A phase I clinical trial was initiated to treat patients with stage IV B-derived chronic lymphocytic leukemia (CLL) with the IgG₄ murine monoclonal antibody T101. This antibody binds to a 65,000-mol wt (T65) antigen found on normal T lymphocytes, malignant T lymphocytes, and B-derived CLL cells. All of the patients had a histologically confirmed diagnosis of advanced B-derived CLL and were refractory to standard therapy, and more than 50% of their leukemia cells reacted with the T101 antibody in vitro. The patients received T101 antibody two times per week, over two to 50 hours by intravenous administration in 100 mL of normal saline containing 5% human albumin. Twelve patients were treated with a fixed dosage of 1, 10, 50, or 100 mg, and one patient was treated with 140 mg of antibody. It was demonstrated that patients given two-hour infusions of 50 mg developed pulmonary toxicity, with shortness of breath and chest tightness. This toxicity was eliminated when infusions of 50 or 100 mg of T101 were prolonged to 50 hours. All dose levels caused a rapid but transient decrease in circulating leukemia cell counts. In vivo binding to circulating and bone marrow leukemia cells was demonstrated at all dose levels with increased binding at higher dosages. Antimurine antibody responses were not demonstrated in any patients at any time during treatment. Circulating free murine antibody was demonstrated in the serum of only the two patients treated with 100 mg of antibody as a 50-hour infusion and the patient treated with 140 mg of antibody over 30 hours. Antigenic modulation was demonstrated in patients treated at all dose levels but was particularly apparent in patients treated with prolonged infusions of 50 and 100 mg of antibody. We were also able to demonstrate antigenic modulation in lymph node cells, which strongly suggests in vivo labeling of these cells. Overall, T101 antibody alone appears to have a very limited therapeutic value for patients with CLL. The observations of in vivo labeling of tumor cells, antigenic modulation, antibody pharmacokinetics, toxicity, and antimurine antibody formation may be used in the future for more effective therapy when drugs or toxins are conjugated to the antibody.

The T101 monoclonal antibody has been reported to bind to a 65,000-mol wt antigen, designated the “T65 antigen,” which is found on circulating normal T lymphocytes, malignant T lymphocytes, and B-derived chronic lymphocytic leukemia (CLL) cells. Normal circulating B lymphocytes do not express this antigen; however, 2% to 3% of normal tonsil and lymph node B lymphocytes express the T65 antigen and may be the cell of origin for CLL. Previous studies with T101 and anti-Leu-1, which also binds to the T65 antigen, have demonstrated transient responses in patients with CLL and cutaneous T cell lymphoma (CTCL). We report the first fixed multiple-dose phase I trial of monoclonal antibodies using T101 to treat 13 patients with B-derived CLL.
Patients were carefully monitored for clinical response and toxicity, in vivo localization of antibody on tumor cells, antibody pharmacokinetics, antigenic modulation, and antimirum antibody formation. Results of an identical trial in patients with CTCL (mycosis fungoides and Sézary cell leukemia) will be reported in detail separately and have been presented in a preliminary report.14

MATERIALS AND METHODS

Patients

All patients had a histologically confirmed diagnosis of Rai classification stage IV (thrombocytopenia) B-derived CLL that had proved to be resistant to standard therapy, and all were required to have greater than 50% of their CLL cells react in vitro with TlOl. The patients had normal renal and hepatic function with no active severe infections, collagen vascular diseases, vasculitis, arthritis, glomerulonephritis, or cardiac or pulmonary failure. Cytotoxic chemotheraphy, radiation therapy, corticosteroids, and immunosuppressants were not given for at least four weeks before entry of patients into this trial. Patients were fully ambulatory, with a Karnofsky performance status greater than or equal to 70% and a life expectancy of two months or more.

Study Plan

Patients received TlOl antibody by intravenous administration over two hours in 100 mL of normal saline containing 5% human albumin. When it was demonstrated that two-hour infusions at a 50-mg dosage led to pulmonary toxicity, the period of infusion was prolonged to 50 hours. Patients entered the study at each sequential dose level in ascending order from 1 to 10, 50, and 100 mg. Therapy was given twice weekly and most patients received eight doses over four weeks. Oral allopurinol, 300 mg/d, was started 12 hours before therapy.

Antibody Preparation

The TlOl antibody used in the study was supplied by Hybritech, Inc (San Diego). The TlOl monoclonal antibody was produced for this clinical trial by injecting hybridoma cells into the peritoneal cavity of BALB/c mice. The ascitic fluid was concentrated by sodium sulfate precipitation and dialyzed against sodium phosphate. The concentrated ascitic fluid was centrifuged at 10,000 rpm for 20 minutes, sterile filtered through a 0.22-µm filter, and applied to a diethylaminoethyl (DEAE) Sephacel (Pharmacia Fine Chemicals, Uppsala, Sweden) column. The antibody fraction was collected and formulated to 2 mg/mL. The preparation was then tested for bacterial endotoxin with the Limulus lysate assay (Microbiological Associates, Bethesda, Md) and cultured to ensure absence of microbial contamination. The preparation was at least 90% pure immunoglobulin G (IgG). An investigational new drug (IND) application with the Bureau of Biologics of the US Food and Drug Administration was approved before the start of this protocol.

Cell Preparation

Peripheral blood and bone marrow samples were obtained from patients in heparinized syringes, and mononuclear cells were separated on Ficoll-Hypaque gradients. Lymph nodes were mechanically dissociated and filtered through wire mesh to form single-cell suspensions. All peripheral blood, bone marrow, and lymph node specimens used in these studies contained more than 80% leukemia cells.

RESULTS

Immunofluorescence

Immunofluorescent analysis was performed by flow cytometry with a Cytofluorograf 50H immune flow cytometer (Ortho Diagnostic Systems, Westwood, Mass). Anti-Leu monoclonal antibodies (a gift from Becton-Dickinson Co, Mountain View, Calif) and TlOl (a gift from Hybritech, Inc) were used at concentrations of 1.25 to 2.50 µg of antibody protein per 0.5 x 10⁶ cells. Fluorescein isothiocyanate (FITC)-conjugated goat anti-mouse IgG secondary antibody was obtained from Tago, Inc (Burlingame, Calif). Purified mouse myeloma protein of the IgG₁ class served as a negative control (RPC-5, Bethesda Research Laboratories, Rockville, Md). Preparation of cells for immunofluorescence has been previously described.16 Determination of in vivo binding of TlOl was performed by adding the FITC-conjugated goat anti-mouse immunoglobulin directly to the cells without the addition of antibody in vitro.

Measurement of Free Antibody

Circulating murine IgG levels were quantitated in the patients’ serum samples by an enzyme-linked immunosorbent assay (ELISA). Vinyl, 96-well, round-bottomed plates (Dynatech, Alexandria, Va) were coated with 100 ng per well of goat anti-mouse IgG plus IgM antibody (Kirkgeard & Perry Laboratories, Gaithersburg, Md) and then blocked with 0.1 mol/L TRIS buffer containing 0.01% bovine serum albumin and 5.00% chicken serum. They were then incubated with dilutions of patient serum or a mouse γ₅ standard (TlOl), washed, incubated with an appropriate dilution of alkaline phosphatase-conjugated goat anti-mouse IgG (Sigma Chemical Co), and finally developed using p-nitrophenyl phosphate substrate (Sigma Chemical Co). The optical density was determined at 405 nm using a micro-ELISA reader (Dynatech). For each assay, a standard curve of absorbance versus concentration of TlOl was generated, and serum values were determined by linear regression and interpolation.

Measurement of Human Antimurine Antibody Formation

Human antimurine immunoglobulin titers were determined by ELISA as described above. Plates were coated with TlOl (100 ng per well), blocked with 5% chicken serum, incubated with dilutions of patient sera followed by alkaline phosphatase conjugates of goat anti-human γ₅, μ₅, or α-chain-specific antibodies (Tago) and finally developed with p-nitrophenyl phosphate substrate. The titer was defined as the dilution required to obtain a given absorbance value (usually 0.2) as determined by linear regression and interpolation.

Clinical Response

Thirteen patients with Rai classification stage IV CLL were treated on this protocol. Infusions of the TlOl monoclonal antibody had a definite but transient effect on the number of circulating leukemia cells in all but one patient. Circulating leukemia cell counts routinely dropped to 25% to 80% of pretreatment levels immediately after a two-hour infusion of monoclonal antibody except for one patient treated with 1 mg of antibody. These reductions appeared to be independent of the dose of antibody. By 24 to 48 hours,
circulating leukemic cell count throughout the four weeks of therapy. TiOl antibody maintained a pretreatment level (Fig 1). Two patients (H.H. and L.M.) treated with two-hour infusions of 10 mg of TiOl antibody over 50 hours (0. D. J; (B) three patients treated with 10 mg of antibody over 50 hours (0. D. J; (C) two patients treated with 50 mg over two hours (0. D. J; and (D) two patients treated with continuous infusions of 50 mg over 50 hours (0. D. J and two patients treated with 100 mg (b, b) of antibody over 50 hours.

However, the cell count generally returned to the pretreatment level (Fig 1). Two patients (H.H. and L.M.) treated with two-hour infusions of 10 mg of TiOl antibody maintained a 50% reduction in their circulating leukemic cell count throughout the four weeks of therapy.

We were also interested in determining what effect TiOl therapy had on normal T lymphocytes independent from CLL cells. This was accomplished by studying normal T lymphocytes with an independent T cell marker that is not shared with CLL cells. The anti-Leu-4 antibody (equivalent to OKT3) identifies a 19,000-mol wt T cell antigen that is not found on B-derived CLL cells. Our patients with CLL generally had between 2% and 18% of anti-Leu-4–positive cells in their peripheral blood; this remained proportionally the same after therapy with TiOl. This meant that, similar to the reduction in CLL cells, the absolute number of normal T lymphocytes initially declined after therapy and by 24 hours was back to baseline. In contrast to CLL cells, however, in which the intensity of staining with TiOl decreased after the infusion of TiOl because of possible antigenic modulation (via intra), the intensity of staining of T lymphocytes with anti-Leu-4 did not change after the infusion of TiOl.

Because of the pulmonary toxicity (vide infra) that developed in patients treated with two-hour infusions of 50 mg of TiOl, we prolonged subsequent infusions of 50 and 100 mg of TiOl over 50 hours. The responses in patients treated with prolonged infusions of 50 and 100 mg of antibody were quite different than the responses of those who received two-hour infusions. These patients demonstrated a 20% to 50% decrease in their cell counts immediately after the initiation of monoclonal antibody infusion, which reached its nadir approximately 12 to 24 hours into the infusion (25 to 50 mg of antibody administered). Cell counts gradually increased over the next hours, and by the time the infusion was completed, cell counts had recovered to pretreatment levels in most patients. None of the patients treated with TiOl demonstrated a reduction in the size of lymph nodes, spleen, or liver, or improvement in platelet counts or hemoglobin levels. Clinical results of all the patients treated in this trial are summarized in Table 1.

### Table 1. T101 Monoclonal Antibody Therapy of Chronic Lymphocytic Leukemia (Rai Stage IV)

<table>
<thead>
<tr>
<th>Patient* (Isotype)</th>
<th>T101 Positive (%)</th>
<th>Dosage of T101 (mg)</th>
<th>Period of Therapy (h)</th>
<th>Lymphocyte Count (per μL)</th>
<th>Nodes</th>
<th>Liver, Spleen</th>
<th>Toxicity</th>
</tr>
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<tbody>
<tr>
<td>K.K. (μκ)</td>
<td>91</td>
<td>1</td>
<td>2</td>
<td>25,000</td>
<td>Stable</td>
<td>Stable</td>
<td>None</td>
</tr>
<tr>
<td>A.T. (μκ)</td>
<td>72</td>
<td>1</td>
<td>2</td>
<td>59,000</td>
<td>Stable</td>
<td>Stable</td>
<td>None</td>
</tr>
<tr>
<td>L.K. (μκ)</td>
<td>84</td>
<td>1</td>
<td>2</td>
<td>50,000</td>
<td>Stable</td>
<td>Stable</td>
<td>None</td>
</tr>
<tr>
<td>H.H. (λ)</td>
<td>84</td>
<td>10</td>
<td>2</td>
<td>34,000</td>
<td>Stable</td>
<td>Stable</td>
<td>Urticaria, fever</td>
</tr>
<tr>
<td>L.M. (γδ)</td>
<td>91</td>
<td>10</td>
<td>2</td>
<td>5,000</td>
<td>Stable</td>
<td>Enlarged</td>
<td>Urticaria, fever</td>
</tr>
<tr>
<td>A.W. (μκ)</td>
<td>84</td>
<td>10</td>
<td>2</td>
<td>133,000</td>
<td>Enlarged</td>
<td>Enlarged</td>
<td>Fever</td>
</tr>
<tr>
<td>A.R. (μκ)</td>
<td>72</td>
<td>50</td>
<td>2</td>
<td>70,000</td>
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<td>Stable</td>
<td>Pulmonary</td>
</tr>
<tr>
<td>A.O. (μκ)</td>
<td>92</td>
<td>40</td>
<td>2</td>
<td>80,000</td>
<td>Stable</td>
<td>Urticaria, pulmonary; fever</td>
<td></td>
</tr>
<tr>
<td>I.F. (μκ)</td>
<td>73</td>
<td>50</td>
<td>50</td>
<td>290,000</td>
<td>Stable</td>
<td>Stable</td>
<td>Urticaria, fever</td>
</tr>
<tr>
<td>A.V. (μκ)</td>
<td>73</td>
<td>50</td>
<td>50</td>
<td>11,000</td>
<td>Stable</td>
<td>Stable</td>
<td>Fever</td>
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<tr>
<td>H.J. (μκ)</td>
<td>84</td>
<td>100</td>
<td>50</td>
<td>179,000</td>
<td>Stable</td>
<td>Stable</td>
<td>Fever, urticaria</td>
</tr>
<tr>
<td>M.H. (μκ)</td>
<td>98</td>
<td>100</td>
<td>50</td>
<td>310,000</td>
<td>Stable</td>
<td>Stable</td>
<td>Fever</td>
</tr>
<tr>
<td>S.G. (μκ)</td>
<td>87</td>
<td>140</td>
<td>50</td>
<td>257,000</td>
<td>Stable</td>
<td>Stable</td>
<td>Urticaria, hypotension</td>
</tr>
</tbody>
</table>

*All of our patients had previous therapy with alkylating agents and none of them had had a splenectomy.
†Nadir lymphocyte count during or immediately after T101 therapy.
‡Post-study was two days after the last dose of T101.
Toxicity

Temperatures to 38.5 °C were seen in most patients treated with monoclonal antibody at all of the dose levels greater than 1 mg. These fevers did not cause significant problems and did not require antipyretic therapy. Urticaria was seen in approximately 50% of the patients treated with T101 antibody at some point during the course of therapy. In all cases, this promptly responded to antihistamines (50 mg diphenhydramine) and was generally prevented during subsequent courses by pretreatment with antihistamines. None of the patients required cessation of therapy because of urticaria. One patient who received 140 mg of T101 in his first treatment developed urticaria and hypotension (systolic blood pressure of 60 mm Hg) that rapidly responded to antihistamines and fluids.

Pulmonary toxicity was seen in all three patients treated with two-hour infusions of 50 mg of T101. Pulmonary toxicity included shortness of breath with chest tightness without changes in chest films or blood gases. However, two patients with CTCL who developed shortness of breath and chest tightness during a T101 infusion demonstrated transient abnormalities on chest x-ray films and lung scans. The shortness of breath, while uncomfortable, was not severe and occurred at any time point during the infusion of T101 and usually completely resolved without residual problems within 30 to 60 minutes after stopping therapy, with or without treatment with antihistamines. When the infusions were slowed to 1 to 2 mg/h, this toxicity was not witnessed in any patients who were treated at the slow infusion rate.

In Vivo Binding of T101 Antibody and Antigenic Modulation of the T65 Antigen

We studied the number of circulating leukemia cells that were labeled in vivo with the varying doses of T101. To assess in vivo binding of T101 to circulating leukemia cells, fluorescein-labeled goat anti-mouse immunoglobulin was added directly to the cells without supplying additional T101 in vitro. In this way, only cells that bound with T101 in the circulation after an intravenous infusion of T101 would be labeled. Positive results were obtained in all patients. Immediately after a 1-mg infusion of T101, circulating cells demonstrated up to 25% staining; after a 10-mg infusion, up to 60% of circulating cells were stained. From 25% to 80% of circulating cells were stained in vivo with either rapid or prolonged infusions of 50 and 100 mg of T101 antibody.

To determine the maximum ability of cells removed from T101-treated patients to label with T101, excess T101 antibody was added to cells in vitro followed by addition of fluorescein-labeled goat anti-mouse antiserum. In actuality, this procedure permits detection of T101 bound both in vivo and in vitro, although we simply use the term in vitro here for clarity. This curve was compared with a curve of the same cells (at the identical time-point after infusion of T101) which were treated with the fluorescein-labeled goat antiserum only. In this way we could determine the number and intensity of cells being stained with T101 in vivo and compare this with the same cells treated with excess T101 in vitro. The difference between these curves was the result of the T65 antigen not bound in vivo. Figure 2 shows a comparison of in vivo (lower curve) v in vitro staining (upper curve) for one representative patient from each treatment group. At 1 mg, a small proportion of circulating cells was stained in vivo (25%), while excess antibody stained 80% of the cells in vitro. Immediately after a two-hour infusion of 10 mg of antibody, there was a maximum number of circulating cells that stained in vivo, which equaled the number stained in vitro. This declined over the next few hours, either because T101 was lost from the cell surface membrane or because these cells were removed from the circulation. After a rapid infusion of the 50-mg dose of T101 (actually 40 mg, since the infusion was discontinued because of pulmonary toxicity), there was again maximum in vivo binding of T101 to circulating cells immediately after therapy. However, when 50 mg of T101 was added over a prolonged infusion (1 mg/h), the in vivo and in vitro binding did not coincide until 24 hours after the initiation of therapy, and at that time only about 50% of the circulating cells expressed the T65 antigen, most likely because of antigenic modulation.

Bone marrow samples were obtained to determine in
vivo localization of T101 antibody in leukemic bone marrow cells. As indicated in Fig 3, bone marrow cells were removed before infusion, as well as two and 24 hours after infusion of 10 mg of T101 antibody. Before therapy, 90% of the cells stained in vitro with T101 antibody. Eighty-one percent of the leukemic bone marrow cells were labeled in vivo two hours after infusion of T101, with only 10% additional cells labeled when excess T101 was added in vitro. When excess antibody was added in vitro to the two-hour specimen, staining was less intense than when excess antibody was added to the sample taken before therapy (Fig 3, compare dotted lines, top and middle curves), indicating a decrease in T65 antigen density, most likely as a result of antigenic modulation. Twenty-four hours after therapy, the bone marrow cells no longer had in vivo-bound T101 on the cell surface membrane and the cells had fully recovered expression of the T65 antigen.

In contrast to the results obtained after 10-mg infusions over two hours, prolonged infusions of 50 and 100 mg of T101 monoclonal antibody (1 to 2 mg/h) resulted in less in vivo binding to the cell surface membranes of circulating and bone marrow cells. By five hours into the infusion, no greater than 15% of the cells demonstrated in vivo binding, and at the end of the 50-hour infusion, virtually no in vivo binding was observed. This was a result of nearly 100% modulation of the T65 antigen secondary to this prolonged infusion. Furthermore, while T65 antigen expression declined, the expression of B cell antigens (B1, BA1) and immunoglobulin remained unchanged throughout the course of therapy.

Lymph nodes were removed from one patient five hours after a two-hour infusion of 10 mg of T101 and from another patient at the completion of a 50-hour infusion of 50 mg of antibody. In both cases, more than 70% of the lymph node cells stained with T101 when it was added in vitro, but in neither case could we detect in vivo labeling of tumor cells or antigenic modulation of lymph node cells. These data strongly suggest that at these doses T101 did not reach the lymph node cells in vivo. This was not surprising, since we could not detect circulating free T101 in the serum of either of these patients at any time, suggesting that all of the available antibody was bound to circulating and bone marrow cells and none was available to label lymph node cells.

To increase the amount of free T101 in the serum to label lymph node cells in vivo, one patient was treated with a 50-mg infusion of T101 over 25 hours to modulate his circulating and bone marrow leukemia cells. Immediately after this therapy, when his circulating cells had lost more than 75% of the T65 antigen from their surface membrane, the patient was given 5, 10, 50, and 100 mg of T101 antibody, with each increment infused during a one-hour period. Therapy was stopped because of hypotension 15 minutes (25 mg) into the 100-mg dose of T101. At this point, 5.4 μg/mL of free T101 was measured in the patient’s serum. A lymph node biopsy specimen obtained one
hour after the completion of therapy demonstrated nearly 100% modulation of lymph node cells (Fig 4). These cells were cultured in vitro without TIOI; after five days, 83% of the cells expressed the T65 antigen. Parallel results were demonstrated with his peripheral blood lymphocytes.

**Antimouse Antibody Response**

Serum specimens were quantitated for human IgG, IgM, or IgA anti-TIOI titers using an ELISA. None of the patients experienced significant increases in anti-globulin titer over the period of the study (data not shown).

**Circulating Levels of TIOI**

Measurable quantities of circulating mouse IgG (TIOI) were not detected in patients receiving doses of less than 100 mg. The detection limit for the assay used was in the range of 0.2 to 0.4 μg/mL. The presence of circulating TIOI was critical because it was only when free TIOI was detected in the serum that we could be sure that excess antibody was available to label tumor cells in extravascular compartments in vivo. The only patients who demonstrated measureable levels of circulating mouse IgG were the two patients who received 100-mg doses of TIOI over 50 hours and the one patient who received 140 mg. For the two patients who received 100 mg of TIOI over 50 hours, circulating levels of murine IgG were first detected at 24 hours and reached maximal concentrations of 0.99 and 3.50 μg/mL, respectively, in the two patients. Our assay measured total mouse immunoglobulin and did not assess the quantity of immunoreactive TIOI that was in the serum. Circulating levels of murine IgG were again assessed after the third infusion of TIOI and rose to a peak of 4.2 and 5.0 μg/mL, respectively, by 12 hours and remained on a plateau until after cessation of the infusion. The data for one of these patients are summarized in Fig 5.

**DISCUSSION**

We treated 13 patients with stage IV B-derived CLL with the TIOI monoclonal antibody. Patients were treated at fixed dosages twice weekly ranging from 1 to 100 mg (except for one patient treated with 140 mg of antibody). All but one patient demonstrated a rapid reduction of circulating leukemia cells. This was only a transient response in most patients, however, because their counts returned to pretreatment levels within 24 to 48 hours. Interestingly, two patients treated with two-hour infusions of 10 mg of TIOI had a sustained reduction in circulating leukemia cells throughout the course of therapy that only returned to pretreatment levels after the discontinuation of TIOI therapy. Neither of these patients demonstrated a change in their bone marrow, hemoglobin, platelet counts, lymph nodes, liver, or spleen. When we attempted to treat patients with higher doses of TIOI over two hours, they developed a potentially serious pulmonary toxicity. When we prolonged the infusion rate at higher doses of TIOI (1 to 2 mg/h), we no longer witnessed pulmonary toxicity; however, antigenic modulation of the T65 antigen on bone marrow and circulating cells prevented binding of TIOI to the cells and we saw no further reduction in cell counts. These data present a number of critical issues that bear on successful monoclonal antibody therapy.

Most of the patients treated with TIOI developed mild fevers, and at least 50% of the patients developed urticaria. Neither of these toxicities proved to be critical, because they both promptly responded to therapy and urticaria could usually be prevented during subsequent TIOI infusions by pretreatment with antihistamines. We did not witness hepatic or renal toxicity in any of the patients treated with this protocol. Patients treated with two-hour infusions of 50 mg of TIOI developed shortness of breath and chest tightness. This toxicity appeared to be independent of the number of circulating leukemia cells and was also seen
in patients with CTCL who did not have detectable circulating Sézary cells but had low numbers of normal T lymphocytes (that bound to T101). One patient with CTCL treated with 50 mg of T101 developed a small pulmonary infiltrate coincident with shortness of breath and chest tightness. This infiltrate resolved over the next two weeks. Another patient with CTCL treated with a two-hour infusion of 10 mg of T101 also developed shortness of breath and chest tightness coincident with an abnormality of his lung scan, which resolved within two days. These data suggest that this toxicity may be related to microaggregation of circulating leukemia or normal T lymphocytes as a result of in vivo labeling with T101, leading to pulmonary microemboli. This conclusion is further supported by the fact that this toxicity was no longer witnessed when the antibody infusion was slowed to 1 to 2 mg/h because at the slower rate of infusion there was probably less aggregation of cells since fewer cells were labeled with antibody simultaneously. Furthermore, one patient was treated with a 24-hour infusion of 2 mg/h of T101. When antigenic modulation on circulating cells was near 100% and they could no longer bind to additional T101, hourly infusions of 5, 10, 50, and 100 mg of T101 did not cause pulmonary symptoms. Finally, patients treated with an antimelanoma monoclonal antibody that does not react with any circulating cells have received up to 500 mg of murine monoclonal antibody over a two-hour period without any pulmonary toxicity.

Because of the pulmonary toxicity witnessed during the two-hour infusions of 50 mg of antibody described above, patients were treated with prolonged infusions of 50 and 100 mg of T101 (1 to 2 mg/h). This slow infusion led to nearly 100% modulation of circulating and bone marrow leukemia cells. This demonstrated that this prolonged infusion was an ineffective way to infuse antibody, because modulation prevented binding of antibody to cells with their subsequent removal from the circulation. However, there may be a possible role for slow infusion of antibody when drug and toxin immunoconjugates are used therapeutically. Antigenic modulation with T101 is accompanied by internalization of antigen–antibody complexes that may be ideal for effective conjugate therapy.

Another objective of this study was to demonstrate in vivo labeling of tumor cells. We demonstrated localization of antibody on circulating leukemia cells and bone marrow cells at all dosages studied. We were not able to identify antibody localization on lymph node cells five hours after infusion of 10 mg of T101 in one patient or at the completion of a 50-hour infusion of 50 mg of antibody in another patient. These lymph node cells were determined in vitro to be T101 positive, yet we could not label them by in vivo infusion with T101 antibody. No antigenic modulation was noted, which strongly suggests that the T101 antibody did not reach the lymph node cells in vivo. One possibility was that antibody rapidly saturated bone marrow and circulating cells, and none was available to the lymph nodes. This seems likely, since circulating free T101 antibody could only be detected in patients treated with 100 mg of antibody by prolonged infusion. Another possible explanation was that the lymph node blood supply does not allow antibody accessibility to these cells. This, however, was not the case in a guinea pig model, in which we demonstrated in vivo labeling of lymph node tumor cells with a monoclonal antibody. To study this more thoroughly, we treated one patient with a 50-mg infusion over 50 hours to modulate his circulating and bone marrow cells. At the end of this infusion, when we could demonstrate modulation, we began an hourly dose escalation of 5, 10, 50, and 100 mg. The patient did not develop shortness of breath but developed urticaria and hypotension 15 minutes into the 100-mg dosage, and therapy was stopped (a total of 140 mg). A lymph node biopsy specimen obtained within one hour of the termination of the infusion, when the circulating free T101 level was 5.4 μg/mL, demonstrated 100% modulation of the T65 antigen on lymph node cells. After five days of in vitro culture, these cells reexpressed the T65 antigen. These data strongly suggest that T101 did label the lymph node cells in vivo, causing modulation of the T65 antigen, which subsequently was reexpressed in vitro when the cells were no longer exposed to the T101 antibody. We have identified nearly identical results by treating CLL cells in vitro with T101 and similar results have been shown for the common acute lymphoblastic leukemia antigen (CALLA) using the J5 monoclonal antibody.

None of our patients developed increased levels of murine-reactive antiglobulin during their course of therapy. Although some patients had detectable levels of antiglobulin before initiation of therapy, these levels did not increase after therapy and proved not to be murine specific because they also reacted with a variety of immunoglobulins from other species. Using an ELISA, we demonstrated that all of the normal controls, as well as the CTCL patients studied, had preexisting antiglobulin that reacted with T101 and antibodies from other species. These preexisting antiglobulin levels in controls and CTCL patients were significantly higher than the levels found in CLL patients. In the CTCL patients, however, after treatment with T101 there was a rapid increase in their antiglobulin levels (ten- to 50-fold). This was primarily IgG, suggesting that this was a secondary response.
The CLL patients treated with T101 were Rai stage IV and severely hypogammaglobulinemic; therefore, it would not be expected that they would mount a normal humoral immune response to foreign protein as did the CTCL patients. The subject of antiglobulin responses in patients receiving monoclonal antibody therapy is presented in greater detail in a separate article.21

We have demonstrated that patients with B-derived CLL treated with T101 antibody have a consistent reduction of circulating leukemia cells after the infusion of antibody, but in most cases this returned to the pretreatment levels 24 to 48 hours after this therapy. The transient decrease in cell count was either the result of cell destruction or transient sequestration of cells. If there was cell destruction, it seems unlikely that this reaction was caused by complement fixation and lysis, because while T101 lyses cells with rabbit complement in vitro, it does not lyse cells with human complement. Interestingly, Ritz et al5 demonstrated deposition of C3 on antibody-coated cells after infusion of the J5 murine antibody (anti-CALLA) into patients with acute lymphoblastic leukemia. While this antibody does not lyse cells in vitro with human complement, it appears that it was capable of fixing human C3 in vivo, which may have enhanced cell removal by the reticuloendothelial system of the liver, spleen, and lungs or possibly even cause some event in vivo cell lysis. Miller et al3 and Dillman et al6 labeled leukemia cells in vitro with 111In-oxine and 51Cr, respectively, and determined that after reinfusion of these cells into patients treated with monoclonal antibody, there was sequestration of cells in the liver, spleen, and lungs.

The lack of a therapeutic response in our patients is similar to the cases of two patients with CLL treated with T101 reported by Dillman et al.6 Miller and Levy reported an excellent but transient response in a patient with CTCL treated with the anti–Leu-1 monoclonal antibody that also binds to the T65 antigen.4 After treatment with T101, we have seen minor skin responses in five of eight patients with CTCL and one lymph node regression.14

Miller et al demonstrated an excellent and durable response in a lymphoma patient treated with antidiotype monoclonal antibody.22 They speculated that antidiotype monoclonal antibody therapy led to activation of the immune system, which subsequently assisted in the elimination of the tumor. An additional explanation for the differences in response to antidiotype monoclonal antibody and T101 antibody is that the antidiotype antibody is very likely bound to the dividing tumor stem cell, whereas T101 might not have.23 This would explain why the effects of these antibodies were very different despite the fact that they both appeared to bind to the malignant cell population. Most malignant cells and end-stage cells are not capable of dividing. The critical cell to eliminate in any successful therapy is the tumor stem cell, which is capable of self-replication. If T101 does not bind to the stem cells, then therapy with this antibody will at best lead to minor responses. Whether T101 binds to tumor stem cells might be better evaluated when it is made into a more effective cytotoxic agent by covalently linking it to toxins, drugs, or radionuclides. Animal studies suggest that immunotoxins may have a far greater therapeutic effect than unconjugated free antibody,24 and our future clinical trials will address this issue.

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Effects of monoclonal antibody therapy in patients with chronic lymphocytic leukemia

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