Monoclonal Antibody 1F5 (Anti-CD20) Serotherapy of Human B Cell Lymphomas

By Oliver W. Press, Frederick Appelbaum, Jeffrey A. Ledbetter, Paul J. Martin, Joyce Zarling, Pamela Kidd, and E. Donnall Thomas

Four patients with refractory malignant B cell lymphomas were treated with continuous intravenous (IV) infusions of murine monoclonal antibody (MoAb) 1F5 (anti-CD20) over five to ten days. Dose-dependent levels of free serum 1F5 were detected in all patients. Two patients had circulating tumor cells and in both cases 90% of malignant cells were eliminated from the blood stream within four hours of initiation of serotherapy. Antigenic modulation did not occur, and sustained reduction of circulating tumor cells was observed throughout the duration of the infusions. Serial bone marrow aspirations and lymph node biopsies were examined by immunoperoxidase and immunofluorescence techniques to ascertain MoAb penetration into extravascular sites. High doses (100 to 800 mg/m²/d) and high serum 1F5 levels (13 to 190 μg/mL) were required to cost tumor cells in these compartments in contrast to the low doses that were adequate for depletion of circulating cells. Clinical response appeared to correlate with dose of MoAb administered with progressive disease (52 mg), stable disease (104 mg), minor response (1,032 mg), and partial response (2,380 mg) observed in consecutive patients. The patient treated with the highest 1F5 dose achieved a 90% reduction in evaluable lymph node disease, but the duration of this remission was brief (six weeks). This study demonstrates that high doses of 1F5 can be administered to patients with negligible toxicity by continuous intravenous infusion and that clinical responses can be obtained in patients given >1 g of unmodified antibody over a ten-day period.

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MONOCLONLAL ANTIBODY (MoAb) serotherapy of malignancy represents a theoretically attractive, potentially nontoxic approach for the treatment of neoplastic disease. Preliminary animal experimentation has demonstrated both the effectiveness and limitations of MoAbs that recognize tumor-associated antigens in preventing growth of murine hematologic malignancies. Early human trials have shown that infusion of antibodies recognizing lymphoid cell differentiation antigens is a well-tolerated therapy capable of coating tumor cells and causing tumor regression in some patients. However, the antitumor effectiveness of MoAbs has been limited by the presence of circulating free antigen, antitumor modulation, development of human antihomogeneous antibodies (HAMA), emergence of antigen-negative tumor cell variants, and the inadequacy of host effector cell mechanisms.

Here we present findings in four patients with B cell lymphomas treated with a murine IgG2a MoAb (1F5) chosen to avoid many of the previously encountered obstacles. MoAb 1F5 recognizes a 35,000 dalton antigen (Bp35, CD20) present on the surface of normal and malignant B cells that is not shed from the cell surface (unpublished observations), does not modulate in response to MoAb binding, and does not bind to any other normal tissues. Consequently, prolonged continuous MoAb 1F5 therapy can be administered without inducing the unresponsiveness to therapy that has necessitated intermittent bolus therapy in previous trials. We have administered 1F5 by continuous intravenous (IV) infusion (52 to 2,380 mg over five to ten days) to determine toxicity, kinetics, penetration to extravascular tissues, and efficacy. Our studies have shown 1F5 to be a minimally toxic therapy capable of depleting circulating tumor cells at low doses and lymph node tumor cells at high doses. However, responses were transient, suggesting that antibodies conjugated to toxins or radioisotopes might afford more lasting clinical benefit than unmodified antibody.

MATERIALS AND METHODS

Antibody preparation. Murine MoAb 1F5 (IgG2a) was produced in BALB/c mice and purified as previously described. Antibody 1F5, along with the B1 antibody, has been assigned to the CD20 (anti-Bp35) cluster group by the Second International Workshop on Human Leukocyte Differentiation Antigens. The reactivity of antibody 1F5 with normal and malignant B cells has previously been reported. MoAb 1F5 was purified from ascites by saturated ammonium sulfate precipitation followed by diethyl aminoethyl (DEAE)-Sephacyl (Pharmacia, Piscataway, NJ) column chromatography. Testing of the purified antibody by Microbiological Associates (Bethesda, MD) has shown it to be free of bacterial, viral, or endotoxin contamination. Preclinical testing in two macaques (M. fascicularis) injected with 1F5 IV showed that this antibody was capable of eliminating circulating B cells and penetrating lymph nodes without causing any acute toxicity (J. Ledbetter, unpublished observations, 1983). A battery of normal human autopsy tissues was screened for reactivity with antibody 1F5 by an indirect immunoperoxidase method. No reactivity was seen with any tissue except those known to be rich in B lymphocytes (tonsils, lymph nodes, spleen). Tissues failing to bind 1F5 included heart, thyroid, adrenal, lung, muscle, kidney, testis, skin, colon, breast, and brain.

Patient selection. Adult patients with histologically confirmed B cell lymphomas shown by immunoperoxidase or immunofluorescence techniques to be reactive with the 1F5 antibody were eligible.
for this study if they had failed previous conventional therapy (chemotherapy and/or radiotherapy), if they had normal renal and hepatic function (creatinine <2.0 mg/dL, bilirubin <1.5 mg/dL), had evaluable disease, had not received any other treatment for ≥ four weeks, had no other active medical problems, and signed an informed consent approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center.

Study design. Premeditation blood, marrow, and lymph node specimens were obtained and analyzed by conventional histopathology and by an indirect immunoperoxidase method (Vectastain, Vector Laboratories, Raritan, NJ) for evidence of tissue involvement with tumor cells capable of binding 1F5. Cell suspensions of these tissues were analyzed by two-color flow cytometry using a panel of fluorescein and phycoerythrin antibody conjugates to determine the baseline immunologic phenotypes of the resident cell populations (see below). Intradermal skin testing with 10 μg of antibody 1F5 in 0.1 mL of normal saline was performed, and no hypersensitivity responses were observed. Allopurinol (300 mg/d) was given throughout antibody administration. A bolus loading dose was given over one to two hours IV to rapidly achieve steady state serum antibody levels. The loading dose was calculated from the following equation: Loading dose = 1.4 x elimination half-time (in days) x daily maintenance dose. Preclinical studies of murine anti-CD20 antibody infusions in nonhuman primates (Ledbetter, unpublished data) and clinical trials of murine MoAbs administered to patients with graft-versus-host disease (GVHD) suggested an elimination half-time of 1.2 days, and this figure was used in calculating the loading doses. Patients were assigned a predetermined maintenance antibody dose that was diluted in 500 mL normal saline and administered by continuous IV infusion for five to ten days. (Patient 1 had premature discontinuation of his infusion after five days due to rapidly progressive lymphoma.) The maintenance antibody doses administered to the patients are summarized in Table 1. The dose escalation range was chosen to progress from safe low doses (5 mg/m²/d) known to be well tolerated for other MoAbs to high doses (400 to 800 mg/m²/d), which we felt were more likely to result in good tissue penetration. We initially planned to escalate doses between patients. However, because of the absence of toxicity, poor penetration of low doses of antibody into patients 1 and 2, and slow patient accrual, doses were escalated progressively in patients 3 and 4 (from 10 mg/m²/d to 800 mg/m²/d) to achieve high circulating antibody levels in blood that felt more likely to achieve extravascular tissue penetration.

<table>
<thead>
<tr>
<th>Table 1. Summary of Patient Characteristics</th>
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<tbody>
<tr>
<td>Patient</td>
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<tr>
<td>Age/Sex</td>
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<tr>
<td>Type of Lymphoma</td>
</tr>
<tr>
<td>Stage</td>
</tr>
<tr>
<td>Total Dose of 1F5</td>
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<tr>
<td>Duration of Therapy</td>
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<tr>
<td>Response</td>
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<td>Disease</td>
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Abbreviations: DML, diffuse mixed small and large lymphoma; WDLL, diffuse well-differentiated lymphocytic lymphoma (small lymphocytic, working formulation); DPDL, diffuse, poorly differentiated lymphocytic lymphoma (diffuse small cleaved cell, working formulation); DHL, diffuse "histiocytic" lymphoma (diffuse large cell lymphoma, working formulation).

Patient monitoring. Pretreatment tests included a history and physical examination, relevant radiographic studies and computed tomographic scans, chemistry batteries, uric acid levels, complete blood cell counts (CBCs) and differentials, prothrombin time, partial thromboplastin time, serum complement levels (CH50, C4, C3), immune complex levels (C1Q binding assay), urinalysis, ECG, and cell surface marker analysis. Patients were examined twice daily during antibody infusion. Serial serum specimens for 1F5 levels and antismouse antibody levels, blood counts, chemistries, and blood specimens for surface marker studies were obtained four hours after initiation of 1F5 therapy and daily thereafter. Patients were discharged at the termination of antibody infusions. Blood samples were obtained on an outpatient basis for the above tests on days 1, 2, 7, and 21 after cessation of therapy and monthly thereafter. Serum complement and immunoglobulin levels were tested pretreatment and on days 1, 5, and 10 and then at roughly monthly intervals for six months.

Response criteria. Standard response criteria were employed as follows: Complete response—disappearance of all measurable and evaluable disease; Partial response—reduction by ≥ 50% of leukemic cell counts and >50% reduction in the size of a measurable lesion, and no increase in the size of any measurable or evaluable lesions or appearance of new lesions; Stable disease: Less than a partial response without an increase of >25% in leukemic cell count and ≤ 25% increase in any measurable lesion. Progression: Increase in leukemic cell count (>25%), appearance of new lesions, or an increase of 25% or greater in any measurable lesion.

Measurement of free 1F5 and human antismouse antibody. Serum 1F5 levels and human antismouse antibody levels (HAMA) were measured by solid phase competitive inhibition radioimmunoassay (RIA) as previously described.

Detection of cell-bound 1F5. Assessment of tumor cell coating by infused antibody 1F5 was accomplished on serial specimens of peripheral blood, bone marrow, and lymph nodes by indirect immuneroxidase and immunofluorescence techniques. Peripheral blood and bone marrow mononuclear cells were obtained by Ficoll-Hypaque (LSM, Litton Bionetics, Kensington, MD) density gradient centrifugation. Lymph node biopsies were divided in thirds: one portion was minced into a single cell suspension, another part was fixed in formalin for routine histologic staining, and another portion was frozen in liquid nitrogen for immunohistochemical staining. Lymph node frozen sections were fixed to gelatin-coated glass slides and stained with rabbit antihuman immunoglobulin (Vectastain, Vector Laboratories, Raritan, NJ) using an indirect avidin-biotin technique. Pretreatment biopsies served as controls.

Cell suspensions of blood, bone marrow, and lymph nodes from antibody-treated patients were examined by flow cytometry (FACS IV, Becton Dickinson, Sunnyvale, CA) for the presence of surface 1F5 by using fluorescein-conjugated goat antimouse immunoglobulin (FITC-GAM1g; TAGO, Burlingame, CA). The mean fluorescence intensity of cells stained with FITC-GAM1g was compared to the intensity of cells incubated with excess 1F5 in vitro before staining to assess the saturation of binding sites in vivo. Relative CD20 surface antigen density was estimated for normal and malignant B cells by measuring the mean fluorescence intensity of cells stained in vitro with saturating quantities of fluorescein-conjugated anti-CD20 antibody after correcting for nonspecific fluorescence with a control reagent. Tumor cell surface antigen phenotypes were determined by both immunofluorescence and immuneroxidase methods using peroxidase, fluorescein, or phycoerythrin conjugates of MoAbs 10.2 (anti-CD5), HB10a (anti-DR), G1-4 or 3E10 (anti-kappa), and 2C3 (anti-μ) as previously described. Serial monitoring of these tumor cell markers demonstrated that CD20-negative tumor cells were not
generated during 1F5 therapy by antigenic modulation (data not shown).

CASE HISTORIES

Patient 1 was a 42-year-old man with stage IVB diffuse, mixed, small and large cell lymphoma who presented in 1982 with fever, diffuse lymphadenopathy, and hepatosplenomegaly. Previous therapy included CHOP (cyclophosphamide, adriamycin, vincristine, and prednisone) chemotherapy, intrathecal methotrexate, whole brain irradiation, splenectomy, sequential upper and lower hemibody irradiation, and four cycles of bleomycin, cytotoxic arabinoside, vincristine, procarbazine, and prednisone. He was referred for 1F5 therapy in July, 1984 because of refractory disease. He did not respond to low-dose 1F5 and was taken off study after five days of infusion because of progressive bone marrow (BM) and liver replacement with tumor. Salvage CHOP chemotherapy was given, but the patient died of progressive lymphoma on March 16, 1985.

Patient 2 is a 64-year-old man with stage IVA diffuse, small lymphocytic lymphoma diagnosed by lymph node (LN) and BM biopsy in 1976. He received multiple chemotherapeutic regimens (CVP, CHOP, chlorambucil, and CCNU, etoposide, and methotrexate) with partial responses. He was referred for 1F5 serotherapy in December 1984, 11 months after his last course of chemotherapy. He did not respond to low dose 1F5 but had stable disease that did not require therapy until the summer of 1985 when he was begun on bleomycin, etoposide, BCNU, and Decadron, to which he remains partially responsive.

Patient 3 was a 63-year-old man with stage IVA diffuse, small, cleaved-cell lymphoma involving lymph nodes, marrow, and spleen. Previous therapy included splenectomy, chlorambucil, and CVP. He was referred for 1F5 therapy in December 1984, one month after his last cycle of CVP because of the development of refractory disease with rapidly progressive adenopathy and lymphocytosis (>30,000 cells/μL). He showed a minor response to intermediate dose 1F5 therapy. ProMACE/MOPP chemotherapy was given in January and February 1985 without response. A partial response occurred after therapy with high-dose cytotoxic arabinoside, but the patient died with marrow aplasia in July 1985.

Patient 4 was a 45-year-old man with sclerosing, diffuse large-cell lymphoma presenting in January 1983 with bowel and lymph node involvement. Therapy included eight cycles of CHOP, intrathecal methotrexate, involved field abdominal irradiation, prophylactic cranial irradiation, and allogeneic marrow transplantation (in March 1984). He was referred for 1F5 therapy because of refractory lymphoma in late October 1985. He had been on dexamethasone (4 mg/d) for many months as symptomatic therapy for myalgias, and this was continued during serotherapy. After treatment with 1F5 there was a partial response that lasted six weeks. He then redeveloped progressive lymphoma and refused further treatment. He expired on December 21, 1985.

RESULTS

Serum 1F5 levels. Circulating free antibody levels were detectable by RIA in all patients throughout the period of infusion (Fig 1). Patients 1 and 2 received MoAb doses of 5 mg/m²/d and consistently had 1F5 serum concentrations of 0.3 to 1.0 μg/mL. Patients 3 and 4 received escalating antibody doses and had corresponding increases in 1F5 levels. For comparable antibody dosing the patients with circulating antigen-positive tumor cells (1 and 3) had lower serum levels of 1F5 than the patients who did not have significant numbers of circulating malignant lymphocytes (2 and 4, see Fig 1), probably reflecting the effect of antibody binding to tumor cells. Antibody remained detectable in the serum for as long as three weeks after termination of infusion in patient 4 (peak concentration 190 μg/mL). However, 1F5 levels fell into the undetectable range within three days of termination of infusion in patients 1 and 2 (peak levels 1 μg/mL) and within two weeks in patient 3 (peak level 13.4 μg/mL). Rough estimates of the serum elimination half-times were calculated to be 24 hours for patient 1 (from 0.93 μg/mL to 0.22 μg/mL in the 48 hours after termination of infusion), 42 hours for patient 3 (from 13.4 μg/mL to 9.0 μg/mL in 24 hours), and 52 hours in patient 4 (from 179 to 35.6 μg/mL over five days). Data for patient 2 were insufficient for estimation of a serum half-life. These elimination half-times are in good agreement with previous studies of murine anti-CD20 antibodies in nonhuman primates (J. Ledbetter, unpublished results) and studies of murine anti-T cell antibodies in patients with GVHD.19

CD20 antigen density on tumor cells. Table 2 summarizes the relative Bp35 surface antigen densities on patient lymphoid cells from blood, bone marrow, and lymph nodes as determined by direct immunofluorescent analysis.13,17 The density of this antigen on normal B lymphocyte populations is also listed for comparison. Patients 1 and 4 had CD20 densities on their malignant cells comparable to those seen on normal, resting B lymphocytes (eg, peripheral blood B cells and tonsil mantle zone B cells19). Patient 3 had a much higher surface antigen density on his lymphoma cells, comparable to that observed on normal, activated B cells (tonsil germinal center cells13). Patient 2 had a very low Bp35 antigen density on his lymph node and bone marrow tumor cells with a mean fluorescence intensity only 3.5 times higher than control cell populations lacking the antigen. (This degree of staining was unequivocally greater than control, however.) Patients 2 and 4 had negligible numbers of circulating tumor cells morphologically, confirming the negligible staining with FITC-1F5 seen by immunofluorescence (nearly all circulating lymphocytes were T cells in these patients). Of interest, the bone marrow of patient 4 was grossly involved with tumor but failed to bind FITC-1F5, suggesting that an antigen-negative tumor cell variant was responsible for infiltration of this tissue. With this single exception, the different sites of lymphomatous involvement within a given patient showed similar CD20 antigen densi-
Table 2. Relative CD20 Antigen Densities on Normal and Malignant Lymphoid Cells

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Relative Antigen Density</th>
</tr>
</thead>
<tbody>
<tr>
<td>Normal Tissues</td>
<td></td>
</tr>
<tr>
<td>1. Peripheral Blood T cells</td>
<td>2†</td>
</tr>
<tr>
<td>2. Peripheral Blood B cells</td>
<td>82</td>
</tr>
<tr>
<td>3. Tonsil Mantle Zone B cells</td>
<td>70</td>
</tr>
<tr>
<td>4. Tonsil Germinal Center B cells</td>
<td>256</td>
</tr>
<tr>
<td>Lymphoma Patients</td>
<td></td>
</tr>
<tr>
<td>1. Patient 1 a. Blood lymphocytes</td>
<td>96</td>
</tr>
<tr>
<td>2. Patient 2 a. Bone marrow</td>
<td>7</td>
</tr>
<tr>
<td>b. Lymph node cells</td>
<td>7</td>
</tr>
<tr>
<td>3. Patient 3 a. Blood lymphocytes</td>
<td>301</td>
</tr>
<tr>
<td>b. Bone marrow cells</td>
<td>301</td>
</tr>
<tr>
<td>c. Lymph node cells</td>
<td>235</td>
</tr>
<tr>
<td>4. Patient 4 a. Blood lymphocytes (uninvolved)</td>
<td>2</td>
</tr>
<tr>
<td>b. Bone marrow cells</td>
<td>2</td>
</tr>
<tr>
<td>c. Lymph node cells</td>
<td>84</td>
</tr>
</tbody>
</table>

*Expressed as the linear channel number of the mean fluorescence intensity measured on a FACS IV cell sorter for cells stained with saturating concentrations of fluorescein-conjugated anti-CD20 antibody by the method of Ledbetter and Clark.†

†Negative control (unstained) cells also showed a mean fluorescence intensity of 2.

‡Patient 1 had an unaspirable marrow and no accessible adenopathy, so immunofluorescent studies were done solely on circulating malignant cells.

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Regression of marrow lymphoma was not seen in any of these three patients.

The marrow of patient 4 was unusual in that it appeared to contain tumor cells that did not express the Bp 35 antigen. Although marrow aspirates and biopsies contained unequivocal large cell lymphoma, no tumor cells reactive with FITC-1F5 were detected by flow cytometry. In contrast, tumor cells in cervical and inguinal lymph nodes had the same morphology as the cells in the marrow but reacted strongly with antibody 1F5 as assessed by both immunoperoxidase and immunofluorescence techniques (see Table 2). As would be anticipated, infusion of antibody 1F5 had no effect on the antigen-negative tumor cells in the marrow of this patient.

**Effects of 1F5 on lymph nodes.** Patients 2, 3, and 4 had evaluable adenopathy that was biopsied before treatment and on the last day of antibody infusion. Immunoperoxidase and immunofluorescent analyses showed no penetration of antibody 1F5 into the nodes of patient 2 (who received 105 mg over ten days). There was minor perivascular penetration detectable only by immunoperoxidase methods in patient 3 (1,032 mg over ten days). In contrast, significant coating of tumor cells detectable by both immunoperoxidase and immunofluorescence (Figs 5 and 6) was present in patient 4 (2,380 mg over seven days) with 69% saturation of available binding sites. In vitro studies showed that an ambient 1F5 antibody concentration of 24 μg/mL was necessary to achieve 69% saturation of cell-surface binding sites. Since the serum 1F5 concentration in patient 4 at the time of his lymph node biopsy was approximately 190 μg/mL, we estimate that a 1F5 antibody gradient of 8:1 existed between serum and lymph node interstitial fluid.

No clinical response was observed in the nodes of patient 2. Some inguinal nodes regressed by 25% in patient 3, but most lymph nodes were unaffected. There was marked regression of all nodes in patient 4 with a calculated >90% reduction in tumor burden (Fig 7). Of note, the diminution of LN size did not begin until day 5 of antibody infusion, and progressive node shrinkage continued for three weeks after cessation of 1F5 infusion. The response duration was brief, however, with regrowth of LN occurring six weeks after therapy.

**Overall clinical response.** Patient 1 had diminution of...
circulating tumor cells, but progressive liver and marrow lymphoma required premature termination of 1F5 therapy and institution of salvage chemotherapy. Patient 2 had no response of his evaluable marrow or LN disease. Patient 3 had a minor response consisting of transient 90% reduction of circulating tumor cells and 25% shrinkage of some but not all LN but no response in the marrow. Patient 4 had a partial response consisting of 90% reduction of all evaluable LN.

Toxicity. No clinically significant toxicity was observed in any of our patients. Patients 1 and 4 had asymptomatic, intermittent, low-grade fever (38 to 39 °C) lasting six days and two hours, respectively. Transient decrements of platelet and neutrophil counts to 50% to 75% of baseline levels were observed in all four patients. These changes were rapidly reversible in all cases except patient 1, where progressive cytopenias were clearly due to marrow replacement with tumor. In the other three instances, the blood counts stabilized after one to two days and often demonstrated some recovery, even before discontinuation of antibody infusion. No bleeding or infectious episodes occurred during antibody treatment. No allergic, pulmonary, renal, hepatic, or cutaneous sequelae occurred. Renal function as measured by 24-hour creatinine clearance was unchanged after completion of antibody therapy. Complement consumption was observed in patients 1 (40% reduction of CH50 and C4 levels) and 3 (97% reduction of CH50, 94% reduction of C4 levels), but circulating immune complexes could not be detected at any time in any patient.

Effects of 1F5 on normal B cells. Attempts to monitor numerical changes and functional alterations of normal B cells in patients receiving 1F5 serotherapy (by assessing proliferative responses to polyclonal B cell mitogens and in vitro Epstein-Barr virus (EBV)-induced immunoglobulin synthesis) were precluded by the extremely low numbers of normal B cells that could be harvested from these patients following antibody infusion. The paucity of B cells was due to two factors: baseline deficiency of normal B lymphocytes (a common feature of advanced, refractory B cell malignancies) and depletion of normal as well as malignant B cells by 1F5 therapy (which has also been documented in normal nonhuman primates infused with anti-CD20 antibodies, [Jeffrey Ledbetter, unpublished results]). Quantitative immunoglobulin levels were carefully monitored in all patients before, during, and for up to five months after 1F5 therapy. Baseline hypogammaglobulinemia was present in patients 1 and 3, but no patient demonstrated a decline in any subclass of immunoglobulin consequent to 1F5 therapy.

Human antinmouse antibody levels. IgM HAMA were undetectable by RIA in all patients. Low levels of IgG HAMA (2 x pretreatment control levels) became detectable five months after serotherapy in patient 1 only.

**DISCUSSION**

This report summarizes our findings in four patients with refractory malignant B cell lymphomas treated with MoAb 1F5 (anti-CD20) by continuous IV infusion for five to ten days. Our study differs from previous serotherapy trials of hematologic malignancies by employing an antibody directed against a nonmodulating antigen. This feature allowed us to maintain continuous high serum antibody levels without inducing the tumor refractoriness generally encountered with modulating antibodies. The continuous infusion mode of administration allowed delivery of very high doses of antibody (up to 800 mg/m²/d in patient 4) without the significant pulmonary toxicity that is often observed following bolus injection of high MoAb doses. Serial kinetic measurements revealed a dose-dependent relationship between the amount of antibody infused and the concentration of free MoAb in the blood stream. We found that even low doses of 1F5 (5 to 10 mg/m²/d) were capable of depleting circulating tumor cells from the blood stream analogous to observations made using murine MoAbs T101 (for chronic lymphocytic leukemia) and J5 (for acute lymphocytic leukemia). However, in contrast to the studies with modulating antibodies T101 and J5 given by prolonged or repeated administration, the responses induced by the nonmodulating 1F5 were sustained throughout the duration of the infusion (five to ten days).

Although small doses of 1F5 sufficed to deplete circulating tumor cells, penetration of antibody into extravascular sites such as bone marrow and lymph nodes proved to be much more problematic. Intravenous administration of 400 to 800 mg/m²/d was required to achieve 69% saturation of binding sites on lymph node tumor cells. Even at these doses the intranodal distribution of antibody was heterogeneous. The immunoperoxidase staining patterns observed in LNs...
suggested passive diffusion of antibody down a concentration gradient from small blood vessels into the LN parenchyma. The clinical responsiveness observed appeared to correlate with IF5 dose administered, peak-serum MoAb concentration achieved, and degree of extravascular tissue penetration obtained. A total dose of 52.4 mg was associated with progressive disease (patient 1). 104.8 mg resulted in stable disease in patient 2, 1,032 mg caused a minor response in patient 3, and 2,380 mg produced a partial response in patient 4.

These observations have been made on a very small group of patients, each of whom had a different type of B cell lymphoma. The degree to which our findings might be applicable to other lymphoma patients is unclear, since each patient has a different tumor burden and distribution, different numbers of circulating malignant cells acting to absorb infused MoAb, and different surface densities of the Bp35 antigen. Consequently, the dose levels and kinetic data found in our patients can only serve as a rough guideline for the management of other patients with this antibody. Generalizations from our findings with IF5 to other MoAbs should be made with caution in light of recent studies demonstrating dramatic, unpredictable kinetic and functional differences among different antibodies recognizing the same antigen.

The toxicity seen in these four patients was insignificant. Minor fever and moderate cytopenias were the only adverse effects observed despite administration of massive doses of antibody IF5 to patients 3 and 4. Since neither platelets nor neutrophils express the Bp35 antigen or label with FITC-IF5, the exact mechanism for the decrement in neutrophil and platelet counts is uncertain. It is of interest that similar decrements in blood counts have also been observed in patients treated with anti-idiotypic antibodies. Small quantities of antibody might be absorbed via Fc receptors to these cells, which may then be removed from the circulation by the reticuloendothelial system. Although the development of HAMA has been a major problem in some reported series, antimeouse antibodies were detected in only one of our four patients, and in this patient they did not appear until five months after IF5 therapy was completed. These findings are in accord with other studies demonstrating that patients with B cell malignancies undergoing monoclonal serotherapy seldom develop HAMA, whereas patients with T cell malignancies or solid tumors receiving similar treatment often develop antimeouse antibodies.

Previous trials of MoAb serotherapy have also generally encountered minimal toxicity. The major adverse events described to date involved anaphylactoid reactions in patients with large circulating tumor cell burdens and/or high circulating antigen levels given high doses of antibody by rapid bolus injection. These episodes have been ascribed to pulmonary leukostasis resulting from sequestration of antibody-coated tumor cells in the pulmonary vasculature leading to wheezing, dyspnea, and hypotension. The absence of circulating antigen and the prolonged duration of antibody administration in our trial were mitigating factors that probably helped avoid these untoward sequelae in our patients.

The relative merits of continuous infusion of MoAbs compared with intermittent bolus therapy remain debatable. For many antibodies continuous infusion is not feasible because of antigenic modulation. In such circumstances intermittent therapy is necessary to allow regeneration of cell surface antigen. The bolus method is less cumbersome than continuous infusion and achieves higher peak MoAb concentrations for equivalent doses. Whether the maintenance of uniform high-circulating antibody levels and reduction in toxicity achievable with continuous infusion are sufficiently advantageous to offset the inconveniences remains unanswered. Nevertheless, maintenance of steady state antibody levels in this trial has afforded an advantageous setting for kinetic measurements and for the assessment of the serum concentrations required for MoAb penetration into the extravascular space.

The mechanisms by which unmodified MoAbs might cause elimination of tumor cells in vivo remain controversial. Most workers currently view antibody-dependent cellular cytotoxicity and reticuloendothelial system phagocytosis of MoAb-coated cells as the most likely processes involved. Mutine MoAbs (including IF5) fix human complement poorly in vitro, and consequently complement-mediated tumor cell lysis is not thought to be of major significance in vivo. The significant consumption of complement in two of our four patients was unexpected and suggests a possible role for complement in eliminating tumor cells.

The short, incomplete responses obtained with serotherapy using unmodified MoAbs have been of minimal clinical benefit (with the notable exception of the patient described by Miller et al). Consequently, innovative MoAb administration schedules, testing of new antibodies, and administration of antibody conjugates will be necessary if the promise of monoclonal serotherapy is to be realized. Badger et al have already convincingly demonstrated cures of lymphomas in mice treated with radioiodinated MoAbs in a setting in which unmodified MoAbs were ineffective. Recent clinical reports of responses in patients with Hodgkin's disease or hepatoma treated with radioidinated antiferritin antibodies suggest that this approach will also be useful in man. Radiolabeled MoAbs can potentially kill not only the tumor cells to which they bind but could also kill neighboring cells that do not bind antibody by virtue of poor tissue penetration, antigenic modulation, or somatic mutation ("antigen-negative variants"). Toxin-antibody conjugates are similarly promising, although antigens such as CD20, which are not endocytosed after ligand binding, might not be good targets for this approach, since immunotoxin internalization is generally required for cell killing. The findings of our current pilot study should assist in the rational design of subsequent trials employing such radiolabeled or toxin-conjugated immunotoxins.

ACKNOWLEDGMENT

We thank Mary Gallagher, Marsha Bolton, and Terri McLaren for competent assistance in performance of the immunofluorescence and immunoperoxidase studies and Anajane Smith for help in performance of the radioimmunoassays. We are grateful to Drs H. Straley, A. Keller, and S. Speckart for referring patients for these trials.
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Monoclonal antibody 1F5 (anti-CD20) serotherapy of human B cell lymphomas

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