Murine Monoclonal Antibody Therapy in Two Patients With Chronic Lymphocytic Leukemia


We infused the murine monoclonal antibody T101 into two patients with advanced refractory chronic lymphocytic leukemia (CLL) after confirming its reactivity with their CLL cells. One patient received doses of 1.3 and 12 mg; the second patient received 10 mg. Antibody was delivered over 10–15 min. The major observations were: (1) T101 murine monoclonal antibody did bind to cells with T65 surface antigen and saturated these cells in vivo; (2) cells that bound T101 disappeared from the circulation by 2 hr after treatment, as evidenced by a marked drop in lymphocyte counts; (3) T101 serotherapy resulted in some intravascular cell injury associated with sequestration and probably destruction in the liver and lung; (4) free serum T101 was demonstrable, but disappeared by 2–4 hr after infusion; (5) rapid infusion of T101 did not induce significant modulation of T65; (6) rapid infusion of greater than 10 mg of T101 was associated with significant systemic reactions. Monoclonal antibodies may someday have an application in leukemia therapy, but additional experimental trials are clearly indicated.

Anticancer therapy with heterologous and isologous immunoglobulin preparations has been attempted through the years with varying results. Most recently, equine antithymocyte globulin was found to be clinically effective with tolerable toxicity in T-cell malignancies. Patients with chronic lymphocytic leukemia (CLL) have responded to treatment with plasma from volunteers who had been immunized with normal lymphocytes and to sheep anti-CLL antiserum. Unfortunately, the use of such preparations has been limited by problems of specificity, availability, and purity. Large-scale production of pure murine monoclonal antibodies (MoAbs), which react with tumor-associated antigens, offers new potential for antitumor serotherapy.

Trials of MoAb serotherapy have produced promising results in animal tumor model systems. AKR mice with a transplantable leukemia/lymphoma benefitted from treatment with a murine monoclonal antibody that reacted with a thymic-dependent differentiation antigen. In these mice, IgG2a MoAbs were more effective than IgG, or IgM. Serotherapy with antico- lorectal carcinoma MoAbs have produced tumor responses in athymic mice bearing transplanted human colon cancer.

Based on the reported safety of heterologous antiserum in humans and the efficacy of MoAbs in animal models, we have embarked on preliminary trials to evaluate the use of MoAbs in man. Such trials will be significant even if MoAbs are ineffective as anticancer agents by themselves, since they may still have great utility as carriers of toxins, radioisotopes, or chemotherapeutic agents. In this article, we report the results of treatment of two patients with end-stage chronic lymphocytic leukemia (CLL) using MoAb T101.

Materials and Methods

Patients

The first patient was a 45-yr-old white man who was diagnosed in 1977. In subsequent years he was treated with melphalan-prednisone, cyclophosphamide-vincristine-adriamycin-prednisone (CHOP), vinblastine, splenectomy, and total body irradiation. On physical examination he had retinal exudates and hemorrhages, erythematous skin lesions, bulky lymphadenopathy, and hepatomegaly. Hematocrit was 9%, WBC 640,000/μl with 95% lymphocytes, and platelet count 15,000/μl. Repeated leukaphereses were performed to lower the WBC to 1–2 × 10⁹ cells/μl. He subsequently received 3 infusions of antibody at doses of 1, 3, and 12 mg. The first 2 doses were given on successive days, and the last dose was given 2 wk later.

The second patient was a 58-yr-old Mexican-American diagnosed in 1975. He had been treated with chlorambucil, cyclophosphamide-vincristine-prednisone (CVP), and had undergone splenectomy. At the time of antibody treatment, he had diffuse lymphadenopathy with massive abdominal adenopathy. Hematocrit was 20%, WBC 12,000/μl, and platelet count 6000/μl. He subsequently received a 10 mg infusion of antibody.

Prior to treatment, peripheral blood lymphocyte marker typing was performed on lymphocytes from both patients.10 In each case the CLL cells were surface immunoglobulin positive (slg+), did not form rosettes with sheep erythrocytes, but did bear the T65 antigen detected by T101 (T101'), and Ia antigen as determined by reactivity with an anti-Ia MoAb. Approval for treatment was granted by the Human Subjects Committees of the University of California, San Diego, and the San Diego VA Medical Center, and written informed consent was obtained from each patient.

Isolation of Lymphocytes

The patients' peripheral blood lymphocytes (PBL) and CLL cells were isolated on a Ficoll-Hypaque (Pharmacia, Piscataway, N.J.) density gradient. Monocytes were removed by an iron-magnet
MONOCLONAL ANTIBODY THERAPY IN CLL

Lucas, H. L., et al. The use of red fluorescence for slg... hypophosphatemia.}

In vitro complement-mediated cytotoxicity (CMC) was determined as previously described.3 Other controls were run in 96-well, round-bottom microtiter plates in triplicate. One-hundred microliter dilutions of T101 antibody were mixed with 10 6 3Cr-labeled target cells (CLL or 8402) and 5 x 10 4 normal peripheral blood effector cells in a final volume of 200 g. The plates were centrifuged at 120 g and incubated at 37°C. After 4 hr the plates were centrifuged 10 min at 400 g and 100 g of each supernatant were counted in a gamma counter. Percent chromium release was as in the CMC assay. Anti-HLA and rabbit anti-T-cell heteroantiserum were positive controls. The T-cell heteroantiserum was made to the MOLT-4 leukemia cell line.10 Other controls were target cells incubated with media alone, antibody alone, or effector cells only.

**Monoclonal Antibody Administration**

T101 is secreted by a murine/murine hybridoma.13 Large quantities can be isolated from ascitic fluid of BALB/c mice that have been previously inoculated intraperitoneally with hybridoma cells. Ascitic fluid was precipitated with 18% sodium sulfate, centrifuged at 30 min at 1000 g, and the pellet redissolved in 0.01 M borate-buffered saline, pH 8.2. After a second precipitation with 15% sodium sulfate, the resulting solution was diazylized against 0.01 M borate buffer, then centrifuged and filtered. This material was diluted in sterile phosphate-buffered saline and human albumin, and sterilized by filtration through a 0.22 pm filter. The final antibody solution was tested for bacterial pyrogen with a Limulus lysate assay (Malin-}

**Experimental cpm - Spontaneous cpm**

**Maximal cpm - Spontaneous cpm**

where cpm is counts per minute. Target cells were lysed by Triton X100 to determine maximum release. Spontaneous release was that of target cells incubated in media alone.

Antibody-dependent cellular cytotoxicity (ADCC) assays were run in 96-well, round-bottom microtiter plates in triplicate. One-hundred microliter dilutions of T101 antibody were mixed with 10 6 3Cr-labeled target cells (CLL or 8402) and 5 x 10 4 normal peripheral blood effector cells in a final volume of 200 g. The plates were centrifuged 5 min at 120 g and incubated at 37°C. After 4 hr the plates were centrifuged 10 min at 400 g and 100 g of each supernatant were counted in a gamma counter. Percent chromium release was as in the CMC assay. Anti-HLA and rabbit anti-T-cell heteroantiserum were positive controls. The T-cell heteroantiserum was made to the MOLT-4 leukemia cell line.10 Other controls were target cells incubated with media alone, antibody alone, or effector cells only.

**In Vitro Cytotoxicity**

In vitro complement-mediated cytotoxicity (CMC) was determined prior to infusion of T101 antibody. Ten microliter samples of chromium-51-labeled ( 51Cr) target cells were mixed with 10 g of antibody in 96-well, round-bottom microtiter plates. After 1 hr at room temperature, 50 g of complement was added to each well and the plates incubated at 37°C in triplicate. One hour later, 130 g of cold media (RPMI plus 10% gamma-globulin-free horse serum) was added and the plates were centrifuged 10 min at 250 g. One hundred microliter of each supernatant were removed and counted in a gamma counter.

Target cells were 2.4 x 10 6 CLL or normal human peripheral blood lymphocytes or cell line 8402 T cells. Various dilutions of T101 were used, as well as control antibodies, which included a MoAb anti-HLA (a gift from Dr. Ian Trowbridge) and a 1:150 dilution of a mouse anti-human lymphocyte antiserum produced in our laboratory.

The percent of 51Cr release was expressed by:

**Experimental cpm - Spontaneous cpm**

**Maximal cpm - Spontaneous cpm**

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**Study Parameters**

Blood samples were obtained prior to treatment, immediately upon completion of the infusion, and 0.25, 0.5, 1, 2, 4, and 12 hr after each infusion and daily thereafter. Samples included EDTA anticoagulated blood for CBC, and platelet count and plasma, clotted blood for serum, and heparinized blood for PBL typing. CBC, electrolytes, liver, and renal function tests were monitored daily for 3 days after treatment, and then weekly for at least 2 wk.

(A) The third and fourth components of complement were measured using standard C3 and C4 Reagent Test Kits (Beckman Instruments, Fullerton, Calif.). In addition, C3a and C5a were also determined.

(B) Direct determination of T101 by radial immunodiffusion was not possible because of the small quantities of antibody in the patients' sera. Relative estimates of serum T101 were determined by incubating serum samples with 8402 cells followed by incubation with fluorescein-conjugated F(ab')2 rabbit anti-mouse antibody as above. One million 8402 T cells or 8392 B cells (control) were incubated with serial dilutions of the patient's sera for 30 min at 4°C, washed, and then incubated with the fluorescein-conjugated anti-mouse antibody. These cells were subsequently analyzed for fluorescence intensity on the Cytofluorograf.

(C) Similar indirect immunofluorescence assays were used to test serum for free antigen or other factors that could inhibit the binding of T101. Samples of the patients' sera and equal volumes of various dilutions of T101 were incubated together for 1 hr and then added to 8402 target cells or 8392 control cells. Free antigen or endogenous anti-mouse antibody in the patients' sera should have inhibited binding of T101 to the target cells.

(D) In the first patient, levels of hypoxanthine were measured in plasma samples as an indirect measure of cell death. This product of purine degradation was measured rather than uric acid because of the concurrent administration of allopurinol during therapy. Fresh plasma was extracted with perchloric acid, then neutralized with alamine and freon, and the liquid phase assayed for hypoxanthine by high pressure liquid chromatography. In the second patient, cells were incubated with fluorescein diacetate and ethidium bromide to estimate cell viability. Injured cells allowed ethidium bromide to traverse the cell membrane and exhibit red fluorescence, while viable cells exhibited green fluorescence.

(E) In the second patient, autologous 51Cr-labeled lymphocytes were infused 24 hr prior to the T101 infusion. Counts per minute were measured over various organs using an external gamma counter for 1-min counts. Additionally, 3 cc of whole blood was removed at various times and cpm determined. Radioactivity measurements were made before, immediately following, and then 0.25, 0.5, 1, 2, 4, 8-12 and 24 hr following the infusion of autologous cells on 1 day and following T101 infusion 24 hr later. The specimens were centrifuged, and the cpm in plasma and in the cell layer were measured in a gamma counter.

(F) Attempts were made to identify any human IgG, IgM, or IgE anti-mouse antibodies circulating in the first patient's sera after repeated treatment with T101. The 8402 cells were coated with T101, then incubated with the patient's sera, washed, and finally incubated with fluorescein-conjugated goat anti-human IgG, IgM, and IgE (Tago, Burlingame, Calif.). Any endogenous human anti-mouse antibodies would have bound to the T101 attached to 8402 cells. The degree of such binding was estimated by the intensity of fluorescence produced. In addition, we attempted to demonstrate serum IgE anti-mouse activity using a radioallergosorbent test (RAST) in which cyanogen-bromide-activated discs bound with T101 were used.

**RESULTS**

**Cell Surface Markers**

Multiple analyses of the first patient's PBL before therapy demonstrated that greater than 90% of his circulating cells were slg-, T101+, and la+. Less than 1% of cells formed E-rosettes with sheep red cells and less than 1% reacted with OKT3 antibody. In the second patient, prior to therapy, 32% of the cells stained with OKT3 (normal T cells) and 42% stained simultaneously for T101 and slg (CLL cells).

**In Vitro Cytotoxicity**

Chromium release assays confirmed that the first patient's CLL cells could be lysed in the presence of rabbit complement by T101, anti-HLA, and mouse anti-human leukocyte antibodies. However, none of the antibodies was lytic in the presence of the patient's own serum or other human sera as sources of complement. The antibodies were also lytic for 8402 and normal PBL, but again, only in the presence of rabbit complement. Maximum cytolysis of all target cells occurred at 10 dilution of T101, which represented 2 ng antibody/10^6 cells. This figure, and the number of circulating CLL cells per milliliter of blood in vivo, were used to estimate an initial therapeutic dose of 1 mg of T101. Neither the patient's CLL cells nor 8402 target cells were lysed by T101 and normal PBL in ADCC assays. Very little lysis was effected by the murine monoclonal anti-HLA antibody, and then only in the 8402 target system, while cytolysis was effected by the rabbit and anti-T-cell antiserum.

**Effect of T101 on Circulating Lymphocytes**

Figure 1 illustrates the effect of antibody therapy on the first patient's lymphocytes. There was a marked drop in lymphocyte number after each treatment that was maximal at 2 hr. However, the WBC had increased by 4 hr after injection and by the following day had surpassed the pretreatment WBC. This rapid increase in WBC, after the brief posttherapy drop in WBC, was in sharp contrast to the gradual recovery of WBC after cell removal with leukapheresis.

Figure 2 shows the effect of therapy on the circulating lymphocytes from the second patient. There was an immediate drop in lymphocytes following the infusion that reached its nadir 2 hr postinfusion. By 4 hr, the lymphocytes were increasing in the circulation, and by 24-48 hr the lymphocyte number was approaching pretreatment values. The OKT3+ cells decreased immediately following the infusion, while the CLL cells decreased shortly thereafter. This may have been due to the fact that OKT3+ cells express a greater quantity of T65 antigen than OKT3- T101+ CLL
cells. Both cell types recovered at the same rates to near baseline levels by 48 hr.

Measures of Cell Injury

In the first patient, hypoxanthine levels were normal; so, we had no direct evidence for cell lysis. In the second patient, we looked for cell injury by two methods: ethidium bromide uptake and $^{51}$Cr release into the plasma. The circulating lymphocytes showed no evidence of cell injury at any time except immediately following the infusion (Fig. 3). At that time, 10% of the cells exhibited red fluorescence and only 85% of the cells stained with fluorescein diacetate. However, as Fig. 4 shows, although the $^{51}$Cr cpm decreased in the cell fraction of the peripheral blood immediately following T101 infusion, there was no concomitant increase in the plasma cpm as one would expect in the setting of intravascular cytolysis.

T101 and OKT3 Reactivity

Table 1 shows the comparison of in vivo binding of T101 as compared to the effect of incubating with additional T101 in vitro in the second patient. Similar results were obtained in the first case. Infusion of T101 was followed by immediate binding of T101 to the CLL cells, which was maximal by completion of the infusion (Fig. 5). Based on the proportion of cells binding T101 and the fact that the median intensity of
fluorescence was not increased after in vitro incubation, it appeared that lymphocytes were saturated with T101 in vivo. After 2 hr, T101 cells had disappeared from the circulation, but a population of lymphocytes bearing T65 antigen were returning by 4 hr (Fig. 3). At no time was there a substantial proportion of lymphocytes that failed to express T65 antigen—based on in vitro detection by T101. Intensity of fluorescence staining in vitro was only mildly decreased after the lymphocyte level began to increase; so, there was no evidence of significant in vivo antigen modulation (Fig. 6).

**Distribution of \( ^{51} \text{Cr} \) Label**

Figure 4 shows the distribution of \( ^{51} \text{Cr} \) label after infusion of autologous \( ^{51} \text{Cr} \)-labeled lymphocytes. Standard error bars are not shown because they were smaller than the figures used as data points. Immediately following infusion of these cells, there was a rapid drop in plasma \( ^{51} \text{Cr} \), which probably represented free \( ^{51} \text{Cr} \). There was also a drop in cpm of the circulating cells, probably due to removal of damaged cells. There was an initial localization of activity in the liver that decreased over time, perhaps representing removal of damaged cells by the reticuloendothelial system. There

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Fig. 3. Top is percent of viable circulating cells as determined by ethidium bromide uptake. Bottom two curves compare the proportion of lymphocytes reacting directly with T101 in vivo versus reactivity following incubation with additional T101 in vitro.
Table 1. Comparison of T101 * for Direct Incubation With CLL Cells In Vivo Versus Incubation With T101 In Vitro*

<table>
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<tr>
<th>Hours After</th>
<th>No.</th>
<th>Percent Cells</th>
<th>Median Intensity</th>
<th>Fluorescence for T101</th>
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<td>Lymphs/µl</td>
<td>In Vivo</td>
<td>In Vitro</td>
<td>In Vivo</td>
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<td>77</td>
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</table>

*In vivo determined by incubating cells directly with fluorescein-conjugated horse anti-mouse immunoglobulin. In vitro included incubation with a saturating level of T101 before incubation with fluoresceinated anti-mouse preparation.

was an initial increase in activity in the lung, which subsequently declined as well. By 4 hr after infusion of the labeled lymphocytes, cpm had stabilized for all tissues. However, immediately following infusion of T101, there was again a dramatic increase in cpm in the liver and a less rapid increase in the lung as well. These elevations were sustained for only 1–2 hr, and then cpm returned to pre-T101 levels. Simultaneous with the increase in cpm in the liver and lung, there was a decrease in the cpm of the circulating pool of lymphocytes. There was no return of 51Cr-labeled cells into the circulation during 24 hr of follow-up, even though the lymphocyte count recovered markedly during that period. Also of note was the fact that plasma cpm did not increase at any time. Significant intravascular cytolysis presumably would have been associated with a brief increase in plasma cpm. Urinary cpm were relatively constant throughout several 12-hr periods. These observations suggest that antibody administration was associated with removal and destruction of 51Cr-labeled lymphocytes in the liver and lung in the same manner as damaged 51Cr cells were removed immediately following the infusion of autologous labeled cells 24 hr earlier.

Antibody Levels

Figure 7 displays the median intensity of indirect immunofluorescence of free serum T101 bound to 8402 target cells versus time after each antibody treatment. In both patients, peak serum T101 levels were reached immediately following completion of the infusion. Two to four hours after the infusion of any dose, free T101 could not be detected in either patient. This was a qualitative assay, so very small levels of antibody could have persisted beyond this point, but have been undetectable in our test system.

Absence of Blocking Factors

Figure 8 displays titration curves that indirectly reflect the amount of T65 antigen or any other block-
ing factor present in the patients' sera. Presence of a significant amount of T65 antigen or anti-mouse antibody would have caused a sharp drop in the titration curve. The similarity of these curves provides no evidence for the presence of free T65 antigen or anti-mouse antibodies. A positive control for antigen levels was not possible because we had no pure free antigen.

In the second patient (Fig. 8B), the presence of antigen was examined at 1 hr postinfusion, at a time when free antibody was still present in serum. This was reflected in the patient's serum titration curve, which remained flat while the control curves were dropping at higher dilutions of T101. Fluorescent assay tests for endogenous human IgG, IgM, and IgE anti-mouse antibodies were negative in the first patient 2 wk after his initial treatment, and following his third course of therapy. The RAST assay also revealed no evidence of serum IgE anti-T101 activity.

**Complement Levels**

Serum levels of C3 and C4 were normal throughout the observation periods during and after therapy in both patients. C3a and C5a were never elevated. Thus, there was no evidence of activation of the classical or
Fig. 8. Results of blocking experiments to detect circulating antigen or endogenous anti-mouse antibodies. T101 titration curves were generated using 8402 target cells in various sera. The similarity of all curves suggests blocking factors were absent. (A) obtained using a pretreatment serum from the first patient 2 wk after previous T101 therapy. (B) The second patient 1 hr after 10 mg T101 infusion—at a time when free serum T101 was still present (hence failure of patient serum to titrate at T101 dilutions shown).

alternate complement pathways to account for the biologic effects which were seen.

Clinical Observations

In the first patient, the first two injections were uncomplicated, but the third infusion was followed by an anaphylactoid reaction that included urticaria, diarrhea, dyspnea, cough, and hypotension. This occurred about 20 min after completion of the antibody infusion. The reaction responded promptly to epinephrine, steroids, and volume expansion. Subsequently, there was no evidence of immune complex disease such as vasculitis, arthritis, serum sickness, or glomerulonephritis. Clinically, the patient’s lymphadenopathy and skin rash varied markedly in size before and after antibody therapy. However, immediately following the 12-mg infusion (and prior to the onset of the anaphylactoid reaction), he complained of a strange tingling and burning sensation in his lymph nodes.

During the 1-mo interval from the initiation of leukapheresis to 30 days following completion of serotherapy, no steady trend of clinical improvement or deterioration occurred. Fluctuating lymphadenopathy and episodic worsening of his pruritic skin rash persisted. No further therapy was given. Thirty days after the last dose of antibody, his hematocrit was 34, WBC 220,000 with 95% lymphocytes and platelet count 33,000.

In the second patient, hypotension and dyspnea developed within 15 min of completion of the antibody infusion, and subsequently he experienced rigors and fever as well. There was a prompt response to epinephrine and volume expansion. He had no rash and developed no other acute problems. Fever resolved without therapy.

The second patient had mild liver abnormalities, including a bilirubin of 1.8 mg/dl, SGOT of 58 U, and alkaline phosphatase of 131 U/liter prior to T101 therapy. Within 2 wk, he developed ascites, malaise, and jaundice. Bilirubin was 6.4, SGOT 246, and alk phosph 214. Abdominal ultrasound showed massive ade-
nopathy but no hepatic duct or common duct obstruction. Liver scan showed no specific abnormality. Hepatitis B surface antigen tests were negative. IgG hepatitis-A-related antibodies were present, but IgM hepatitis-A-related antibodies were not suggesting prior rather than active hepatitis A infection. These abnormalities subsequently resolved, and the clinical diagnosis was non-A, non-B viral hepatitis presumed secondary to one of the innumerable blood transfusions he had received in the months prior to antibody therapy. He was subsequently treated with additional chemotherapy and supportive care without response, and expired 2 mo later.

DISCUSSION

We have treated two CLL patients with i.v. infusions of MoAb T101, which reacts with an antigen common to T cells and CLL cells. The in vivo biologic effects were promising and included antibody binding and reduction of circulating tumor cells. Reversible systemic complications occurred following 10-mg doses administered by rapid infusion.

The ability to decrease circulating CLL cells with an antibody preparation has been demonstrated in the past, but this is the first evidence that similar results can be achieved with a MoAb in that disease. The fact that T101 did bind to the CLL cells suggests that the antibody binding was directly related to the disappearance of the lymphocytes. The mechanism of lymphocyte removal involved direct cell injury (as evidenced by ethidium bromide uptake), as well as removal of cells in the liver and lung (based on $^{51}$Cr uptake). These results were achieved in spite of the inability to produce cytolysis in vitro with the antibody in either an ADCC or a human complement cytotoxicity assay system. This cell reduction occurred in the absence of any decrease in complement levels in vivo. The results suggest the reticuloendothelial system was responsible for cell removal.

The brevity of lymphocyte suppression was probably the result of an outpouring of new lymphocytes from lymph nodes, bone marrow, and perhaps other tissue sites. Other groups have recently made similar observations with regard to the transient decrease in circulating lymphocytes caused by MoAbs. The recovery of lymphocyte count correlated with the disappearance of T101 from the serum. Thus, a more substantial clinical effect might be obtained by utilizing repeated or continuous infusions of the MoAb. Also, a more efficient antitumor effect might be obtained by: (1) using a combination of MoAbs that detect multiple antigenic determinants on the surfaces of CLL cells; (2) using MoAbs linked to radioisotopes; (3) using MoAbs linked to chemotherapy agents; or (4) using MoAbs linked to natural toxin subunits. These are questions that will be pursued in future trials in various tumor models.

Some investigators have had difficulty demonstrating serum levels of antibody or in vivo binding to tumor cells. One explanation for this problem is the secretion of antigen into the circulation. This was not a problem in our CLL patients in whom there was no evidence of free circulating antigen prior to T101 infusion. However, there are numerous tumor systems in which therapeutic or diagnostic use of MoAbs may be difficult because of circulating antigen. Another potential obstacle to binding antibody to cell surface antigens is the presence of endogenous anti-mouse antibody. We saw no evidence of such a problem in our first patient 2 wk after his initial treatments.

Surface antigen modulation occurs in vitro and in vivo, and could account for the increase in lymphocyte count 4 hr after treatment. However, since the lymphocytes continued to express T65, and bound T101 with a similar intensity of fluorescence, modulation was apparently not a significant factor in our patients. It is possible, however, that modulation of T65 could be a problem in the setting of continuous therapy, since we have observed in vitro modulation with this antigen during prolonged incubation.

The systemic complications that occurred after the rapid administration of greater than 10 mg of MoAb is of concern. There are multiple potential explanations for this, including anaphylactoid reactions due to: (1) murine antigens and IgE, (2) microaggregates of immunoglobulin and C3a and C5a, or (3) kinins released by lysed tumor cells, or a reaction to leukoagglutination of antibody coated cells. We have been unsuccessful in demonstrating endogenous anti-mouse IgE in patients' sera. There was no decrease in complement levels in either patient after the onset of T101 treatment, and no increase in C3a or C5a. Hyperkalemia, increased xanthine, or uric acid were not seen. Release of $^{51}$Cr into the circulation did not occur in spite of ethidium bromide evidence of cell injury. Thus, we found no direct evidence for any of these mechanisms.

Recently, Hamblin et al. observed similar blood pressure and pulmonary complications in a CLL patient treated with a rapid infusion of anti-CLL idiootype sheep antiserum. This raises the possibility that such problems occur primarily in the setting of circulating cells with relevant antigen following rapid infusion of large amounts of antibody. Clearly, the problem is not specific for monoclonal preparations. It appears that such problems can be avoided by adminis-
tering the MoAb more slowly in a large volume of fluid. We had no complications at the 1 and 3 mg doses and have subsequently seen no problems in three melanoma patients treated with 10-mg infusions of an antimelanoma antibody over 2 hr. We have also treated a third CLL patient with 10 mg T101 infused over 2 hr, and he had no complications. This lack of complication with MoAb infusions given over 2–6 hr has also been observed by other investigators treating other malignancies with doses in excess of 10 mg. It appears reasonable to proceed with more extensive trials in CLL using the slow infusion of MoAbs and to reexamine the scheduling of MoAb therapy.

ACKNOWLEDGMENT

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