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Treatment of primary central nervous system lymphoma with induction of complement-dependent cytotoxicity by intraventricular administration of autologous-serum-supplemented rituximab

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We describe an immunocompetent 19-year-old man with CD20-positive primary central nervous system (CNS) lymphoma refractory to chemotherapy and irradiation. After intraventricular administration of rituximab, a chimeric anti-CD20 monoclonal antibody, supplemented with autologous serum, a remarkable response developed to the CNS parenchymal lymphoma. Cytotoxicity assays showed that untreated patient's serum with rituximab, but not that of heat-inactivated patient's serum with rituximab or rituximab alone, induced potent rituximab-mediated cytotoxicity against tumor cells in the patient's cerebrospinal fluid, suggesting induction of complement-dependent cytotoxicity against CNS lymphoma. (*Cancer Sci* 2006; 97: 80–83)

Despite the high complete remission rate achieved with the most advanced current therapies, 70–80% of patients with primary central nervous system (CNS) lymphoma are treatment refractory or experience relapse.^(1–3) Patients with this condition are less likely to have durable remission with survival after progression of 2–4 months.^(3,4)

Rituximab, a human–mouse chimeric anti-CD20 monoclonal antibody, induces potent complement-dependent cytotoxicity (CDC) and antibody-dependent cell-mediated cytotoxicity (ADCC) in extra-neural non-Hodgkin's lymphoma of B-cell origin.^(5–10) It might also be efficacious for the treatment of CNS lymphoma, as more than 90% of primary CNS lymphomas are B-cell neoplasms expressing CD20,⁽¹¹⁾ and CD20 is not expressed on normal neurons or glia in the brain. However, the potential efficacy of intravenous rituximab in the treatment of primary CNS lymphoma is limited by the high molecular weight of rituximab (146 kDa), which prevents its entry into the CNS though the blood–brain barrier.^(12,13) Pharmacokinetic studies have estimated that levels of rituximab in cerebrospinal fluid (CSF) are 1% or less of those in serum for patients with primary CNS lymphoma or systemic non-Hodgkin's lymphoma.^(12,13) Furthermore, rituximab given intrathecally or intraventricularly has not been shown to have substantial therapeutic effects on CNS parenchymal lymphoma,⁽¹³⁾ suggesting that such an approach alone is unable to produce beneficial cytotoxic activity against CNS lymphoma

refractory to conventional therapy. To overcome this dilemma, a patient with refractory primary CNS lymphoma was intraventricularly administered autologous serum in addition to rituximab, which produced a remarkable tumor response, probably by induction of CDC.

Case Report

A 19-year-old man presented in March 2003 with a 2-week history of diplopia and headache that continued until resection of a brain tumor. Computed tomographic (CT) scanning of the brain followed by brain and spinal magnetic resonance imaging showed an enhancing mass in the left parietal lobe with surrounding vasogenic edema and right midline shift. Histology from a subtotal tumor resection revealed a CD20-positive diffuse large B-cell lymphoma that was negative for CD3, CD5, CD10 and Epstein–Barr virus-encoded small RNA. There was no evidence of congenital immunodeficiency in this patient, nor was there history of exposure to immunosuppressive drugs. The patient was also seronegative for human immunodeficiency virus. CT scans of the chest and abdomen, pelvis bone marrow biopsy sample, and positron emission tomography imaging revealed no lymphoma outside the CNS, confirming the diagnosis of primary CNS lymphoma. There had been no evidence of leptomeningeal lymphoma involvement throughout the overall course. Brain CT at the initiation of chemotherapy in April 2003 showed a residual tumor in the left lateral ventricle. Four cycles of high-dose methotrexate, 8 g/m² every 14 days,⁽¹⁴⁾ failed to produce a response, prompting salvage therapy. The patient received whole brain irradiation with a dose of 3600 cGy followed by involved field irradiation with 900 cGy, and systemic and intrathecal chemotherapy (methotrexate, cytarabine [Ara-C] and prednisolone). Despite intensive treatment, CNS lymphoma progressed with development of hydrocephalus that required external ventricular drainage using an Ommaya reservoir (Fig. 1A–C).

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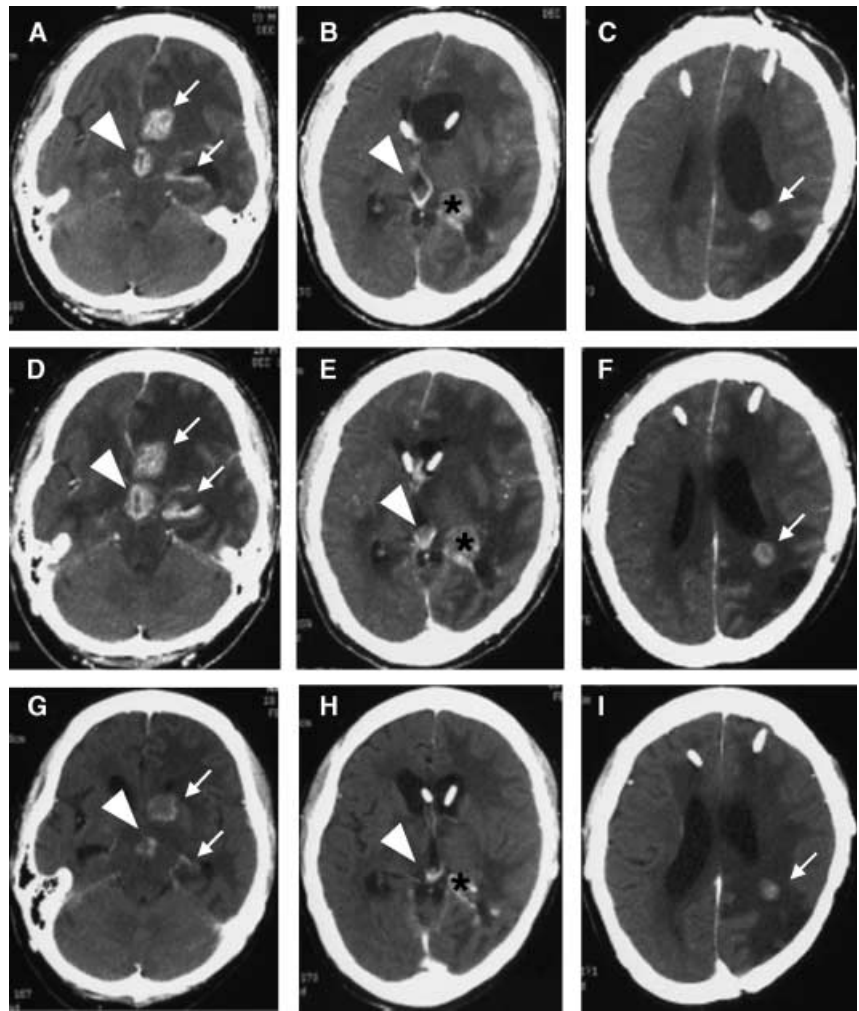


Fig. 1. Response of primary central nervous system lymphoma to chemotherapy, radiation therapy and autologous serum-supplemented intraventricular rituximab therapy. (A–C) Computed tomography (CT) after 7 months of chemotherapy and radiation therapy shows multiple enhancing masses in the left lateral ventricle (arrow), third ventricle (arrowheads) and left caudate nucleus (*) with hydrocephalus and adjacent edema. (D–F) CT obtained 4 weeks after treatment with intraventricular rituximab together with autologous serum shows the brain masses increasing in size. (G–I) CT obtained 8 weeks after treatment show a substantial decrease in the size of the tumor, resolution of the hydrocephalus and improvement of brain edema.

In view of the low probability of response to other forms of systemic therapy, intraventricular administration of rituximab along with autologous serum was planned, based on our *in vitro* studies showing that coadministration of patient's serum is required for effective treatment of CNS lymphoma with intracranial rituximab.⁽¹⁵⁾ The patient gave written informed consent to participate in this investigational protocol, which was approved by the institutional review board. In November 2003, the patient received intraventricular administrations of rituximab; 10 mg (1 mL), 40 mg (4 mL) and 50 mg (5 mL) on days 1, 3 and 6, respectively, concomitant with 8 mL of autologous serum taken several days before initiation of rituximab therapy. No diluting agents except for autologous serum were added. Intraventricular rituximab injections (10 mg/9 mL, 40 mg/12 mL and 50 mg/13 mL, respectively) were carried out over 5 min via Ommaya reservoir. Prior to administration of intraventricular rituximab and autologous serum, an equivalent volume of CSF was removed. While there were no adverse events, including neurotoxicity, the patient's clinical condition continued to deteriorate over the next 4 weeks, leading to obtunded consciousness and bradycardia. Disease progression was confirmed radiologically by CT (Fig. 1D–F). At that time, the

patient and his family declined additional therapy, and he was provided the best supportive care.

Unexpectedly, the patient recovered consciousness 8 weeks after intraventricular rituximab treatment. CT showed a partial radiographic response with resolution of hydrocephalus and improvement of brain edema (Fig. 1G–I), which remained until 12 weeks after intraventricular rituximab treatment. Unfortunately, because the patient developed serious aspirating pneumonia immediately after we became aware of the disease response, he was not given additional intraventricular treatment with rituximab and autologous serum. His consciousness worsened again around 10 weeks after intraventricular rituximab treatment, and brain CT 14 weeks after rituximab treatment confirmed regrowth of the CNS lymphoma. The patient died of lymphoma 121 days after the course of intraventricular rituximab treatment.

Materials and Methods

Cell lines

Epstein–Barr virus-immortalized lymphoblastoid B-cell line (LCL) and CD20-positive lymphoma cell line IM9 were used in the present study. Cell lines were maintained in RPMI

1640 (Gibco Laboratories, Grand Island, NY, USA), 10% heat-inactivated fetal calf serum (Gibco Laboratories), 2 mmol/L L-glutamine (Gibco Laboratories) and penicillin–streptomycin (Gibco Laboratories). Cells in the late logarithmic phase of growth were used for cell-killing assays.

Antibodies and flow cytometry

The chimeric anti-CD20 monoclonal antibody rituximab was purchased from Roche Pharmaceuticals (Basel, Switzerland). Mouse fluorochrome-conjugated isotype control antibodies were purchased from Becton Dickinson (Franklin Lakes, NJ, USA). Three-color flow cytometric phenotyping of target cells was carried out on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA, USA). Saturating amounts of antibodies were added to cells for 30 min at 4°C, before extensive washing and analysis.

Rituximab-mediated cell lysis in the presence of CSF

Target cells (1×10^6 /mL) were incubated in CSF obtained from the present patient prior to intraventricular rituximab administration. The CSF was passed through 0.22- μ m filters (Millipore, Bedford, MA, USA) before use. Cultures containing either 10 μ g/mL rituximab or no rituximab were supplemented with autologous patient serum that was heat inactivated at 56°C for 30 min or non-heat inactivated (untreated). After various times at 37°C, lysis of cells was determined flow cytometrically by uptake of propidium iodide (Becton Dickinson).

Pharmacokinetics

Rituximab concentrations were determined by enzyme-linked immunosorbent assay.⁽¹⁶⁾ Analysis of rituximab concentrations was carried out in FALCO biosystems (Kyoto, Japan) but not in our institute.

Results and Discussion

To test the *in vitro* cytotoxicity of rituximab in the presence of patient CSF, we carried out kinetic and dose–response experiments using LCL cells and the CD20-positive lymphoma cell line IB9 as targets. In the presence of patient CSF, the addition of untreated patient's serum along with rituximab induced time-dependent killing of both target cells, whereas the addition of heat-inactivated autologous serum along with rituximab or rituximab alone induced no considerable killing of target cells (Fig. 2A,B). Both target cells were lysed with only 10% serum-supplemented rituximab, and cytotoxicity against target cells appeared to be independent of increasing concentrations of serum (Fig. 2C). Levels of complement proteins C3 and C4 in the patient's serum were within reference intervals, in contrast to those in the patient's CSF that were lower than detectable levels. These findings suggest that concomitant administration of serum with rituximab into the craniospinal axis, but not the use of rituximab alone, activates rituximab-mediated CDC, leading to killing of tumor cells.

The CSF concentration of rituximab just before the third intraventricular injection in our patient was 12.8 μ g/mL, an amount sufficient to induce lysis of lymphoma cells according to our *in vitro* studies (Fig. 2). These findings support the

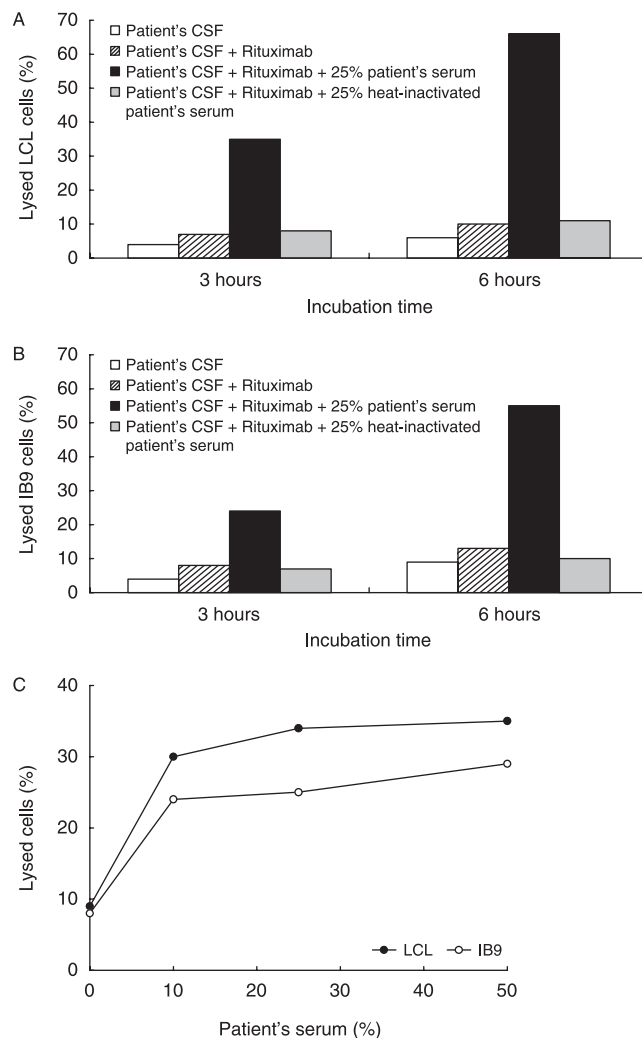


Fig. 2. Rituximab-mediated cell lysis in the presence of the patient's cerebrospinal fluid depends on the presence of autologous non-heat-inactivated serum. (A) CD20-positive lymphoblastoid B-cell line (LCL) cells or (B) CD20-positive lymphoma cell line IB9 cells were incubated for the indicated time in patient cerebrospinal fluid (CSF) supplemented with either heat-inactivated or non-heat-inactivated patient serum in the presence or absence of 10 μ g/mL rituximab. (C) CD20-positive LCL cells (●) or CD20-positive lymphoma cell line IB9 cells (○) were incubated for 3 h in patient CSF in the presence of 10 μ g/mL rituximab with increasing concentrations of patient serum.

possibility of *in vivo* development of CDC in the patient's CNS, because concentrations of rituximab within the cerebrospinal axis were likely to have surpassed 10 μ g/mL at least for 72 h.

Besides CDC, a potent mechanism of rituximab-dependent lymphoma cell killing is ADCC. Monocytes, natural killer cells and polymorphonuclear leukocytes have all been shown to kill opsonized lymphoma cells.^(5,6) Therefore, primary CNS lymphoma cells could be cleared by intraventricular or intrathecal administration of such cellular effectors in addition to rituximab. This approach may be another treatment option for primary CNS lymphoma, in addition to the combination of serum-supplemented rituximab and other chemotherapeutic drugs within the cerebrospinal axis.

To our knowledge, this is the first report of a positive response in the treatment of primary CNS lymphoma following administration of autologous-serum-supplemented rituximab within the cerebrospinal axis. The response is particularly remarkable given the disease progression during prior treatment with cytotoxic chemotherapy and irradiation, and the comatose state of the patient at the start of rituximab therapy.

Our observations form a striking contrast to a previous study,⁽¹³⁾ which reported that intraventricular administration of rituximab alone was ineffective against a parenchymal lesion, despite its substantial efficacy in leptomeningeal disease. One possible explanation is that effector mechanisms such as CDC and ADCC may be mandatory to eliminate parenchymal lymphoma cells by rituximab, while leptomeningeal lymphoma cells may be eradicated by direct induction of apoptosis⁽⁵⁾ via contact with rituximab in the CSF without the effector mechanisms.

It is notable that it took 8 weeks after the intraventricular administration of rituximab until our patient developed clinical improvement and tumor reduction following the transient exacerbation. The clinical data cannot easily be agreeable with immediate killing in short-term *in vitro* assays (Fig. 2). However, this may be explained by a previous report showing the occasional lag between rituximab infusion and therapeutic effect.⁽¹⁷⁾ Of 49 patients who received rituximab as a single first-line therapy, 10 patients had clinical responses between 2 and 11 months after rituximab, whereas 36 patients responded within 2 months after rituximab treatment. These findings suggest the involvement of late effectors such as dendritic cells and T lymphocytes in the effector

mechanisms of rituximab. Selenko *et al.*⁽¹⁸⁾ demonstrated that rituximab treatment of CD20⁺ lymphoma cells induces uptake and cross-presentation of lymphoma cell-derived peptides on dendritic cells, which finally allows an efficient activation of specific T lymphocytes. Although it is unknown whether these reactions could occur in the CNS, the hypothesis may be supported by several observations that glial cells such as microglia can gain antigen-presenting capacity through the expression of major histocompatibility complex molecules in patients with brain tumors,⁽¹⁹⁾ that rituximab promotes release of proinflammatory cytokines such as tumor necrosis factor (TNF)- α ,⁽²⁰⁾ and that resident brain cells can synthesize TNF- α capable of allowing invasion of T lymphocytes into the affected brain tissue.⁽¹⁹⁾

There were no adverse events resulting from treatment with intraventricular autologous-serum-supplemented rituximab, which offers new hope for a disease that until now has been associated with a poor prognosis.^(2,4,11,13) Further refinements of this therapy might lead to effective treatment of primary CNS lymphoma refractory to conventional therapy.

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