Clinical Stabilization and Effective B-Lymphocyte Depletion in the Cerebrospinal Fluid and Peripheral Blood of a Patient With Fulminant Relapsing-Remitting Multiple Sclerosis

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Background: The anti-CD20 monoclonal antibody rituximab effectively depletes B lymphocytes and has been successfully used in the therapy of immune-mediated disorders of the peripheral nervous system. A limited effect of rituximab on B lymphocytes in the cerebrospinal fluid compartment of patients with primary progressive multiple sclerosis (MS) was recently reported.

Objective: To determine the effect of rituximab on clinical, magnetic resonance imaging, and immunological variables in a patient with relapsing-remitting MS.

Design: A patient with relapsing-remitting MS was treated with rituximab. The patient was repeatedly examined clinically and by magnetic resonance imaging. The frequency of peripheral blood and cerebrospinal fluid B lymphocytes was assessed by flow cytometry before, during, and after rituximab therapy.

Results: Rituximab monotherapy resulted in significant clinical improvement. Inflammatory surrogate markers on magnetic resonance imaging were also reduced. B lymphocytes were depleted in the cerebrospinal fluid and peripheral blood.

Conclusions: Our data demonstrate beneficial clinical effects of rituximab in relapsing-remitting MS, mediated through modulation of humoral systemic and central nervous system intrinsic immune responses. Clinical trials should determine optimal therapeutic strategies for patients with relapsing-remitting MS.

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MULTIPLE SCLEROSIS (MS) is an inflammatory autoimmune disorder of the central nervous system (CNS).1 There is emerging evidence that B-lymphocyte responses against yet unidentified antigens may contribute to the immunopathogenesis of MS.2-4 B lymphocytes, plasmablasts, and plasma cells are present in active and late MS lesions and in the cerebrospinal fluid (CSF); ectopic B-lymphocyte follicles were identified within the meninges; and intrathecally produced IgG and oligoclonal bands are detectable in the CSF of patients with MS.5-8 Not surprisingly, B lymphocytes have become a target of novel pharmacotherapies in those with MS.

Rituximab (Roche Pharma AG, Reinach, Switzerland) is a chimeric IgG1κ monoclonal antibody that targets the B-lymphocyte surface antigen CD20. In humans, CD20 is expressed on most B lymphocytes, but not on plasma B lymphocytes.9,10 It is our understanding that rituximab depletes B lymphocytes via antibody-dependent cytotoxicity and complement-dependent cytolysis. Rituximab exhibits clinical efficacy in neoplastic B lymphocyte–mediated diseases.11 In addition, case reports12-14 showed beneficial clinical effects in immune-mediated demyelination of the peripheral nerve. Recently, some of the immunological effects of rituximab in 4 patients with primary progressive MS were reported.15 The researchers found that B-lymphocyte depletion by rituximab in the CSF compartment was only incomplete.

METHODS

PATIENT HISTORY

In October 2002, a 25-year-old right-handed white man developed left-sided optic neuritis. The re-
sults of his neurological examination were otherwise normal. Vi-

Figure 1. Time course of relapse frequency, clinical disability, and chosen therapeutic approaches. The initial clinical symptoms became apparent in October 2002. Arrows indicate confirmed clinical relapses; EDSS, Expanded Disability Status Scale; and stars, time points when the frequencies of B lymphocytes were analyzed.

NEUROIMAGING AND THERAPY DATA

Disease activity was monitored by frequent MRI on a 1.5-T whole-body scanner (Magnetom Vision; Siemens AG, Erlangen, Germany). Standard protocols and gadolinium contrast agent (Magnevist; Schering, Berlin, Germany) were used. The patient was treated with a dose of rituximab, 1 g, intravenously every 4 weeks at 3 consecutive time points.

ASSESSMENT OF B LYMPHOCYTES

Peripheral blood and CSF samples were obtained prior, during, and after rituximab therapy. Peripheral blood was aseptically collected by standard venipuncture into vacuum tubes containing heparin sodium as anticoagulant at each sampling. At the same time points, CSF was obtained. Fresh PB and CSF cells were stained with an allophycocyanin-labeled anti-CD19 monoclonal antibody (Becton Dickinson, San Jose, Calif) to enumerate B lymphocytes in both compartments. CD19+ and CD20 are coexpressed on B lymphocytes, and binding of anti-CD20 to its receptor could potentially produce false-negative results in detection assays. The staining was performed as described previously.3 The cells were analyzed on a flow cytometer (FACSCalibur; Becton Dickinson) using computer software (CellQuest; Becton Dickinson). B lymphocytes were analyzed in a lymphocyte/monocyte gate that was set according to forward and sideward scatter properties. The results are given as percentage of all gated cells.

RESULTS

EFFECT OF RITUXIMAB ON THE CLINICAL COURSE

Before the induction of rituximab therapy, the patient experienced 9 relapses within 21 months. Following initiation of rituximab therapy, he has remained relapse free for 9 months. His EDSS score improved from 6.0 immediately before rituximab therapy to 4.0, which has been stable for 9 months. The clinical course is mirrored in the MRI, in which no further gadolinium-enhancing lesions on T1-weighted images were observable with rituximab therapy. After 2 infusions, gadolinium enhancement was no longer detectable and has been absent after 6 months of treatment. The lesion load on T2-weighted images has remained stable since the therapeutic regimen with rituximab was initiated (Figure 2).

EFFECT OF RITUXIMAB ON B-LYMPHOCYTE FREQUENCY

The PB and CSF samples were collected before, during, and after rituximab therapy. Before the first application
of rituximab, the patient had 2.7% CD19+ B lymphocytes in the periphery and 2.5% CD19+ B lymphocytes in the CSF. CD19+ B lymphocytes were effectively depleted after 2 courses of rituximab in the periphery and in the CSF. In both compartments, no CD19+ B lymphocytes were detectable 8 weeks after the first application of rituximab, before the third infusion of the drug. This B-lymphocyte depletion remained complete: 6 months after the first infusion, no CD19+ B lymphocytes could be found in PB and CSF samples (Figure 2).

**COMMENT**

This case demonstrates that a complete depletion of CD19+ B lymphocytes through targeting of the coexpressed CD20 molecule can significantly reduce the relapse rate and the
progression of clinical disability in patients with relapsing-remitting MS. This observation is particularly important, because our patient did not respond sufficiently to approved immunomodulating agents or chemotherapy. While the definition of a nonresponsiveness to therapy in those with MS remains controversial, the ongoing high relapse rate and the rapid clinical decline required a change in our therapeutic regimen. During the initial 4 relapses, the patient responded well to intravenous methylprednisolone. After the fifth relapse, the clinical response to corticosteroids was incomplete, but there was a dramatic improvement with plasma exchange.

This clinical observation suggested that in this particular individual at that particular time, a humoral immune response was a critical factor in the inflammatory cascade. From the present data, it seems evident that a complete depletion of CD19+ B lymphocytes is obtainable in PB and CSF samples within 8 weeks after rituximab administration. This depletion was still detectable 6 months after the first application of the drug. Rapid depletion within the PB compartment has been described in various disease entities before and has been seen in patients with primary progressive MS as well. However, in contrast to this recent report, in which a mild decrease of CD19+ B lymphocytes was observed, we induced a complete and sustained depletion of CD19+ B lymphocytes in the CSF of our patient. There may be several biological mechanisms that would explain the variations observed by different groups with regard to B-lymphocyte depletion, including the distinct histopathological characteristics of diverse MS phenotypes.

Inflammation of the CNS with breakdown of the blood-brain barrier is observed less frequently in primary progressive MS than in clinically active forms of relapsing-remitting MS. Thus, the bioavailability of rituximab in the CSF of patients with primary progressive MS may be significantly lower than that in patients like the one presented herein, in whom a high number of gadolinium-enhancing lesions was evident on brain MRI. The effect of inflammation on drug distribution seems especially evident by the fact that the total dose of rituximab given intravenously to our patient before the second lumbar puncture was lower (a total dose of 2 g) than that in the other 2 reported cases (375 mg/m² per week for 4 consecutive weeks).

The clinical efficacy of anti-CD20 therapy with rituximab has been reported in immune-mediated demyelinating disorders of the peripheral nervous system. However, its long-term efficacy remains unknown. Similarly, it is impossible to predict how long the clinical status of our patient will remain stable. It is possible that a decrease of biologically active rituximab levels may reduce of biologically active rituximab levels may

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