Chimeric (murine/human) anti-CD4 monoclonal antibody was infused into seven patients with mycosis fungoides. Successful patients received doses of 10, 20, 40, and 80 mg of antibody twice a week for 3 consecutive weeks. All patients had some clinical improvement, but responses were of relatively short duration. Serum levels of chimeric antibody varied as a function of dose. At the 80-mg dose level, antibody was readily observed in biopsied skin lesions. Although there was coating by antibody of most CD4 positive cells in the blood, there was no significant depletion of CD4 positive cells. Low-level antibody responses against the mouse Ig variable region and human Ig allotypic constant region determinants were observed in several patients, but none were of clinical significance. All but two patients made primary antibody and T-cell proliferative responses to a simultaneously administered foreign protein test antigen. However, there was marked suppression of the mixed lymphocyte reaction. We conclude that at the dose levels studied, a chimeric anti-CD4 monoclonal antibody (1) had some clinical efficacy against mycosis fungoides; (2) was well tolerated; (3) had a low level of immunogenicity; (4) had immediate immunosuppressive effects; and (5) did not induce tolerance to a co-injected antigen.

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CHIMERIC ANTI-CD4 IN MYCOSIS FUNGOIDES

Mycosis fungoides (MF) is a T-cell lymphoma characterized by infiltrates in the skin and eventually other organs composed primarily of CD4 positive cells. Current therapy for this disease consists of topical and systemic chemotherapy, radiation therapy, and PUVA (photosenstizing psoralesin with subsequent exposure to long wavelength ultraviolet radiation), but unfortunately many patients develop recurrent and progressive disease. Dermal disease may be indolent for years, but once the disease involves lymph nodes, bone marrow, and/or visceral organs, median survival is usually 2 years or less despite treatment with systemic chemotherapy. Clearly there is a need for new therapeutic modalities in this disease. Recent innovative approaches have included acyclovir, interferon, extracorporeal photochemotherapy, retinoids, 2-deoxycoformycin, and monoclonal antibodies (MoAbs).

Therapeutic effects have been observed in trials using mouse MoAbs directed to CD5 (T101 and anti-Leu 1) in MF patients, but these responses were limited largely by the development of immune responses to the treatment antibody. We have now conducted a clinical trial using a chimeric anti-CD4 MoAb. This antibody was of interest because of its potentially therapeutic and immunosuppressive properties. The chimeric antibody was composed of human constant regions (IgG, k and murine variable (V) regions (anti-CD4/Leu 3a). The antibody was administered intravenously (IV) two times a week for 3 consecutive weeks. The dose was escalated throughout the study between patient groups, and individual patients received either 10, 20, 40, or 80 mg per dose. The objectives of the study were to: (1) measure the pharmacokinetics of chimeric anti-CD4; (2) determine the immunogenicity of chimeric anti-CD4; (3) evaluate the immunosuppressive and tolerogenic properties of chimeric anti-CD4 by monitoring a variety of T-cell functions; (4) monitor escalating doses of chimeric anti-CD4 for possible toxicity and define a maximally tolerated or optimal biologic dose; and, (5) assess the antitumor effects, if any, of chimeric anti-CD4 in mycosis fungoides.

MATERIALS AND METHODS

Patients. This study was approved by the Food and Drug Administration (IND BD0411) and the Stanford University Institutional Review Board. Seven patients were enrolled in the study. Before entry on the study, patients had to meet the following eligibility criteria: (1) histologic diagnosis of MF with a CD4 positive phenotype; (2) advanced disease (persistent or progressive disease following conventional therapy), with at least one measurable or evaluable disease site, unlikely to require standard therapy during the 15-week study period; (3) serum creatinine less than 1.5 mg/dL; (4) SGOT within normal limits; (5) white blood cell count (WBC) greater than 3,500/mm³; (6) platelets greater than 100,000/mm³; (7) Karnofsky performance status ≥ 70%; (8) no active infections or other concomitant active malignancy (except basal or squamous cell carcinoma of the skin); (9) no prior MoAb therapy; and (10) at least 4 weeks since the last treatment with any other biologic agent, systemic chemotherapy (CTX), or topical CTX, radiation therapy, or UV therapy to any designated measurable or evaluable site(s) of disease. Informed consent was obtained from all patients before initiation of therapy. The extent of disease in these patients and their history of prior treatment have been summarized in Table 1. Three of the patients were women and four were men, ranging in age from 43 to 76 years.

Chimeric anti-CD4. The antibody was produced by the Becton-Dickinson Immunocytometry Systems Research and Development Department (Mountain View, CA), and is composed of human...
IgG, and κ constant regions, and mouse V-regions. It was made by cloning the genes for the V-region of murine anti-CD4 (Leu 3a), and the constant regions of human IgG, and κ as previously described.10,11 Briefly, the murine gene segment for the variable region of the murine MoAb SK3 (anti-Leu 3, CD4) was selected using specific pathogen-free mice. Appropriate V-region genes were cloned from a λ phage genomic library constructed with SK3 DNA. Plasmid expression vectors for this gene segment and for the human gene segments of the human IgG constant region were produced and amplified in Escherichia coli (HB 101 strain). Once adequate amplification had taken place, E. coli protoplasts were made. These protoplasts were fused with a mouse myeloma cell line and stable antibody producing cell lines were selected. These cell lines were grown in RPMI medium and fetal calf serum. The antibody-producing cells were cultured in specific pathogen-free mice.

The antibody was purified by anion exchange chromatography (Q sepharose). The antibody was concentrated, subjected to sodium acetate and phosphate-buffered saline (PBS), respectively. A final buffer exchange was performed with 25 mmol/L mes(morpholino-ethanesulfonic) acid. The antibody was free of pyrogen, bacterial and viral contamination, and had an immunoreactivity identical to that of murine anti-CD4. Antibody was administered IV two times a week for 3 consecutive weeks at either the lo-, 20-, 40-, or 80-mg dose level.

Clinical parameters monitored. During the treatment course and at 3-week intervals during a 12-week follow-up period, a number of parameters were followed. These included weekly chemistry panels and urinalyses, and blood counts before and after antibody infusion. Physical exams were performed at least twice weekly. Disease activity was followed by measuring skin lesions designated at the beginning of study entry in three dimensions, and by measuring lymphadenopathy by physical exam, computed tomography (CT) scans, and abdominal x-rays following lymphangiogram. Chest x-rays, chest, abdominal, and pelvic CT scans, as well as Sezary cell counts were performed when indicated. Additionally, in patient 3, who had extensive involvement of joints, synovial fluid aspiration and biopsy were performed pre- and posttreatment.

Pharmacokinetics. Serum levels of infused antibody were measured by Particle Concentration Fluorescence Immunoassay (PCFIA).14 Serial dilutions (in PBS, 0.1% bovine serum albumin [BSA], 0.1% sodium azide) of serum were incubated with polystyrene particles (Pandex beads) coated with the anti-CD4 idiotype monoclonal IDF1 (produced by Dr Sherie Morrison, Department of Microbiology, University of California at Los Angeles) to capture chimeric anti-CD4. Then, 20 μL of bead suspension was added to each well of the reaction plate, followed by 20 μL of diluted patient serum. The plates were incubated at room temperature for 30 minutes and washed with PBS. Finally, 20 μL of a fluorescein-labeled anti-CD4 (produced by Dr Masato Sugi, Becton-Dickinson), was added to each well and incubated 30 minutes. Samples were run in triplicate and data were read as fluorescence intensity. Serum antibody levels were quantitated by comparison with a chimeric human IgG, anti-CD4 standard run in parallel.

Immunopathology. Pretreatment and several posttreatment skin biopsies were obtained on each patient for immunophenotyping and determination of chimeric anti-CD4 penetration in skin lesions. Four to six-millimeter punch skin biopsies were obtained. Half of the biopsy was processed in paraffin block and stained with routine hematoxylin and eosin (H&E). The remainder was frozen. After the final specimen was obtained, all biopsies on any given patient were cut and sections were stained in parallel with a panel of MoAbs including (1) chimeric anti-CD4 (BD0411), mouse anti-Leu3a, and L71 (binds distinct epitope that is different from Leu 3a; defined by its inability to block anti-Leu3a binding); (2) IDF1 (produced by Dr Sheri Morrison), γ2b anti-idiotypic mouse MoAb that binds to the chimeric anti-CD4 in either free or bound form and is detected by using a labeled biotin avidin method; (3) anti-Leu 2a (CD8) and anti-Leu 4 (CD3); (4) anti-Leu 7 (CD56), anti-Leu 11b (CD16), and anti-Leu 19; (5) anti-Leu 6 (CD1); (6) anti-Leu M3 (CD14) and anti-Leu M5 (CD 11c); (7) anti-Leu I2 (CD19); and (8) anti-Leu 8 and anti-Leu 9 (CD7), which detected CD4 cells, CD4 cells with administered BD0411 bound to them, T cells, natural killer cells, dermal macrophages, other macrophages, B cells, and tumor cells, respectively. Results were recorded both as intensity of cellular staining and as percent of positively staining cells. Epidermal versus dermal localization of cells was noted as well as any features unique to the particular biopsy. Controls for staining included skin biopsies from two patients with MF who had not yet received the chimeric anti-CD4, and one patient each with B- and T-cell lymphoma. Chimeric anti-CD4 could be detected in a concentration as low as 0.1 μg/mL, approximately 1:200 the dilution normally used in tissue staining.
Peripheral Blood Immunophenotyping

Fluorescent conjugated antibodies. All MoAbs were produced by Becton-Dickinson Immunocytometry Systems, purified from ascites fluid and labeled with fluorescein isothiocyanate (FITC) or R-phycocerythrin (PE). The following MoAbs were used: anti-CD3 (Leu 4™), anti-CD4 (Leu 3a), anti-CD7 (Leu 9), anti-CD8 (Leu 2), anti-CD14 (Leu M3), anti-CD19 (Leu 12), anti-CD45RA (Leu 18), anti-CD45 (Hle-1), IDFl (anti-idiotypic anti-CD4), L71 (antibody directed against a different epitope of CD4 than targeted by Leu 3a), anti-Leu8, antihuman IgG (HulIgG), and antihuman λ.

Immunofluorescence staining. Blood was collected into EDTA (Vacutainers; Becton-Dickinson, Rutherford, NJ) and was processed within 6 hours of collection. Mononuclear cells were prepared by density gradient separation on Ficoll Hypaque (Pharmacia LKB Biotechnology Inc, Piscataway, NJ). Separated cells, 1 x 10^6, were stained at room temperature for 15 minutes, washed once in modified Dulbecco's PBS (without Ca^2+ and Mg^2+) pH 7.4, and fixed overnight in 0.5% (wt/vol) parafomaldehyde in PBS before flow cytometric analysis. Conjugated mouse IgG MoAbs to keyhole limpet hemocyanin (KLH) and human IgG, myeloma protein served as isotype-matched controls. Anti-CD45 (Hle-1) FITC and anti-CD14 (Leu M3) PE (LeucoGATE SimulTEST; Becton-Dickinson, San Jose, CA), together with SimulSET software (Becton-Dickinson) provided automatic lymphocyte scatter gating. A panel of MoAbs was used in the following two-color combinations: anti-CD45 FITC/anti-CD14 PE, anti-CD3 FITC/anti-CD19 PE; anti-CD8 FITC/anti-CD7 PE; L71 FITC/anti-HuIgG PE; L71 FITC/anti-Hu λ PE; L71 FITC/anti-CD7 PE; L71 FITC/anti-HuIgG PE; control PE.

Flow cytometry. Immunofluorescent stained cells were analyzed on a FACScan flow cytometer (Becton-Dickinson). List mode data for 10,000 events was stored and subsequently analyzed using CONSORT 30 software (Becton-Dickinson). Lymphocytes were identified by gating on forward (low angle) and side (90°) light scatter parameters using markers set with LeucoGATE SimulTEST.

To estimate the amount of chimeric anti-CD4 bound to the cells, an experiment was performed in which normal peripheral mononuclear blood cells were incubated with chimeric anti-CD4 at levels varying from 10^5 to 10^5 µg/10^6 cells. The cells were then stained with L71 FITC/anti-HuIgG PE. The PE mean channel fluorescence was determined and plotted against the antibody level. Patient sample values were estimated from this standard curve.

Immunogeneity. Antivariable and anticonstant region antibody levels were determined using enzyme-linked immunosorbent assays (ELISAs). Ninety-six well microtiter plates were coated with (1) murine anti-Leu 3a (same V-regions as chimeric anti-CD4); (2) chimeric anti-dansyl; or (3) chimeric anti-CD4, to detect antibodies in the test serum directed to the V-region, C-region, and both, respectively. Fifty microliters of the coat antigen solution was dispensed in each well of a flat-bottom microtiter plate (Dynatech [Immunon] Lab, Inc, Chantilly, VA). Plates were washed five times with 0.05% Triton X 100 in PBS before use. Nonspecific protein binding sites were blocked by filling wells with 2% BSA in PBS. Following incubation for 15 to 20 minutes at room temperature, plates were washed with 0.05% Triton X 100 in PBS. Serum samples were plated in serial dilutions. For antivariable region assays, monkey serum containing anti-Leu 3a variable region antibodies was used as a positive control at a 1:100 or 1:500 dilution into the pretreatment sera of each patient. For anticonstant region assays, mouse antihuman IgG (Caltag, San Francisco, CA) was used as a positive control at a final concentration of 50 µg/mL in patient pretreatment sera. Plates were incubated at room temperature for 1 hour and washed four times. Antihuman λ labeled with horseradish peroxidase (HRP) (Becton-Dickinson) was added (50 µL) to each well at a final concentration of 1 µg/mL in 2% BSA-PBS. After a second incubation for 1 hour, plates were washed four times, and 100 µL of enzyme substrate solution (H2O2, 30% [J.T. Baker Chemical Co, Phillipsburg, NJ; 3.5 µL/10 mL], ABTS [2,2’ Azino bis (3-ethylbenzthiazoline sulfonylic acid) (Sigma, St Louis, MO; stock 15 mg/mL, 10 µL/mL) in citrate buffer, pH 4.0) was added to the plates. Plates were incubated at room temperature in the dark and read in an automatic ELISA microtiter plate reader (Dynatech) at OD 405 nm. Chimeric anti-dansyl coated plates were then washed again, and 50 µL of a second detector goat antimouse IgG (Southern Biochem, Birmingham, AL) was added at a final concentration of 1:2,000. Plates were incubated, developed, and read as above.

Antimouse IgG antibody responses were similarly determined using plates coated with 10 different IgG, irrelevant antibodies at a final concentration of 10 µg/mL. Serum from a patient with a known antimouse IgG response was used as a positive control.

Tolerogeneity. Patients were immunized with KLH subcutaneously 24 hours after the first antibody infusion. An ELISA was used to monitor anti-KLH responses. Plates were coated with KLH (Calbiochem, San Diego, CA) as above at a final concentration of 10 µg/mL. Serum from a monkey immunized with KLH was used as a positive control at a dilution of 1:25 into patient pretreatment sera.

T-Cell Functional Assays

Mixed lymphocyte culture. Peripheral blood mononuclear cells from heparin anticoagulated blood were isolated by density gradient centrifugation using Histopaque-1077 (Sigma). Each one-way mixed lymphocyte reaction (MLR) was performed in triplicate in a microculture system using round-bottom plates with 5 x 10^5, 5 x 10^6, and 2.5 x 10^6 responder lymphocytes per well if available. In addition, each well contained 5 x 10^5 stimulator cells (irradiated with 4,000 rad), and 20% heat-inactivated pooled human male serum in RPMI 1640 (Mediatech) culture medium supplemented with 20 mmol/L HEPES, 20 mmol/L L-glutamine, and antibiotics. After 120 hours of incubation (5% CO2, 37°C) cultures were labeled with 2 µCi of 3H-thymidine (specific activity 5 Ci mmol/L) for an additional 18 hours. The labeled cells were harvested onto glass fiber filters, dried, and counted in a liquid scintillation spectrometer. The responder capacity of the patients' lymphocytes was tested by incubation with stimulator cells obtained from three unrelated healthy donors. All results are expressed as the mean of the three stimulation indices on each sampling day to allow comparison between groups and between sampling times. Whenever possible, cells from the same subjects were used as stimulators for each patient at each time point.

T-cell proliferation assay. Peripheral blood lymphocytes were obtained as above. Peripheral blood cells, 5 x 10^7, were placed into a flat-bottom 96-well microtiter plate. The culture medium consisted of RPMI 1640 supplemented with 10% heat-inactivated human male AB serum, 5 x 10^5 mol/L mercaptoethanol, 2 mmol/L glutamine, 100 U/mL penicillin, and 10 µg/mL of streptomycin. Triplicate assays were performed using KLH (Sigma) at 100 µg, 10 µg, and 1 µg/mL, and tetanus toxoid (Department of Public Health, Boston, MA) at a final concentration of 1:500. Cultures were pulsed with 1 µCi of tritiated thymidine at 96 hours and harvested 16 hours later. The mean cpm of thymidine incorporation was calculated for triplicate cultures. All responses were calculated as stimulation indices (cpm stimulated culture/cpm background).
RESULTS

Clinical Responses/Toxicity

Two patients were treated at each dose level with the exception of the 10-mg dose level, at which some clinical efficacy and no toxicity was observed. Both patients at each dose level completed the 3-week course of therapy without severe acute toxicity before any additional patients were treated at the next highest dose. All patients treated to date had some transient clinical improvement in their MF during the treatment period with a reduction in one or more of the following parameters: erythema and induration of lesions, erythroderma, and adenopathy. Examples of some of the observed clinical responses are shown in Fig 1 through 3. Interestingly, in patient 3 there was almost complete resolution of gross and microscopic CD4 positive synovial infiltrate on repeat arthroscopy 3 weeks after the completion of treatment (Fig 4). The most dramatic response was seen in patient 2 with almost complete resolution of generalized erythroderma in less than 24 hours after the first antibody infusion (Fig 1). In general, the other observed responses were modest and have been summarized in Table 2. There were two partial responses (≥50% regression of tumor at all sites), one minor response (↓ ≥ 25% but < 50%), three mixed responses with site dependent tumor regression ranging from complete resolution of erythroderma to stable disease (↓ < 25%), and one patient with progressive disease (↑ ≥ 25% or appearance of any new lesions), but the responses were of relatively short duration: ranging from 9 days to more than 12 weeks (end of follow-up period) after the completion of therapy, with a median duration of approximately 2 weeks. The best overall responses were seen in the two patients who received the 80-mg dose level, and persisted for 9 days and greater than 12 weeks, respectively. Only patient 8 had a reduction greater than 50% in the product of three diameters of all measurable lesions that persisted for over 30 days.
Fig 3. Patient 6 with a small plaque lesion of the dorsal wrist before (A) and after (B) treatment showing decreased erythema and induration. Note that the wrist is more pronated in (A) than in (B).

Fig 2. Patient 4 with a large facial plaque lesion pretreatment (A), and with a marked reduction of erythema and induration following completion of therapy (B).
Fig 4. Biopsies of right knee synovium from patient 3 (stained with H&E, original magnification ×600). (A). Biopsy 2 weeks before therapy shows a lymphocytic infiltrate consisting of a mixed population of large and small lymphocytes, some with hyperchromatic, “cerebriform” nuclei. Immunoperoxidase studies showed predominantly T4 lymphocyte staining. (B). Biopsy 3 weeks after therapy shows a marked decrease in cellular infiltrate, again predominantly T4 lymphocytes by immunoperoxidase studies.

following the completion of therapy. The treatment was tolerated well with no significant acute toxicity either during or immediately after the antibody infusions.

No significant changes in any routine laboratory parameters (serum chemistries, urine analyses, and blood counts) were noted either during or following the treatment course, with the exception of mild to moderate transient anemia secondary to phlebotomy. Patient 2 died shortly after the 3-week follow-up with extensive progressive systemic disease (including the central nervous system), and infectious complications including both *Herpes simplex* and *Pneumocystis carinii* (both of which, in retrospect, predated the chimeric anti-CD4 treatment). Patient 6 developed acute cytomegalovirus, *Herpes simplex*, oral candidiasis, and *Entamoeba histolytica* infections in rapid succession after the sixth antibody infusion, but subsequently made a full recovery from these infections. Although anti-CD4 conceivably could exacerbate underlying immunosuppression, it is not known whether the antibody contributed significantly to either of these patients’ infections.

**Pharmacokinetics**

Serum levels of chimeric anti-CD4 varied as a function of dose as shown in Table 3, with the highest serum levels occurring in the patient that received the highest absolute dose (1.83 mg/kg). Of note, on the second day (42 hours post) following antibody infusions, there was no detectable chimeric anti-CD4 in the sera of patients 1 through 5 (data not shown). Patient 7 had 42-hour postinfusion levels of approximately 5 μg/mL that dropped to zero by day 3, just
before the next antibody infusion. Only patient 6 had progressively increased 42- and 66-hour postinfusion levels of 25 to 50 μg/mL and 10 to 30 μg/mL, respectively. The half-life of chimeric anti-CD4 ranged from approximately 21 to 42 hours in different patients.

**Immunopathology**

The presence of administered chimeric anti-CD4 was detected in patients who received more than 0.58 mg/kg. In patients 1, 2, and 3, no chimeric antibody was detected in any of the postinfusion biopsies by immunostaining of tissue sections, nor were any consistent changes observed in the normal infiltrating cells in any of the biopsies. In patient 4, antibody was detected in the final biopsy, taken 24 hours after the sixth infusion. In patients 6 and 7, antibody was detected in all three postinfusion biopsies, but was never detected in any of the preinfusion biopsies, nor in any of the biopsies taken 21 to 48 days after the last infusion. Patient 5 could not be completely assessed as preinfusion, and some postinfusion biopsies were not taken, but the antibody was detected in the final biopsy taken 24 hours after the sixth antibody infusion. Interestingly, the numbers of anti-Leu 6 (CD1, Langerhans cells), anti-Leu 2a (CD8, cytotoxic/suppressor T cells), and anti-Leu M3 (anti-CD4 macrophages) positive cells appeared to increase in some biopsies. Although this increase was not consistently found from one biopsy to another, it was most prominent in the last postinfusion biopsies from the patients receiving the highest dose of chimeric anti-CD4.

**Peripheral Blood Immunophenotyping**

There was no significant alteration in circulating T- and B-cell subpopulations in peripheral blood after treatment with chimeric anti-CD4 as evidenced by stable staining patterns with anti-CD3, anti-CD7, anti-CD8, anti-CD19, anti-CD45RA, and anti-Leu 8. Of note, there was no significant depletion of CD4 positive cells in the blood of any of the patients, although coating of CD4 positive cells by chimeric anti-CD4 was readily observable. There was a positive correlation between serum levels of chimeric antibody and the amount of anti-HuIgG PE detected by immunostaining of peripheral blood mononuclear cells. Only in patients treated with 80 mg/infusion was HuIgG detectable on CD4 T cells between all infusions (0.45 ng chimeric antibody per 10⁶ cells on average). Furthermore, at this dose level anti-CD4 stained cells became undetectable with Leu 3a after the first infusion. However, this did not represent a depletion of anti-CD4 positive cells because the percentage of L71 (non-crossblocking anti-CD4) stained cells remained unchanged. Coating of CD4 positive lymphocytes with chimeric anti-CD4 resulted in decreased mean channel fluorescence of L71 FITC, secondary to either modulation of the antigen or steric hinderance of L71 binding by chimeric anti-CD4. Only in patient 6 were Leu 3a stained cells undetectable between infusions, presumably secondary to the presence of serum excess of chimeric antibody between treatments as shown in Table 3.

**Immune Responses**

Antibody responses to anti-CD4 variable region, human constant region, mouse IgG, and to KLH were measured and are summarized in Table 4. With the exception of the three late responses that occurred between 6 to 12 weeks following the completion of therapy, most of the antibody responses were first observed between days 8 and 11. There were two antivariable responses that occurred in patients treated at the 20- and 40-mg dose levels between days 10 and 18 with relatively low titers (1:64, 1:256), approximately 1/1,000 that of the positive hyperimmunized monkey control serum. Patient 3 made a late anticonstant region (allotypic) response, and patient 1 made an antimouse IgG, response by day 8. Mouse IgG is a minor contaminant in the treatment preparation, present at approximately 0.1 μg/mL.

KLH (0.5 mg) was administered subcutaneously to patients 24 hours after the first antibody infusion to determine whether chimeric anti-CD4 would induce tolerance to a coadministered antigen. All patients, with the exception of

### Table 2. Clinical Responses

<table>
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<th>Patient No.</th>
<th>Absolute Dose (mg/kg)</th>
<th>At Completion of Treatment</th>
<th>3 wk F/U</th>
<th>≥ 6 wk F/U</th>
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<td>7</td>
<td>0.88</td>
<td>ER</td>
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</table>

Abbreviations: CR, disappearance of all clinical evidence of malignancy at all sites; PR, ↓ ≥ 50% in amount of tumor at all sites; MR, ↑ ≥ 25% but <50% in amount of tumor at all sites; SD, ↑ ↓ < 25% in ≥ 1 site of disease, no new lesions; PD, ↑ ≥ 25% in size of any lesion, or appearance of any new lesion at any site; sx, symptoms.

*SD overall, but site-dependent CR and/or PR.

†PR: Four of six lesions.
patient 2 (anergic in all assays) and patient 6 (s/p total lymphoid irradiation 5 years prior), made an anti-KLH antibody response. Of note, patient 1 made a detectable anti-KLH response only after a boost (0.5 mg KLH) was given at the 9-week follow-up visit.

All of the above antibody responses generally decreased in titer with time as patients were seen in subsequent follow-up visits after the completion of therapy. None of the observed antibody responses were clinically significant, as evidenced by the absence of acute antibody reactions and absence of any significant change in chimeric antibody peak serum values following subsequent infusions (Table 3).

### T-Cell Function

To assess potential interference of chimeric anti-CD4 on in vitro immunoreactivity, the responder capacity of patient lymphocytes was measured in MLR and T-cell proliferative assays using a panel of normal donor lymphocytes, tetanus toxoid, and KLH (with which the patients had been previously immunized). The results of the MLR are summarized in Fig 5, where the mean of three stimulation indices is shown for each patient (with the exception of patient 7 from whom insufficient numbers of cells were harvested) before initiation of therapy and following the third infusion of chimeric anti-CD4. This time point was selected because it was midway through the treatment course and corresponds to the time at which there had been sufficient exposure to antibody to potentially alter function. After the third antibody infusion all patients previously responsive at the 2.5 \times 10^6 responder cell concentration (response = stimulation index > 2.5) had a marked decrease in the stimulation index. This effect was also seen in patient 1 at a lower concentration of responder cells (5 \times 10^5). When followed sequentially for 12 weeks after the completion of therapy, the MLR reactivity of patients 1 and 5 gradually returned to normal. However, this was not true for patient 3, but she was subsequently treated with prednisone for exacerbation of her arthritic symptoms during the follow-up period. The other initially unresponsive patients did not show a major change in the stimulation index at this concentration.

With the exception of patient 1, all patients were relatively unresponsive to tetanus toxoid pretreatment and remained so. Patient 1 had a significant reduction in T-cell proliferation to tetanus toxoid during the treatment course that gradually recovered following the completion of therapy. Five of seven patients made a T-cell proliferative response to KLH. Patients 2 and 3 failed to make T-cell proliferative responses to KLH, presumably because of profound immunosuppression that predated administration of chimeric anti-CD4. Both patients had previously responded to KLH. Patients 4 and 5 gradually returned to normal. However, this was not true for patient 3, but she was subsequently treated with prednisone for exacerbation of her arthritic symptoms during the follow-up period. The other initially unresponsive patients did not show a major change in the stimulation index at this concentration.

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received chemotherapy. Thus, chimeric anti-CD4 depressed the MLR response but was not tolerogenic at the dose levels studied, and did not suppress the ability of patients to mount an immune response to a coadministered antigen.

DISCUSSION

Partial and minor responses of relatively short duration have been reported in patients with cutaneous T-cell lymphoma treated with anti-CD5, but were limited in part by the development of antimouse Ig antibodies as well as anti-idiotypic antibodies in 50% to 100% of the patients. Although 95% of the host antimouse Ig responses following administration of anti-CD5 (anti-Leu 1) were directed against mouse Ig constant region determinants, a small but significant component was found to be anti-idiotypic. The most extensive clinical experience to date with in vivo murine MoAb has been obtained with OKT3 (anti-CD3) in patients treated for acute rejection of cadaveric renal transplants. Antibody responses to the infused OKT3 occurred in 75% to 100% of patients treated with OKT3, and were directed to idiotypic and/or idiotypic determinants in 60% and 44% of patients, respectively.

Chimeric MoAbs are potentially advantageous compared with murine MoAbs because the human constant region domains should decrease immunogenicity, increase biologic half-life, and facilitate interaction with host effector cells. Encouraging results have been reported with chimeric CAMPATH-1A and 17-1A. Two patients with B-cell lymphoma treated with chimeric CAMPATH-1A responded clinically and did not make an antoglobulin response. However, this is not necessarily attributable to the chimeric property of the antibody because in our experience B-cell lymphoma patients treated with mouse antibodies do not commonly make human antimouse antibody (HAMA) responses. The immunoincompetence of B-cell lymphoma patients is further compounded by prior chemotherapy. LoBuglio et al have administered chimeric 17-1A to 10 patients with metastatic colon cancer. Only one of their patients made an antivariable region response that was first detected on day 63. None of these patients had a toxic or allergic reaction, and the circulation time of the chimeric antibody was approximately sixfold that of the murine counterpart. In our experience reported here, chimeric anti-CD4 was immunogenic in some of the patients, though less so than murine anti-Leu 1 in a similar group of patients, both in terms of titer and the percentage of patients making an immune response to the MoAb during the treatment course. These antibody responses were clinically insignificant as evidenced by the absence of antibody reactions and lack of effect on peak levels of chimeric anti-CD4.

The antibody infusions were well tolerated and there was no associated acute toxicity. All patients had some clinical improvement with a reduction in one or more of the following parameters in at least one site of disease: erythema and induration of lesions, erythroderma, adenopathy, and CD4 positive synovial infiltrate. Nevertheless, these responses were relatively minor and of short duration, ranging from 9 days to more than 12 weeks (end of follow-up period) after the completion of therapy. The most dramatic response was seen in erythroderma, occurring in one patient within 24 hours of the first antibody infusion, which suggests that this effect may be mediated by soluble mediators. The best overall responses were seen in the two patients who were treated at the 80-mg dose level, and persisted for 9 days and more than 12 weeks, respectively, but it was not possible to more clearly define the dose-response relationship. With data from only seven patients thus far, it is not possible to draw any definite conclusions about the response rate, nor to define a maximally tolerated or optimal biologic dose, which is the goal of ongoing and future studies.

Anti-CD4 may be immunosuppressive. This is evidenced by suppression of the MLR and T-cell proliferative responses to tetanus toxoid in those patients that were reactive in these assays before therapy. The majority of patients were relatively unresponsive before antibody therapy in both of these assays. This baseline unresponsiveness is not surprising because cells from patients with Sezary syndrome have been reported to be relatively unresponsive/hyporesponsive in MLR and mitogenic proliferation assays. Furthermore, neoplastic CD4 positive cells and cells from Sezary syndrome patients are known to be deficient in interleukin-2 production and fail to support pokeweed mitogen-driven Ig synthesis. The occurrence of opportunistic infections in patients 2 (present in retrospect before treatment) and 6 could have been the result of an immunosuppressive effect of the antibody. On the other hand, both of these patients were relatively immunosuppressed before initiation of MoAb therapy. Depression of proliferative responses to mitogens and antigen in patients with multiple sclerosis and suppression of pokeweed mitogen-induced Ig synthesis in patients with rheumatoid arthritis has been reported following treatment with murine anti-CD4. Some of the patients with rheumatoid arthritis have shown clinical improvement. In animal models anti-CD4 has been effective in the treatment of autoimmune diseases.

We found no significant alteration of the phenotype of peripheral blood lymphocytes, and no depletion of CD4 positive cells after treatment with the chimeric anti-CD4, although most of the CD4 positive cells were coated with the chimeric MoAb. The coating of CD4 positive cells with chimeric anti-CD4 MoAb may interfere with the ability of these cells to function normally, and may be responsible for the suppressed MLR reactivity and T-cell proliferative responses observed following treatment. Similarly, Hafer et al failed to show lysis of CD4 positive T cells following treatment of multiple sclerosis patients with murine anti-CD4, while Reiter et al observed a brief reduction of circulating CD4 positive T cells in rheumatoid arthritis patients treated with an antibody of similar specificity. It is more difficult to explain the antitumor effects of the chimeric anti-CD4 MoAb, which may be secondary to (1) cell killing at the tumor site and/or peripheral destruction with simultaneous recruitment of CD4 positive cells from
other sites into the circulation, and/or (2) indirect effects mediated by factors released by T cells.

Chimeric anti-CD4 was not tolerogenic at the dose levels studied. This was tested because the coadministration of antigen with anti-CD4 in experimental animals has resulted in selective immunologic unresponsiveness to that antigen.\textsuperscript{4,57} It failed to do so in this instance, but the doses used in this study were much lower on a per kilogram basis than were used in the animal model. Furthermore, the chimeric anti-CD4 does not bind to the analogous epitope of the CD4 molecule as do the antibodies used in animal studies, nor does it have the same degree of interference with lymphocyte activation that has been seen in the experimental animals.

The preliminary results reported here suggest that in the future, chimeric anti-CD4 may prove to be of use for the treatment of CD4 positive lymphoid malignancies. Clearly, the maximally tolerated or optimal biologic dose needs to be defined and the treatment regime optimized. If the antibody was administered differently, at higher doses, or in a radiolabeled form, it may be more efficacious. The associated in vitro and clinical immunosuppressive effects of anti-CD4 therapy suggest that this antibody may be useful in the treatment of autoimmune diseases, or possibly in the field of transplantation, for the purpose of induction of tolerance. However, there is no evidence that at the dose levels reported here the antibody is tolerogenic in humans. The chimeric antibody does appear to be less immunogenic than murine anti-Leu 1 in a similar patient population, but a direct comparison with murine anti-CD4 (preferably against the same epitope) is needed to make a definitive statement about relative immunogenicity. If this proves to be true, then the chimeric version of the antibody will be advantageous for repetitive administration. Future studies will be designed to determine the relative immunogenicity of chimeric anti-CD4 compared with the murine version of the antibody, to define a maximally tolerated or optimal biologic dose, to optimize the treatment regime, and to elucidate the mechanism of action of anti-CD4 MoAbs.

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Observations on the effect of chimeric anti-CD4 monoclonal antibody in patients with mycosis fungoides

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