Monoclonal Antibody T101 in T Cell Malignancies: A Clinical, Pharmacokinetic, and Immunologic Correlation


Eight patients with cutaneous T cell lymphomas (CTCL) and five with various other T cell malignancies were treated with mouse monoclonal antibody (MoAb) T101. Doses of 1 to 500 mg were administered weekly over a two-hour period and resulted in one complete remission (convoluted T cell lymphoma) and one partial remission (CTCL). Remission duration was 6 weeks and 3 months, respectively. Frequent toxicities were pruritis, hives, flushing, and shortness of breath. Supraventricular arrhythmias and blood pressure instability were also observed. Complete targeting of peripheral blood T cells was achieved with 1 mg of MoAb in the nonleukemic patients (WBC < 10,000/μL), and free, bioavailable antibody was present at the next (10-mg) dose level. Even higher doses resulted in substantial antibody excess that persisted for as long as 6 weeks. Serum concentrations of MoAb decreased with increasing number of peripheral blood T cells, and 25 to 35 mg of T101 were required for induction of antibody excess in leukemic patients. Excess antibody induced antigenic modulation, which was of consequence only if MoAb excess persisted to the next treatment. In the original treatment, the rapidly administered MoAb was able to target and remove peripheral blood T cells before the development of antigenic modulation. Anti-mouse antibodies developed in three patients. Their presence rendered further therapy ineffective and was associated with an anaphylactic reaction in one patient. Development of these antibodies could not be predicted by lymphoproliferative assays. In these assays, however, the T101 protein strongly stimulated the mononuclear cells of the patient who reached the only complete remission of this trial. Immunologic stimulation by the MoAb thus might have played a role in this patient’s antitumor response. In summary, therapy with MoAb T101 was specific but only modestly efficacious. Rapid infusion of nonmodulating doses of antibody provided excellent targeting and removal of peripheral blood T cells and might be a valid approach in future trials with immunoconjugated T101.

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T CELL MALIGNANCIES are a heterogeneous group of diseases. They include the cutaneous T cell lymphomas, T cell–derived acute lymphoblastic leukemia (T-ALL), and subsets of poorly differentiated lymphocytic lymphoma and histiocytic lymphoma. These last two entities are now known as convoluted T cell lymphoma and T cell immunoblastic sarcoma, respectively.1,2 Cells from many of these T cell disorders express a 65,000-dalton antigen (T65) that is a normal T cell differentiation antigen.3 The monoclonal antibodies (MoAb) T101, L17F12, and anti-Leu-1 are directed against this T65 antigen4 and were used clinically in a small number of patients with various T cell malignancies.5,4 They were also used in B cell–derived chronic lymphocytic leukemia (CLL),5,10 which also expresses the T65 antigen.11 Encouraging antitumor activity was noted in some of these patients. Antigenic modulation, however, severely interfered with MoAb-induced peripheral blood T cell removal9,10,12 and was particularly severe with prolonged (24-hour or longer) infusion schedules.

We present here our experience with the MoAb T101 in patients with T cell malignancies. A two-hour infusion schedule was adopted for this trial in an attempt to minimize antigenic modulation. Toxicity, in vivo pharmacokinetics, and antitumor activity of this short-term MoAb infusion are reported. Factors preventing interaction of MoAb with its target such as formation of antimouse antibodies, therapy-induced liberation of cell surface antigens, and antigenic modulation were also carefully determined. These findings were correlated with observed degree of peripheral blood T cell removal and serum concentrations of T101 MoAb. Lymphoproliferative assays were also performed serially during and after each treatment in all patients to examine the potential influence of MoAb on this parameter of host immunity.

MATERIALS AND METHODS

Patients. Patients with histologically confirmed T cell malignancies (12 patients) or B cell CLL (one patient) were enrolled in this study. Important clinical and laboratory characteristics are given in Table 1. Almost all patients were heavily pretreated and were refractory to standard therapies. Patients were staged prior to enrollment in the study with computerized tomography (CT) of the chest, abdomen, and pelvis. Bone marrow aspiration and biopsies were also performed in all patients prior to the beginning of MoAb therapy, and skin biopsy specimens were obtained from the CTCL patients. None of the patients had received chemotherapy or radiotherapy within 4 weeks of the beginning of this trial or during antibody therapy. Patients evaluable for response also received no therapy for 4 weeks after completion of MoAb therapy. Informed consent was obtained from all patients according to institutional and National Cancer Institute (NCI) policies.

Antibody preparation and administration. MoAb T101 produced by Hybritech Inc (San Diego) and provided by NCI was administered intravenously for a two-hour period. Treatments were given once weekly for a total of four doses. The initial antibody dose was 1 mg, and subsequent dose levels were 10, 50, 100, and 500 mg, each given to three previously untreated patients. Dose escalation within a given patient was also permitted provided there was no severe toxicity noted at the previous dose. Antibody doses were diluted to 500 mL with normal saline and were administered with continuous-flow monitoring.
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<th>Site of Disease</th>
<th>No. of Treatments</th>
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<th>WBC x 10⁹/µL</th>
<th>Percentage of Ly</th>
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Abbreviations: CTCL, cutaneous T cell lymphoma; T-IBS, immunoblastic T cell sarcoma; BM, bone marrow; PB, peripheral blood; NR, no response; PR, partial remission; CR, complete remission; PD, progressive disease; NE, not evaluable; Ara-C, cytosine arabinoside; L-ASP, L-asparaginase; BLEO, bleomycin; 6-MP, 6-mercaptopurine; MTX, methotrexate; 6-TG, 6-thioguanine; CHOP, cytosan, Adriamycin, vincristine; CVP, cytosan, vincristine, prednisone; MOPP, nitrogen, mustard, vincristine, procarbazine, prednisone; ATG, antithymocyte globulin; RT, electron beam irradiation; Ly, lymphocytes.
Therapy was discontinued after administration of four MoAb doses regardless of the outcome of therapy. Tumor measurements were obtained weekly from patients with CTCL and those with measurable peripheral disease. CT scanning and bone marrow biopsies were repeated in all patients at the end of therapy. Skin biopsies were repeated in patients with CTCL who had signs of antitumor response. Antitumor responses were classified according to the World Health Organization criteria. Patients who had obtained at least stabilization of disease were eligible for retreatment at the time of relapse.

**Monitoring for toxicity.** Vital signs were obtained every 15 minutes during the infusion period and then every 30 minutes for the next four hours. All patients were also evaluated by continuous electrocardiographic bedside monitoring. Complete blood counts (CBC), serum electrolyte determinations, and blood studies for determining renal and hepatic functions were performed weekly during the treatments and for 4 weeks after completion of therapy.

**Monitoring for in vivo effects of T101 antibody.** Blood for CBC and T cell subset determinations and sera for blocking assays, T101 serum concentration determinations, and endogenous antitumor antibody levels were obtained at 0, 1, 3, 5, 7, 12, 24, 48, 72, and 96 hours during initial treatment. On subsequent treatments, the seven- and 12-hour blood specimens were omitted. Bone marrow aspirations were obtained immediately prior to and 24 hours after MoAb administration in three patients. Skin biopsies performed on two and 12-hour blood specimens were omitted. Bone marrow aspirates were repeated in patients with CTCL who had signs of antitumor response. Antitumor responses were classified according to the World Health Organization criteria. Patients who had obtained at least stabilization of disease were eligible for retreatment at the time of relapse.

**Immunofluorescence assays.** These assays were performed immediately after therapy on whole blood specimens and again after completion of this trial simultaneously on cryopreserved cells of all patients. One hundred microliters of whole blood was diluted 1:1 with a lysis solution containing MgCl (Ortho Diagnostics, Westwood, Mass), and after ten minutes, 10 µL of the MoAb T3, T4, T8, T11 (Ortho) or 10 µL of a 1:100-diluted T101 MoAb was added. Specimens were incubated for one hour at 4°C and washed, and thereafter 20 µL of a 1:40-diluted fluorescein isothiocyanate (FITC)-conjugated goat antimouse F(ab')2 fragments was added (Cappel Laboratories, Cochranville, Pa). After another one-hour incubation period at 4°C, cells were washed again and resuspended in 1 mL of 0.1 mol/L phosphate-buffered saline (PBS) containing bovine serum albumin (BSA) and sodium azide, and the resulting fluorescence was determined on a cytofluorograph 5 OH (Ortho). Purified mouse myeloma protein of the IgG2a subclass served as a negative control (RPC-5, Bethesda Research Laboratories, Rockville, Md). The immunofluorescence assays with cryopreserved cells were performed in an identical fashion except that 1 x 10⁶ purified mononuclear cells were used instead of whole blood. These cells were isolated by Ficoll-Hypaque gradient centrifugation and then frozen at a constant rate of 1°C/min. Cells were stored in the vapor phase of liquid nitrogen.

In vivo binding of the T101 antibody after therapy was determined by the immediate addition of 20 µL of the 1:40-diluted FITC–goat antimouse F(ab')2 fragment to 1 x 10⁶ purified mononuclear cells. The intensity presented for all measured fluorescence is the mean channel of fluorescence intensity of the Ortho cytofluorograph 5 OH.

**Immunoperoxidase staining procedure.** Fresh biopsy specimens were embedded in ortnithine carbamyl transferase (OCT) (Miles Scientific, Naperville, Ill) and snap-frozen in a liquid nitrogen–isopentane mixture (−12°C to −15°C). These tissues were stored at −70°C until sectioned. Cryostat sections were cut at 7 µm and allowed to air-dry overnight. Immediately before staining, the slides were fixed in acetone for ten minutes at 25°C and then washed with PBS (pH 7.4) for three minutes. The MoAbs and secondary linking reagents used for staining were also prepared with this PBS solution. The tissue sections were incubated with the primary antibody for 30 minutes at 25°C in a humidified chamber and then washed in PBS for ten minutes. The peroxidase-labeled secondary antibody (peroxidase-conjugated goat antimouse IgG, Tago Inc, Burlingame, Calif) was applied to the section for 30 minutes. The slides were washed, and the colored substrate aminoethyl carbazole (AEC, Sigma, St Louis) was applied to the tissue and incubated for ten minutes. The slides were then rinsed in tap water for five minutes, stained with Mayer's hematoxylin for ten minutes, and mounted with Aquamount (Lerner Labs, Sewickley, Pa). Controls included replacement of the primary antibody with a non-specific antibody (ascites) and omission of the primary antibody altogether.

**Determination of the serum concentration of T101 MoAb.** Serum concentrations of T101 were determined in all patients by a cell-binding competitive inhibition radioimmunoassay that measures immunoreactive T101. This assay was performed by adding 50 µL of variously diluted sera to 5 x 10⁶ of 8402 cells (a T lymphocytic leukemia cell line that expresses the T65 antigen in high concentrations) followed by overnight incubation at 4°C. Cells were then washed and resuspended in 50 µL PBS containing 1% BSA, and 50 µL of iodinated T101 MoAb (1 to 2 x 10⁶ cpm) was added. After an additional four-hour incubation period, cells were washed extensively and bound radioactivity determined. Serum concentrations of T101 MoAb were determined from a standard curve created with various concentrations of purified T101.

This cell-binding assay was accurate to approximately 400 ng of T101/mL. Above this concentration, the binding capacity of the T cells was exhausted, and a solid-phase radioimmunoassay was used concomitantly to minimize sequential dilution errors. This assay detected up to 6 µg of T101/mL and was performed by overnight coating of the wells of polystyrene microtiter plates with 10 µg/mL of purified goat antimouse Ig (Cappel). In the morning, plates were washed, and 50 µL of serially diluted sera was added and incubated for one hour. Thereafter the plates were washed again. Fifty microliters of ¹²⁵I-labeled T101 was added, plates were incubated and washed, and the amount of bound radioactivity was determined in a gamma counter. Various dilutions of T101 were used to create a standard curve for quantitative measurement.
solid-phase radioimmunoassay was not reliable in the presence of antomouse antibodies or other blocking serum activities and was principally used to confirm the measurements obtained by the cell-binding assay.

**Assay for blocking activity in serum.** To determine whether blocking activity, ie, the T65 antigen or antomouse antibodies, was present, 100 µL of patient serum was incubated with 100 µL of variously diluted T101 antibody (1:2, 1:5, 1:50) for one hour at 4°C. Subsequently, 2 x 10^5 of 8402 cells was added and incubated for one hour at 4°C. Cells were then washed and 50 µL of 1:40-diluted FITC-labeled goat antomouse antibody added (Cappel) and incubated for one hour at 4°C. The resulting fluorescence was determined with a FACS III analyzer (Becton Dickinson, Mountain View, Calif) after cells were washed again with PBS containing 1% BSA and sodium azide. If blocking activity was present in the patient’s serum, the assay for endogenous antomouse antibody was performed.

**Assay for endogenous antomouse antibody.** An immunoprecipitation method was used for these measurements. Five to fifty microliters of T101 (1 x 10^6 cpm) was added to 50 µL of variously diluted sera and incubated for eight hours at 4°C. Thereafter, 50 µL of goat antihuman Ig (Cappel) coupled to Sepharose 4B was added and incubated overnight at 4°C. The resulting precipitate was then washed and counted in a gamma counter. Sera from patient 10, who had an anaphylactic reaction because of the presence of antomouse antibodies, served as the positive control in all assays.

**Assay for lymphoproliferation.** Mononuclear cells from normal individuals and all patients were prepared from heparinized venous blood by Ficoll-Hypaque density gradient centrifugation. Cells of patients and normal individuals (alloantigen) were dispensed into microtiter wells at a concentration of 2 x 10^5/well each in 100 µL of RPMI 1640 medium. Twenty microliters of normal serum or patients’ pretreatment or posttreatment sera was then added to the wells. In certain experiments, concanavalin A (20 µg/well) was used instead of the normal mononuclear cells. Plates were cultured in a humidified atmosphere of 5% CO₂ in air at 37°C. Wells were then pulsed with 1 µCi/well of 'H-thymidine. Cells were harvested 18 hours later onto glass fiber strips, and thymidine uptake was measured by scintillation counting. Data are expressed as the means of quadruplicate wells.

**RESULTS**

Thirteen patients were entered into this trial, including eight patients with CTCL and one patient each with convoluted T cell lymphoma, T-ALL, T cell CLL, T cell immunoblastic sarcoma, and B cell CLL. Their clinical and laboratory characteristics are given in Table 1. All patients were evaluated for toxicity and efficacy. Doses of T101 MoAb ranged from 1 to 500 mg, and a total of 39 doses was administered.

**Toxicity of T101 therapy.** Toxic effects were noted in all patients during all treatments, and their frequency of occurrence is listed in Table 2. Pruritus, hives, flushing, and facial edema were the most common side effects of this trial and occurred at all dose levels. These toxicities were usually seen within 30 to 60 minutes after start of the infusion and regressed spontaneously within 24 hours. Mild to moderate dyspnea was observed in eight patients, requiring supplemental oxygen in all and the use of bronchodilators in one. This side effect usually developed one hour after the beginning of the antibody infusion and was most pronounced during each patient’s first treatment regardless of the initial MoAb dose. Life-threatening anaphylaxis leading to study termination occurred in patient 9 with the third T101 MoAb dose (500 mg). Administration of this dose was delayed for 2 weeks because of patient noncompliance. Antimouse antibodies were present at that time and may have caused the anaphylactic reaction.

Blood pressure instability and cardiac arrhythmias occurred at or above the 10-mg dose level and prevented administration of the four MoAb doses in five patients. Rapidly changing blood pressures were noted in patients 3 and 13. Both individuals were normotensive prior to therapy but had blood pressure readings as high as 210/130 mm Hg in the course of therapy. Blood pressures began to rise approximately 30 to 60 minutes after the beginning of the antibody infusion. With continuation of therapy, blood pressures fluctuated widely, and readings as low as 90/50 mm Hg were observed. Blood pressure changes occurred in patient 3 at the 10-mg dose level and caused termination of therapy during the third (50 mg) treatment. In patient 13, antibody infusion had to be terminated prematurely in both attempted treatments.

Premature atrial contractions (PACs), which were observed in seven patients, were accompanied in all instances by sinus tachycardia and were clinically asymptomatic. They were especially frequent in patient 8. Study was terminated in this patient as a precautionary measure after one treatment.

In patient 12, who had no known preexisting cardiac disease and a normal on-study ECG, a second-degree atrioventricular block developed approximately one hour after the start of the first MoAb treatment (100 mg). The pulse rate decreased concomitantly from 130 to 80 beats/min. An identical arrhythmia occurred with the second MoAb dose, which had been reduced to 50 mg. With both treatments, normal sinus rhythm promptly ensued 60 minutes after termination of the infusion. This patient was subsequently withdrawn from the study. Atrial fibrillation developed in patient 6 during the first treatment (10 mg T101). The relationship of this toxicity to T101 therapy, however, was uncertain since this patient had previous episodes of intermittent atrial fibrillation. Study was continued, and cardiac side effects were absent in the subsequent three treatments.

Gastrointestinal toxicity including nausea, vomiting, diarrhea, and abdominal cramps was seen in a minority of patients. It occurred at doses of 10 mg or above.

The dose-limiting toxicity was reached with an initial T101 dose of 100 mg. At that dose level, all treated patients were pale, tachycardic, and profoundly diaphoretic. Interestingly, doses of 100 mg or higher (500 mg) could be delivered if therapy was begun at a lower dose level. This decrease of toxicity with dose escalation was most prominent in patients who either had persistent antigenic modulation or in whom antomouse antibodies were present.

**In vitro targeting of MoAb T101 and presence of blocking serum factors prior to therapy.** Before enrollment in this study, each patient’s peripheral blood lymphocytes were isolated and reacted with MoAb T101. Most patients with
CTCL had a small percentage of T65-positive cells (ranging from 18% to 87%). In the leukemic patients, 65% or more of the lymphoblasts carried the T65 phenotype (Table 1). Identical results were obtained when whole blood instead of isolated, washed lymphocytes were used. This finding indicates that blocking materials such as tumor antigen or antimouse antibodies were absent prior to Tl01 therapy and was also confirmed in independent assays.

Kinetics and efficacy of MoAb Tl01-mediated peripheral blood lymphocyte removal. The kinetics of T cell targeting and removal were very similar in all nonleukemic patients (peripheral white blood count less than 10,000/μL). A steep decline in peripheral blood T cells occurred approximately one to three hours after beginning the antibody infusion, and the nadir was reached shortly thereafter (Fig 1A). Lymphocyte depletion, however, was only transient, and pretreatment levels were reached within 24 to 72 hours. The T3-, T4-, T8-, and Tl1-positive lymphocyte subsets were equally well removed. This finding may not be surprising since the T65 antigen is present on almost all mature T cells. The 1-mg dose caused a 63% mean reduction in peripheral blood lymphocyte counts (Table 3). Higher doses occasionally removed as much as a 96% of the T65-positive cells. The observed degree of reduction, however, was highly variable from patient to patient and within an individual patient. Dose escalation overall resulted in a steady decline in the mean reduction of peripheral blood lymphocytes, and the 10-mg MoAb dose provided the best mean peripheral blood T cell reduction.

The nadir of the leukocyte count in leukemic patients (peripheral white blood more than 30,000/μL) occurred three to five hours after the beginning of the antibody infusion (Fig 1B). Optimal removal was observed with 25 to 35 mg of MoAb Tl01, and as with the nonleukemic patients, dose escalation beyond this level did not seem to enhance T cell removal (Table 3).

Influence of antigenic modulation on in vivo targeting and removal of peripheral blood lymphocytes. Antigenic modulation, ie, the selective, antibody-induced loss of a surface antigen, was studied initially in vitro by incubating lymphocytes from two patients and two normal individuals with various doses of MoAb Tl01 for as long as 24 hours. Concentrations of the T65 antigen began to decline two hours after addition of the antibody, and almost all incubated cells had lost their T65 antigen at the end of the incubation period. Antigenic modulation proceeded slowly for the initial 12 hours and rapidly thereafter.

The in vivo pattern of antigenic modulation is demonstrated in Fig 2 (patient 5). The initial 10-mg dose already resulted in substantial serum levels of Tl01 MoAb. Antigenic modulation occurred at approximately five hours. Antigenic modulation did not interfere with the initial phase of targeting and lymphocyte depletion. An almost identical pattern was seen with the second (50 mg) dose. Profound antigenic modulation developed, which again did not appreciably impair T cell binding of Tl01 and lymphocyte depletion. Subsequent treatments (100 mg and 500 mg) seemed ineffective. Tl01-positive cells were undetectable even at the beginning of therapy. Persistence of bioavailable MoAb Tl01 had caused sustained antigenic modulation and pre-

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![Fig 1. Effect of Tl01 antibody on peripheral blood lymphocytes. The two-hour antibody infusion was begun at time 0. (A) represents the observed changes in the absolute number of lymphocytes (Ly) and the T cell subsets T3, T4, T8, Tl1, and Tl01 in patient 1 (convoluted T cell lymphoma) after administration of a 50-mg dose. (B) provides this information for patient 12 (T cell CLL) following a 25-mg dose.](image-url)
were administered at time 0 by a two-hour intravenous infusion. The effect of these doses on the absolute numbers of the T11 and T101 T cell subsets is shown. Serum concentrations of T101 during each of the treatments are indicated in the inserts in each panel.

vented targeting and removal of peripheral blood lymphocytes. In this patient and also in patients 7 and 10, antigenic modulation persisted during almost the entire month of therapy and caused a progressive decline of the T101 subset (Fig 3). The rapid infusion schedule thus could not prevent sustained antigenic modulation after the repeated administration of large MoAb T101 doses.

Although peripheral blood T cells usually declined after administration of MoAb T101, a marked increase in circulating lymphocytes occurred in two patients with CTCL after several treatments. These cells were T3- and T11-positive but negative for the T65 antigen. Antigenically modulated T cells thus had entered the peripheral circulation.

Detection of T101 MoAb in malignant tissue. Skin biopsy specimens from two patients with CTCL were evaluated by the immunoperoxidase technique for expression of the T101 and T3 antigens and for the presence of in vivo bound T101 antibody. Biopsy specimens were obtained at four hours and 24 hours after administration of 50 mg of T101. At four hours in vivo bound T101 was present in perivascular areas of the biopsy specimens. Addition of exogenous T101 MoAb revealed the T65 antigen to be abundant in the unstained areas. After 24 hours, however, neither in vivo bound T101 antibody nor the T65 antigen was detectable. The T3 antigen, however, was present in concentrations similar to those observed in the four-hour specimen. Thus, MoAb T101 had entered the target tissue by four hours, and by 24 hours its concentration was sufficient to cause antigenic modulation.

Liberation of blocking materials during T101 therapy. Although none of our patients' sera prevented in vitro targeting of peripheral blood cells with T101 prior to therapy, such blocking activity transiently developed in patient 6 and to a lesser degree in patients 1 and 12 during their initial treatment. This blocking activity significantly lowered T101 antibody concentrations in patient 6 during the first and second treatment (Fig 4) and persisted for at least 48 hours (data not shown). Removal of peripheral blood lymphoblasts was impaired in the first (10 mg) treatment and was still less than optimal in the second (50 mg) treatment. In subsequent treatments no more blocking activity was detectable, and excess MoAb had induced antigenic modulation. Since assays for antimouse antibodies were negative, this blocking activity in the serum is assumed to represent liberated T65 antigen.

Determination of antimouse Ig antibodies. Antimouse antibodies were detected in three patients approximately 3 to 4 weeks after initiation of therapy. Two patients (no. 9 and 11) had CTCL, and the third patient (no. 1) had convoluted T cell lymphoma. The presence of these antibodies led to a severe anaphylactic reaction in patient 9 immediately after the start of the third antibody infusion (500 mg). Administration of this dose was delayed for 2 weeks because of noncompliance, and this interruption of therapy might have facilitated the immunization process. Interestingly, antimouse antibodies developed in patient 11 shortly after a medically necessary 2-week delay of therapy. No adverse reactions were noted clinically, but all subsequently administered MoAb doses were ineffective. In patient 1, low levels of antimouse antibodies were present at completion of the four antibody treatments. They increased to very significant levels in the 2-month follow-up period, and at the time of retreatment, antibody excess could not be achieved, and there was no therapeutic benefit.

Determination of serum concentrations of T101 antibody. Serum concentrations of immunoreactive MoAb T101 were determined in all 13 patients serially, both during and after each treatment. The highest and most consistent
serum concentrations were measured in patients 2, 5, and 10, who had minimal tumor burdens and low peripheral blood counts. In these three patients, serum concentrations of the T101 antibody ranged from 0.2 \( \mu \text{g/mL} \) for the 1-mg dose to almost 20 \( \mu \text{g} \) for the 100-mg dose. T101 serum levels for patient 2 are shown in Fig 5. The 1-mg dose was only transiently detectable, whereas 10 mg of antibody was present for as long as 96 hours. Serum levels of T101 were still detectable seven days after administration of the 50- and 100-mg doses and were measured at 170 ng/mL and 750 ng/mL, respectively. In patient 5, 480 ng of T101 was present in the serum 2 weeks after administration of the final 500-mg dose. T101 serum concentrations decreased with increasing numbers of peripheral blood T blasts and a minimum of 25 to 35 mg of MoAb were required for induction of antibody excess in the leukemic patients.

The time-concentration curves of MoAb T101 were subjected to pharmacokinetic analysis. The antibody was found to follow a single compartment distribution. Serum half-life was determined to be 23, 27, and 36 hours for the 10-, 50-, and 100-mg doses, respectively.

Influence of T101 antibody on lymphoproliferation. Lymphoproliferative assays with concanavalin A or alloantigen were performed serially in all patients during and after therapy to examine the potential influence of MoAb T101 on this parameter of immunity. Lymphoproliferation was absent in the four patients with the various leukemias. Low levels were present in almost all patients with CTCL (patient 5, Fig 6). A transient decline of lymphoproliferation occurred in all responsive patients during the first three hours of antibody treatment, which corresponded with the peak of T101-induced cell removal. Proliferation usually returned to baseline at 24 hours but did not improve with the four courses of therapy. Similar low levels of lymphoproliferation were observed in patient 1 prior to each therapy (Fig 7).

Antitumor response to T101 antibody. Antitumor activity of various degrees was observed in four patients. A complete remission was obtained in patient 1 with convoluted T cell lymphoma. This patient had minimal bone marrow

Fig 5. Serum concentrations of T101 antibody in patient 2 after administration of various antibody doses.

Fig 6. Effect of T101 therapy on the proliferative response of mononuclear cells from patient 5. Mononuclear cells were isolated before T101 therapy and at 1, 2, 5, and 24 hours after the beginning of the antibody infusion. Isolated cells were incubated either with alloantigen (mixed lymphocyte reaction) or with T101.
involvement as the only site of disease. After completion of therapy with T101 MoAb, bilateral bone marrow aspirates and biopsy specimens revealed a normal morphology and a blast count of 3% (Fig 8). The bone marrow biopsy was repeated shortly thereafter to minimize the risk of a sampling error, but was again negative for tumor. Remission was sustained for 8 weeks as documented before T101 therapy and at 1, 3, 5, and 24 hours after the beginning of the antibody infusion. Isolated cells were incubated either with alloantigen (mixed lymphocyte reaction) or with T101.

Fig 7. Effect of T101 therapy on the proliferative response of mononuclear cells from patient 1. Mononuclear cells were isolated before T101 therapy and at 1, 3, 5, and 24 hours after the beginning of the antibody infusion. Isolated cells were incubated with alloantigen (mixed lymphocyte reaction) or with T101.

Fig 8. Representative bone marrow sections of patient 1 prior to (A) and after completion of therapy (B).

Patient 6. A reduction in circulating leukemic/Sezary cells lasting 2 weeks was observed in patients 6, 12, and 13.

DISCUSSION

We have investigated the toxicity, pharmokinetics, and clinical efficacy of the T101 MoAb in 13 patients with various T cell malignancies. MoAb was administered as a short-term, two-hour infusion, a schedule intended to minimize the negative effects of antigenic modulation. It was based upon the in vitro observation that T101-exposed lymphocytes required at least 12 hours for conversion to complete antigen negativity. It thus was hoped that targeting and removal of peripheral blood lymphocytes occurred prior to the development of antigenic modulation.

The findings of this trial clearly support this concept. Every patient had excellent targeting and removal of peripheral blood T cells in the initial treatment regardless of the administered dose. With large MoAb doses (50 mg or above), however, bioavailable antibody persisted to the next treatment and kept peripheral blood T cells in the modulated stage. In some patients, antigenic modulation persisted during the entire month of treatment and caused a progressive decline of the T101 subset. This persistence of antigenic modulation after administration of larger doses explains why 10 mg of MoAb was the most effective dose in nonleukemic patients. Serum concentrations of MoAb T101 decreased with increasing numbers of peripheral blood T cells, and 25 to 35 mg of T101 was the equivalent most effective dose in leukemic patients. Since antigenic modulation was insignificant during the original treatment, consecutive use of higher doses would have been equally effective provided spacing of these treatments would allow for reexpression of the T65 phenotype.

Antigenic modulation was not restricted to the peripheral blood but was also present in skin biopsy specimens of patients with CTCL. Similar observations were obtained by other investigators and confirmed the ability of MoAb T101 to enter peripheral tissues in appreciable concentrations. Besides antigenic modulation, blocking serum factors interfered with targeting and removal of peripheral blood T cells by MoAb T101. Antimouse antibodies were present in three patients of this study. This is less than reported by other investigators and might be related to our treatment schedule, which consisted of only two-hour treatments for each patient. Desensitization to the mouse protein was recently observed after administration of very high MoAb doses but seems a more remote possibility since much smaller MoAb doses were administered in this trial.

An anaphylactic reaction developed in one of these three patients after administration of the third MoAb dose. There were no such side effects, however, in the other two patients, and the pathophysiologic base for this discrepancy is presently unknown. We examined possible differences in immunoreactivity with lymphoproliferative assays but found similar responses to concanavalin A or alloantigens in all three patients. Lymphoproliferative assays were also not able to predict the development of antimouse antibodies in general, since all patients with CTCL had similar low levels of
lymphocyte reactivity. The presence of antimouse antibodies usually rendered further therapy with MoAb ineffective, although some lymphocyte removal occurred in one patient in the early stage of the immunization process.

Blocking materials other than antimouse antibodies were detected in several of our patients during therapy. Blocking of T101 activity occurred early in the treatment course and caused lowered serum concentrations of administered MoAb T101. The blocking apparently was due to tumor antigens in the serum and seemed to be generated by MoAb-mediated cell destruction because antigenic modulation was not present. A similar blocking activity was detected by Miller et al in one patient after administration of a 1-mg dose and was identified as the T65 antigen.

Aside from pruritus, hives, and other effects commonly associated with therapy with mouse MoAbs, previously unreported toxicities occurred during this trial. Substantial blood pressure changes, premature atrial contractions, and atrial fibrillation and atrioventricular conduction disturbances were noted. These toxicities occurred at the 10-mg dose level but were more frequent with doses of 50 mg or higher. They seemed to be associated with the rate of antibody infusion, since administration of identical MoAb doses by a continuous 24-hour infusion was much less toxic. This assumption is also supported by a trial of T3 MoAb in patients with renal graft rejection (Goldstein et al, unpublished observation) where administration of large quantities of MoAb over a short time period resulted in similar toxicities. MoAb T101 is thus only poorly suited for rapid administration. In the proper clinical setting, however, doses of up to 50 mg can be delivered without life-threatening toxicity.

A poorly understood side effect was moderate and occasionally severe shortness of breath, which occurred in almost all patients of this trial. A similar pulmonary toxicity was noted in the T101 trials of Dillman et al and Foon et al. In two of these patients, transient defects were noted either on a perfusion lung scan or chest x-ray. MoAb T101 thus might have formed lymphocyte aggregates that served as microemboli. Prolongation of the antibody infusion from two hours to 24 hours or 50 hours abolished this side effect, and the rapid rate of infusion seemed the cause for this toxicity. Antigenic modulation that invariably occurred with prolongation of the infusion might be an alternative explanation. In our trial, shortness of breath was most pronounced during each patient’s initial treatment, and subsequent use of immuno-modulating doses either diminished or altogether abolished this side effect.

Various degrees of antitumor responses were observed in this trial. They ranged from transient reductions in peripheral T cell counts to sustained remissions in two patients, with disease restricted to bone marrow (patient 1) or skin (patient 5). The best antitumor responses thus were not obtained in leukemic patients but in those with tissue-based disease (bone marrow, skin). This seems surprising since these patients’ malignant T cells were not directly available to the filtering action of the reticuloendothelial system (RES). Effector systems other than the RES thus might have mediated the antitumor activity, and the marked improvement in the mixed lymphocyte reaction in patient 1 supports this notion. We, however, also noted an influx of antigenically modulated T cells into the circulation in two patients with CTCL. This included patient 5 and occurred after each treatment. Dillman et al found a similar phenomenon in one patient with CTCL, and it thus appears that tissue-based T cells were able to enter the peripheral blood stream. These cells might have undergone subsequent removal by the RES.

In summary, this trial provided an exciting opportunity to analyze the action of MoAb T101 at a molecular level. Therapy was specific but only modestly efficacious. Rapid infusion of doses that did not cause antigenic modulation provided excellent targeting and removal of peripheral blood T cells and might be a valid approach in future trials, particularly with immunoconjugated T101.

REFERENCES


Monoclonal antibody T101 in T cell malignancies: a clinical, pharmacokinetic, and immunologic correlation

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