Treatment of Cutaneous T-Cell Lymphoma With Chimeric Anti-CD4 Monoclonal Antibody

By Susan Knox, Richard T. Hoppe, David Maloney, Iris Gibbs, Sherry Fowler, Carol Marquez, P. JoAnne Cornbleet, and Ronald Levy

Chimeric anti-CD4 monoclonal antibody was administered intravenously as a single dose to eight patients with mycosis fungoides. The dose was escalated throughout the study between patient groups, and individual patients received 50, 100, or 200 mg per dose. Seven of eight patients responded to treatment with an average freedom from progression of 25 weeks (range, 6 to 52 weeks). The treatment was well tolerated, and there was no clinical evidence of immunosuppression. Following treatment, there was significant suppression of peripheral blood CD4 counts in all patients for 1 to 22+ weeks. Only one patient made a very low titer human antichimeric antibody response. All but two patients made primary antibody and T-cell proliferative responses to a foreign antigen administered 24 hours after antibody infusion. However, there was generally marked, but temporary suppression of T-cell proliferative responses in vitro to phytohemagglutinin (PHA), tetanus toxoid, and normal donor lymphocytes. We conclude that at the dose levels studied, this antibody (1) had clinical efficacy against mycosis fungoides; (2) was well tolerated; (3) had a low level of immunogenicity; (4) decreased T-cell proliferative responses in vitro, and (5) did not induce tolerance to a foreign antigen.

MYCOSES FUNGOIDES (MF) is a cutaneous T-cell lymphoma (CTCL) that is composed primarily of CD4 positive cells. It is characterized by CD4 positive infiltrates in the skin and can eventually involve lymph nodes, visceral organs, and bone marrow. The management of recurrent, progressive, and often refractory disease can be a difficult problem. Once the disease involves sites other than skin, despite treatment with systemic chemotherapy, median survival is approximately two years. These patients are generally considered to be incurable, and new therapies are needed for this disease. Innovative therapeutic approaches have included the use of photopheresis, interleukin-2, fusion toxin, cyclosorpine, interferons, retinoids, adenosine analogues, other novel biologic response modifiers or chemotherapeutic drugs, and monoclonal antibodies.

Clinical responses have been reported in studies using murine monoclonal antibodies (MoAb) directed against the T-cell antigen CD5, but the development of immune responses to the murine MoAb limited the effectiveness of this therapy. In a previous study using a chimeric (murine/human) anti-CD4 MoAb (SK3; anti-Leu3) in patients with MF, low level antibody responses against the mouse Ig variable region and human Ig allotypic constant region determinants were observed in two of seven patients, but were of no clinical significance. The antibody was well tolerated and had some clinical efficacy against MF. Given these encouraging results, we conducted a second Phase I clinical trial using a different chimeric anti-CD4 MoAb (chimeric M-T412) directed to a different epitope on CD4. Like chimeric SK3, the avidity and epitope specificity of the chimeric M-T412 is derived from the variable region of its murine counterpart antibody. Chimeric M-T412 has high-affinity binding to the CD4 antigen and depletes CD4+ T lymphocytes from the circulation via Fc mediated mechanisms unlike chimeric SK3. Chimeric M-T412 antibody contains human constant regions (IgG1, k) and murine variable (v) regions (anti-CD4). It was administered intravenously (IV) as a single dose. The dose was escalated throughout the study between patient groups, and individual patients received 50, 100, or 200 mg per dose. The objectives of the study were: (1) monitor escalating doses of chimeric anti-CD4 for possible toxicity and define a maximally tolerated or optimal biologic dose, (2) measure the pharmacokinetics of chimeric anti-CD4, (3) determine the antigenicity of chimeric anti-CD4, (4) evaluate the immunosuppressive and tolerogenic properties of chimeric anti-CD4 by monitoring a variety of T-cell functions, and (5) assess the antitumor effects of chimeric anti-CD4 in patients with MF.

MATERIALS AND METHODS

Patients

Eight patients were treated in the study. Four of the patients were women and four were men, ranging in age from 41 to 77 with a mean of 57 years. The clinical profile of the patients including the extent of disease before treatment and their history of prior therapy has been summarized in Table 1. Seven patients had disease limited to the skin (six with plaque stage disease involving <10% of the total skin surface and one with plaque stage disease involving >10% of the total skin surface). One patient had pathologic adenopathy without measurable skin disease. All patients had failed at least one form of conventional therapy, with a mean of 2.4 prior therapeutic modalities (range, 1 to 5). 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

From the Department of Radiation Oncology, Clinical Laboratories and the Department of Pathology, Department of Internal Medicine, Division of Medical Oncology, Stanford University School of Medicine, Stanford, CA; Oregon Health Sciences University, Department of Radiation Oncology, Portland, OR; and the Fred Hutchinson Cancer Research Center, Clinical Division, Seattle, WA.

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Address reprint requests to Susan Knox, PhD, MD, Stanford University Medical Center, Department of Radiation Oncology (A-093), Stanford, CA 94305-5105.

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therapy during the 12-week study period; (3) serum creatinine less than 1.5 mg/dL; (4) SGOT within normal limits; (5) white blood cell count (WBC) >4,000/mm²; platelets >100,000/mm²; granulocytes ≥1,500/mm³; Hgb ≥10 gm/dL and CD4 count >300/mm³; (6) human immunodeficiency virus (HIV) negativity; (7) physiologic age ≥20 and ≤70; (8) Karnofsky performance status ≥70% and life expectancy ≥15 wk; (9) no active infections or other concomitant active malignancy (except basal or squamous cell carci-noma of the skin); (10) no prior MoAb therapy; (11) at least 4 weeks since the last treatment with any other biologic agent, systemic chemotherapy (CTX), or topical CTX, radiation therapy, or ultraviolet (UV) therapy to any designated measurable or evaluable site(s) of disease; (12) no significant underlying cardiac or respiratory disease; and (13) no underlying medical or psychiatric condition that would compromise the ability of the patient to give informed consent or to complete the study. Informed consent was obtained from all patients before initiation of therapy. The study was approved by the Food and Drug Administration (BB IND #3719) and the Stanford University Internal Review Board.

Chimeric Anti-CD4 MoAb

The antibody was produced by Centocor (Malvern, PA). The chimeric anti-CD4 antibody (cM-T412) is the product of genetic reconstruction and is composed of human IgG1, and κ constant regions and the murine M-T412 heavy and light chain variable region. The binding specificity of the antibody has been localized to conformational epitopes in the extracellular V'V' domains of the CD4 receptor protein. The antibody was supplied in single dose an immunoreactivity identical to that of murine anti-CM. Before administration using a double antigen radioimmunoassay with a detection limit of 0.05 μg/mL as previously described. At the completion of therapy (18 to 24 hours postinfusion) and at 3-week intervals during a 12-week follow-up period, a number of clinical parameters were monitored. These included blood counts, chemistry panels, and urine analyses. Physical examinations were performed at each follow-up visit. Disease activity was followed by measuring skin lesions designated at the beginning of study entry in three dimensions and by measuring lymphadenopathy by physical examination and computed tomography (CT) scans. Patients were similarly followed beyond 12 weeks if they had persistent laboratory abnormalities or ongoing responses. Response criteria were defined as follows. A complete response (CR) was the disappearance of all clinically detectable tumor. A partial response (PR) was a reduction by at least 50% from baseline in the overall tumor size of sentinel lesions (the sum of the products of three dimensions: height and the two largest perpendicular diameters of each of the sentinel lesions so designated before therapy), without the appearance of new lesions at any sites. A minor response (MR) was a decrease of at least 25%, but less than 50%, from baseline in overall tumor size of sentinel lesions without the appearance of new lesions. Progressive disease (PD) was defined as a 25% or greater increase in the size of any lesion as measured by the product of three dimensions or the appearance of any new lesion. Freedom from progression (FFP) was measured from the treatment date to the date that PD was first documented.

Immunopathology

Pretreatment biopsies were obtained from each patient for pathological review and immunophenotyping. Four- to 6-mm punch skin biopsies were obtained. Half of each biopsy was fixed in formalin and processed routinely for hematoxylin and eosin (H&E) stained sections. The remaining tissue was snap frozen for immunologic studies. Biopsies were stained with a panel of MoAb including: CD2 (leu 5), CD3 (leu 4), CD4 (leu 3), CD5 (leu 1), CD7 (leu 9), CD8 (leu 2), Ki-67, and BF1 (alpha/beta T-cell receptor). Antibody binding was detected by a three-stage antibody-biotin-avidin method as previously described.

Peripheral Blood Immunophenotyping

Blood was collected into EDTA (Vacutainers; Becton Dickinson, Rutherford, NJ) and was processed within 6 hours of collection. Flow cytometry was performed on the EDTA-anticoagulated whole blood using a FACScan (Becton Dickinson, Mountain View, CA) flow cytometer with LYSIS II software. Cells, up to 1 × 10⁶, were stained at 4°C for 30 minutes in the dark, then lysed with ammonium chloride EDTA and analyzed by measuring skin lesions at 5 and 20 minutes after incubation at room temperature. Anti-CD45 fluorescein isothiocyanate (FITC) and anti-CD14 R-phycoerythrin (PE) (Becton Dickinson, San Francisco, CA) were used as markers for T and B lymphocytes, respectively.
Jose, CA) were used to define the lymphocyte analysis gate, from which the percent positive cells was calculated. The leucocyte count and percent lymphocytes obtained from the Coulter STKS were used to compute the absolute number of positive cells. The panel included anti-CD3 FITC/anti-CD8 PE, anti-CD20 FITC/anti-CD16, and CD56 PE (Becton Dickinson), and a saturating concentration (1 pg) of Pneumovac 23 and serum from a monospecific anti-human IgG MoAb that does not competitively inhibit the binding of M-T412), along with appropriate murine isotype-matched controls. To assess the number of CD4-positive lymphoid cells coated with M-T412, washed buffy coat cells (up to 1 x 10^6) were mixed with equal amounts of heat-inactivated rabbit serum and stained with 2.4 pg of FITC-conjugated polyclonal rabbit IgG antimurine M-T412 and matched rabbit isotype control (Fab'; J. T. Biochemicals, San Diego, CA). To estimate the amount of chimeric anti-CD4 bound to the cells, an experiment was performed in which normal blood was incubated with M-T412 at a final concentration of 50 pg/mL. Mononuclear cells were prepared by density gradient separation on Ficoll Hypaque (Pharmacia LKB Biotechnology, Inc, Piscataway, NJ), then stained with M-T412 FITC and antimurine M-T412 FITC. Patient sample values were estimated from a standard curve.

Human Antichimeric Antibody Responses

Human antichimeric antibody (HACA) levels were determined using an enzyme-linked immunosorbent assay (ELISA). Ninety-six well microtiter plates were coated with chimeric anti-CD4 MoAb. Fifty microliters of the coat antigen solution (10 pg/mL in carbonate buffer pH 9.6) was dispensed in each well in a flat-bottom microtiter plate (Dynatech Immunon) Lab, Inc, Chantilly, VA). Plates were washed five times with 0.05% Triton X 100 in phosphate-buffered saline (PBS) before use. Nonspecific protein binding sites were blocked by filling wells with 2% bovine serum albumin (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 in 2% BSA in PBS. Goat antimonospecific (y chain specific) MoAb (Sigma, St Louis, MO) was used as a positive control. Plates were incubated at room temperature for 1 hour and washed four times. Horse serum peroxidase (HRP)-conjugated chimeric anti-CD4 MoAb (final dilution 1/166) or HRP conjugated goat antihuman lambda (Sigma) and k (Caltag Lab, S. Francisco, CA) chain antibodies were added (50 pg) to each well at a final concentration of 1 pg/mL in 2% BSA-PBS. After a second incubation for 1 hour, plates were washed four times, and 100 pg of enzyme substrate solution (H2O2 30% J.T. Baker Chemical Co, Phillipsburg, NJ; 3.5 pg/mL), ABTS (2,2'-Azino bis (3-ethylbenzthiazoline sulfonic acid) (Sigma, St Louis, MO; stock 15 ng/mL, 10 pg/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 kinetic micro plate reader (Molecular Devices, Palo Alto, CA) or ELISA microplate reader (Dynatech) at optical density (OD) 405 nm.

Tolerogenicity

Patients were immunized with Pneumovac 23 (Merck and Co, Inc, Westpoint, PA) and in some cases with keyhole limpet hemocyanin (KLH) (Calbiochem, San Diego, CA) subcutaneously 24 hours after the first antibody infusion. An ELISA was used to monitor antipneumococcal Type III polysaccharide (Ell Lilly Laboratory, Indianapolis, IN) and anti-KLH responses. Plates were coated with KLH as above at a final concentration of 10 pg/mL, or Type III pneumococcal polysaccharide at a final concentration of 1.0 pg/mL in 0.1 mol/L HEPES buffer pH 3.5,15 Undiluted serum of normal volunteers previously immunized with Pneumovac 23 and serum from a monokley immunized with KLH (at a dilution of 1:25 into patient pretreatment sera) were used as positive controls.

T-Cell Functional Assays

Mixed lymphocyte culture. Peripheral blood mononuclear cells from hepatic 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 7.5 x 10^5 responder lymphocytes per well. In addition, each well contained 7.5 x 10^5 stimulator cells (irradiated with 4,000 cGy), and 10% heat-inactivated fetal calf serum (Gemini Bioproducts, Inc, Calabasas, CA) serum in RPMI 1640 (Mediatech) culture medium supplemented with 20 mmol/L HEPES, 20 mmol/L L-glutamine, and antibiotics. After 10 hours of incubation (5% CO2, 37°C) cultures were labeled with 1 pg of 3H-thymidine (specific activity 5 Ci mmol/L; Amersham, Arlington Heights, IL) 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 two unrelated healthy donors. All results are expressed as the mean of the three stimulation indices (cpm stimulated culture/cpm background) on each sampling day to allow for 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 10^6 were placed into a flat-bottom 96-well microtiter plate. The culture medium consisted of RPMI 1640 supplemented with 10% heat-inactivated fetal calf serum, 2 mmol/L glutamine, 100 U/mL penicillin, and 10 µg/ml of streptomycin. Triplicate assays were performed using KLH (Sigma) at 100 µg and 10 pg/mL, tetanus toxoid (Connaught Laboratories Limited, Willowdale, Toronto, Canada) at a final concentration of 1/1000 and 1/1500, 7.5 µg phytohemagglutinin/well (PHA; Sigma) and Pneumococcal polysaccharide Type III at 5.8 and 11.5 µg/well. 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.

Assessment of Baseline Immunocompetence

Pretreatment mixed lymphocyte culture (MLC) and T-cell proliferation assay results using PHA and tetanus toxoid, as well as skin tests to measure delayed type hypersensitivity to Tuberculin Purified Protein Derivative (PPD) (Connaught, Willowdale, Ontario, Canada), mumps (Connaught, Swiftwater, PA), candida (Miles, Inc, Elkhart, IN), trichophyton (Berkeley Biologicals, Berkeley, CA) and coccidioidin (Berkeley Biologicals, Berkeley, CA) were used to assess the general level of the immunocompetence of the patients before treatment.

RESULTS

Clinical Responses

The clinical responses for patients treated with 50 to 200 mg of chimeric anti-CD4 MAB are shown in Table 2. The dose administered (expressed in terms of mg and mg/kg) is shown for each patient as well as the subsequent best response and FFP in months measured from the treatment date to the date that PD was first documented or reported. The clinical responses consisted of decreased size and/or induration of the skin lesions. All patients with skin lesions had
dose and clinical response. However, the data suggest that tumor burden is inversely related to response.

Patient no. 3 had an MR with an erythema/induration/size ranged from 6 to 52 weeks, with an average of 25 weeks. Only patient no. 4, who was treated on a compassionate use basis and had extensive and progressive adenopathy at the time of treatment, failed to show any improvement and continued to have PD. Although the sample size is small, there does not appear to be a significant correlation between dose and clinical response. However, the data suggest that tumor burden is inversely related to response.

Toxicity

All of the patients tolerated the treatment well without any significant problems or complications. Nevertheless, some patients complained of fatigue postinfusion and others reported symptoms that included myalgia, headache, nausea, chills, and fever. The adverse events observed following administration of the chimeric anti-CD4 MoAb have been summarized in Table 3. All of these with the exception of a skin reaction that occurred in patient no. 5 after treatment and a headache that occurred in patient no. 7 days after treatment were thought to be either possibly or probably related to administration of the MoAb. There was no Grade 3 or 4 toxicity. All reported symptoms responded to acetaminophen, antihistamines and/or nonsteroidal antiinflammatory drugs as indicated. There was no clinical evidence of immunsuppression. There were also no significant changes in any routine laboratory parameters (serum chemistries, urine analyses and red blood cell and platelet counts) attributable to the MoAb therapy following treatment.

Peripheral Blood Immunophenotyping

Pretreatment and nadir CD4 and CD8 counts are shown for each patient in Table 4. The time of onset and duration of suppression of CD8 counts was less than that observed for CD4 counts. The nadir of CD8 counts occurred 24 hours postinfusion, and in all but one patient (no. 4) persisted for only 1 week. There was some waning and waning of counts during and after the recovery period. The two patients treated with 200 mg had transient decreases in CD14 (macrophage) counts and one patient treated with 100 mg and both patients treated with 200 mg had transiently decreased CD20 (B lymphocyte) counts that returned to normal posttreatment levels within 1 week of treatment (data not shown). All eight patients had depressed CD16 counts (NK cells) 24 hours postinfusion (data not shown). Again, there was no obvious dose response relationship, and all CD16 counts had returned to normal or pretreatment levels 1 week posttreatment, with the exception of patient no. 3's counts which took 3 weeks to recover. Antibody coated cells were transiently detectable at very low levels 24 hours postinjection in three of four patients treated with 50 mg of antibody. Maximal numbers of antibody coated cells were detected in the other dose groups 24 hours postinjection, with staining of an average of 24% and 41% of peripheral blood mononuclear cells with antichimeric anti-CD4 idiotyp MoAb in patients treated with 100 and 200 mg, respectively. Antibody coated cells were still detectable 1 and 3 weeks posttreatment in patients treated at these two dose levels (data not shown).

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Event</th>
<th>Toxicity Grade</th>
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<tbody>
<tr>
<td>1</td>
<td>Fatigue</td>
<td>1</td>
</tr>
<tr>
<td>2</td>
<td>Leukopenia</td>
<td>2</td>
</tr>
<tr>
<td>3</td>
<td>Fatigue</td>
<td>1</td>
</tr>
<tr>
<td>4</td>
<td>Leukopenia</td>
<td>2</td>
</tr>
<tr>
<td>5</td>
<td>Nausea</td>
<td>1</td>
</tr>
<tr>
<td>6</td>
<td>Headache</td>
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</tr>
<tr>
<td>7</td>
<td>Nausea</td>
<td>2</td>
</tr>
<tr>
<td>8</td>
<td>Vomiting</td>
<td>2</td>
</tr>
</tbody>
</table>

* In the absence of infection.
† Probably not related to the MoAb administration as this occurred 2 to 12 days after MoAb infusion.
Table 4. Peripheral Blood Immunophenotyping

<table>
<thead>
<tr>
<th>Pt No.</th>
<th>MoAb Dose (mg)</th>
<th>Pretreatment</th>
<th>Nadir</th>
<th>Onset (hrs)</th>
<th>Duration† (wks)</th>
<th>CD4 Counts*</th>
<th>Pretreatment</th>
<th>Nadir</th>
<th>Onset (hrs)</th>
<th>Duration† (wks)</th>
<th>CD8 Counts*</th>
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<tbody>
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<td>50</td>
<td>290</td>
<td>90</td>
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<td>150</td>
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<td>3</td>
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<td>160</td>
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<td>1,180</td>
<td>300</td>
<td>24</td>
<td>1</td>
<td>550</td>
<td>270</td>
<td>24</td>
<td>1</td>
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</table>

CD4 and CD8 counts are expressed in units of cells/μL. The normal range for CD4 is 540 to 1,660/μL. The normal range for CD8 is 270 to 930/μL.

† Total time required for recovery of counts to within approximately 10% of the pretreatment level (if counts pretreatment were below normal).
‡ The patient had progressive disease and died before the counts returned to pretreatment levels.
§ The patient developed progressive disease and required other therapy before the count returned to the pretreatment level.
‖ Lost to follow-up with CD4 count of 350/μL.

Pharmacokinetics

Mean serum levels ± standard deviation (SD) of the chimeric anti-CD4 MoAb are shown in Table 5 as a function of dose. Higher serum levels of MoAb were achieved with higher doses of administered MoAb, although there was not a linear relationship between administered dose and subsequent serum levels. By 1 week postinfusion at the highest dose level, the level of MoAb remaining in the serum was <1 μg/mL.

Antibody Responses

Antibody responses to the chimeric anti-CD4 MoAb, KLH and type III pneumococcal polysaccharide (PPS III) were measured and are summarized in Table 6. Only one patient (no. 5) made a significant, but very low titer HACA response that was only detectable 3 weeks posttreatment. All patients were immunized with a pneumococcal vaccine, and five were also immunized with KLH 24 hours after antibody infusion to determine whether the chimeric anti-CD4 would induce tolerance to a coadministered antigen. Six patients made an antibody response to one or both antigens. Patients no. 4 and no. 8 failed to make antibody responses and were either anergic or poorly responsive to a variety of skin test antigens before therapy (Table 7).

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 studies are summarized in Table 8. Most patients had a temporary suppression of these responses. The level of reactivity in these assays generally returned to baseline levels within 1 to 6 weeks posttreatment. There was no direct relationship between dose or the extent of duration of the suppressed responses. In addition, in two patients with pretreatment reactivity to pneumococcal polysaccharide, there was also suppression of their proliferative responses to this antigen following treatment. Despite the fact that preexisting responses to pneumococcal antigens were suppressed by the anti-CD4 antibody, five patients were able to make new T-cell proliferative responses

Table 5. Pharmacokinetics: Serum Levels of Chimeric Anti-CD4 MoAb

<table>
<thead>
<tr>
<th>Dose Level</th>
<th>Time* (h)</th>
<th>Mean MoAb Concentration ± SD (μg/mL)</th>
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<tr>
<td>50 mg</td>
<td>1</td>
<td>12.6 ± 1.3</td>
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<tr>
<td></td>
<td>18-24</td>
<td>8.0 ± 1.1</td>
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<tr>
<td>100 mg</td>
<td>1</td>
<td>19.7 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>18-24</td>
<td>9.2 ± 4.1</td>
</tr>
<tr>
<td>200 mg</td>
<td>1</td>
<td>28.5†</td>
</tr>
<tr>
<td></td>
<td>18-24</td>
<td>35.4 ± 18.9</td>
</tr>
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* Time post-MoAb infusion.
† Based on one patient only.

Table 6. Antibody Responses

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Dose Level</th>
<th>HACA*</th>
<th>Anti-KLH</th>
<th>Anti-PPS III</th>
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<tr>
<td>1</td>
<td>50 mg</td>
<td>-</td>
<td>NA</td>
<td>+</td>
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<td>50 mg</td>
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<tr>
<td>5</td>
<td>200 mg</td>
<td>+†</td>
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<tr>
<td>6</td>
<td>200 mg</td>
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<td>+</td>
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<tr>
<td>7</td>
<td>50 mg</td>
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<tr>
<td>8</td>
<td>50 mg</td>
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* HACA: Human antichimeric antibody response.
† Very low titer at 3 weeks only.
to pneumococcal immunization or to Pneumovac 23 and four patients made T-cell proliferative responses to KLH. Thus, chimeric anti-CD4 depressed the MLR and preexisting T-cell proliferative responses to tetanus toxoid and PPS III, 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**

In this dose escalation study of single doses of 50 to 200 mg of chimeric anti-CD4 MoAb in patients with MF, five of eight patients had a PR and the overall response rate was approximately 88%. The FFP ranged from 6 to 52 weeks, with an average of 25 weeks. This response rate is superior to response rates reported for therapy of CTCL with multiple doses of unlabeled murine MoAb with brief response durations of =4 months, and is also better than response rates obtained in CTCL patients following radioimmunotherapy. This relatively high response rate may be due in part to the relatively low tumor burden in the group of patients treated in this study. Interestingly, there was no obvious dose response relationship.

Although clinical responses of fairly short duration have been reported in patients with CTCL treated with murine MoAb, these responses were limited in part by the development of antimouse Ig antibodies as well as antiidiotypic antibodies in approximately 50% to 100% of the patients. In the study reported here, only one patient made a significant, but very low level HACA response. The rate of HACA formation (12.5%) was lower than previously observed (28.5%) in a similar study using a different chimeric anti-CD4 MoAb. This relatively high response rate may be due in part to the relatively low tumor burden in the group of patients treated in this study.

The antibody infusions were well tolerated. The mild side effects (e.g., fever, fatigue, and nausea) reported are not uncommon during or after antibody infusions. Importantly, there was no dose-limiting toxicity observed at any of the dose levels studied. This is in contrast to other unlabeled MoAb studies in which more significant toxicities were observed, and to radioimmunotherapy studies in which myelosuppression is the dose-limiting toxicity unless bone marrow or peripheral stem cell reinfusion is used. Although there was a marked temporary reduction in CD4 and CD8 counts and in vitro T-cell proliferative responses following treatment, there were no clinical indications of myelosuppression. Specifically, none of the patients developed opportunistic infections, whereas two of seven patients previously treated with a different chimeric anti-CD4 MoAb developed opportunistic infections (although in one of these patients, the infections in retrospect predated the MoAb therapy). Interestingly, the extent and duration of CD4 and CD8 count suppression in this study in this patient population was considerably less than that previously observed in multiple sclerosis and rheumatoid arthritis patients treated with similar doses of the same antibody (personal communication, Dr. Richard McCloskey, May 1995). As previously reported in a similar patient population treated with a different chimeric anti-CD4 MoAb, treatment with chimeric anti-CD4 MoAb (c-MT412) was not tolerogenic at the dose levels studied and did not suppress the ability of patients to mount an immune response to a new antigen administered 24 hours after MoAb infusion.

The results reported here are encouraging and demonstrate that this chimeric MoAb was safe, very tolerated, and efficacious in the patients studied. These results suggest that the epitope specificity of the MoAb is an important determinant of toxicity and efficacy, and that this chimeric anti-CD4 MoAb may be useful for the treatment of CD4 positive lymphoid malignancies. This MoAb might be even more efficacious if administered as multiple doses or radiolabeled. It is the least immunogenic of any chimeric MoAb studied to date in patients with CTCL, but a direct comparison with murine anti-CD4 against the same epitope is needed to make a definitive statement about relative immunogenicity. Future studies are needed to determine the relative immunogenicity of this chimeric anti-CD4 compared with the murine version of the antibody, to better define an optional biologic dose, to optimize the treatment regimen, and to elucidate the mechanism of action of anti-CD4 MoAb in patients with CTCL.

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REFERENCES

Treatment of cutaneous T-cell lymphoma with chimeric anti-CD4 monoclonal antibody

S Knox, RT Hoppe, D Maloney, I Gibbs, S Fowler, C Marquez, PJ Cornbleet and R Levy