Depletion of B Lymphocytes From Cerebral Perivascular Spaces by Rituximab

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Background: Rituximab is a recombinant chimeric monoclonal antibody against CD20, a molecule expressed on cells of the B-cell lineage. A phase 2 clinical trial recently provided strong evidence of the beneficial effects of rituximab in patients with relapsing-remitting multiple sclerosis. We and other investigators previously demonstrated that rituximab therapy depletes B lymphocytes from peripheral blood and cerebrospinal fluid of patients with relapsing-remitting multiple sclerosis.

Objective: To determine the effect of rituximab on the presence of B cells in cerebral perivascular spaces.

Design, Setting, and Patients: Case report from a tertiary academic medical center. Cerebral white matter from autopsy material of a patient with gastrointestinal mantle-cell lymphoma who developed progressive multifocal leukoencephalopathy following rituximab therapy was evaluated by immunohistochemistry. Location-matched brain sections of patients with multiple sclerosis not treated with rituximab, patients without central nervous system disease, and patients with progressive multifocal leukoencephalopathy not associated with rituximab were used as controls.

Main Outcome Measures: Assessment of the number of B lymphocytes in cerebral perivascular spaces in a patient with gastrointestinal mantle-cell lymphoma treated with rituximab, patients with multiple sclerosis, patients with progressive multifocal leukoencephalopathy not associated with rituximab, and healthy control subjects.

Results: We were unable to detect B cells in cerebral perivascular spaces of the patient who developed progressive multifocal leukoencephalopathy following rituximab therapy 8 months after her last dose. In contrast, B cells were detectable in all control brain tissues.

Conclusions: To our knowledge, this is the first report to show B-lymphocyte depletion from brain tissue following rituximab therapy. A reduction in B-cell numbers may be an important contributing factor in the pathogenesis of central nervous system infections.

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RITUXIMAB (RITUXAN; Genentech, Inc, South San Francisco, California, and Biogen Idec, Cambridge, Massachusetts) is a recombinant chimeric IgG1 monoclonal antibody that binds to the surface antigen CD20, which is expressed on cells of the B-cell lineage. B-cell depletion is likely the result of antibody-dependent cytotoxicity, complement-dependent cytolysis, and activation of proapoptotic pathways. In a recent phase 2, double-blind, placebo-controlled clinical trial, the safety and efficacy of rituximab were evaluated in patients with relapsing-remitting multiple sclerosis (MS), a demyelinating inflammatory disorder of the central nervous system (CNS). Rituximab was significantly more effective than placebo with regard to all clinical and paraclinical outcome measures.

We and other investigators recently showed that rituximab therapy significantly decreases the number of B cells in peripheral blood and cerebrospinal fluid (CSF) of patients with MS. This study was designed to determine the effect of rituximab on the presence of B cells in cerebral perivascular spaces (CPVS), the CNS compartment in which antigen presentation occurs.

METHODS

PATIENTS

The index patient is a 69-year-old right-handed woman who was diagnosed with a gas-
trointestinal mantle-cell lymphoma in December 1999. Following chemotherapy with fludarabine phosphate and cyclophosphamide, the patient went into remission until November 2004, when a neoplastic recurrence was detected in the oropharynx. Treatment with 4 cycles of fludarabine, cyclophosphamide, and rituximab was initiated, and a final cycle of rituximab was given on May 1, 2006. Following therapy, there was no evidence of lymphoma clinically or on computer tomographic images of the gastrointestinal tract. On October 10, 2006, the patient reported confusion and memory problems. The patient was evaluated at a tertiary academic medical center and noted to have left facial weakness and a left homonymous hemianopsia. There was hypoesthesia in the right hand distal to the wrist and a mild paresis in the left upper extremity. Her gait showed shortened footsteps on the left, and she was unable to do tandem gait.

A magnetic resonance image of the cerebrum obtained in November 2006 showed confluent subcortical white matter changes bilaterally. The diagnosis of progressive multifocal leukoencephalopathy (PML) was established based on her clinical presentation, her magnetic resonance images, and a JC virus polymerase chain reaction of CSF that showed a copy number of 320/mL. Her peripheral blood CD4+ T-cell count was 126/µL, and the peripheral blood CD8+ T-cell count was 84/µL. The patient elected to begin cytarabine pharmacotherapy and completed a single 5-day treatment course.

The patient was reassessed in an ambulatory clinic setting on December 10, 2006. All of her prior neurological impairments had continued to progress. In addition, there was now evidence of a constrictive apraxia and dysphagia with liquids, and her speech was dysarthric with labial, lingual, and guttural sounds. There was saccadic intrusion on smooth pursuit as well as decreased hearing bilaterally. The patient had generalized muscle weakness. Following her ambulatory assessment, her neurological functioning continued to decline rapidly, she was cared for by hospice, and she died of progressive neurological dysfunction on December 28, 2006.

The figure shows a cerebral magnetic resonance image of the index patient obtained 6 months after the last dose of rituximab. The area that was histopathologically evaluated included periventricular white matter and basal ganglia from the right cerebral hemisphere. Approximately 50% of the tissue obtained from the index patient who developed PML associated with rituximab therapy included an area that was normointense on magnetic resonance imaging, whereas the remainder of the tissue was hyperintense. Immunohistochemical analyses of anatomically matched tissue specimens from 3 control groups were obtained: (1) healthy CNS tissue; (2) inflammatory CNS tissue from patients with MS; and (3) JC virus–infected CNS tissue. Specifically, 1 patient carried a diagnosis of Burkitt lymphoma without any detectable CNS involvement, 4 patients had a diagnosis of MS and were not treated with rituximab therapy, and 2 patients had been diagnosed with PML not associated with rituximab (1 with human immunodeficiency virus and 1 with myelogenous leukemia). In both patients with PML that was not associated with rituximab treatment, most of the examined tissue was affected by the disease. Cerebral samples in all 4 patients with MS showed evidence of chronic demyelination throughout the tissue sections. Brain specimens from the patient with PML following rituximab therapy were provided by the Department of Pathology, University of Washington, Seattle. Consent had been obtained prior to this study. The control cases included in this study were provided by the Brain Bank, Department of Pathology, University of Colorado Health Sciences Center School of Medicine, Denver. All of the procedures were approved by the respective institutional review boards.

**HISTOLOGICAL ANALYSES**

Formalin-fixed, 5-µm, paraffin-embedded sections were processed for immunohistochemistry using a biotin, avidin, and peroxidase technique visualized with diaminobenzidine. The primary antihuman antibodies in this study included CD20 (clone L26; Dako Corp, Glostrup, Denmark) for B lymphocytes and β2 microglobulin (polyclonal; Dako Corp) for class I major histocompatibility complex. Rituximab and the detection antibody L26 recognize different epitopes of the CD20 molecule. Specifically, rituximab binds to an epitope that comprises 2 distinct regions found within the extracellular domain of CD20, whereas L26 binds to cytoplasmic epitopes. Because a down-regulation of CD20 by rituximab cannot be completely precluded, anti-CD19 (clone LE-CD19; Dako Corp) was used to confirm the presence of B cells. Pervasively located single positive cells were counted in a minimum of 40 fields from corresponding sections in all groups. Class I major histocompatibility complex staining in conjunction with morphological evaluation was used to identify CPVS. Automated image analysis was used to quantitate class I major histocompatibility complex expression as described. Images were acquired at ×40 magnification using the AxioVision AC software (Carl Zeiss MicroImaging GmbH, Göttingen, Germany) on an Axiophot microscope (Carl Zeiss MicroImaging GmbH).

**FLOW CYTOMETRY**

Detection of CD19+ and CD20+ B cells was performed in the Hematopathology Laboratory, University of Washington. After lysis of erythroid cells, lymphocyte subsets, monocytes, and granulocytes in peripheral blood and CSF were identified by cell-specific surface markers.

**STATISTICAL ANALYSIS**

Correlations between continuous and categorical variables were assessed using the Mann-Whitney U test. The means of 2 normally distributed samples were compared by t test. P < .05 was considered statistically significant. The standard error of the mean is shown.

**RESULTS**

**RITUXIMAB DEPLETES B CELLS IN PERIPHERAL BLOOD AND CSF**

At 6.5 months after the last dose of rituximab, no mature CD19+ B cells were identified in peripheral blood (Figure, A) or CSF (Figure, B) in the patient with gastrointestinal mantle-cell lymphoma who developed PML following rituximab therapy.

**RITUXIMAB COMPLETELY DEPLETES B CELLS FROM CPVS**

The area of the brain that was histopathologically evaluated in the index patient is shown (Figure, C). We were unable to detect any CD20+ B cells or CD19+ B cells in brain sections of this patient (Figure, D and E). The difference between other patient cohorts was significant (Figure, D). B lymphocytes were readily detectable in autopsy tissues of 1 patient with healthy brain tissue (Figure, D and F), 4 patients with a diagnosis of MS not treated with rituximab therapy (Figure, D and G), and 2 patients diagnosed with...
PML not associated with rituximab (Figure, D and H). The number of B cells in CPVS of patients who developed PML not associated with rituximab therapy was also significantly decreased compared with that in healthy cerebral tissue and tissue from patients with MS not treated with rituximab (Figure, D).
Results from a recently published phase 2 trial indicate that rituximab is an effective therapy for patients with relapsing-remitting MS. It remains unclear how exactly rituximab mediates its beneficial clinical and paraclinical effects. It was originally assumed that B-cell depletion will eventually result in a reduction of antibody-producing plasma cells in peripheral tissues. However, recently published data generated in an animal model suggest that the number of long-lived plasma cells is not affected by prolonged B-cell depletion with anti-CD20 monoclonal antibody therapy.

B cells are also capable of presenting antigen in the context of the major histocompatibility complex to T cells. Thus, depletion of B cells in the CNS after rituximab therapy may significantly impair the reactivation of antigen-specific T lymphocytes. The CPVSs are the main CNS compartment in which antigen presentation and T-cell reactivation occur. In healthy CNS tissue, the frequency of activated B lymphocytes appears to be very low. In a recent study, other investigators were not able to identify B cells in CPVS. In patients with MS and other CNS inflammatory disorders, B cells can readily be detected in CPVS and active lesions. In this study, there was no significant difference in the number of B cells in CPVS between the patient with healthy CNS tissue and the patients with MS. In either cohort we were able to detect B lymphocytes in only a fifth of all CPVS. Thus, the number of B cells is significantly lower than that of dendritic cells and macrophages in this compartment, confirming previously published data by our group.

In this study, we demonstrate for the first time to our knowledge that rituximab therapy can deplete CPVS-associated B cells. Interestingly, the patient had received the last dose of rituximab 8 months prior to her death. Our data suggest the following: (1) B-cell depletion of CPVS after rituximab therapy can be complete, and (2) repopulation of B cells in the CPVS may be significantly delayed following treatment cessation. Our observation does not explain whether B-cell depletion in CPVS is a consequence of B-cell depletion in peripheral blood and CSF or whether rituximab directly eliminates B cells in the CPVS compartment.

Because the patient had no obvious risk factors for PML, such as an infection with the human immunodeficiency virus or an active malignant neoplasm, it cannot be excluded that rituximab therapy was a contributing factor. Progressive multifocal leukoencephalopathy has also been associated with fludarabine or cyclophosphamide treatment, drugs that our patient received 2 years prior to her death. However, patients develop neurological signs and symptoms of PML typically during or within 6 months after cessation of chemotherapy. Interestingly, one case of PML was reported recently in a patient who had been treated with rituximab and fludarabine combination therapy. Thus, there may be an increased risk associated with the combination of multiple immunosuppressive agents. While there is a theoretical concern that treatment with fludarabine, cyclophosphamide, or both may have contributed to the depletion of B cells from CPVS in this patient, the pharmacological properties of both drugs make this extremely unlikely.

Our data also suggest that some of the pathogenic mechanisms underlying PML associated with rituximab therapy may be different from PML not associated with rituximab therapy. However, in the 3 patients with PML whom we evaluated, a reduction in B-cell numbers may be an important contributing factor.

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REFERENCES


Error in Figure. In the Observation article titled “Depletion of B Lymphocytes From Cerebral Perivascular Spaces by Rituximab” by del Pilar Martin et al, published in the August issue of the Archives (2009;66[8]:1016-1020), part B of the Figure on page 1018 was incorrect. The corrected figure appears here.

**Figure.** Rituximab depletes B cells from peripheral blood, cerebrospinal fluid, and cerebral perivascular spaces (CPVS). At 6.5 months after the last dose of rituximab, no mature CD19+ B cells were identified by flow cytometry in peripheral blood (A) or cerebrospinal fluid (B) in a patient with gastrointestinal mantle-cell lymphoma who developed progressive multifocal leukoencephalopathy (PML) following rituximab therapy. C, A cerebral magnetic resonance image of the index patient obtained 6 months after the last rituximab dose. D and E, No CD20+ B cells or CD19+ B cells were detectable in CPVS of the index patient (indicated as PML with rituximab) (original magnification x40). In contrast, B cells were present in autopsy tissue of 1 patient with healthy brain tissue (D and F), 4 patients with a diagnosis of multiple sclerosis (MS) not treated with rituximab therapy (D and G), and 2 patients diagnosed with PML not associated with rituximab (indicated as PML) (D and H) (original magnification x40). D, The number of B cells in CPVS of patients who developed PML not associated with rituximab therapy was also significantly decreased compared with that in healthy cerebral tissue and tissue from patients with MS not treated with rituximab. VF indicates visual field; error bars, SEM. In E-H, an anti-CD19 monoclonal antibody (clone LE-CD19; Dako Corp, Glostrup, Denmark) was used to confirm the presence of B cells in CPVS. In F-H, arrows indicate B cells in CPVS.