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Brief Report

B Cell Depletion Therapy for New-Onset Opsoclonus-Myoclonus

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Video 

Abstract: **Twelve immunotherapy-naïve children with opsoclonus-myoclonus syndrome and CSF B cell expansion received rituximab, adrenocorticotropic hormone (ACTH), and IVIg. Motor severity lessened 73% by 6 mo and 81% at 1 yr ($P < 0.0001$). Opsoclonus and action myoclonus disappeared rapidly, whereas gait ataxia and some other motor components improved more slowly. ACTH dose was tapered by 87%. Reduction in total CSF B cells was profound at 6 mo (-93%). By study end, peripheral B cells returned to 53% of baseline and serum IgM levels to 63%. Overall clinical response trailed peripheral B cell and IgM depletion, but improvement continued after their levels recovered. All but one non-ambulatory subject became ambulatory without additional chemotherapy; two relapsed and remitted; four had rituximab-related or possibly related adverse events; and two had low-titer human anti-chimeric antibody. Combination of rituximab with conventional agents as initial therapy was effective and safe. A controlled trial with long-term safety monitoring is indicated.** © 2009 Movement Disorder Society

Key words: ACTH; anti-B cell agent; dancing eyes; Kinsbourne syndrome; neuroblastoma; paraneoplastic syndrome; rituximab

The unique combination of opsoclonus and myoclonus has come to connote a paraneoplastic syndrome and B cell pathology within the CNS.[1] Treatment failure, partial response, and neurological relapse compromise the outcome of opsoclonus-myoclonus syndrome (OMS).[2] Rituximab, the prototypic chimeric anti-B cell monoclonal antibody (anti-CD20), has been applied to the therapy of various autoimmune neurological disorders.[3] We demonstrated previously that its adjunctive use treats the characteristic CSF B cell expansion in pediatric OMS[4] with clinical benefit,[5–7] as recent case reports attest.[8–11] This study was designed to look at the feasibility and safety of combining rituximab together with adrenocorticotropic hormone (ACTH) and IVIg, the two most commonly used conventional therapies for OMS,[2] as initial therapy for untreated patients. Such an approach to gaining more complete neurological remission and preventing relapse of OMS is new.

PATIENTS AND METHODS

Study Design

This was a 1-yr, investigator-sponsored, open- and off-label, prospective study, with video-documented evaluations and blinded scoring of clinical efficacy. The primary study end point was preset at 6 mo after the final rituximab, because cerebrospinal fluid (CSF) testing also was done then. "Relapse" was defined as distinct OMS worsening or symptom reappearance lasting at least 72 h. Failure to walk within 6 mo or

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respond to measures for relapse by 1 mo indicated a need for additional immunotherapy. For comparison, the OMS relapse frequency in patients treated only with conventional agents is 50 to 70%.[2,12]

Subjects

Children with OMS were recruited to the National Pediatric Myoclonus Center and examined by the principal investigator (M.P.). New referrals for OMS average 30 to 40 annually and about 25% are untreated. Parents of 12 untreated children meeting inclusion and exclusion criteria (all those approached) signed informed consent for this Institutional Review Board approved study (SCRIHS protocol 04-112), which was conducted from 2004 to 2007 (IND 11,771). The demographic data (means ± SD) were as follows: age 1.9 ± 0.4 yr, range 1.3 to 2.6 yr; OMS onset age 1.6 ± 0.5 yr; OMS duration 0.3 ± 0.2 yr; OMS score 23 ± 5. The categorical subgroups included five boys and seven girls, seven acute and five subacute cases, six moderate and six severe cases, two neuroblastomas, and four prior relapsers.

Treatments

Subcutaneous IV ports were placed in eight toddlers with poor venous access. The doses of rituximab, ACTH, and IVIg were chosen to match those used in our adjunctive trial.[5] ACTH and IVIg were started before rituximab, with 3 to 7 d in between the sequential introduction of agents. Rituximab (Rituxan; South San Francisco, CA)/Biogen IDEC (San Diego, CA) was given IV (1 mg/mL in D5¼ NS) once weekly for four consecutive weeks at a dose of 375 mg/m^2. Patients were premedicated with oral acetaminophen (15 mg/kg), diphenhydramine IV (1.5 mg/kg up to 25 mg), and dexamethasone IV (0.05–0.08 mg/kg up to 1 mg).[13] Rituximab was infused at 20 mL for 30 min, 40 mL for 30 min, and then 60 mL/h. A 52-week protocol for $ACTH_{1–39}$, extending our previous protocol,[14] was initiated. Acthar Gel (80 IU/mL; Questcor Pharmaceuticals, Union City, CA) was injected IM at 75 IU/m^2 twice a day for 1 week, daily for 1 week, on alternate days for 2 weeks, then slowly tapered to a final dose of 5 IU/m^2 at 1 yr. IVIg was induced at 2 g/kg (divided over 2 days) and maintained at 1 g/kg once a month with acetaminophen and diphenhydramine pretreatments. Clinical evaluations were made about 1 mo after IVIg. Concomitant prophylactic treatments were trimethoprim-sulfamethoxazole, ranitidine HCl, calcium with vitamin D, and a 2-g low sodium diet. Patients received speech, occupational, and physical therapy.

Repeated Measures

Lumbar puncture was performed at baseline and 6 mo after completion of rituximab, using methods to obtain CSF atraumatically in children.[4] In fresh CSF and corresponding blood, the expression of lymphocyte surface antigens was investigated by flow cytometry, using a comprehensive panel of monoclonal antibodies to adhesion proteins in combination with anti-CD3 and anti-CD45 antibodies.[4] CSF-quantitative Ig was measured by Nephelometry at Specialty Labs (Santa Monica, CA).

Blood for complete blood count, quantitative Ig, blood lymphocyte subsets, human anti-chimeric antibody (HACA) was collected at baseline and at intervals. The HACA assay, a proprietary bridging ELISA assay of Genentech, was performed by Covance Laboratories (Chantilly, VA), using rituximab as the capture reagent and biotinylated rituximab and streptavidin-horseradish peroxidase (Jackson ImmunoResearch Laboratories, West Grove, PA) for detection. A calibrator curve was prepared with proprietary goat polyclonal antibodies to rituximab. Serum Ig was quantitated by the Tina-quant antigen-antibody turbity assay in the clinical laboratory (St. John's Hospital, Springfield, IL).

Clinical outcome was rated by the co-investigator (E.T.) from videotapes using a validated 12-item motor evaluation scale.[15] Each item was scored in increasing severity from 0 to 3.[1] Subscores were converted to a total score to designate mild (0–12), moderate (13–24), and severe (25–36) categories. The rater was blinded to the order (pretreatment vs. treatment) in all subjects.

Statistical Procedures

The level of significance was $P < 0.05$. Time-course data were analyzed on the Statistical Analysis System by one-way analysis of variance (ANOVA) with repeated measures, and follow-up comparisons of means were made by the least square means procedure. Bonferroni corrections were made for multiple comparisons. CSF data were analyzed by paired t tests, and correlation analysis by Pearson correlations.

RESULTS

OMS Motor Severity

Treatment reduced the total score (ANOVA, $P < 0.0001$; Fig. 1A). At the 6-mo evaluation, all subjects

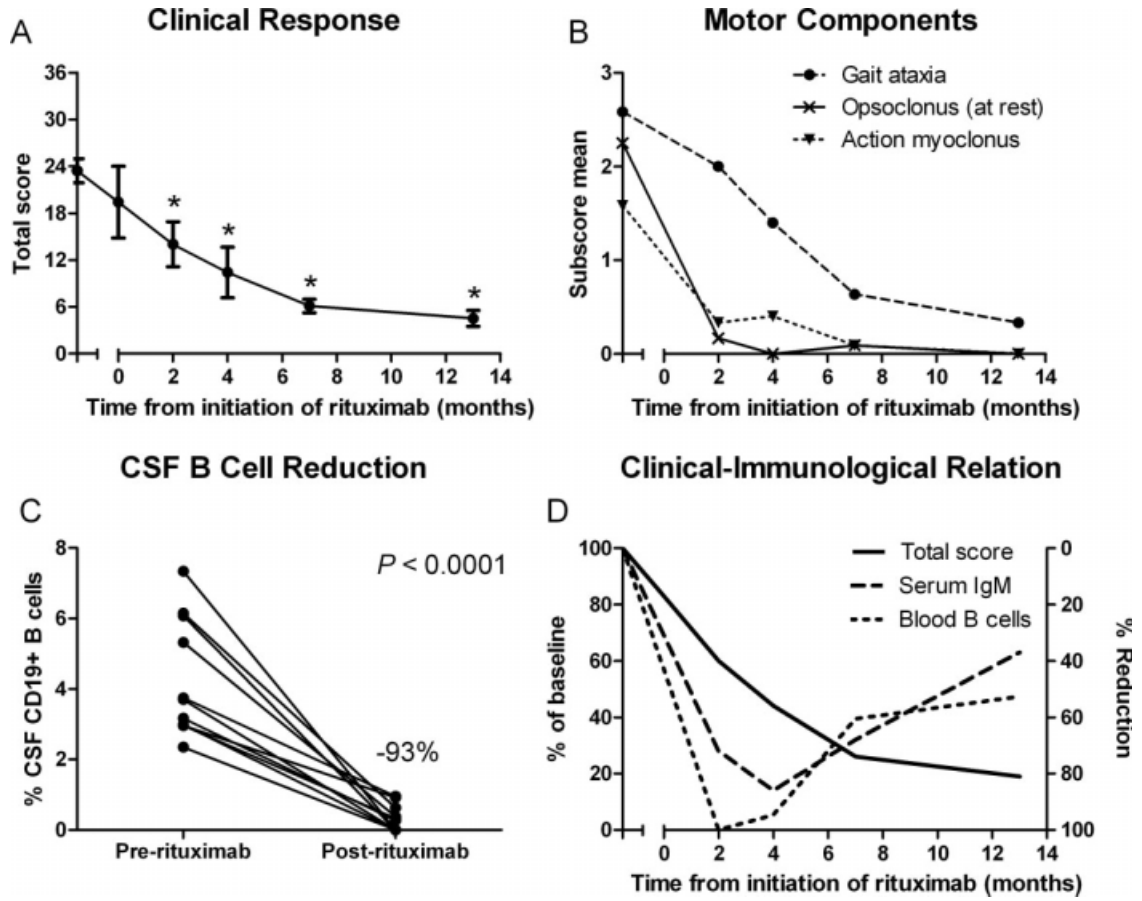

FIG. 1. (A) Clinical efficacy (reduction in motor severity) and effect on immunological measures. Data are total score means ± SEM. Asterisks indicate significant differences compared with the score at initial evaluation. The initiation of rituximab is denoted by time = 0, and time = 2 represents the 1-mo evaluation after completion of rituximab. Compared with baseline, mean total score decreased by 43% at 1 mo, 59% at 3 mo, 73% at 6 mo, and 81% at 1 yr ($P < 0.0001$). (B) Mean subscores for key motor components of OMS Scale (scale items 1, 7, and 11). Upper extremity action myoclonus was scored as reaches target with no jerks (0), minimal jerks (1), moderate to severe jerks (2), or unable to reach target due to jerks (3). (C) Dot plots of $CD19^+$ B cell frequency in CSF. For pediatric neurological controls of mean age (±SD) 9.3 ± 1.2 yr (n = 16), the median CSF B cell frequency was 0.71% (National Pediatric Myoclonus Center database). (D) The relation between changes in total score, blood B cells, and serum IgM concentration is shown. IgM was still below the reference range in five of nine subjects at 1 yr.

had improved by ≥6 scale points, 8 by ≥12 points, 7 by ≥18 points, and 1 by ≥ 24 points. Opsoclonus and action myoclonus disappeared rapidly in parallel with B cell depletion, but some other motor components, such as gait ataxia, improved more slowly (Fig. 1B). Clinical improvement continued even as blood B cells and serum IgM began to recover (Fig. 1D). Treatment had functional impact. Seven children were not ambulating independently at the initial visit, but 6 mo after completion of rituximab, only one was not walking. He received additional immunotherapy and was ambulatory at 1 yr. ACTH dose was tapered steadily, a total decrease of 87% ($P = 0.0001$) by 1 yr. The subsequent time points differed significantly from the first.

CSF Immunophenotype

At baseline, the frequency of total B cells was 4.4 ± 1.6% (normal ≤ 1%). Six mo after the final rituximab, there was significant reduction in total B cell frequency (Fig. 1C) and 83% reduction in the CSF-to-blood B cell ratio ($P = 0.001$). CSF B cells were undetectable in five of 11 children. $CD19^+$ and $CD20^+$ B cells (Fig. 1E) were correlated ($r = 0.87$, $P < 0.0001$).

Blood Immunophenotype

Treatment with rituximab depleted both relative and absolute blood B cell pools. Total blood B cells ($CD3^-CD19^+$) plummeted to 0% by 1 mo after the

last rituximab infusion, corresponding to a reduction in total B cell counts from 1,377 (1,001–1,753, 95% CI) to 1.7 (0.2–3.1) per mm^3 ($P = 0.0002$). Six mo after the last rituximab infusion, the reduction in B cell frequency (−61%) was still significant ($P < 0.0001$).

Quantitative Immunoglobulins

Serum IgM concentration declined rapidly and returned to 63% of the pretreatment values by 12 mo ($P < 0.0001$). Serum IgG and IgA levels did not change significantly. Baseline CSF IgG concentrations were normal at 0.79 mg/dL (0.7–0.9, 95% CI) and not reduced by rituximab. Six of 11 subjects initially had detectable CSF IgM; by 6 mo, only one had detectable levels (NS).

Serum HACA

HACA was measured in eight children. Two children (25%) developed HACA antibodies, one at 6 mo and the other at 12 mo (Table 1). The antibody concentrations were low (<100 ng/mL) and showed no clear relationship to efficacy or safety.

Relapse and Other Therapies

During the study, two subjects (25%) relapsed, one with a prior history (Table 1). No consistent relapse profile was identified. A partial responder had a complete response to additional immunotherapy given outside the study protocol.

Safety Data

The frequency of relapse or need for treatment of side effects was low (Table 1). Adverse events caused no lasting difficulties and were proportionate among the three immunotherapies.

TABLE 1. *Relapse, partial-responders, adverse events, and HACA*

Results	n
Relapse[a]	
Onset	
First 6 months	2
Second 6 months	0
Type	
ACTH tapering-induced	1
Infection-induced	1
Severity	
Mild	2
Moderate	0
Severe	0
Partial-responders	
Because not ambulatory[b]	1
AE	
Rituximab-related	
Treatment emergent (SAE)[c]	1
Other treatment emergent[d]	2
Rituximab/ACTH related	
Bacterial infection (SAE)[e]	1
ACTH-related	
Cushingoid[f]	4
Hypertension, treated[g]	1
GERD, treated	0
IVIg-related	
Postinfusion syndrome[h]	3
HACA[i]	2

[a]One responded to ACTH increase by 10 IU/m^2, a dose doubling, the other to treatment of infection alone. Both were girls and had peripheral B cell repletion. One was severe at baseline; the other had a prior tumor. Neither was HACA positive.
[b]Received cyclophosphamide and became ambulatory.
[c]Cough on first infusion. Rituximab was stopped and then resumed without recurrence.
[d]Reduced diastolic blood pressure (asymptomatic) was managed by stopping rituximab for 30 min or giving fluids while continuing.
[e]*E. coli* urinary tract infection 5 mo after rituximab despite pentamidine prophylaxis.
[f]Excessive weight gain or facial puffiness.
[g]Asymptomatic, occurring on high-dose end of ACTH schedule.
[h]Fever, headache, nausea, vomiting, or malaise.
[i]Concentrations (ng/mL): 8.4, 20. One with HACA at 6 months was seronegative at 12 months. AE, adverse event; SAE, serious adverse event; IVIg, intravenous immunoglobulins; HACA, human anti-chimeric antibodies.

DISCUSSION

Three-pronged initial immunotherapy is based on the strategy that delivering adequate treatment at the onset of OMS with agents working by various mechanisms will induce faster neurological remission, lower the relapse rate, and proffer the best long-term prognosis. It was rapidly acting, very efficacious, and feasible in OMS, with only slightly more side effects than with conventional agents alone. B cell targeting resulted in remarkable CSF B cell depletion. The clinical response was sustained in most patients and better than anticipated for ACTH and IVIg alone, based on a survey study of 105 cases.[2] In a disorder notorious for relapse, the 17% relapse rate in this study is one-third to one-quarter lower than reported for conventional agents alone[2,12] and merits long-term follow-up.

Despite the small sample size that characterizes studies of rare orphan diseases, this response has implications for OMS in adults and other paraneoplastic syndromes in which CSF B cells are expanded.[16] It is pertinent to movement disorder specialists, especially those dealing with children. The patient who responded to additional immunotherapy emphasizes how retrievable children with OMS can be.

Open-label methodology leaves concerns of placebo effects. However, the authors have data on a negative double-blind trial in OMS with the same scale,[15] sug-

gesting that our observations will withstand a double-blind study. Also, not all immunotherapy for OMS is clinically effective.[2]

The safety profile of combination therapy was good. Each agent caused adverse events, but they were reversible. Occurrence of bacterial infection, although low, emphasizes the need for taking prophylactic steps. The risk of more serious infection was perhaps also offset by preserved serum IgG levels from long-term plasma cells (they lack CD20). The frequency of HACA in our study was typical, and titers were not high. These results are conducive to encouraging a further, controlled, larger cohort study with long-term safety monitoring.

LEGENDS TO THE VIDEO

Segment 1. Representative before-and-after videotapes were excerpted to illustrate response in four patients. Treatment status is indicated as "Prerituximab" (labeled in white letters and displayed on the left of each frame) or "Postrituximab" (labeled in yellow letters on the right). Opsoclonus was elicited during tracking of a small object held above the frame. To evoke action myoclonus with or without dysmetria, children were instructed to bring a drinking cup to their mouth, stack small wooden blocks into a straight tower, and put a paperclip into a small bottle with arms outstretched. Ataxia was demonstrated on testing of station and gait.

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Author Roles: M.P. and E.T. designed and organized the study; all authors contributed to its execution. E.T., M.P., and J.S. collected data. J.S. videotaped the patients and made the movies, which were edited by E.T. and M.P. Statistical analysis was performed by J.S, J.C., and S.V. The manuscript was drafted by M.P. and reviewed and critiqued by all authors.

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