

Featured Article

Pretreatment with Rituximab Does Not Inhibit the Human Immune Response against the Immunogenic Protein LMB-1

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Abstract

Purpose: Rituximab, a humanized monoclonal antibody directed to the CD20 antigen present on B lymphocytes, could potentially abrogate the humoral immune response to murine monoclonal antibodies or immunotoxins by depleting antibody-producing B cells.

Experimental Design: A Phase II study of LMB-1, an immunotoxin targeting the Lewis Y tumor antigen, in combination with rituximab was conducted to test the hypothesis that rituximab could abolish or diminish the development of human antibodies to LMB-1. Five patients were treated in this study and received 375 mg/m² rituximab on days 1 and 7 followed by 45 µg/kg/day LMB-1 on days 10, 12, and 14. The development of human antibodies against LMB-1 was detected using a serum neutralization and ELISA.

Results: All five of the patients had a total suppression of circulating CD20/CD19 B-cell population before the administration of the first dose of the immunotoxin. Before rituximab treatment, the mean percentage of CD20/CD19-positive B cells in the five treated patients was 19.8% (range, 4.5–29.8%) of the total peripheral lymphocytes. After two doses of rituximab, CD20/CD19-positive B lymphocytes constituted ≤0.1% of the total peripheral lymphocytes. Despite absent circulating antibody-producing B cells, before and during LMB-1 treatment, all of the patients developed neutralizing antibodies to the immunotoxin by day 21 of drug administration, which prevented retreatment.

Conclusions: Even though rituximab caused complete depletion of circulating CD20/CD19-positive B cells, it had no effect in suppressing the human antibody response to LMB-1 and may be of limited utility in suppressing human antibody responses to other immunogenic proteins.

Introduction

A limitation of monoclonal antibody and immunotoxin based therapies for cancer treatment is the development of human antimouse antibody (HAMA) and human antitoxin antibody (HATA) responses that prevent readministration of the drug (1). Approximately 90% of patients with epithelial carcinomas and 30% of the lymphoma patients have detectable human antimouse antibody and human antitoxin antibody after one cycle of immunotoxin therapy (1–4). Although the formation of human antimouse antibody can be partly overcome by the use of humanized or chimeric monoclonal antibodies, the formation of antitoxin antibodies continues to represent a major problem. Immunosuppressive agents such as cyclophosphamide and cyclosporin have been tested in cancer patients, with limited success, treated with immunotoxins (5, 6). A potentially useful agent for inhibiting the human antibody response to foreign proteins is rituximab, a humanized murine monoclonal antibody that binds the CD20 antigen present on the surface of normal and malignant B lymphocytes, but not on hematopoietic stem cells (7). In a Phase II study of patients with non-Hodgkin's lymphoma treated with rituximab at 375 mg/m² weekly for 4 weeks, Rituximab rapidly and effectively depleted B cells from the peripheral blood circulation, with maximum effect within 3–4 days and the B-cell levels remained nearly undetectable until approximately 6 months posttreatment (8). Besides its role in the treatment of lymphomas, Rituximab has also been shown to have some efficacy in the treatment of patients with antibody-mediated autoimmune diseases (9). However, the value of rituximab in suppressing the humoral immune response in patients being treated with protein-based therapies has not been reported.

LMB-1 is an immunotoxin consisting of the B3 monoclonal antibody (a murine IgG1k directed against a carbohydrate antigen of the Lewis Y family) chemically linked to a truncated form of *Pseudomonas* exotoxin. A Phase I study of this agent showed some clinical activity that was limited by the development of neutralizing antibodies to LMB-1 in 43 of the 47 patients treated with only one cycle of therapy (2). Development of antibodies to LMB-1 impedes repeat administration of the drug to patients, as these antibodies will bind to LMB-1 in the bloodstream with very little drug reaching the tumor site. Because repeated administration of LMB-1 could potentially result in increased antitumor activity, a Phase II study of rituximab administered in combination with LMB-1 was conducted at the National Cancer Institute, Bethesda, to test the hypothesis that giving LMB-1 at the time of maximum B-cell depletion after rituximab treatment will reduce or eliminate the host antibody response against LMB-1.

Materials and Methods

Treatment Plan. The institutional review board of the National Cancer Institute approved the study, and all of the patients who participated in the study signed a written informed consent. Patients with Lewis Y antigen-positive tumors whose

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Notes: Rituximab was provided by Genetech Inc./IDEC Pharmaceuticals Corp.

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Table 1 Effect of rituximab on circulating CD20/CD19-positive B cells^a

Patient	Day 1 ^b	Day 7 ^b	Day 10	Day 43 ^c
1	4.5	<0.1	0	<0.1
2	14.9	<0.1	nd ^d	nd
3	20.5	0.1	0.04	nd
4	29.8	nd	0.1 ^e	1.5
5	29.5	<0.01	<0.1	<0.1

^a CD20/CD19-positive B cells as percentage of circulating lymphocytes. Rituximab (375 mg/m²) was administered on days 1 and 7.

^b ±2 days.

^c ±8 days.

^d nd, not done.

^e Day 16.

serum did not contain pre-existing antibodies to LMB-1 were eligible for the study. The patients were treated with 375 mg/m² rituximab on days 1 and 7, followed by the administration of LMB-1 at a dose of 45 µg/kg on days 10, 12, and 14. Patient blood was collected on days 1, 7, and 10 and more than 1 month after the first dose of rituximab for flow cytometry to detect CD20/CD19-positive B cells. In addition, serum to detect antibodies to LMB-1 was obtained on days 7, 14, 21, and 28 after the first dose of LMB-1. Patients who did not develop antibodies to LMB-1 by day 28 and had no evidence of disease progression were eligible for a second cycle of LMB-1 treatment.

Detection of Human Immune Response to LMB-1.

The detection of antibodies to LMB-1 was done using a serum neutralization and ELISA, as described previously, except that in the ELISA, the 96-well microtiter plates were coated with LMB-1 (2). For the serum neutralization assay, a positive antibody response against LMB-1 was defined as the ability of the serum to neutralize ≥75% activity of LMB-1 at a concentration of 100 ng/ml. Using the ELISA, we considered samples positive when the absorbance was twice the background.

Results

Four male and one female patient with tumors expressing the Lewis Y tumor antigen were treated in this study. The median age of patients was 41 years with a range of 40–72

years. The histological diagnosis of the five patients was: colon adenocarcinoma (two patients), carcinoma of unknown primary, breast adenocarcinoma, and esophageal adenocarcinoma. As shown in Table 1, all five of the patients had complete suppression of circulating CD20/CD19-positive B cells after rituximab infusion and before starting the LMB-1 therapy. Before rituximab treatment, the mean percentage of CD20/CD19 positive B cells in the five treated patients was 19.8% (range, 4.5–29.8%) of the total peripheral lymphocytes. After two doses of rituximab, CD20/CD19-positive B lymphocytes constituted ≤0.1% of the total peripheral lymphocytes.

Development of human antibodies against LMB-1 that were detected by using the serum neutralization and ELISA are shown in Table 2. By day 14 of starting LMB-1 therapy, three of the patients had developed neutralizing antibodies; and by day 21, all of the patients had developed neutralizing antibodies to LMB-1. Using the ELISA, we found that four of the five patients had an increase in their antibody titer, including patient 5 who had a low-level antibody titer before treatment. Patient 3, who did not have an increased antibody titer by the ELISA, did develop neutralizing antibodies on day 21 of LMB-1 treatment. All of the patients received only one cycle of LMB-1 because of the development of antibodies to LMB-1.

Discussion

Our results show that rituximab does indeed rapidly deplete peripheral circulating CD20/CD19-positive B cells after a single dose of 375 mg/m² and this depletion was present for more than 1 month in all three of the patients tested at this time point. However, despite the complete absence of circulating CD20/CD19-positive B cells before treatment with the immunotoxin, all five of the patients developed antibodies to LMB-1 by day 21 of treatment.

Rituximab has been shown to have clinical benefit in several diseases characterized by the development of antibodies to self antigens. These include hematological disorders such as idiopathic thrombocytopenic purpura and autoimmune hemolytic anemias, as well as other autoimmune disorders such as rheumatoid arthritis and Wegener's granulomatosis (10–13). It seems that the beneficial effect of rituximab in these diseases is

Table 2 Human antibody response to the immunotoxin LMB-1^a

Antibodies to LMB-1 were detected by a neutralization (Neut.) and ELISA on days 7, 14, 21, and 34 after the first dose of LMB-1. LMB-1 was given by i.v. injection on days 1, 3, and 5.

Patient	Pre ^a		Day 7 ^b		Day 14 ^b		Day 21 ^b		Day 34 ^c	
	Neut. assay	ELISA titer	Neut. assay	ELISA titer	Neut. assay	ELISA titer	Neut. assay	ELISA titer	Neut. assay	ELISA titer
1	–	–	–	–	+	1/2400	+	1/9000	+	1/6300
2	–	–	–	–	+	1/4000	+	1/60,000	+	1/100,000
3	–	–	–	–	–	–	+	–	nd ^d	nd
4	–	–	–	–	–	–	+ ^e	1/1300 ^e	+	1/800
5	–	1/700	–	1/540	+	1/700	+	1/2500	+	1/3300

^a Pre, assays done prior to starting LMB-1 treatment; –, absence of neutralizing antibodies or an ELISA titer less than twice the background; +, presence of anti-LMB-1 neutralizing antibodies. For the positive ELISA results, the titers are shown.

^b ±2 days.

^c ±8 days.

^d nd, not done.

^e Day 26.

probably due to the elimination of autoreactive B cells (14). However, the benefit of rituximab in suppressing the humoral immune response to alloantigens is not clear. A study of rituximab in nonhuman primates showed that pretreating with rituximab blocks the humoral response to a simple hapten, but its value in suppressing this response to an antigen was not described (15). However, a study of rituximab in baboons undergoing xenotransplantation, showed that, despite the rapid depletion of circulating B cells by rituximab, there was no significant decrease in the production of anti- α -Gal antibodies (16). There are no published reports of rituximab inhibiting the human antibody response to exogenously administered proteins.

Development of human immunogenic response to foreign antigen is a complex process with the final step involving the secretion of antibodies by antibody-producing B cells. Although rituximab is very effective in eliminating circulating B cells, it does not kill all B cells in other body compartments such as the bone marrow and lymph nodes, which could explain the generation of humoral antibodies to foreign proteins such as LMB-1 (7, 16). It has also been suggested that the response to a foreign antigen might also involve CD20-negative subpopulations that would escape targeting by rituximab (14, 16).

Although the number of patients treated in our study was small, all of the patients developed antibodies to LMB-1 despite absent CD20/CD19-positive B cells before and during treatment. These results suggest that rituximab is unlikely to be effective in suppressing the antibody response to immunogenic proteins, and alternate strategies are needed to reduce the immunogenicity of these agents. However, we cannot exclude that higher dose and/or more frequent administration of rituximab could be beneficial by eliminating B cells from lymph nodes, bone marrow, and other sites that may not have been eliminated by two doses of rituximab at 375 mg/m². Also, the schedule of administration of immunogenic protein after treatment with rituximab may be important. In our study, LMB-1 was administered 3 days after the last dose of rituximab when CD20/CD19-positive B cells constituted $\leq 0.1\%$ of the total peripheral lymphocytes. It is possible that delayed administration of LMB-1 after pretreatment with rituximab could limit the immunogenicity of LMB-1.

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