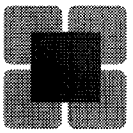

Treatment of chronic lymphocytic leukemia with an anti-idiotypic monoclonal antibody

MICHAEL J. CAULFIELD, PHD; SIVA MURTHY, MD; RAYMOND R. TUBBS, DO; JAMES SERGI, MSN; RONALD M. BUKOWKSI, MD

■ A monoclonal antibody (H-99) was prepared that reacts specifically with the leukemia cells from a patient with stage IV chronic lymphocytic leukemia of B-cell origin. Escalating doses of the antibody were administered to the patient in two courses of therapy. The first course did not result in any significant change in total lymphocyte count; however, the platelet count (which was initially abnormal) rose steadily during treatment to a level > 250,000 per mm^3. A second course of treatment was begun approximately three months after the first course, and the protocol was modified to consist of five doses of antibody given over a 10-day period, with the highest dose being 500 mg per day. The second course of treatment did not result in any change in total lymphocyte or platelet levels, and there was no significant toxicity associated with the treatment. The failure to induce an anti-tumor response in the patient was probably related to the large concentration (approximately 400 μg/mL) of free serum idiotype in addition to the severely compromised immune system of the patient at the time of treatment.

□ INDEX TERMS: ANTIBODIES, MONOCLONAL; CASE REPORTS; IMMUNOTHERAPY; LEUKEMIA, LYMPHOCYTIC □ CLEVE CLIN J MED 1989; 56:182–188

AN IMPORTANT consideration in the use of monoclonal antibodies in patients is to identify a "tumor-specific" antigen that can be used as a target for therapy. The approach taken by Miller et al[1] and Meeker et al[2], and adopted by our group, has been to prepare antibodies against immunoglobulins integrated into the membrane of the tumor cell. In the case of B-cell malignancies, this approach is feasible because most tumors of this type show membrane or surface immunoglobulins. The immunoglobulin molecules on each cell of a given B-cell tumor are identical; however, the "tumor immunoglobulins" are slightly different from every other immunoglobulin in the immune system. This difference reflects changes in the amino acid sequence of the tumor immunoglobulin such that a unique structure, called an idiotype, is formed. Since this idiotype is unique, it is also antigenic because the immune systems of animals of the same species recognize it as "foreign" and make antibodies against it. Thus, the idiotype expressed on a B-cell tumor can serve as a tumor-specific antigen, and antibodies prepared against such an antigen could serve as a reagent to treat a particular B-cell tumor without toxicity to host tissues.

Chronic lymphocytic leukemia (CLL) is characterized by the progressive accumulation of mature-appearing small lymphocytes in peripheral blood and various tissues. A standard diagnostic criterion involves demonstration of >15,000 lymphocytes/mm^3 of peripheral blood. Cell kinetic studies involving CLL indicate that

From the Departments of Immunology and Cancer Research (M.J.C., J.S.), Hematology and Medical Oncology (S.M., R.M.B.), and Pathology (R.R.T.), The Cleveland Clinic Foundation. Submitted Nov 1987; accepted June 1988.

Address reprint requests to M.J.C., Department of Immunology and Cancer Research, The Cleveland Clinic Foundation, One Clinic Center, 9500 Euclid Avenue, Cleveland, Ohio 44195.

the fractional production rate of CLL lymphocytes in peripheral blood and possibly lymph nodes is lower than normal, but that an increased lymphocyte life span may occur.[3] Clinically, CLL is found in older patients (median age, 60 years) and predominantly in males. Lymphadenopathy and hepatosplenomegaly are common findings. Morphologically, small, uniform, mature-appearing lymphocytes are found in the peripheral blood and lymph nodes. Occasionally, patients may have larger, less mature lymphocytes with nuclei demonstrating folding lobation and indentation. These are often termed prolymphocytes and, when present in large numbers, result in a variant called lymphosarcoma cell leukemia or prolymphocytic leukemia. Other clinical features include a paraprotein (5%), positive direct Coombs test (10%–12%), and hypogammaglobulinemia (50%–75%).

More than 99% of CLL is now thought to be derived from a monoclonal proliferation of B cells. Studies by Brouet and Seligmann[4] have further demonstrated that the surface immunoglobulin present on these B cells is usually IgM and IgD. In addition, typing of these immunoglobulins has shown they contain identical light chains, similar idiotypic specificities, and similar antibody activity. In a single patient, therefore, both surface IgM and surface IgD will have similar variable regions and differ only in the constant portions of the heavy chains. Occasionally, patients have tumor cells showing IgA or IgG on their surfaces, but this is unusual. When a monoclonal serum immunoglobulin is present in a patient with CLL, it will usually be IgM and will be identical to the surface IgM in all respects. Finally, and most importantly for this study, CLL lymphocytes in a single patient are usually clonal and in the rare instance when acute leukemia develops in patients with CLL, the peripheral blood blasts carried the same surface IgM as did the CLL lymphocytes.[5] In view of this, it would be expected that monoclonal antibodies prepared against surface immunoglobulin idiotypes would be specific only for the malignant lymphocytes of an individual patient without the cross-reactivity associated with monoclonal antibodies produced against differentiation antigens.

Miller et al[1] have pioneered the treatment of patients with B-cell lymphoma using anti-idiotypic monoclonal antibodies. In their first patient, increasing doses of the anti-idiotypic monoclonal antibody were administered over a three-week period, during which time the patient's fevers and sweats disappeared and his hemoglobin level and platelet count began to rise. Over the next three weeks, the patient's lymph nodes gradually became smaller, his spleen and liver returned to normal size, and the tumors on his scalp disappeared. After eight doses of anti-idiotype over a four-week period, treatment was discontinued. The patient has remained in remission for more than five years. Based on this early success, 10 more patients with B-cell lymphomas were treated with tumor-specific anti-idiotypic antibodies by Meeker et al.[2] Five of the 10 additional patients had objective responses; however, the remissions were not complete and were of short duration.

Most of the patients studied by Meeker et al[2] had nodular B-cell lymphomas rather than CLL. We wanted to determine whether monoclonal anti-idiotypic antibodies could be effective in the treatment of a patient with CLL of B-cell origin. The patient, who initially had thrombocytopenia, was treated with two courses of the anti-idiotypic antibody. During the first course, therapy was accompanied by a steady increase in the number of platelets well into the normal range. However, in neither course of therapy was the number of circulating lymphocytes significantly reduced. Treatment with the anti-idiotypic antibody was complicated by a high concentration of circulating free serum idiotype (> 350 μg/mL). Sustained levels of free anti-idiotypic antibodies could only be achieved transiently with high doses of the anti-idiotypic monoclonal antibody because of complex formation with free serum idiotype.

MATERIALS AND METHODS

Case report

The patient was a 58-year-old man with an eight-year history of CLL. Prior therapy included total body irradiation, intermittent administration of cyclophosphamide, administration of chlorambucil (Leukeran) and high-dose corticosteroids, and a splenectomy. Recent problems included increasingly severe anemia, neutropenia, thrombocytopenia, chylous pleural effusions, and recurrent fevers. The initial physical examination demonstrated a temperature of 102°F (38.9°C), bilateral cervical lymph nodes measuring 1 × 1 cm, and a left-sided pleural effusion. Initial studies included a complete blood count, which demonstrated a hemoglobin level of 7.1 g/dL; a platelet count of 114,000/μL; and a white blood cell count of 164,000/μL. A differential smear demonstrated 99% lymphocytes and 1% granulocytes. His initial serum creatinine level was 2.4 mg/dL and BUN, 52 μg/dL. A chest radiograph showed a large left pleural effusion, and an ultrasound examination of the abdomen disclosed retroperitoneal adenopathy. All urine and blood cultures were sterile.

Initial therapy included periodic transfusion and em-

TABLE 1
TREATMENT SCHEDULE

Course	Day of study	Dose of mAb (mg)
I	1	1
	4	5
	8	15
	11	50
	15	100
	18	100
II	1	5
	3	20
	6	100
	8	500
	10	500

piric antibiotics, including tobramycin, piperacillin, and nafcillin. The antibiotics were continued for 14 days. The patient's serum idiotype concentration was 390 µg/mL. Once he underwent study, he received twice weekly treatments with the monoclonal antibody at the following dose levels: 1, 5, 15, 50, 100, 100, and 100 mg each given as six-hour intravenous infusions in a 5% albumin solution (*Table 1*). No toxicity was observed at any dose level. The first course of treatment did not result in any significant change in the total lymphocyte count; however, the platelet count rose steadily during the treatment to a level >250,000 per mm^3. Beginning with the 15-mg dose, marked decreases in the serum idiotype level occurred, then returned to their original levels within three days. After each 100-mg dose, the monoclonal antibody could be detected in the serum at a level of approximately 1 µg/mL. No significant changes in renal function occurred, and following completion of the first course of treatment, the patient's creatinine level was 2.4 mg/dL.

A second course of therapy was begun approximately three months after the first course, and the protocol was modified to consist of five doses of antibody given over a 10-day period, with the highest dose levels being 500 mg/day. The second course did not result in any change in the total lymphocyte or platelet levels, and there was no significant toxicity associated with the treatment. The patient was rehospitalized four weeks later with increasing respiratory distress secondary to increasing pleural effusion. His blood count at that time included a hemoglobin level of 10.0 mg/dL, platelet count of 50,000/µL, and white blood cell count of 80,000/µL. A chest radiograph demonstrated increasing mediastinal lymphadenopathy. Gram-negative septicemia developed and, despite therapy with antibiotics and ventilating support, the patient died five months following initial therapy with the monoclonal antibody.

Preparation of anti-idiotypic monoclonal ntibodies

Our strategy for the preparation of hybridomas was to immunize mice using intact leukemia cells that showed the tumor-specific idiotype on the surface immunoglobulin and then to assay hybridomas for production of specific antibodies using tumor immunoglobulin isolated from cultured CLL cells. Using the immunization schedule of Cianfriglia et al,[6] BALB/c mice were injected intraperitoneally with 10^6 CLL cells (in Freund's complete adjuvant) from the patient on day 0 and challenged with 10^6 cells in Freund's incomplete adjuvant on day 7. On days 12, 13, and 14, the mice were injected intraperitoneally with 10^6 cells in saline. On day 15, the spleen cells from the mice were removed and fused with the nonsecreting myeloma cell line X63-Ag 8.653,[7] as described by Goding.[8]

Supernatants from hybridoma cultures were screened for antibody production using an ELISA assay in which goat anti-mouse immunoglobulin (GAMIg) was used to "trap" the tumor immunoglobulin from cultured CLL cells. Thus supernatant from the cultured CLL cells (tumor immunoglobulin) was added to plates coated with GAMIg and incubated overnight at 4° C. After washing the plates four times, supernatant from the hybridoma cells was added to provide the third layer of this sandwich assay. After an overnight incubation at 4° C, the plates were washed four times and then alkaline phosphatase-conjugated GAMIg was added as the fourth layer to bind with any hybridoma antibody that may have bound to the preceding layer. Finally, the substrate for alkaline phosphatase (*p*-nitrophenyl phosphate) was added to detect antibodies binding to the tumor immunoglobulin. All hybridoma samples were assayed in parallel with plates in which normal human IgM replaced the tumor immunoglobulin. Positive anti-idiotypic antibodies were those that reacted with the tumor immunoglobulin and not with the normal human IgM. The positive hybridoma that was isolated in this manner (H-99) was cloned three times by limiting dilution[9] and then grown as ascites in mice to obtain large amounts of anti-idiotypic antibody for subsequent treatment of the patient.

Purification of the anti-idiotypic antibody

The ascites fluid containing the IgM class anti-idiotypic antibody was partially purified by ammonium sulfate precipitation (50% saturation). The precipitate was dissolved in 0.85% saline and dialyzed against saline to remove residual ammonium sulfate. Subsequently, the euglobulin fraction (containing IgM) was prepared by dialysis against 2% boric acid buffer. The precipitate was

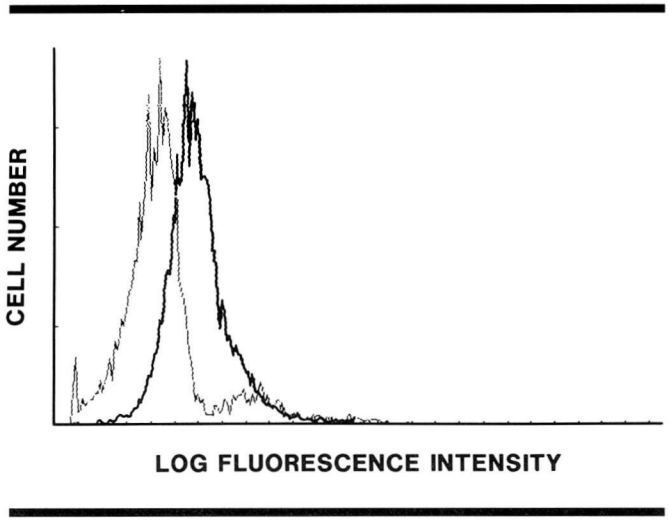

FIGURE 1. Flow cytometry analysis of peripheral blood cells from the CLL patient, using a control IgM monoclonal antibody (left) and the IgM anti-idiotype (H-99) (right).

dissolved in 0.85% saline and filter sterilized through a 0.45-μm filter. Sterility testing showed no bacterial growth using thioglycolate broth, and endotoxin levels were less than 0.1 ng/mL using the limulus amoebocyte lysate assay. This final preparation of anti-idiotypic antibody contained approximately 50% mouse IgM as determined using our ELISA procedure.

Assay for serum idiotype levels

A multi-layer ELISA assay was used to determine the level of idiotype in the patient's serum. Microtiter plates were first coated with GAMIg to "trap" the mouse anti-idiotypic monoclonal antibody (H-99). Thus hybridoma supernatant containing approximately 2 μg/mL of H-99 monoclonal antibody was added to plates coated with GAMIg and incubated overnight at 4° C. The plates were washed four times, and then serial dilutions of the patient's serum were added to the wells of the plates and incubated overnight at 4° C. Alkaline phosphatase-conjugated goat anti-human immunoglobulin was then added and incubated overnight at 4° C. Finally, the reaction was developed using the substrate, *p*-nitrophenyl phosphate. The optical density at 405 nm was determined using a Dynatech MR 580 MicroELISA reader. Antibody concentrations were calculated using a computer program developed here.[10]

Assay for mouse (anti-idiotypic) antibodies in human serum

Serial dilutions of the patient's serum were added to the wells of plates precoated with GAMIg. After an overnight incubation at 4° C, the plates were washed and alkaline phosphatase-conjugated GAMIg was added to the wells. After another overnight incubation at 4° C, the plates were washed and the reaction developed with *p*-nitrophenyl phosphate. The optical density at 405 nm was read, and the antibody concentrations were determined as described above.

Assay for human anti-mouse immunoglobulin antibodies

Human anti-mouse immunoglobulin antibodies were assayed using an ELISA in which serial dilutions of the patient's serum were added to plates coated with mouse IgM (41-H11) or IgG (FLOPC-21) monoclonal antibodies. After an overnight incubation at 4° C, alkaline phosphatase-conjugated goat anti-human immunoglobulin was added. The plates were incubated at 4° C overnight. The reaction was then developed with *p*-nitrophenyl phosphate. Concentrations were determined by measuring the optical density at 405 nm, as described previously.

Immunocytometry

Peripheral blood lymphocytes were obtained by gradient centrifugation over Ficoll-Paque in the usual manner, washed with tissue culture medium, and counted. A total of 1×10^6 cells were incubated with anti-idiotype supernatant for 30 minutes at 4° C, washed twice with cold modified RPMI, and incubated for 30 minutes at 4° C with phycoerythrin-conjugated $F(ab)_2$ fragment GAMIgM. The cells were then washed twice at 4° C with modified RPMI and fixed with 2% paraformaldehyde. Controls included the use of an irrelevant mouse IgM class hybridoma supernatant in place of anti-idiotypic antibody followed by the phycoerythrin-conjugated secondary antiserum at the same dilutions. An additional control consisted of normal peripheral blood lymphocytes incubated with anti-idiotypic antibody in an identical manner. The immunostained cells were analyzed with an upgraded FACS II equipped with Consort 40 software.

Immunocytometry of peripheral blood lymphocytes for lymphocyte differentiation antigens

Aliquots of washed peripheral blood lymphocytes were placed in test tubes (1×10^6 cells per tube) and allowed to incubate at 4° C in the dark with FITC-CD2 and PE-CD20 monoclonal antibodies. Appropriate FITC-PE isotypic control conjugates were included as controls. Analysis was done as stated above.

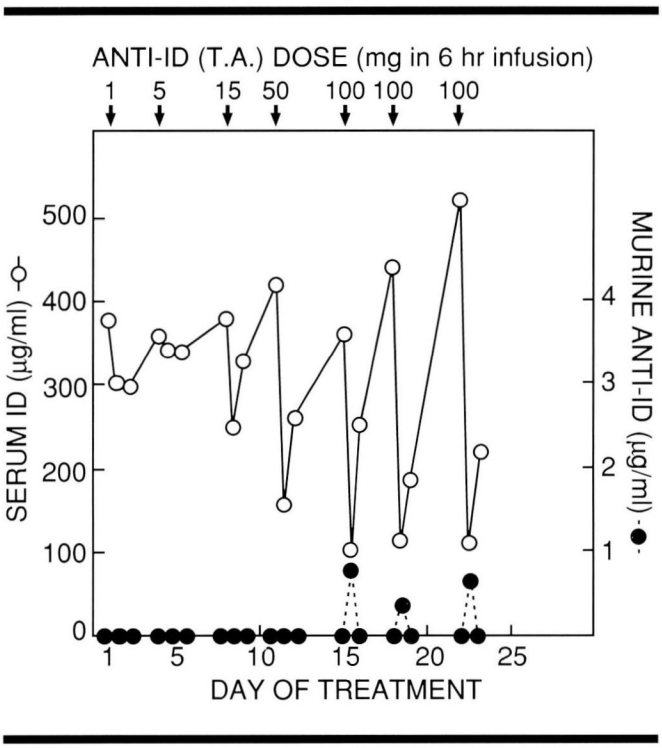

FIGURE 2. Pharmacokinetic analysis of the patient's serum idiotype (open circles) and the monoclonal antibody (closed circles) during course 1 of treatment.

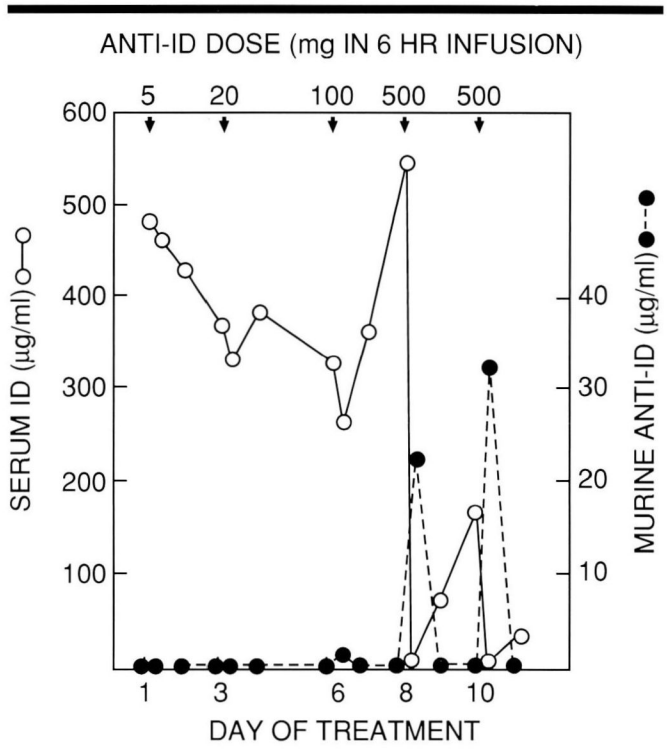

FIGURE 3. Pharmacokinetics of serum idiotype (open circles) and anti-idiotype monoclonal antibody (closed circles) during course 2 of treatment.

RESULTS

Reactivity of H-99

H-99 was derived and tested for idiotypic specificity as described previously. The monoclonal antibody exhibited strong binding to idiotypic determinants on the tumor immunoglobulin from the patient, with no cross-reactivity to normal human IgM. The antibody was used to stain CLL cells of the patient, which were then analyzed by flow cytometry. As shown in *Figure 1*, the entire population of CLL cells appears to be stained since there is a shift in the fluorescence intensity of the cells stained with the anti-idiotype compared with cells stained using a control mouse IgM. These results indicate that the CLL in this patient is clonal and that all of the cells show the surface idiotype. Thus, this antibody had the required selectivity and reactivity to be used for treatment of the CLL patient.

Pharmacokinetics of the anti-idiotype monoclonal antibody and serum idiotype levels

As shown in *Figure 2*, after each infusion of the anti-idiotypic monoclonal antibody during the first course, there was a transient decrease in the serum idiotype level. With the 100-mg doses of H-99, the serum idiotype levels decreased from approximately 400 µg/mL to approximately 100 µg/mL. Immediately after infusion, the mouse anti-idiotypic monoclonal antibody was detectable at a level of approximately 1 µg/mL. Within two days of each infusion of the anti-idiotype, the serum idiotype levels returned to the previous levels of approximately 400 µg/mL.

During the second course of therapy (*Figure 3*), administration of the anti-idiotype had similar effects on serum idiotype levels as in the first course. With the highest dose levels (500 mg), the serum idiotype levels were reduced almost completely; however, within 24 hours, the serum idiotype was again increasing. At the end of each infusion with the 500-mg dose of monoclonal antibody, mouse anti-idiotype could be detected at approximately 20–30 µg/mL indicating that a significant level of free anti-idiotype capable of binding to circulating leukemia cells was achieved. Following the final treatment, the anti-idiotypic monoclonal antibody was detected on a small number of circulating cells (3%).

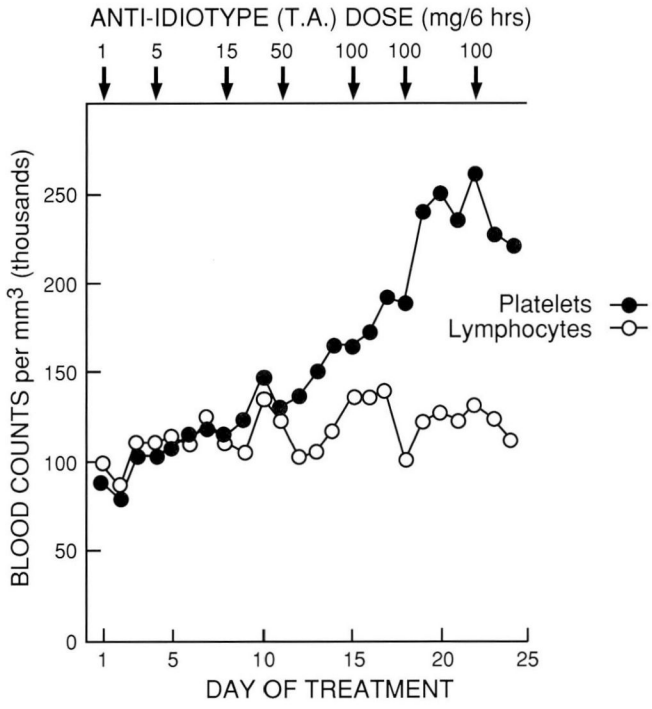

FIGURE 4. Changes in peripheral blood levels of platelets (closed circles) and lymphocytes (open circles) during course 1 of treatment.

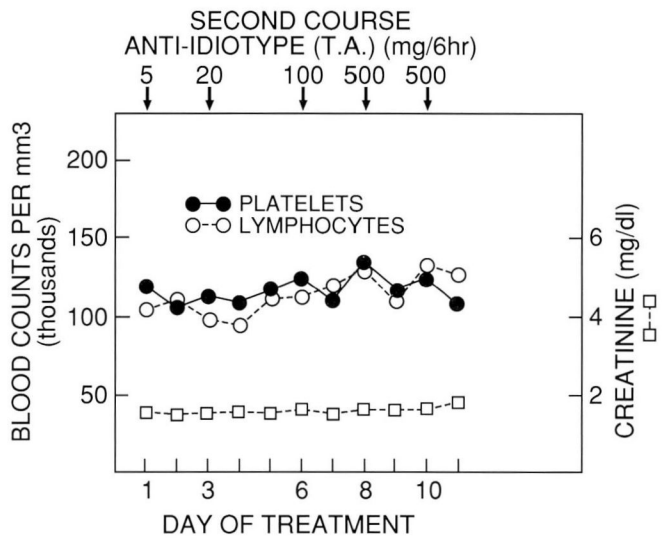

FIGURE 5. Peripheral blood counts during course 2 of therapy with H-99 monoclonal antibody.

Effect of anti-idiotype therapy on peripheral blood counts

The results from the first course of therapy (*Figure 4*) indicated that the treatment had no effect on the number of circulating lymphocytes (>95% of which were leukemia cells); however, the results were somewhat encouraging as the platelet levels increased dramatically during the course of therapy, then returned to normal levels. During the second course of therapy (*Figure 5*), however, there was no increase in the level of platelets and no decrease in the lymphocyte count.

DISCUSSION

At the time of treatment, the patient had stage IV disease with thrombocytopenia (platelets <100,000 per mm^3) and a lymphocyte count >100,000 per mm^3. Nearly all of the lymphocytes were from the malignant B-cell clone since they stained with the anti-idiotype antibody. Less than 1% of the peripheral blood cells expressed the pan T-cell marker, CD3. Thus, the patient had marked impairment of his immune system, which would explain the recurrent episodes of fevers due to bacterial infections. The impairment of the patient's immune system was further seen in his inability to make an antibody response against the infused mouse monoclonal antibody even after two courses of therapy over a three-month period. The functional capacities of T helper cells in CLL patients has been shown by others to be impaired[11,12]; however, in our patient, the lack of immune function may simply be due to a lack of total T cells related to the advanced stage of the disease.

A beneficial aspect of therapy with the anti-idiotype was the dramatic increase in platelet levels during the first course of therapy. It seems unlikely that this effect was due to a specific effect of the anti-idiotypic monoclonal antibody since it was not seen during the second course of therapy. Instead, the increased levels of platelets with our monoclonal antibody may be related to the intravenous infusion of immunoglobulin, which results in increased platelet levels in patients with idiopathic thrombocytopenia purpura.[13,14]

The possible mechanisms of antibody-mediated killing include complement-mediated lysis, antibody-dependent cell cytotoxicity, and phagocytosis of antibody-coated cells. The initial patient treated by Miller et al[1]

showed a dramatic and long-lasting response to anti-idiotype therapy; however, the mechanism of anti-idiotype therapy remains obscure since the anti-idiotype did not activate human complement. In addition, clinical responses to monoclonal antibody therapy often occur long after the monoclonal antibody had been cleared from the circulation.[1,15] Thus, the efficacy of therapy may require activation of host immuno-regulatory mechanisms. Indeed, a host immune response may preexist in certain patients with follicular lymphoma since T-cell "infiltrates" were present within the neoplastic follicles.[16,17] Garcia et al[18] and Lowder et al[19] have evidence suggesting that a clinical response of lymphoma patients to anti-idiotype therapy is correlated with the degree of host T-cell infiltrates within the lymphoma.

The failure of anti-idiotype therapy to effect a clinical response in our CLL patient is consistent with the results shown by Meeker et al[2] in the treatment of B-cell lymphoma patients. Although one of the patients studied by this group has remained in remission for several years, clinical remissions seen in five of 10 additional patients were of short duration, and in the remaining patients, the disease did not respond. The treatment failures were attributed to several factors:

1. Selection of idiotypically different variants of the lymphoma cells,[20,21]
2. The finding that some lymphomas were biclonal,[22]
3. The development of human anti-mouse immunoglobulin responses,[2] and
4. The presence of large concentrations of serum idiotype capable of neutralizing the anti-idioptypic monoclonal antibody.[2]

The failure to induce an anti-tumor response in our patient was probably related to the large concentration (approximately 400 μg/mL) of serum idiotype in addition to the patient's severely compromised immune system at the time of treatment. Efficacy of anti-idiotype therapy for B-cell malignancies may be improved by treatment of patients at an earlier disease stage.

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REFERENCES

1. Miller RA, Maloney DG, Warnke R, Levy R. Treatment of B-cell lymphoma with monoclonal anti-idiotype antibody. N Engl J Med 1982; **306**:517–522.
2. Meeker TC, Lowder J, Maloney DG, et al. A clinical trial of anti-idiotype therapy for B cell malignancy. Blood 1985; **65**:1349–1363.
3. Strychmans PA, Debusscher L, Collard E. Cell kinetics in chronic lymphocytic leukaemia (CLL). Clin Haematol 1977; **6**:159–167.
4. Brouet J-C, Seligmann M. Chronic lymphocytic leukaemia as an immunoproliferative disorder. Clin Haematol 1977; **6**:169–184.
5. Brouet J-C, Preud'homme JL, Seligmann M, Bernard J. Blast cells with monoclonal surface immunoglobulin in two cases of acute blast crisis supervening on chronic lymphocytic leukaemia. Br Med J 1973; **4**:23–24.
6. Cianfriglia M, Armellini D, Massone A, Mariana M. Simple immunization protocol for high frequency production of soluble antigen-specific hybridomas. Hybridoma 1983; **2**:451–457.
7. Kearney JF, Radbruch A, Liesegang B, Rajewski K. A new mouse myeloma cell line that has lost immunoglobulin expression but permits the construction of antibody-secreting hybrid cell lines. J Immunol 1979; **123**:1548–1550.
8. Goding JW. Antibody production by hybridomas. J Immunol Methods 1980; **39**:285–308.
9. Coller H, Coller BS. Statistical analysis of repetitive subcloning by the limiting dilution technique with a view toward ensuring hybridoma monoclonality. Hybridoma 1983; **2**:91–96.
10. Caulfield MJ, Shaffer D. A computer program for the evaluation of ELISA data obtained using an automated microtiter plate absorbance reader. J Immunol Methods 1984; **74**:205–215.
11. Chiorazzi N, Fu SM, Montazeri G, Kunkel HG, Rai K, Gee T. T cell helper defect in patients with chronic lymphocytic leukemia. J Immunol 1979; **122**:1087–1090.
12. Keller RH, Blake DG, Lyman S, Siebenlist R. Immunoregulatory abnormalities in chronic lymphocytic leukemia. I. Correlation of immunoregulatory subpopulations with stage of disease. J Clin Lab Immunol 1981; **6**:201–209.
13. Imbach P, Barandun S, d'Apuzzo V, et al. High-dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura in childhood. Lancet 1981; **1**:1228–1230.
14. Schmidt RE, Budde U, Schäfer G, Stroehmann I. High dose intravenous gammaglobulin for idiopathic thrombocytopenic purpura (letter). Lancet 1981; **2**:475–476.
15. Houghton AN, Mintzer D, Cordon-Cardo C, et al. Mouse monoclonal IgG3 antibody detecting G_{D3} ganglioside: a phase I trial in patients with malignant melanoma. Proc Natl Acad Sci USA 1985; **82**:1242–1246.
16. Dvoretsky P, Wood GS, Levy R, Warnke RA. T-lymphocyte subsets in follicular lymphomas compared with those in non-neoplastic lymph nodes and tonsils. Hum Pathol 1982; **13**:618–625.
17. Harris NL, Bhan AK. Distribution of T-cell subsets in follicular and diffuse lymphomas of B-cell type. Am J Pathol 1983; **113**:172–180.
18. Garcia CF, Lowder J, Meeker TC, Bindl J, Levy R, Warnke RA. Differences in "host infiltrates" among lymphoma patients treated with anti-idiotype antibodies: correlation with treatment response. J Immunol 1985; **135**:4252–4260.
19. Lowder JN, Meeker TC, Campbell M, et al. Studies on B lymphoid tumors treated with monoclonal anti-idiotype antibodies: correlation with clinical responses. Blood 1987; **69**:199–210.
20. Meeker T, Lowder J, Cleary ML, et al. Emergence of idiotype variants during treatment of B-cell lymphoma with anti-idiotype antibodies. N Engl J Med 1985; **312**:1658–1665.
21. Raffeld M, Neckers L, Longo DL, Cossman J. Spontaneous alteration of idiotype in a monoclonal B-cell lymphoma: escape from detection by anti-idiotype. N Engl J Med 1985; **312**:1654–1658.
22. Sklar J, Cleary ML, Thielmans K, Gralow R, Warnke R, Levy R. Biclonal B-cell lymphoma. N Engl J Med 1984; **311**:20–27.