Phase I Clinical Trial Using Escalating Single-Dose Infusion of Chimeric Anti-CD20 Monoclonal Antibody (IDEC-C2B8) in Patients With Recurrent B-Cell Lymphoma

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The B-cell antigen CD20 is expressed on normal B cells and by nearly all B-cell lymphomas. This nonmodulating antigen provides an excellent target for antibody-directed therapies. A chimeric anti-CD20 antibody (IDEC-C2B8), consisting of human IgG1-κ constant regions and variable regions from the murine monoclonal anti-CD20 antibody IDEC-2B8, has been produced for clinical trials. It lyses CD20$^+$ cells in vitro via complement and antibody-dependent cell-mediated lysis. Preclinical studies have shown that the chimeric antibody selectively depletes B cells in blood and lymph nodes in macaque monkeys. In this phase I clinical trial, 15 patients (3 per dose level) with relapsed low-grade B-cell lymphoma were treated with a single dose (10, 50, 100, 250, or 500 mg/m^2) of antibody administered intravenously. Treatment-related symptoms correlated with the number of circulating CD20 cells and grade II events consisted of fever (5 patients), nausea (2), rigor (2), orthostatic hypotension (2), bronchospasm (1), and thrombocytopenia (1). No significant toxicities were observed during the 3 months of follow-up. Serum C3, IgG, and IgM levels, neutrophils, and T cells were largely unchanged. At the three higher dose levels, pharmacokinetics of the free antibody showed a serum half-life of 4.4 days (range, 1.6 to 10.5). Levels greater than 10 μg/mL persisted in 6 of 9 patients for more than 14 days. No quantifiable immune responses to the infused antibody have been detected. CD20$^+$ B cells were rapidly and specifically depleted in the peripheral blood at 24 to 72 hours and remained depleted for at least 2 to 3 months in most patients. Two-week postinfusion tumor biopsies showed the chimeric antibody bound to tumor cells and a decrease in the percentage of B cells. Tumor regressions occurred in 6 of 15 patients (2 partial and 4 minor responses). The results of this single-dose trial have been used to design a multiple-dose phase I/II study.

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THE TREATMENT OF patients with relapsed non-Hodgkin's lymphoma (NHL) remains a frustrating problem. More than 50% of patients with aggressive lymphomas and the majority of patients with low-grade lymphomas are not cured by current therapies. New treatments with different mechanisms of action and toxicity profiles are needed. Previous work using patient-specific anti-idiotype monoclonal antibodies (MoAbs) has shown that NHLs are accessible to intravenously infused antibody, and that tumor regressions, including durable complete remissions, can be induced in some patients.[1-3] With current technology, because of the difficulty and time required to produce patient-specific antibodies, this approach is not feasible for general application. In contrast, the antigen CD20, a 32-kD nonglycosylated phosphoprotein present on the surface of nearly all B cells provides a more universal target for immunotherapy.[4] CD20 is expressed on the surface of normal B cells from the time of cytoplasmic μH chain expression throughout differentiation until the antibody-secreting plasma cell stage. Importantly, it is not expressed on early pre-B cells, stem cells, or antigen-presenting dendritic reticulum cells.[5] More than 90% of B-cell NHLs express this surface protein.[6,7] It is also expressed at a lower density on B-cell chronic lymphocytic leukemia.[8] Although the function of this molecule is not completely defined, it may aggregate and function as a calcium channel.[9] Antibodies binding to surface CD20 can induce a transmembrane signal[10] that can cause a variety of effects from cell activation to blocking cell cycle progression and differentiation.[11,12]

The CD20 protein has multiple *trans*-membrane domains and does not modulate from the cell surface in response to antibody binding and thus provides an ideal target for immunotherapeutic strategies not depending on internalization for their antitumor effect. Unconjugated murine MoAbs to CD20 have been used for immunotherapy. A trial of the 1F5 murine IgG2a anti-CD20 MoAb in four patients showed antitumor activity with minimal toxicity at the highest dose level.[13] Two trials have been reported using anti-CD20 radioimmunoconjugates. Bone marrow ablative doses of ^{131}I-conjugated B1 (IgG2a murine MoAb anti-CD20) resulted in complete remissions in 84% of patients.[14] The use of non–marrow-ablative doses also resulted in partial and complete remissions in the majority of patients.[15] In both of these trials, antitumor effects were observed during the imaging portion of the trials when trace doses of radiolabeled MoAbs were infused with large amounts of unlabeled antibody, suggesting that the murine anti-CD20 MoAb itself may be contributing to the antitumor effect. Indeed, the relative contributions to the antitumor effect of the targeted radiotherapy, of the cold antibody, and of the nonspecific whole body radiation delivered by the radioimmunoconjugates is difficult to determine.[16,17]

Murine MoAbs have several potential limitations when used in clinical trials. Genetic manipulation has made it possible to engineer chimeric antibodies with murine binding

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sites and human constant regions that have lower immunogenicity, longer half-life, and are able to lyse tumor cells using human complement or antibody-dependent cell-mediated cytotoxicity (ADCC) effector cells more effectively than the murine MoAb.[18-20] A chimeric anti-CD20 antibody has been produced that contains the heavy and light chain variable regions from a murine IgG1 monoclonal anti-CD20 antibody (IDEC-2B8) and human IgG1 κ constant regions.[21] Stable, high-level expression was obtained by transfection of the relevant gene constructs into Chinese hamster ovary (CHO) cells. In vitro studies showed similar binding characteristics between the chimeric and murine anti-CD20 antibodies; however, the chimeric antibody was able to lyse $CD20^+$ B cells using human complement or human effector cells (ADCC) 1,000-fold more effectively than the murine antibody. Preclinical studies in macaque cynomolgus monkeys have shown that repeated doses of the chimeric antibody depleted around 80% of $CD20^+$ B cells in the peripheral blood, lymph nodes, spleen, and bone marrow, with gradual recovery over a period of several months.[21] No toxicity was observed in these studies. We describe here the first phase I clinical trial of single-dose infusion with the chimeric anti-CD20 antibody (IDEC-C2B8) in patients with relapsed B-cell NHL.

MATERIALS AND METHODS

Chimeric monoclonal anti-CD20 antibody. The chimeric monoclonal anti-CD20 antibody (IDEC-C2B8) has been produced and provided for clinical trials by IDEC Pharmaceuticals Co (San Diego, CA) and supplied under an Investigational New Drug Application.

Protocol design. This was a phase I clinical trial of single-dose IDEC-C2B8 chimeric anti-CD20 MoAb administered to patients with relapsed B-cell NHL. Detailed informed consent was obtained from all patients in accordance with the human subjects institutional review board of Stanford University Medical Center. Three patients were treated at each dose level with a single intravenous infusion of 10, 50, 100, 250, or 500 mg/m^2 of MoAb. Patients were evaluated for infusional related toxicity and effect on peripheral blood B cells, T cells, neutrophils and platelets, serum chemistries, Ig, and complement levels. In patients treated at the upper three doses, tumor biopsies were obtained 2 weeks after treatment and examined for evidence of antibody binding and B- and T-cell content. All patients were evaluated for antitumor activity.

Patient selection. On entry to the study, patients were required to have relapsed NHL with measurable disease after at least one prior course of standard therapy. A tumor biopsy was performed to document tumor cell expression of the CD20 antigen and reactivity with IDEC-2B8 or IDEC-C2B8 antibodies using flow cytometry. In addition, baseline hematologic function (1,500 granulocytes and 50,000 platelets/μL), renal function (serum creatinine of $<$2.5 mg/dL), quantitative serum IgG of greater than 600 mg/dL, a negative serology to human immunodeficiency virus, a negative hepatitis B surface antigen, and a life expectancy of at least 3 months without other serious illness was required. Patients previously exposed to murine antibodies were required to have no evidence of a pretreatment human antimurine antibody immune response (HAMA).

Flow cytometry. CD20 antigen expression was determined on all cases before antibody treatment by flow cytometry of fresh or cryopreserved tumor cell suspensions. Tumor cells were obtained from excisional biopsies or from fine needle tumor aspirations and stained for CD20 expression with fluorescein isothiocyanate (FITC)-conjugated IDEC-2B8 or IDEC-C2B8 (IDEC Pharmaceuticals) and Leu-16, an independent anti-CD20 antibody (Becton Dickinson, San Jose, CA). Tumor cells were also analyzed for expression of surface Ig light chains [FITC-goat $F(ab)_2$-antihuman κ or λ; Tago, Burlingame, CA), CD19, CD4, CD3, CD8 (FITC- or phycoerythrin [PE]-conjugated Leu12, Leu3, Leu4, and Leu2; Becton Dickinson), and CD37 (MB1 clone 6A4). Peripheral blood samples were analyzed for the number of cells expressing the CD20 antigen using two-color flow cytometry using PE or FITC conjugates of the above reagents.

Two-week posttreatment tumor biopsies were also evaluated for B- and T-cell content using the same reagents described above. Antibody bound to tumor cells from in vivo administration was detected by a combination of two different methods. In the first method, cells were stained using FITC-labeled anti-CD20 antibodies. The presence of the unlabeled antibody blocked the binding of the labeled antibody, resulting in decreased immunostaining of the B-cell tumor population (as identified using antibodies to additional B-cell antigens CD19, CD37, IgM, IgG, κ or λ). Second, the bound chimeric antibody was detected directly by looking for IgM κ- or λ-positive tumor cells now bearing the human IgG (κ) constant regions of the chimeric antibody (IDEC-C2B8) using an FITC-labeled goat $F(ab)_2$ antihuman IgG γ-chain–specific reagent (Tago). An estimate of the percentage of tumor cells with the chimeric antibody attached was obtained by comparing the staining of the pretreatment and the posttreatment biopsies for human IgG constant regions.

IDEC-C2B8 pharmacokinetics. Serum levels of the chimeric antibody were determined using an enzyme-linked immunosorbent assay (ELISA). Microtiter plates were coated with a purified polyclonal goat anti-IDEC C2B8 idiotype antiserum. After washing and blocking, posttreatment sera were serially diluted. Bound human IgG was then detected using a horseradish peroxidase (HRP)-conjugated polyclonal antihuman IgG reagent, and the plates developed with the substrate 2,2-azinobis(3-ethylbenzthiazoline sulfonic acid) (ABTS). Antibody concentration was determined by comparison of the signal from the patients sera with that obtained from known concentrations of purified chimeric antibody diluted into normal human serum.

Measurement of host anti-IDEC C2B8 antibody response. Posttreatment sera from evaluations at 1, 2, and 3 months were analyzed for evidence of a host antichimeric antibody immune response using a sandwich ELISA with microtiter plates coated with IDEC C2B8, the murine antibody 2B8, or normal murine IgG. Dilution's of the patients sera were added and, after washing, detected with biotin-labeled IDEC C2B8 followed by Avidin-HRP and the substrate ABTS. This assay has a level of quantification of 5 μg/mL.

Study measurements. Patients were evaluated for infusional related toxicity using the National Cancer Institute's Common Toxicity Criteria. Hematologic, renal, and hepatic function was monitored before and after infusion and during monthly intervals after therapy. Sera for evaluation of antibody levels and pharmacokinetics, serum IgG and IgM levels, and CD20 expression on peripheral blood B cells was obtained at each follow-up visit. Tumor response was assessed by evaluation of tumor measurements from physical examination and from radiologic imaging studies. For 3 months after therapy, patients were evaluated at monthly intervals and then followed at 1- to 3-month intervals until disease progression was observed. A complete remission (CR) required complete resolution of all detectable disease. A partial remission (PR) required a greater than 50% reduction in measurable disease persisting more than 30 days. A minor response (MR) was defined as a 25% to 50% reduction in disease. Stable disease (SD) was defined as no significant change in tumor measurements without progression over the period of observa-

Table 1. Patient Characteristics

Patient No./Sex/Age	Dose (mg/m^2)	Tumor Histology	Stage*	Prior Therapy	Disease Bulk†	Maximal Response
001/M/53	10	FM	III	Splenectomy C-MOPP m-BACOD CHOP	+++ Leukemia	
002/M/55	10	FSC	III	XRT (total nodal) CVP Chl	+ Leukemia Splenomegaly	Delayed PR?
003/M/49	10	DILD (mantle zone)	IV	MACOP-B Chl Anti-Id (MoAb) CVP	++	
004/M/63	50	FSC DLC FM	IV	CVP Id-Vac ProMACE/CytaBOM	+	Mixed
005/M/61	50	FSC	III	XRT anti-Lym-1 (MoAb) Chl/P CVP	++	
006/F/59	50	SL	IV	CVP CVP CVP/BVP	+++	
007/F/58	100	DLC, monocytoid B cell	IV	MACOP-B XRT	+ Splenomegaly	PR
008/M/73	100	FM	IV	XRT Chl anti-CD4 (MoAb)	++	MR
009/M/65	100	FM	IV	Chl/P Splenectomy	++	Mixed
010/M/38	250	FSC (diffuse areas)	IV	Chl Velban CVP Anti-Id (IFN) Anti-Id (Chl) MINE/ESHAP ^{90}Y-B1	++ Splenomegaly	
011/M/46	250	DLID (mantle zone)	IV	CVP	+++ Splenomegaly	MR
012/F/52	250	FSC	IV	CHOP/XRT Chl/P Chl/P VACOP-B Fludarabine IFN	+++	
013/M/48	500	FM	IVb	CHOP DHAP CVP ^{90}Y-B1	+	MR
014/F/65	500	FM	IV	CHOP Ctx	+	MR
015/F/38	500	FSC	IV	ProMACE/MOPP XRT IFN	+	PR

Abbreviations: FSC, follicular small cleaved cell; FM, follicular mixed small and large cell; DLID, diffuse lymphoma intermediate differentiation; DLC, diffuse large cell; SL, small lymphocytic; XRT, radiation therapy; IFN, interferon α; ProMACE/MOPP, etoposide, cyclophosphamide, adriamycin, methotrexate, prednisone, nitrogen mustard, vincristine, and procarbazine; ProMACE/CytaBOM, cyclophosphamide, adriamycin, etoposide, prednisone, cytarabine, bleomycin, vincristine, and methotrexate; Ctx, cyclophosphamide; CHOP, cyclophosphamide, adriamycin, vincristine, and prednisone; CVP, cyclophosphamide, vincristine, and prednisone; ^{90}Y-B1, ytrium-labeled B1 (anti-CD20 MoAb); DHAP, cisplatin, cytarabine, and decadron; VACOP-B, VP-16, adriamycin, cyclophosphamide, vincristine, prednisone, and bleomycin; Chl/P, chlorambucil and prednisone; MINE-ESHAP, ifosphamide, novantrone, etoposide, platinum, and cytarabine; Anti-Id, anti-idiotype MoAb; Id-Vac, Idiotype vaccination; BVP, bleomycin, vincristine, and prednisone; Mixed, regression noted in some but not all areas.

* Clinical stage at disease diagnosis.

† Tumor bulk estimated from physical exam and CT scans of the chest, abdomen, and pelvis and graded as follows: multiple areas of adenopathy with largest mass <5 cm (+), nodal mass >5 cm (++), extensive disease with multiple areas >5 cm (+++).

tion. Progressive disease (PD) was noted when there was a 25% increase in measurable disease or the appearance of any new lesion.

RESULTS

Patient characteristics. Fifteen patients were entered into the study. The characteristics of these patients are shown in Table 1. Patients ranged in age from 38 to 73 years, with a mean age of 55 years. Fourteen of the patients had an initial tumor diagnosis of a low-grade histology, with 6 being predominately follicular small cleaved cell, 5 being follicular mixed small cleaved and large cell, 2 being diffuse intermediate cell (mantle zone), and 1 being small lymphocytic

Table 2. Infusional-Related Symptoms During Chimeric Anti-CD20 Antibody Therapy

Patient No.	Dose (mg/m²)	Dose given (mg)	Pre Rx CD20 (cells/μL)	Toxicity
001	10	16	1,630 (tumor)	Fever, rigor, bronchospasm
002	10	22	960 (tumor)	Fever, rigor, nausea
003	10	21	50	None
004	50	100	60	Fever
005	50	100	40	Fever, chill
006	50	90	140	Myalgia
007	100	180	130	Fever
008	100	188	50	Fever
009	100	200	580 (normal B)	Fever, rigor
010	250	520	90	Chill
011	250	575	130	Fever, headache
012	250	400	20	Fever
013	500	1,200	60	Fever orthostatic hypot. nausea
014	500	965	130	Fever, nausea
015	500	760	160	Fever, nausea, headache

lymphoma. One patient had an initial diagnosis of a diffuse large-cell lymphoma; however, a relapsed node biopsy showed a monocytoid B-cell histology. Two patients with an initial diagnosis of a low-grade lymphoma had histologic progression on tumor biopsies performed before antibody therapy (1 follicular large cell and 1 diffuse large cell). The majority of patients (12/15) presented with stage IV disease. Patients had lived with their disease a mean of 5 years (range, 1.7 to 9.5) at the time of antibody therapy. All patients had measurable progressive disease and had received a median of two prior regimens (range, 1 to 5) of conventional therapy within the past 1.5 years. Five patients had previously been treated with murine MoAbs, including 2 patients who had received radiolabeled anti-CD20 antibody therapy (1 with a short PR and 1 with no response). All patients previously exposed to murine antibodies were HAMA-negative at the time of treatment.

Infusional-related toxicity. All patients completed the planned antibody infusion. Infusional-related symptoms are detailed in Table 2. The most frequent side effect was low-grade fever, which was seen in 13 of 15 patients (grade II in 5/15). Three patients additionally developed rigors and 1 developed bronchospasm requiring transient administration of supplemental oxygen. One patient on multiple medications for high blood pressure developed orthostatic hypotension during the antibody administration. In all patients, the antibody infusion was temporarily discontinued when significant side effects were observed. If necessary, the patients were treated as indicated with diphenhydramine and acetaminophen. Antibody infusions were usually restarted within 30 to 45 minutes at 50 to 100 mg/h and then escalated as tolerated to 200 mg/h. No significant further toxicity except fever was observed in any patients during the remainder of the antibody infusion. Three of the 4 patients having more significant reactions were also noted to have the highest levels of pretreatment $CD20^+$ cells in the peripheral blood (Table 2). Interestingly, these cells could be malignant (patients no. 1 and 2) or normal (mixed κ or λ phenotype, as observed in patient no. 9).

Effect on circulating B cells. In all patients, the numbers of B cells present in the peripheral blood before and after anti-CD20 therapy were analyzed using two-color flow cytometry. B cells were identified using the B-cell antigens CD19 and surface Ig, neither of which is blocked by the binding of the chimeric antibody to CD20. Bound antibody from in vivo administration was identified on the B cells by finding human IgG or κ bound to B cells coexpressing IgM and λ, or by the blocking of binding of a directly labeled anti-CD20 antibody. There was a dose-dependent, rapid, and specific depletion of the B cells in all patients, especially those receiving doses of more than 100 mg. In all but 1 patient receiving the higher doses (>50 mg/m²), these depletions persisted for 1 to greater than 3 months. The specificity of the B-cell depletion in a patient receiving 100 mg/m² is shown in Fig 1. After intravenous administration of 180 mg of antibody, there was a rapid and complete disappearance of B cells expressing CD20, CD19, κ, or λ surface antigens. There was no effect on the number of T cells (CD3), and the decrease in the numbers of lymphocytes is accounted for entirely by the decrease in the numbers of B cells. Peripheral blood B-cell levels for the duration of the 3-month study are shown on all 15 patients in Table 3. Three patients had circulating tumor cells, as shown by a clonal expansion of cells containing the same heavy and light chain isotype as identified on cells of their lymphomas obtained from tumor biopsies. Three patients had no detectable peripheral blood B cells at the 3-month evaluation. Follow-up is available

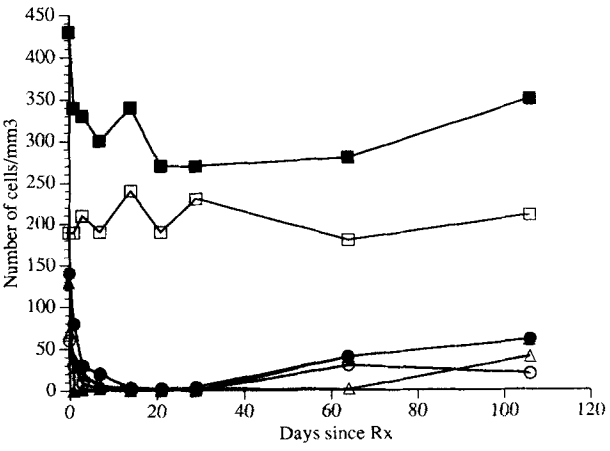

Fig 1. Depletion of peripheral blood B cells after 100 mg/m² chimeric anti-CD20 antibody. Patient no. 007 was treated with 100 mg/m² of chimeric anti-CD20 administered intravenously on day 1. Peripheral blood mononuclear cells were analyzed by flow cytometry for the expression of B- and T-cell antigens. There was a rapid and specific depletion of B cells, as indicated by the loss of cells expressing κ or λ light chains as well as the B-cell antigens CD19 and CD20. (■) Lym; (●) CD19; (▲) CD20; (□) CD3; (△) K; (○) L.

Table 3. Depletion of B Cells From the Blood After Infusion of Chimeric Anti-CD20 Antibody

Patient No.	Dose (mg/m²)	Pre Rx CD19 (cells/μL)	B-Cell Depletion Post-Rx CD19				
			d 3	1 wk	1 mo	2 mo	3 mo
001	10	1,940	995	1,260	ND	ND	ND
002	10	2,240	1,250	1,870	2,150	1,370	1,270
003	10	60	10	10	75	180	290
004	50	60	6	5	5	ND	ND
005	50	50	3	1	6	ND	ND
006	50	80	130	80	50	90	110
007	100	140	30	20	4	40	60
008	100	40	10	1	8	8	10
009	100	600	5	9	10	6	130
010	250	90	1	0	10	0	0
011	250	140	180	165	120	120	ND
012	250	20	5	0	3	0	0
013	500	60	4	5	0	6	20
014	500	130	7	20	3	0	70
015	500	160	7	1	0	0	0

Abbreviation: ND, not determined.

for 1 patient showing a return of B cells 7 months after treatment.

Serum antibody pharmacokinetics. Serum antibody was detected in all patients immediately after the intravenous infusion. Serum levels following 100, 250, and 500 mg/m² infusions are shown in Fig 2. In the 9 patients receiving 100 mg/m² or greater, the mean half-life of the antibody was 4.4 days, with a range from 1.6 to 10.5 days. Antibody levels of greater than 10 μg/mL persisted in the serum of 6 of 9 patients for more than 14 days. In 3 of 6 patients treated with 250 mg/m² or 500 mg/m², antibody levels were detected 1 month after antibody therapy. In one case, the antibody half-life was 10.5 days and serum antibody, capable of binding to pretreatment tumor cells was present more than 1 month after treatment. One patient (patient no. 011) treated at the 250 mg/m² dose had a large tumor burden with splenomegaly and a short half-life of the chimeric anti-CD20 antibody (1.6 days).

Detection of host anti-IDEC-C2B8 immune response. Sera were analyzed at monthly intervals for the 3-month duration of the trial for evidence of an immune response. No quantifiable antibody responses were detected against the chimeric antibody, with a level of quantification of 5 μg/mL. Subsequent analysis of serum from 7 patients at various intervals to 1 year of follow-up were also negative.

Analysis of posttreatment tumor biopsy specimens. In patients receiving 100 mg/m² or greater doses of the chimeric antibody, excisional tumor biopsies were performed 2 weeks after antibody therapy. Tissue sections of these biopsies were examined for histopathology and cell suspensions examined using two-color flow cytometry to determine cellular composition and to look for evidence of the chimeric antibody bound to the tumor cells from the in vivo administration. Successful lymph node biopsies were obtained in 7 of these 9 patients. In 1 patient, a large node was resected; however, histologic examination showed necrosis without any viable

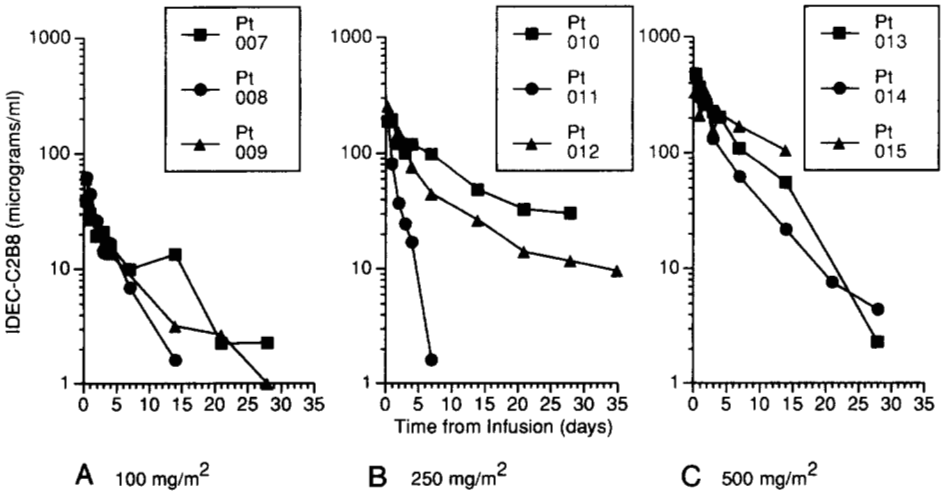

Fig 2. Pharmacokinetics of chimeric anti-CD20 antibody in patients receiving 100, 250, or 500 mg/m² infusions. Serum from 9 patients treated with a single infusion of the chimeric anti-CD20 antibody were analyzed for IDEC-C2B8 by ELISA. Results from each patient treated at 100 mg/m² (A), 250 mg/m² (B), and 500 mg/m² (C) are shown.

Table 4. Summary of Pretreatment and 2-Week Posttreatment Tumor Biopsies

Patient No.	Dose (mg/m²)	Date of Bx	Lymphoma Histology	Analysis by Flow Cytometry		
				% B (CD37)	% T (CD3)	C2B8 on Tumor Cells?*
007	100	10/9/90	Monocytoid B	79	21	—
		5/10/93	F&DMX	54	43	Yes (72)
008		3/24/92	FLC	53	43	—
		5/12/93	No tumor	—	—	—
009		9/16/92	FM	80	12	—
		5/17/93	Necrosis	—	—	—
010	250	6/4/87	FLC	63	26	—
		5/19/93	FM	51	44	Yes (30)
011		1/92	SLID	79	20	—
		5/26/93	SLID	83	16	No
012		5/11/93 fna	—	97	5	—
		6/1/93	FM	77	19	Yes (41)
013	500	10/28/92	FM	57	27	—
		6/1/93	FM	34	35	Yes (83)
014		5/11/93	FSC	85	12	—
		6/9/93	FSC	52	23	Yes (100)
015		6/7/93 fna	—	83	15	—
		6/22/93	FSC	60	40	Yes (93)

Abbreviations: fna, cells obtained by fine needle aspiration; FM, follicular mixed small and large cell; FLC, follicular large cell; FSC, follicular small cleaved cell; SLID, small lymphocytic intermediate differentiation (mantle cell); F&DMX, follicular and diffuse mixed small and large cell.

* Expressed as the percentage (shown in parentheses) of lymphoma cells in the biopsy specimen that stained positive for the expression of human IgG constant regions.

tumor. Flow cytometry also failed to detect any viable cells. A second patient had large bilateral inguinal lymph nodes present before therapy, but attempted surgical excisions in both inguinal regions after treatment failed to find any lymph nodes for biopsy. In the remaining 7 patients, lymph node material was available for analysis. Table 4 details the histologic findings present in the 2-week posttreatment tumor biopsies and compares the B- and T-cell content as determined by flow cytometry with tumor biopsies obtained before antibody therapy. In 3 patients, tumor biopsies were obtained immediately before treatment, and in the remaining patients comparisons were made to earlier cryopreserved cell suspensions obtained during the patient's course (from 8 months to 6 years earlier). Histologic examinations of the posttreatment samples remained diagnostic of lymphoma in the 7 patients. In 1 patient, the histologic appearance of the posttreatment tumor biopsy identified large numbers of hemosiderin-laden macrophages clogging the sinusoids of the lymph node. When the posttreatment samples were compared with the earlier biopsies, there appeared to be a decrease in the percentage of B cells (as determined by the expression of CD37 and CD19, both independent B-cell antigens not blocked by anti-CD20 antibodies) and a corresponding increase in the percentage of T cells seen in all but 1 patient. In addition, chimeric antibody was identified on the surface of the tumor cell population in the 2-week posttreatment biopsy in all but one case. In some cases, the bound antibody nearly completely saturated the available CD20 binding sites.

Effect on serum IgG, IgM, serum complement, and platelets. Sequential quantitative serum Ig levels were obtained pretreatment and monthly during the 3-month follow-up period. As shown in Fig 3, there was no significant change in the serum IgG (Fig 3A) or IgM (Fig 3B) levels over this period. IgA levels also were unchanged (data not shown). The platelet count was largely unaffected by the administration of antibody. One patient who started with a low pretreatment level developed moderate thrombocytopenia (Fig 3C). Two patients developed transient decreases in serum complement (C3) at 24 hours after therapy (Fig 3D).

Clinical antitumor effect. Despite the fact that this trial involved the administration of only a single infusion of antibody, tumor responses were observed. Partial tumor responses were documented in 2 patients and minor responses observed in 4 others. An example of a partial remission is shown in Fig 4. Patient no. 015, who had a follicular small cleaved cell lymphoma previously treated with ProMACE/MOPP, radiation therapy, and interferon was treated with 500 mg/m² of antibody. Computed tomography (CT) images of an abdominal mass pretherapy (Fig 4A and B) and 3 months posttherapy (Fig 4C and D) are shown. This tumor mass substantially decreased after treatment, and other nodes on CT scan and ultrasound examination as well as physical examination disappeared or significantly decreased in size. Disease progression was documented 8 months posttherapy, with recurrence of axillary adenopathy. A second patient treated with 100 mg/m² had a greater than 50% decrease in cervical adenopathy as well as resolution of splenomegaly (CT scan) lasting 9 months posttherapy. Patient no. 002, with follicular small cleaved cell lymphoma previously treated 10 years earlier with total nodal radiation and multiple courses of alkylating agents, was found on pretreatment evaluation to have circulating lymphoma cells and persistent thrombo-

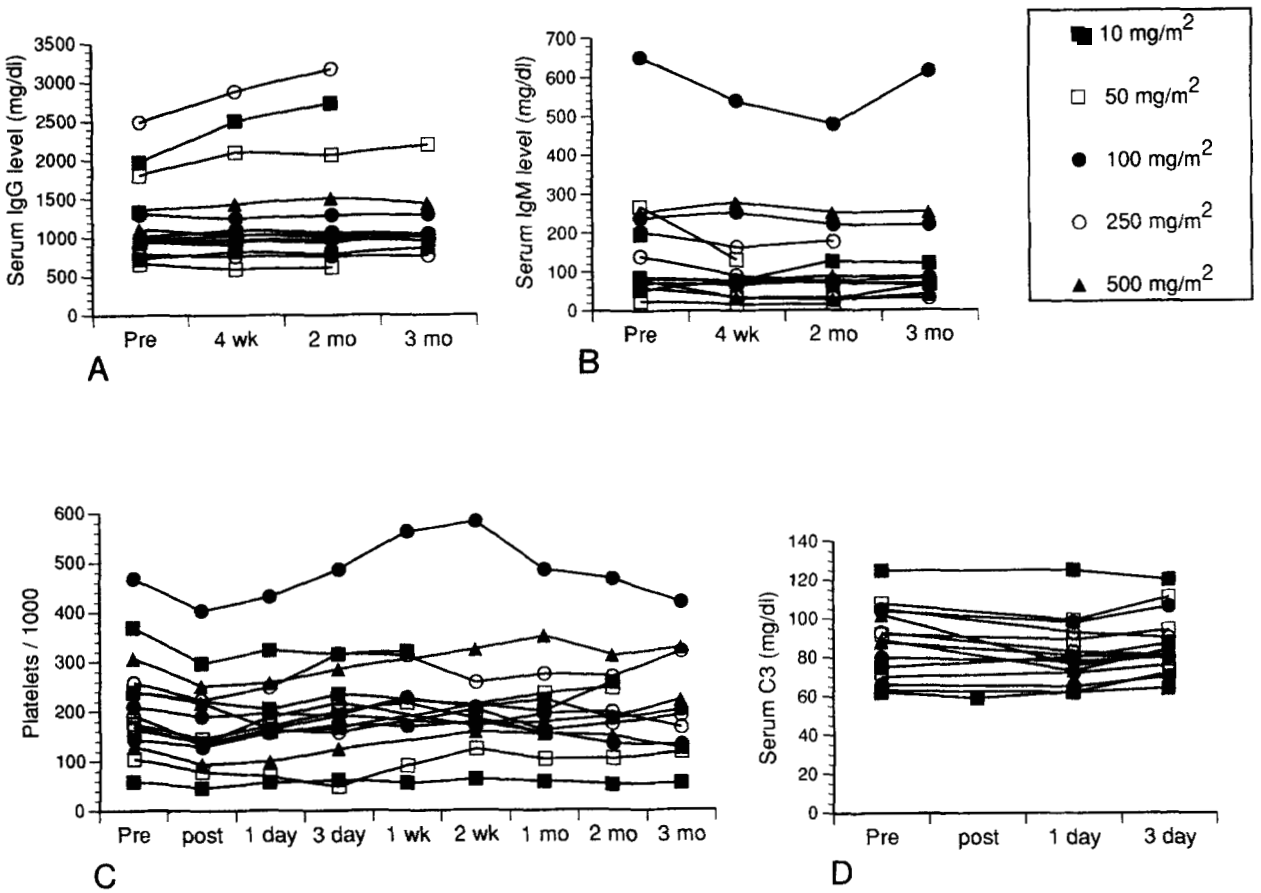

Fig 3. Effect of anti-CD20 antibody therapy on platelets, IgG, IgM, and serum complement levels. After therapy with a single infusion of chimeric anti-CD20 antibody patients were monitored for the effect on serum IgG (A), IgM (B), platelets (C), and serum complement-C3 (D).

cytopenia. He was treated with 10 mg/m^2 antibody. Throughout the 3 months of follow-up there was no significant change in disease measurement or thrombocytopenia. However, evaluation at 7 months showed a significant reduction in disease (lymph nodes and splenomegaly), resolution of thrombocytopenia, and disappearance of tumor cells from the peripheral blood lasting more than 1 year from therapy. Although no other lymphoma therapy had been administered, it is impossible to know whether the clinical improvement in this patient was spontaneous or was indeed related to the antibody treatment. Two patients had attempted excisional biopsies of known disease sites that showed necrosis in one instance and no tumor in a second case. Both patients had evidence of tumor regression with a mixed response and a minor response (25% to 50% decrease in measured lesions), the latter lasting 11 months. Shorter minor responses were also observed in 2 other patients. Additional mixed responses were observed in several patients. One patient with a history of low-grade lymphoma with transformation in bone to diffuse large-cell lymphoma had complete resolution of peripheral disease (last biopsy follicular mixed lymphoma), but progressed 2 months after therapy with relapsed large cell lymphoma again involving bone. He received local radiation therapy to the involved bone, but remains in remission of his peripheral disease.

DISCUSSION

In this phase I clinical trial, patients with relapsed NHL received a single infusion of chimeric anti-CD20 MoAb IDEC-C2B8 in doses ranging from 10 to 500 mg/m^2. All patients received the planned dose and no dose-limiting toxicities were identified. Symptoms were mild to moderate and easily manageable and more commonly observed in the 3 patients with higher numbers of CD20 antigen-bearing B cells (normal or malignant) present in the peripheral blood, suggesting that the destruction or removal of these cells during the early portions of the antibody infusion may contribute to the adverse events observed.

Analysis of antibody pharmacokinetics in patients receiving doses of 100 mg/m^2 or greater showed a mean serum half-life of 4.4 days, with a range from 1.7 to 10.5 days. However, it is difficult to establish the half-life of antibody in patients with widely different degrees of tumor burden receiving a single nonsaturating dose of antibody. It is likely that the true half-life will be longer once sufficient antibody is administered to saturate all tumor and normal CD20 antigenic sites. In the majority of patients receiving these doses, levels of greater than 10 μg/mL were present in the serum 2 weeks after therapy. Lower levels were identified in patients with extensive disease. Two-week postinfusion lymph

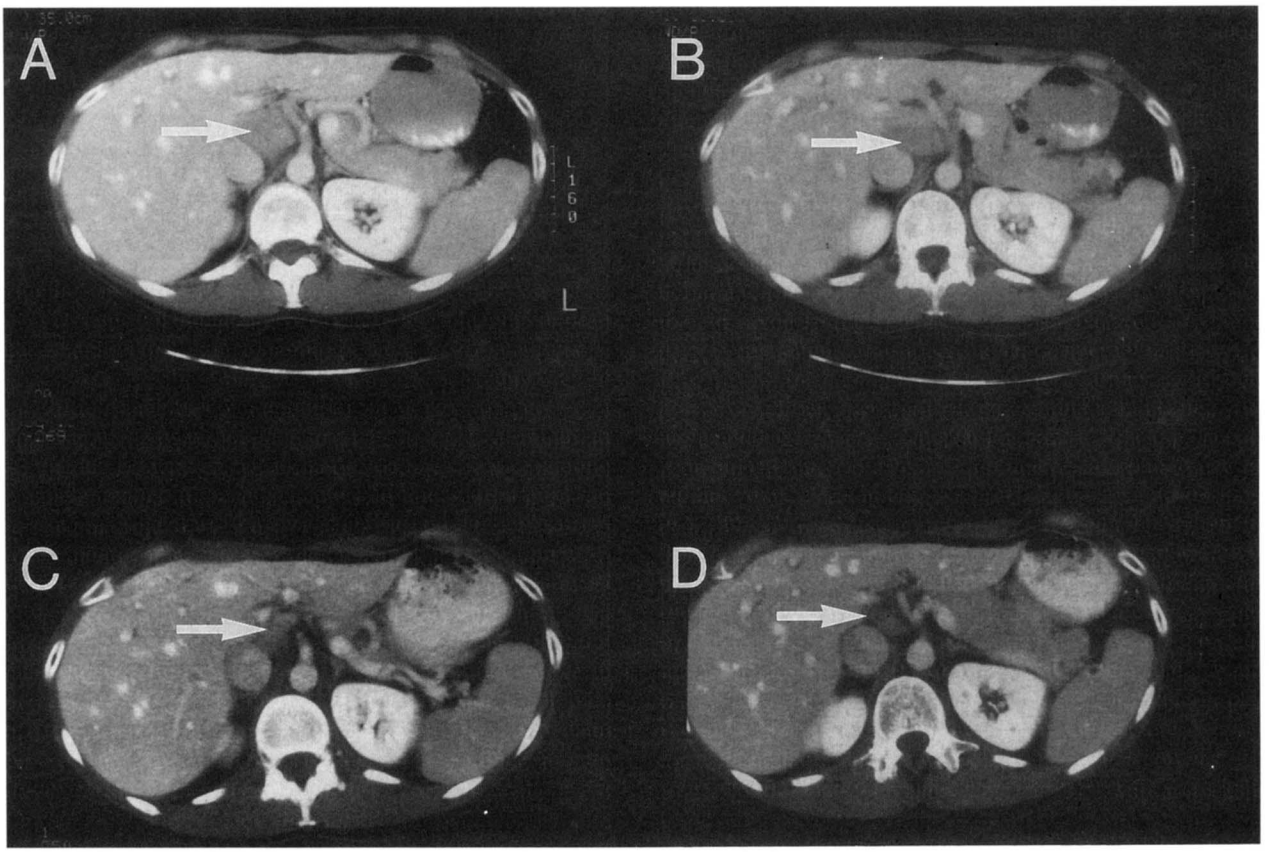

Fig 4. Example of antitumor effect. Patient no. 015 with relapsed follicular small cleaved cell lymphoma was treated with 500 mg/m^2 chimeric anti-CD20 antibody. CT images of an abdominal mass from two contiguous images are shown in (A) and (B). The corresponding images from a scan performed 3 months after antibody therapy are shown in (C) and (D), demonstrating partial regression of the tumor.

node biopsies were performed in the 9 patients receiving doses of greater than 100 mg/m^2. In 7 of the 9 biopsies, tumor was identified on pathologic examination. However, in 2 patients, tumor was not identified; in 1 case a large node that had become smaller after therapy was necrotic and in a second case inguinal lymph nodes present before therapy were not found after surgical exploration of left and right inguinal regions. In some of the biopsies, an increased infiltrate of macrophages was observed. Analysis of the posttreatment lymph nodes by flow cytometry identified tumor cells coated with nonsaturating amounts of the chimeric antibody in 6 of the 7 cases. The single exception was a patient with a large tumor burden, splenomegaly, and a short serum half-life of the administered antibody. In the majority of cases, a general decrease in the percentage of B cells and an increase in the percentage of T cells was observed when comparing pretreatment and posttreatment biopsies.

Treatment caused a selective elimination of the peripheral CD20-expressing B cells in all but 1 patient receiving doses of 100 mg/m^2 or greater. This was observed 24 to 72 hours after MoAb infusion. Over the 3-month follow-up phase, peripheral blood B cells slowly returned to base line in patients treated with the lower doses and partially in patients receiving the higher doses. There was no apparent effect on the serum IgG, IgM, or IgA levels through 3 months of follow-up. The CD20 antigen is not expressed on the early B-cell precursors or on the antibody-secreting plasma cell. The compartment of B cells/plasma cells is much larger than the few cells observed in the peripheral blood, and it is possible that they were not effected by the single dose of antibody. In addition, the long half-life of serum IgG may mean that late effects on serum IgG will be observed. There did not appear to be any increased incidence of opportunistic infections in this group of patients during the 3 months of follow-up. Depletion of normal B cells is common after high-dose chemotherapy and autologous bone marrow reconstitution that slowly resolves 3 to 6 months after transplant.[22,23] Depression of Ig levels can be treated by Ig transfusions.[24] It is possible that longer duration of antibody therapy achieving saturating levels may also cause a greater antitumor effect and a greater effect on the normal B cells and, ultimately, serum Ig.

The mechanism of the antibody-induced antitumor effect is not clear. Serum complement levels (C3) were slightly changed in only 2 patients during therapy. The chimeric antibody is capable of lysing target tumor cell lines in vitro by complement and antibody-dependent cell-mediated lysis.[21] In addition, direct effects of antibody binding to the

CD20 antigen, including growth inhibition, have been reported.[12] It is likely that a combination of these mechanisms is involved in the tumor regressions observed in these patients. Studies using murine anti-CD20 antibodies have also noted antitumor effects.[13] Two recent reports detailing the use of radiolabeled anti-CD20 antibodies describe impressive clinical activity with complete or partial remissions in the majority of patients.[14,15] Both also note tumor regressions associated with the imaging portion of the studies, suggesting clinical activity of the murine antibody.

The use of a chimeric naked antibody offers some advantages over similar trials using toxin-conjugated or radiolabeled antibodies against B-cell NHL. The antibody preparation is used directly for therapy, not requiring conjugation to drugs, toxins, or radiolabels, each of which requires extensive safety testing and may not be stable after formation of the active conjugate. Antibody modification may interfere with antigen binding. Radioiodinated antibodies are unstable, and undergo autolysis, and it is technically difficult to obtain consistent conjugates for large-scale clinical trials. In addition, significant hematologic toxicity is associated with the use of high-dose radiolabeled conjugates, making the application of this approach difficult in patients with impaired bone marrow function or significant involvement by lymphoma. In some studies, immunotoxin conjugates have been associated with significant toxicities.[25] In contrast, this chimeric anti-CD20 antibody is stable and has been engineered to lyse tumor cells through interaction with the patient's own immune system.

The modest tumor responses observed in this trial occurred after the administration of a single infusion of the chimeric antibody. Extension of these studies using multiple doses to achieve prolonged, tumor-saturating levels may lead to responses in patients with more extensive disease. Ultimately, extension of these studies to patients with minimal residual disease, using antibody alone or in combination with conventional therapies, may provide the greatest benefit. Based on these observations of safety and tumor responses to a single infusion of this chimeric anti-CD20 MoAb, a phase I/II trial using four weekly doses of antibody in patients with relapsed B-cell NHL has been initiated.

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