Journal of Neuroimmunology 265 (2013) 128–130

Contents lists available at ScienceDirect

Journal of Neuroimmunology

journal homepage: www.elsevier.com/locate/jneuroim

Short communication

Rituximab ameliorates anti-*N*-methyl-D-aspartate receptor encephalitis by removal of short-lived plasmablasts

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ARTICLE INFO

Article history:
Received 15 July 2013
Received in revised form 17 September 2013
Accepted 19 September 2013

Keywords:
Central nervous system
Anti-NMDAR encephalitis
Rituximab
Flow cytometric analysis

ABSTRACT

We measured anti-*N*-methyl-D-aspartate receptor (NMDAR) autoantibody levels and assessed B cell subsets using multicolor flow cytometry of peripheral blood mononuclear cells (PBMCs) from a recurrent anti-NMDAR encephalitis case to evaluate the effectiveness of rituximab treatment. Rituximab depleted $CD20^+$ fractions of naïve and memory B cell subsets and reduced the number of $CD20^-$ plasmablasts. This study suggests that short-lived plasmablasts are removed by rituximab-induced depletion of the $CD20^+$ B cell population. Increased numbers of plasmablasts in PBMCs may be a candidate predictive factor for unfavorable prognosis of anti-NMDAR encephalitis and an indication of when to commence second-line immunotherapy.

1. Introduction

What this paper adds:

- Short-lived plasmablasts are removed by rituximab-induced depletion of the $CD20^+$ B cell population after rituximab treatment in an anti-NMDAR encephalitis patient.
- Assessing B cell subsets in peripheral blood mononuclear cells using flow cytometry is of benefit for treatment strategies in anti-NMDAR encephalitis.

Production of antibodies against the *N*-methyl-D-aspartate receptor (NMDAR) causes encephalitis in children and adults (Luca et al., 2011; Armangue et al., 2013). Rituximab is a chimeric mouse/human monoclonal antibody directed against the B cell-specific antigen CD20 (Looney et al., 2008) and is recommended as second-line immunotherapy for anti-NMDAR encephalitis (Dalmau et al., 2011). However, this is entirely based on observational data. Whether rituximab should be used in less severe cases, and whether rituximab is superior to other immunosuppressive therapies remains an open question. There is no predictive biological marker for refractory anti-NMDAR encephalitis to first line therapy or relapsing disease, and data regarding peripheral B cell subsets and how they are affected by rituximab in anti-NMDAR encephalitis patients have not been reported. Here, we report the efficient elimination of antibody-producing plasmablasts in peripheral blood after rituximab treatment in a pediatric patient with recurrent anti-NMDAR encephalitis.

2. Case report

A previously healthy 2-year-old Japanese boy received flu immunization 25 days before admission. He had been in excellent health until 10 days before admission, when he suddenly developed transient limb trembling, drowsiness, and reduced activity. Three days before admission, the symptoms became worse, and he was admitted to another hospital. Although he was alert, he developed salivation and a dystonic posture and was referred to our hospital. On admission, he was inactive and exhibited a mask-like face, oral dyskinesia, salivation, dystonic posture (head rotation to the left with right elbow flexion), mutism, postural impairment, and walking disturbances with legs extended. Rigidity of the right upper extremity and spasticity of the lower extremities with Babinski signs were present. He showed involuntary movement during his hospitalization. Blood and urinary analyses were normal. Cerebrospinal fluid (CSF) analysis revealed lymphocytic pleocytosis, normal IgG index, and negative oligoclonal IgG expression. Brain magnetic resonance imaging showed T2 hyperintense lesions in the bilateral basal ganglia with slight swelling. Computed tomography scans of the chest, abdomen, and pelvis were normal. Electroencephalography showed parietal theta frequency activity. Serum and CSF samples were positive for anti-NMDAR antibodies, which supported the diagnosis of anti-NMDAR encephalitis. Following treatment with intravenous immunoglobulin (IVIg) and high-dose methylprednisolone,

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0165-5728/$ – see front matter.
http://dx.doi.org/10.1016/j.jneuroim.2013.09.017

the patient's symptoms gradually and substantially improved. He was discharged on day 75 after hospital admission. Three months after discharge, gait disorder and unusual posture gradually developed, and he had a tendency to fall backward when in a sitting position and showed walking disturbances with legs extended. We re-analyzed his serum and CSF samples on day 235 and compared semi-quantitative titers of serum and CSF using diluted brain tissue samples. On day 1, the CSF antibody titer was <1:4 compared with 1:16 on day 235. The serum titer on day 25 was 1:400 compared with 1:1600 on day 235. These data demonstrated a progressive increase in anti-NMDAR antibody titers in the serum and CSF, and we diagnosed recurrent anti-NMDAR encephalitis. He was treated with L-dopa, trihexyphenidyl hydrochloride, diazepam, IVIg, and high-dose methylprednisolone up to 12 months starting at 8 months after the initial discharge. During this course of therapy, his symptoms showed little improvement. However, rituximab induced clinical improvement starting at 13 months after initial discharge. One week after the first rituximab injection, his oral dyskinesia improved. Three weeks after the first injection, his postural impairment and walking disturbances improved, and salivation was reduced. Six months after rituximab treatment initiation, his mutism gradually improved. Serum and CSF samples were negative for anti-NMDAR antibodies 6 months after the first rituximab injection. Before rituximab treatment, the patient had a modified Rankin Scale (mRS) for children of 3, which improved to 2 during the 5-month follow up period.

Flow cytometric analyses were performed as previously described with slight modification (Chihara et al., 2011). Written informed consent was obtained from the patient's parents. Peripheral blood mononuclear cells (PBMCs) were derived from the patient before and 1 and 5 months after rituximab therapy and from a healthy adult subject as a control. Blood was obtained from the patient and dispensed into EDTA-2Na-containing and plain tubes. PBMCs were isolated from the EDTA-2Na-containing sample using a Ficoll gradient (GE Healthcare Japan, Tokyo, Japan), washed once, and reacted with PE-Cy7 anti-human CD3, Pacific Blue anti-human CD19, APC anti-human CD20, FITC anti-human CD27, and PE anti-human CD38 (all from BioLegend, San Diego, CA, USA). Filtered cells were analyzed by FACS Aria II flow cytometer (BD Biosciences, San Jose, CA, USA).

We used flow cytometry to investigate peripheral blood B cell fractions collected before and after rituximab treatment. $CD19^+$ B cells were almost completely depleted 1 month after treatment (Fig. 1A, B). Three subsets of $CD19^+$ B cells were analyzed: $CD27^-CD38^-$ naïve B cells (Gate 1), $CD27^{dim}CD38^-$ memory B cells (Gate 2), and $CD27^{bright}$ $CD38^+$ plasmablasts (Gate 3) (Fig. 1C). Naïve and memory B cells were $CD20^+$, whereas plasmablasts were $CD20^-$ (Fig. 1D). Memory B cells were completely eliminated 1 month after treatment, but a small number of naïve B cells and plasmablasts remained (Fig. 1C). Rituximab treatment reduced the proportion of all three fractions within total PBMCs (Fig. 1E), but increased the percentage of plasmablasts among total B cells (Fig. 1F). Numbers of peripheral B cells, which were mostly naïve B cells, recovered 5 months after treatment (Fig. 1E). These results demonstrate that rituximab depleted $CD20^+$ fractions of naïve and memory B cell subsets and reduced $CD20^-$ plasmablasts.

3. Discussion

The clinical features of our case are similar to those described previously in the literature. In particular, verbal reduction, akinetic mutism, involuntary movement, and dystonia may be diagnostic clues for anti-NMDAR encephalitis in young children.

In our case, abnormal movement and posture developed 3 months after patient discharge. The repeat analysis of anti-NMDAR antibodies is helpful to determine the appropriate therapeutic strategy for recurrent anti-NMDAR encephalitis. In the recurrent stage, IVIg or ethical problems with off label use delayed the introduction of the prescription for rituximab in this case. Rituximab treatment during the recurrent stage led to a significant improvement in the patient and suggested that patients with recurrent NMDAR encephalitis require early diagnosis and aggressive treatment (rituximab).

Plasma cells are terminally differentiated B-cells that provide humoral immunity by synthesizing and secreting antibodies (Shapiro-Shelef and Calame, 2005). Upon antigen activation, marginal zone and follicular B cells, germinal center B cells, and memory B cells can differentiate into plasmablasts and short-lived plasma cells, both of which can secrete antibodies (Radbruch et al., 2006). Plasmablasts migrate from the lymphoid organs to either inflamed tissue or bone marrow. Those that are home to bone marrow become fully mature, long-lived plasma cells.

Brain samples from patients with anti-NMDAR encephalitis contain numerous $CD138^+$ antibody-secreting cells, suggesting the intrathecal synthesis of pathogenic autoantibodies (Martinez-Hernandez et al., 2011). Thus, B cell-targeting therapies, including rituximab, may be useful for treating anti NMDAR encephalitis. Importantly, rituximab does not deplete long-lived plasma cells because they do not express CD20, thus preventing adverse immunosuppressive effects. In the present case, rituximab treatment eliminated peripheral $CD20^+$ (naïve and memory) B cells and significantly reduced plasmablasts ($CD19^{dim}$ $CD20^-$ $CD27^{high}$ $CD38^+$). However, plasmablasts were relatively enriched in the remaining B cell pool 1 month post-treatment, suggesting that rituximab did not deplete this population. Rituximab may exert its effect by eradicating the source of short-lived plasmablast numbers, which were $CD20^+$. The rapid decline in autoantibody titer after treatment indicates that these antibodies are largely produced by short-lived plasmablasts. Similarly, short-lived plasmablasts are the main effector B cell population in multiple sclerosis patients (Cepok et al., 2005). Despite marked plasma cell infiltration, the central nervous system might not provide a suitable niche for their survival in patients with anti-NMDAR encephalitis. We were also interested in investigating the B cell fractions present in the CSF of this patient. However, the number of mononuclear cells in the CSF, measured by flow cytometry, before rituximab treatment was too low to be analyzed. It is unclear whether B cell depletion in the central nervous system occurred as effectively as in the periphery. In cases with neurological autoimmune disorders, rituximab penetrates the permeable dysfunctional blood–brain barrier and is detectable within the CSF after intravenous application (Petereit and Rubbert-Roth, 2009). It is unlikely, however, that rituximab directly removes infiltrating $CD20^-$ plasmablasts within the central nervous system. We speculate that rituximab treatment disrupted the supply of short-lived plasmablasts from the periphery, resulting in the prompt disappearance of this population from the brain because of their rapid turnover. Collectively, these findings support the usefulness of rituximab to treat NMDAR encephalitis.

Rituximab is recommended as second-line immunotherapy for anti-NMDAR encephalitis to improve outcome (Looney et al., 2008; Dalmau et al., 2011). However, there is no predictive biological marker for refractory anti-NMDAR encephalitis to first line therapy, or relapsing disease. Increased numbers of plasmablasts in patients with acute/chronic anti-NMDAR encephalitis may be a predictive factor for unfavorable prognosis and initiate commencement of second-line immunotherapy. Strategies for targeting antibody-producing cells, such as rituximab and other immunosuppressants may be effective in treating severe types of anti-NMDAR encephalitis. Our study assessment of B cell subsets in PBMCs using flow cytometry was limited by only observing one case, so further studies using greater numbers of patients with less severe, recurrent, and secondary progressive type anti-NMDAR encephalitis are required.

Acknowledgment

We are deeply grateful to Professor Joseph Dalmau (Department of Neurology, University of Barcelona, Spain) for the analysis of the antibodies against NMDA receptor. Dr. Sakuma was supported by a

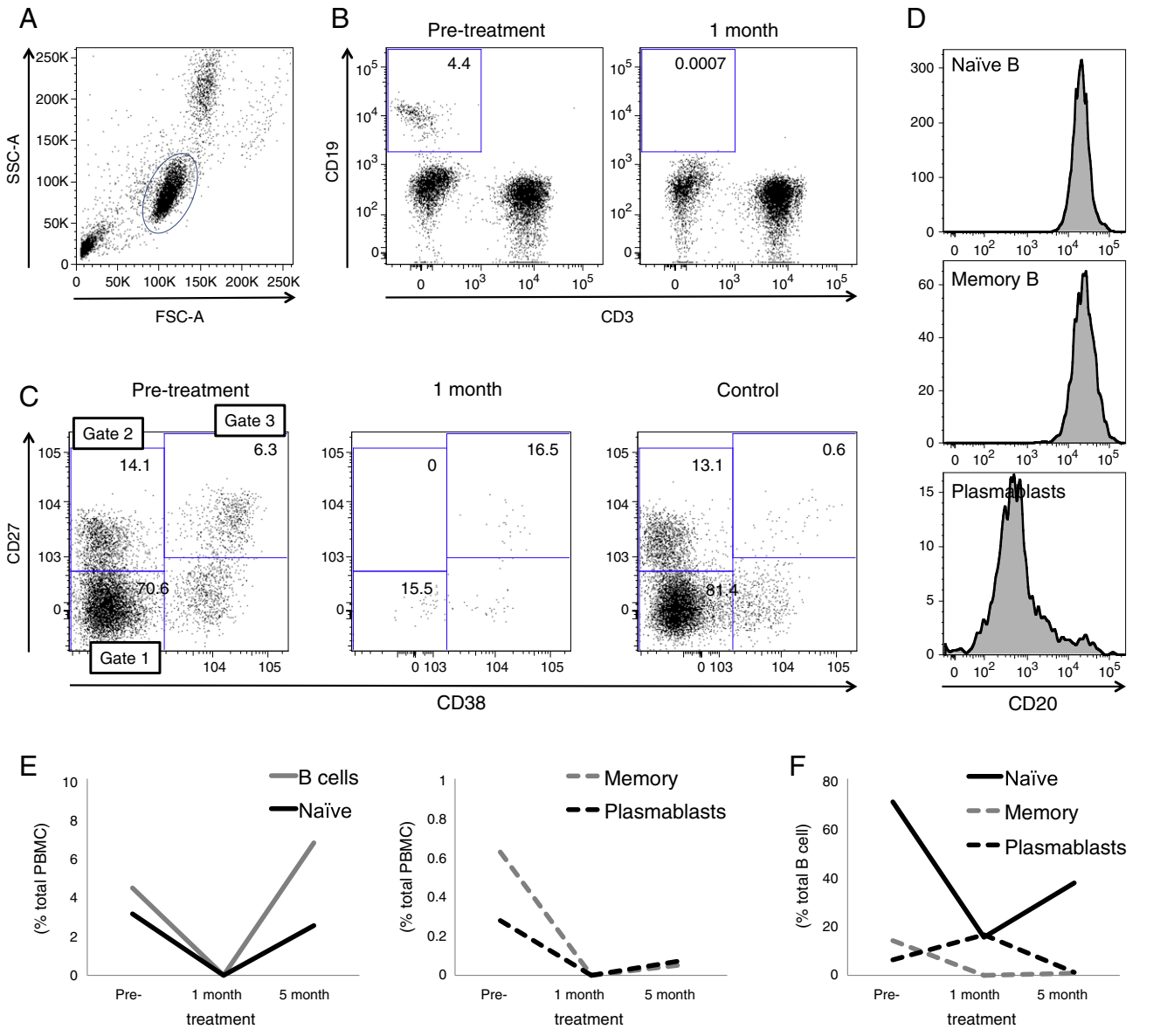

Fig. 1. Peripheral B cell subsets before and after rituximab treatment in a patient with NMDAR encephalitis. A: Lymphocyte gating with forward and side scatter. B: $CD19^+$ B cells were almost completely depleted 1 month after treatment. C: The effect of rituximab on peripheral B cell subsets: PBMCs were first gated on $CD19^+$ B cells and then analyzed for CD27 and CD38. $CD27^-CD38^-$ naïve B cells (Gate 1), $CD27^{dim}CD38^-$ memory B cells (Gate 2), and $CD27^{bright}$ $CD38^+$ plasmablasts (Gate 3). D: Differential expression of CD20 in naïve B cells, memory B cells, and plasmablasts. E: Changes in the proportion of naïve B cells, memory B cells, and plasmablasts among PBMCs after rituximab treatment. Data are shown as the percentage of B cells and each B cell subset per total PBMC count. F: The percentage of plasmablasts per total B cells increased, whereas the percentage of naïve and memory B cell decreased at 1 month after treatment.

Health Labour Sciences Research Grant for Research on Measures for Intractable Diseases (H23-nanchi-ippan-107) from the Japanese Ministry of Health, Labour and Welfare. The funder had no role in the study design, data collection, data analysis, manuscript preparation, or publication decisions.

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