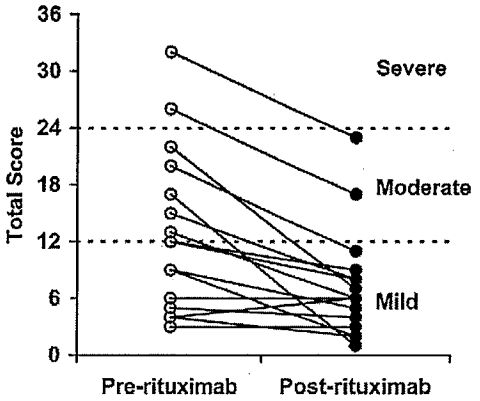

FIGURE 1. Clinical effect of rituximab on total score, denoting neurologic motor impairment on the OMS Evaluation Scale: mild if 0 to 12, moderate if 13 to 24, and severe if 25 to 36. Individual responses are shown. Although there are 16 patients, circles representing some individuals overlap.

were moderately severe at the outset, were not significantly affected.

Several other quantitative parameters for behavior, sleep, and motor control improved significantly after rituximab therapy. Rage score decreased by 64% from almost daily rage to 1 to 2 episodes/wk. Nocturnal awakenings fell by 55%. The number of blocks children could stack into a tower increased by 44%, allowing them to stack an average of 6 of the 8 blocks offered to them.

Rituximab also significantly reduced the number of children with various associated neurologic problems (Fig. 2). Fewer children drooled or had action myoclonus, gait ataxia, opsoclonus, or rage. The degree of reduction ranged from 43% to 78%.

This was not a time-course study, but improvement was reported as early as the second week of treatment. By about 3 months, communication with the families disclosed that 11 of the children had improved in some respect.

Treatment with rituximab allowed the dose of ACTH to be significantly reduced. It was lowered by 51% from $25 \pm 6\,IU/m^2$ to $12 \pm 3\,IU/m^2$ ($P = 0.021$). ACTH was stopped completely in 1 child and tapered in 9 others. However, 2 children (one mild, the other severe) relapsed when the dose of ACTH was reduced rapidly, so the former dose was reinstated and they returned to their baseline level of function. Both were also on IVIg therapy, and no tumor had been found in either.

The posttreatment change (Δ) in total score correlated with the change in the percentage of blood $CD19^+$ B-cells ($r = 0.56$, $P = 0.025$). There was no significant correlation between postrituximab total score and % CSF B-cells, or between the change in total score and % CSF B-cells. Graphically, factors in the lack of correlation included the number of zero CSF values, absence of change in total score in the mildest cases, and disparity in the magnitude of CSF B-cell percentages compared with total scores.

No serious infusion reactions occurred. One child developed urticaria during the first infusion, which resolved after additional diphenhydramine was given. The infusion was resumed and completed uneventfully.

Most children treated with rituximab did not have more infections during the 6-month follow-up; 7 had a minor illness in the 6 months before rituximab treatment, whereas 6 had a minor illness after treatment. One child contracted varicella. As a standard precaution for OMS to prevent relapse, she was treated with acyclovir and a single IVIg 1 g/kg infusion, and had an uncomplicated

TABLE 1. Effect of Rituximab on Quantifiable Clinical Features of OMS

Scale Item	Prerituximab	Postrituximab	*P*
Motor subscores of OMS evaluation scale			
1. Walking, side-to-side imbalance	1.6 ± 0.3	0.8 ± 0.3	0.0047*
2. Walking, front-to-back imbalance	1.3 ± 0.3	0.4 ± 0.3	0.0081*
3. Walking, wide base	1.8 ± 0.3	1.1 ± 0.3	0.013*
4. Instability while standing (feet apart)	1.1 ± 0.3	0.6 ± 0.3	0.17
5. Difficulty achieving standing position	1.5 ± 0.3	1.3 ± 0.3	0.58
6. Truncal instability while sitting	0.3 ± 0.2	0.1 ± 0.1	0.33
7. Targeting difficulty	1.0 ± 0.2	0.7 ± 0.1	0.17
8. Difficulty grasping with 1 hand	0.2 ± 0.2	0.0 ± 0.0	0.33
9. Difficulty with pincer grasp	0.8 ± 0.3	0.2 ± 0.1	0.045*
10. Abnormal eye movements while tracking (fixation)	0.8 ± 0.2	0.1 ± 0.1	0.011*
11. Abnormal eye movements while resting	0.8 ± 0.2	0.1 ± 0.1	0.013*
12. Speech abnormality (dysarthria or anarthria)	2.1 ± 0.2	2.0 ± 0.2	0.50
Other quantifiable clinical features of OMS			
Rage score (n = 16)	2.8 ± 0.5	1.0 ± 0.35	0.001*
No. nighttime awakenings (n = 13)	1.1 ± 0.3	0.50 ± 0.14	0.018*
No. hours slept (n = 13)	8.5 ± 0.5	9.3 ± 0.3	0.054
No. blocks stacked (n = 15)	4.1 ± 0.8	5.9 ± 0.7	0.00061*

Data are means ± SEM.

Rage, nighttime awakenings, and hours of sleep were based on parental reporting, but block stacking was counted by the examiner in clinic.

*Statistically significant by paired *t* test.

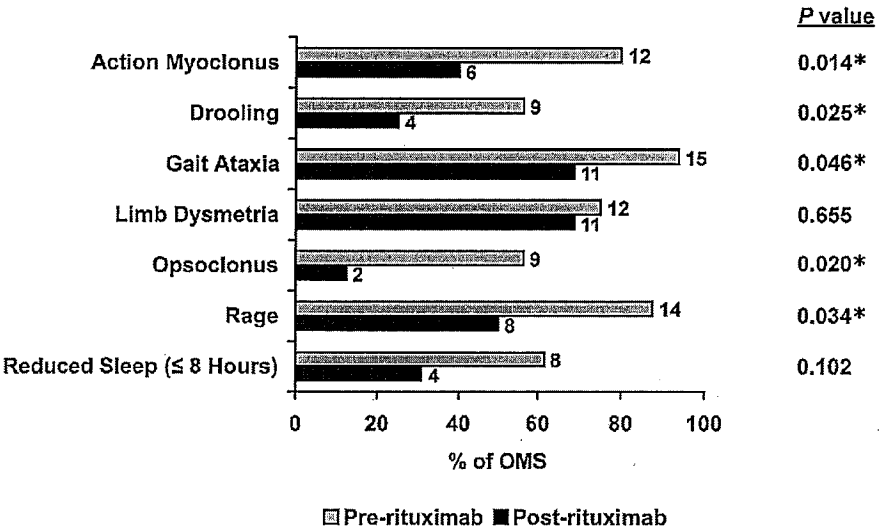

FIGURE 2. Effect of rituximab on the number or percentage of children with OMS and various neurologic problems. Drooling, rage, and reduced sleep were based on parental reporting, whereas other data were obtained from the videotaped neurologic examination. *Statistically significant by McNemar test.

course. Abrupt discontinuation of moderate dose ACTH did not precipitate a neurologic relapse—unexpected in OMS—so it was not restarted; she has not needed it since.

Rituximab Effect on CSF B-Cells

Percentages of $CD19^+$ and $CD20^+$ B-cells were highly correlated ($r = 0.83$, $P = 0.0005$). Before treatment, the percentage of $CD19^+$ and $CD20^+$ B-cells was significantly elevated compared with controls (Fig. 3). Six months after rituximab infusions, CSF B-cell percentages were no longer significantly different than controls. The mean percentage of CSF $CD19^+$ B-cells was reduced by 91% ($P = 0.00003$).

In 1 child (neither of the relapsers), the postrituximab change in % CSF $CD19^+$ B-cells was slight (−6%) and B-cells remained expanded (4.5%). However, the percentage of blood B-cells was 0. Total score decreased by 7.8%. In the 2 relapsers, rituximab reduced CSF $CD19^+$ B-cells into the normal range. Blood B-cells were 0 in 1 (total score decreased by 35%) but had returned to 77% of their pretreatment levels in the mild case (total score increased 1.5-fold).

Frequency analysis also revealed significant effects of rituximab therapy. The percentage of CSF $CD19^+$ B-cells decreased in all patients ($P = 0.021$). $CD19^+$ B-cells were absent in 38% of the cases ($P = 0.014$). The percentage of $CD20^+$ B-cells, which was 0 in 8 rituximab-treated children, was 95% lower than pretreatment values ($P = 0.0036$). The number of controls (5 of 16, 31%) and postrituximab OMS (6 of 16, 38%) with absent CSF B-cells was not significantly different ($P = 0.71$, independent χ^2 test).

Rituximab Effect on Peripheral B-Cells

The CSF:blood $CD19^+$ B-cell ratio (Fig. 3), which was 5.5-fold above controls before rituximab ($P = 0.000004$), decreased after therapy by 77% ($P = 0.00003$) to control values. Rituximab-induced changes in the percentage of CSF and blood $CD19^+$ B-cells were correlated ($r = 0.57$, $P = 0.023$). Relative size of the $CD19^+$ B-cell pool was reduced by 69% ($P = 0.0003$), which was lower than controls ($P = 0.000004$). The absolute number of circulating B-cells also decreased by 71% ($P = 0.03$) below controls ($P = 0.003$); it was 0 in 6 children.

Frequency analysis disclosed that a significant number of children (15 of 16, 94%) still exhibited a reduction in the percentage of blood B-cells ($P = 0.00003$). In most, B-cells had begun to repopulate. The percentage of postrituximab OMS with absent blood B-cells (5 of 16, 31%) was significantly higher than that of controls (0 of 16) ($P = 0.027$, independent χ^2 test).

Humoral Response to Rituximab

Pretreatment CSF and serum Ig concentrations for OMS were well within the laboratory reference ranges (Table 2). Total CSF IgG was not significantly altered by treatment. CSF IgM was undetectable (< 0.03 mg/dL) in 5 of 14 children before rituximab and 6 of them after rituximab therapy; it was undetectable in 10 of 12 controls. Of children with OMS and detectable CSF IgM levels, only 1 was at the top of the reference range of 0.1 mg/dL. In contrast, serum IgM fell by 69% at 6 months postrituximab ($P = 0.00003$), dropping below the lower limit of the reference range. Serum IgG was not significantly affected by treatment, but tended to be lower.

DISCUSSION

Expanding on our previous case reports,[46,47] this study demonstrates that peripherally administered rituximab can eliminate CSF B-cells in an autoimmune disorder. Although our focus is on paraneoplastic disorders, this finding has broad applications for neuroimmunologic disorders of other etiologies in which B-cells play a pathologic role. Longitudinal studies over

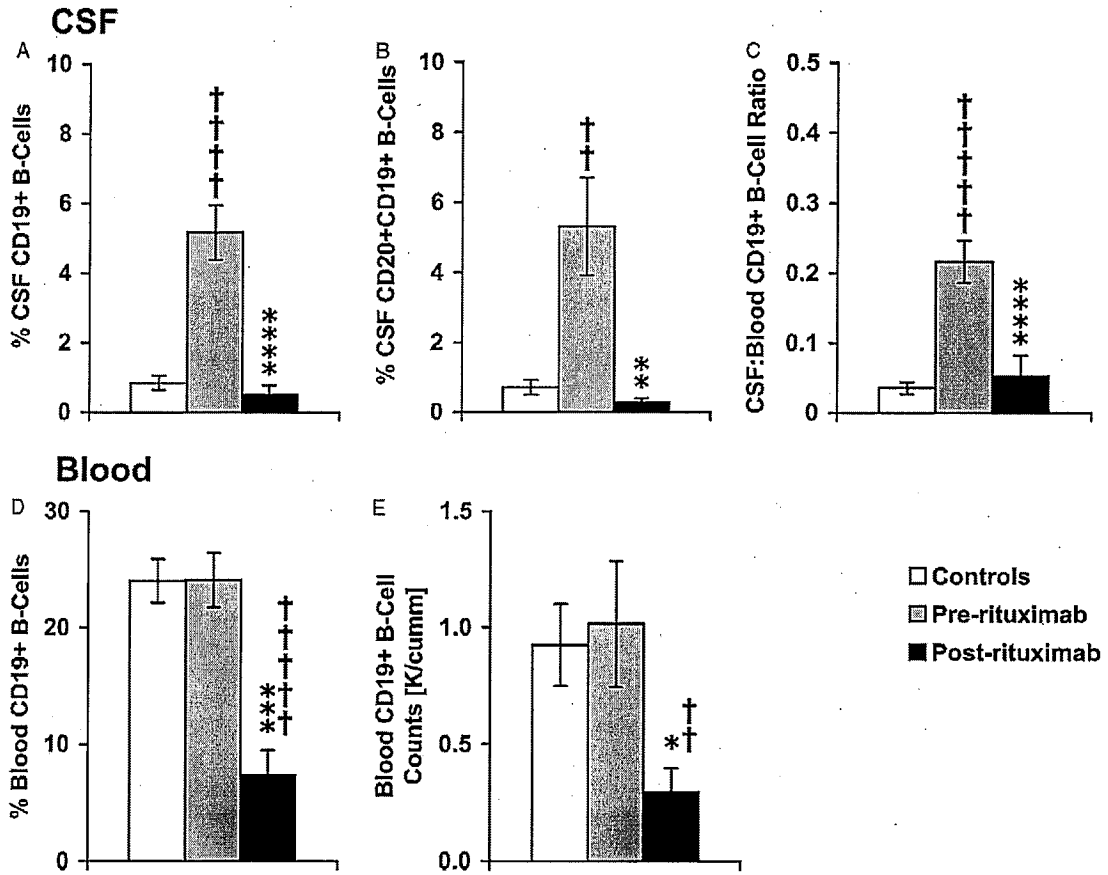

FIGURE 3. Effect of rituximab on the percentage of lymphocytes in CSF (upper tier figures) and blood (lower tier) 6 months after completion of rituximab infusions. Rituximab significantly reduced (A) CSF $CD19^+$ and (B) $CD20^+$ B-cells (n=16), and (C) the CSF:blood $CD19^+$ B-cell ratio derived from percentages of cells (n=13). Both the (D) relative (n=13) and (E) absolute size (n=10) of the blood $CD19^+$ B-cell subset were reduced after rituximab. Data are means ± SEM. *P* values are indicated by an asterisk for paired *t* tests (prerituximab vs. postrituximab) or by a cross for 2-tailed *t* tests (controls vs. OMS) as follows: *$0.01 \leq P < 0.05$, **$0.001 \leq P < 0.01$, ***$0.0001 \leq P < 0.001$, ****$0.00001 \leq P < 0.0001$.

years will be necessary to determine the duration of therapeutic response and long-term effects on B-cell repopulation.

OMS joins a growing list of autoimmune disorders involving various organ systems for which rituximab seems to hold promise, including red cell aplasia,[24] cold

TABLE 2. CSF and Serum Ig in Children Treated With Rituximab

Source	Ig	Rituximab Phase	Ig (mg/dL)	Reference Range (mg/dL)	*P*
CSF†	IgG	Pre	1.0 ± 0.1	(0.5-6.1)	0.054
		Post	0.8 ± 0.07		
	IgM‡	Pre	0.04 ± 0.006	(< 0.10)	0.73
		Post	0.03 ± 0.002		—
	IgA‡	Pre	0.04 ± 0.004	(0.15-0.6)	0.58
		Post	0.04 ± 0.005		
Serum	IgG	Pre	876 ± 125	(441-1135)	0.41
		Post	769 ± 93		
	IgM	Pre	110 ± 13	(47-200)	0.00003*
		Post	34 ± 5		
	IgA	Pre	68 ± 9	(22-159)	0.23
		Post	61 ± 11		

Ig data are means ± SEM.
*Statistical significance, paired *t* test.
†n = 16 for CSF IgG, 14 for IgM, and 15 for IgA.
‡For CSF IgM and IgA, undetectable levels (< 0.03 mg/dL) were recorded as equal to 0.03 rather than 0 for statistical analysis.

agglutinin disease,[23] hemolytic anemia,[19,27,31,33] idiopathic thrombocytopenia,[17,22,31] thrombotic thrombocytopenic purpura,[25,28,36] paraneoplastic pemphigus,[18] rheumatoid arthritis,[26,30,48] graft-versus-host disease,[20] and Wegener granulomatosis.[21] Rituximab also has been used to treat disorders with neurologic manifestations, such as polyneuropathy associated with anti-myelin-associated IgM antibodies[34,35] and lupus,[29,32] and is currently being evaluated in myasthenia gravis.[14] In many reports, the same dosing regimen applied to cancer treatment was used. In several cases, rituximab was added to IVIg and steroids, allowing steroids to be tapered and discontinued.[18,19,23,24]

Clinical synergism between rituximab and ongoing conventional therapies cannot be excluded in an add-on design. The combination may have a synergistic effect on inducing B-cell apoptosis.[5] ACTH also has potent effects that conceivably could have caused cumulative gains during the study period. However, several factors support the conclusion that it was the addition of rituximab that resulted in clinical improvement: ACTH was being tapered, the patients were not on high-end doses of ACTH, and they were enrolled in the study for clinical problems despite ACTH and IVIg. Inclusion of an OMS control group on conventional therapy alone or a monotherapy study would be informative, although not without issues.

Rituximab was most helpful for children with moderate or severe OMS. As reflected in subscores, there also were differences in the responsiveness of motor syndrome components, especially speech, to rituximab therapy. Possible explanations include the chronicity of disease and regional differences in the nature or degree of brain injury, such as to centers or neural networks that subserve different brain functions. In our experience, impairment of expressive language in its various aspects is one of the most difficult areas in OMS to treat. Also, it is likely that more than the 6-month window of this study may be required to appreciate treatment-induced changes in language given the moderately severe pretreatment speech deficits the children exhibited.

This study contained blinded and unblinded, objective and subjective elements. The scorer was blinded to treatment status, as was the flow cytometrist, and used an objective scale to score videotaped motor signs. Counting the number of blocks the children stacked during the clinical evaluation was objective, although not blinded. Parental reports of symptom improvement were subjective and not blinded, so they may have been biased. With regard to sleep, however, parents are usually well aware of sleep habits in their young children, who often get into the parent's bed when they wake up at night.

The clinical and immunologic effects of rituximab were unequal—why? One explanation is that B-cell expansion is not the entire pathophysiology of OMS; T-cell abnormalities are present.[49] Alternatively, Ig persisted in CSF and may have remained at target cells in the CNS. It also could be argued that functional impairment of neurotransmission already in progress as a result of the autoimmune disorder would not be rectified merely by elimination of B-cells. Persistence of CD20-plasmablasts in peripheral blood and sequestration of autoreactive B-cells in lymphoid tissues are other possibilities.[29] These considerations may be useful for future rituximab study designs.

Depletion of peripheral blood B-cells certainly prevents further B-cell trafficking into the CNS,[49] but how rituximab altered the existing CSF B-cell population is unclear. We would assume that, like other IgG, it does not cross the blood–brain-barrier well. If that is the case, CSF B-cells may return to the peripheral blood compartment, as part of trafficking,[50] where they are eliminated. Although egress of B-cells from localized sites of brain inflammation as a result of rituximab therapy would be a therapeutic advantage, there are no supporting data for this speculation, and further studies are needed.

Serum IgM was lowered by rituximab-induced B-cell depletion despite IVIg cotreatment, because most IVIg preparations contain only trace IgM.[51] Reduction in serum IgM, which has been reported in some other studies,[16,48] may be relevant to IgM antibodies found in OMS,[8] but IgG autoantibodies would be unaffected.[9,10] It may seem counterintuitive that IgG levels remained robust despite circulating B-cell depletion, but this phenomenon has been noted previously.[35] Although exogenous IgG could have been a factor, the half-life in vivo for IVIg is about 3 weeks, and by 72 hours after infusion, IgG levels have dropped to 50% of peak levels.[39]

If rituximab does not work by decreasing Ig in the brain, how does it cause clinical improvement? B-cells not only produce Ig, but also participate in autoimmune disease by unconventional mechanisms, such as autoantigen presentation and modulation of other immune cells.[32] Therefore, B-cell depletion may interfere with antigen presentation to T-cells and may have down-stream effects on other lymphocyte populations. Rituximab also may help induce B-cell tolerance by depletion of memory B-cells. Evidence for the latter is decreased response to simple hapten and recall antigen.[31] Studies of these various possibilities are in progress at our center.

Most patients respond well to rituximab,[15] but resistance to monoclonal antibody therapy, though uncommon, has been reported.[52,53] Human antichimeric antibodies are more common in patients with poor B-cell depletion.[32] However, peripheral B-cell depletion was excellent in 1 child whose CSF B-cell percentage was little different 6 months after rituximab. Whether CSF B-cells never were depleted or merely returned early in that child is unknown.

Rituximab was compatible with conventional immunotherapies in our patients and was not associated with serious infusion reactions. It is genetically engineered from murine cells, the proteins of which can cause hypersensitivity. Infusion-related symptoms, such as fever, chills, nausea, hives, fatigue, headache, and itching—all may be due to cytokine release—usually resolve with slowing of the infusion or additional

antihistamine.[14] Rarely, rituximab can cause more serious hypersensitivity reactions, such as hypotension, bronchospasm, and angioedema. Symptomatic treatment with antipyretics, antihistamines, and steroids controls severe reactions in most cases.[14]

The low incidence of life threatening infections[29] may be due to sparing of plasma cells, which lack CD20,[54] or antimicrobial effects of IVIg and trimethoprim/sulfamethazole, which were coadministered in most of our patients. In the few case studies of rituximab use in children with other autoimmune disorders, the risk/benefit ratio has been favorable.[19,24] B-cells repopulate the circulation, reconstituted from stem cells and CD20-precursor B-cells. Rituximab does not produce the severe myelosuppression, alopecia, and other side effects characteristic of chemotherapy.[16]

In summary, rituximab seems to be both effective and safe in treating motor impairment, rage, and sleep disturbance, as well as CSF B-cell expansion, in childhood OMS when added to conventional immunotherapy. These data support a time course and dose-response study.

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