Clinical Response of a Patient With Diffuse Histiocytic Lymphoma to Adoptive Chemoimmunotherapy Using Cyclophosphamide and Alloactivated Haploidentical Lymphocytes

A Case Report and Phase I Trial

PETER C. KOHLER, MD,†‡ JACQUELYN A. HANK, PhD,§ ROBERT EXTEN, MD,∥ DEBORAH Z. MINKOFF, BS,* DOUGLAS G. WILSON, PhD, MD,⁵ AND PAUL M. SONDEL, MD, PhD‡###

Adoptive chemoimmunotherapy has cured experimentally induced tumors in animals, but its clinical use has been limited. Six patients were treated with refractory neoplasms in a Phase I study with cyclophosphamide (CPM) and alloactivated haploidentical lymphocytes. Patients received an immunosuppressive dose of CPM (800 mg/m²) followed by haploidentical lymphocytes primed in vitro with alloantigens in mixed lymphocyte culture (MLC). One week later patients received a second infusion of alloactivated lymphocytes expanded in T-cell growth factor (TCGF). The total number of cells given to each patient progressively increased, with a single patient receiving $35.5 \times 10^9$ cells. Transient febrile responses and delayed-type hypersensitivity reactions at the intravenous sites were the only toxicities noted. A complete clinical response lasting 12 weeks was seen in a single patient with diffuse histiocytic lymphoma. Our experience indicates that adoptive chemoimmunotherapy can be given to patients safely and merits further clinical testing.


Cure of transplanted, experimentally induced animal tumors can be accomplished by the infusion of appropriately sensitized lymphocytes.¹⁻⁴ Animal models have delineated those conditions necessary to obtain a therapeutic response using adoptive immunotherapy. First, adequate numbers of appropriately sensitized lymphocytes must be transferred to animals with a minimal number of residual tumor cells. For example, $2 \times 10^7$ immune lymphocytes given to a leukemic mouse when in a temporary remission can be curative.⁵ Secondly, one must overcome the tumor-generated suppressor T-cell system, which can affect the antitumor activity of either endogenous or adoptively transferred immune cells.⁶ Cyclophosphamide (CPM) given before lymphocyte infusions can eliminate this suppressor population, resulting in an antitumor response not seen with CPM alone.⁷⁻⁸

Extrapolation from murine studies indicates that on the basis of body mass, at least $10^{10}$ immune human lymphocytes would be required to obtain a comparable immunotherapeutic response in patients with cancer. Until recently, _in vitro_ culturing and expansion of this number of cells has been virtually impossible, but with the combined use of leukapheresis, bulk culture priming, and T-cell growth factor (TCGF), it is now possible to obtain $10^{10}$ _in vitro_ activated human T-lymphocytes.

Our pilot study⁹ investigated the use of alloactivated syngeneic lymphocytes from a healthy donor to his identical twin with relapsed acute myelomonocytic leu-

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From the Departments of *Human Oncology, †Genetics, ‡Medicine, § Nuclear Medicine, and #Pediatrics, University of Wisconsin Medical School, Madison, Wisconsin.

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‡ NIH Postdoctoral Trainee.
§ Fellow of the Cancer Research Institute, New York.
** Scholar of the Leukemia Society of America and J. L. and G. A. Hartford Foundation Fellow.

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Address for reprints: Paul M. Sondel, MD, PhD, University of Wisconsin, Clinical Science Center, 600 Highland Avenue, Room K4/448, Madison, WI 53792.

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kemia. Donor lymphocytes were sensitized with alloantigens in vitro, expanded in TCGF, and infused intravenously and intraperitoneally. No immediate toxicities were noted following either infusion, but the patient’s death from sepsis unrelated to the infusion precluded any extensive evaluation of delayed toxicity or therapeutic benefit.

This report presents the results of a Phase I trial of adoptive chemoimmunotherapy in six patients with cancer unresponsive to conventional treatment.

Patients and Methods

Patient and Donor Selection

Patients in this study had histologically documented malignancies unresponsive to conventional and other experimental therapy. The clinical characteristics of the six patients studied and the relationship of the patients to their donors are listed in Table 1. All were ambulatory and had not received treatment for at least 2 weeks before entry. Criteria for acceptance included no clinical evidence of uncompensated congestive heart failure, stable hematologic (absolute neutrophil count ≥ 1000, platelet count ≥ 100,000), renal, hepatic, and pulmonary (FEV₁/FVC ≥ 50%, O₂ saturation ≥ 90%) functions. The availability of a healthy histocompatibility antigen (HLA)-haploidentical first-degree relative willing to undergo leukapheresis was also necessary. In those cases where HLA haplidency could not be based on hereditary factors (i.e., siblings), HLA typing was used. All patients and donors signed informed consent forms approved by the University of Wisconsin Human Subjects Committee.

Lymphocyte Collection

Donor peripheral blood lymphocytes (PBL) were obtained as theuffy coat by-product of plateletpheresis (Badger Red Cross, Madison, WI). Centrifugation at 400 × g separated theuffy coat layer from the platelet-rich plasma. Separation of the lymphocytes from the leukocyte pool was done by Ficoll-Isopaque (Sigma, St. Louis) density gradient followed by washing of the cells with phosphate-buffered saline. The lymphocytes were then resuspended in HS-RPMI (medium RPMI-1640 supplemented with 10% pooled human serum, L-glutamine, penicillin, and streptomycin). This same process was used in obtaining lymphocytes from ten healthy unrelated volunteer platelet donors. These lymphocytes were combined and irradiated (2500 rad) in order to serve as the “pool” of alloantigen-presenting cells.

Generation of T-Cell Growth Factor

Pooled irradiated PBL from six unrelated donors obtained randomly from the Badger Red Cross were cultured at 10⁶ cells/ml in the presence of 1% PHA (Difco, Detroit, MI) and 10⁵ cells/ml of irradiated, allogeneic, Epstein-Barr Virus (EBV)-transformed lymphoblastoid cell line cells. These were cultured in RPMI medium as modified above, but containing only 1% human serum. Supernatants were collected at 48 hours, filtered, and tested for potency as described previously.¹⁰

Bulk Culture and TCGF Expansion

Haploidentical donor lymphocytes were initially stimulated in mixed lymphocyte culture (MLC) with irradiated cryopreserved pooled lymphocytes.¹¹ In 250-ml flasks (Falcon, Oxnard, CA), 25 × 10⁶ haploidentical donor lymphocytes were cultured with 25 × 10⁶ irradiated (2500 rad) pooled stimulating lymphocytes in 50 ml HS-RPMI, modified as described above. Multiple replicate flasks were cultured for each infusion. In the four patients (Patients A, B, C, and D) where cryopreserved autologous tumor cells were available, they were

<table>
<thead>
<tr>
<th>Patient identification</th>
<th>Sex/age</th>
<th>Diagnosis</th>
<th>Previous therapy</th>
<th>Relationship of donor to patient</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>M/16</td>
<td>Osteogenic sarcoma</td>
<td>Surgical amputation Chemotherapy</td>
<td>Mother</td>
</tr>
<tr>
<td>B</td>
<td>M/43</td>
<td>Acute myelomonocytic leukemia</td>
<td>Chemotherapy</td>
<td>Sister</td>
</tr>
<tr>
<td>C</td>
<td>F/35</td>
<td>Recurrent malignant melanoma</td>
<td>Radiation therapy Surgery</td>
<td>Brother</td>
</tr>
<tr>
<td>D</td>
<td>M/46</td>
<td>Recurrent malignant melanoma</td>
<td>Surgery Radiation therapy</td>
<td>Son</td>
</tr>
<tr>
<td>E</td>
<td>F/50</td>
<td>Recurrent malignant melanoma</td>
<td>Surgery Chemotherapy</td>
<td>Son</td>
</tr>
<tr>
<td>F</td>
<td>M/57</td>
<td>Diffuse histiocytic lymphoma Stage IVA</td>
<td>Chemotherapy</td>
<td>(1) Daughter (2) Son</td>
</tr>
</tbody>
</table>
irradiated (4000 rad) and added as stimulating cells (1 × 10^6 cells/flask for Patients A and B; 1 × 10^5 cells/flask for Patient C and 1 × 10^4 cells/flask for Patient D) to flasks containing 25 × 10^6 donor lymphocytes and 25 × 10^6 irradiated pooled stimulating lymphocytes. Following culturing for 6 days at 37°C in 5% CO_2, the lymphocytes were harvested, washed twice, and resuspended in serum-free RPMI. These lymphocytes are designated MLC-activated cells.

In those patients in whom infusion of lymphocytes expanded in TCGF was planned, a portion of the MLC-activated donor cells were continued in culture with HS-RPMI and 25% to 50% TCGF at 10^5 cells/ml. Multiple 250-ml flasks containing 100 ml of this cell suspension were cultured for each patient. After 6 days of culturing, these lymphocytes, designated “TCGF-expanded cells,” were harvested, washed, and resuspended in the same manner as the MLC-activated cells. To assure sterility, microbiologic culturing was done selectively before each infusion.

Experimental Design

Before each infusion of MLC cells, patients received intravenous CPM at a dose of 800 mg/m². Twenty-four hours after CPM, MLC-generated lymphocytes were given by intravenous infusion. Each infusion was carried out over 1 hour. The lymphocytes were delivered through intravenous tubing with the interposition of a blood transfusion filter to remove any small clumps of cells. All patients (except Patient A) received TCGF-expanded lymphocytes by intravenous infusion 7 days later. Four weeks after this first cycle, Patients E and F were given additional cycles of CPM, followed by MLC cells and then TCGF-expanded lymphocytes.

Radioactive Indium (111In) Scanning

With each infusion, 1 × 10^8 cells were cultured with 500 to 600 µCi of indium 111 (111In)-oxide for 30 minutes, washed in sterile normal saline, and mixed with the remainder of lymphocytes to be infused.12 Viability, as judged by dye exclusion, was greater than 90%. Sequential gamma camera scans were done 1 to 2 hours following the completion of each infusion and at approximately 24 and 48 hours postinfusion. Before the TCGF-expanded lymphocyte infusion, scanning was done to account for background counts from the previous MLC infusion.

Toxicity Evaluation

All patients were hospitalized beginning on the day of the CPM infusion and for 2 days following the cell infusion. Pretreatment physical examination, chest roentgenogram, pulmonary function test, and complete blood count with differential, platelet count, and multiphasic blood analysis served as baseline. Ear oximetry for O_2 saturation was performed before and approximately 4 hours after each cell infusion. Physical examination and clinical and laboratory assessment (hemogram and multiphasic blood analysis) were performed daily during hospitalization and at weekly clinic visits.

In Vitro Testing

Donor lymphocytes for each patient were cultured as responding cells for 7 days with media, irradiated autologous lymphocytes (Donor), pooled allogeneic lymphocytes (Pool), lymphocytes from a single unrelated individual (X_i), and the patient's lymphocytes (Patient). Responders and stimulators were both at 1 × 10^5 cells/0.25 cc well (Flow, McLean, VA) and done in quadruplicate. Proliferation was measured by 6-hour ^3HTdR incorporation (New England Nuclear, Boston, MA).

Both MLC-activated and TCGF-expanded cells were tested for cytotoxic function using a 4-hour Chromium 51 (^51Cr)-release assay described elsewhere.13 Targets included lymphocytes from the donor, the patient, an unrelated individual and, when available, the patient’s tumor. Cytotoxicity was determined in 0.25-ml micro-wells with 5 × 10^5 targets and 1.5 × 10^5 effectors per well.

Results

Lymphocyte Recovery From Leukapheresis and Cell Culture

The total number of lymphocytes obtained from each donor following leukapheresis and Ficoll purification ranged from 2.8 × 10^9 to 7.6 × 10^9 (mean, 5.1 × 10^9). Percent recovery following alloactivation in MLC ranged from 75% to 333% (mean, 185%). After expansion with TCGF, recovery ranged from 210% to 925% (mean, 387%) of the initial number of cultured cells. Two donors were required for Patient F. Following alloactivation of the patient’s daughter’s cells, poor expansion with TCGF resulted in insufficient lymphocyte numbers for infusion. The patient’s son was subsequently used for two complete cycles with good in vitro lymphocyte recovery and function.

Cyclophosphamide Dose and Cell Infusion

All patients received CPM (800 mg/m²) on the first day of each cycle. Twenty-four hours after CPM, MLC-generated lymphocytes were given by intravenous infusion (Table 2).

Seven days after the infusion of alloactivated lymphocytes, TCGF-expanded lymphocytes were infused. The first patient (Patient A) was scheduled to receive only
TABLE 2. Cyclophosphamide Dose and Cell Infusion Numbers for Each Patient

<table>
<thead>
<tr>
<th>Patient</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E(1)</th>
<th>E(2)</th>
<th>F(1)</th>
<th>F(2)</th>
<th>F(3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dose of cyclophosphamide (Day = 0 of each cycle)</td>
<td>1.3 g</td>
<td>1.8 g</td>
<td>1.5 g</td>
<td>1.4 g</td>
<td>1.3 g</td>
<td>1.3 g</td>
<td>1.5 g</td>
<td>1.5 g</td>
<td>1.5 g</td>
</tr>
<tr>
<td>No. of MLC-generated cells infused × 10^9 (Day = 1 of each cycle)</td>
<td>0.5</td>
<td>0.5</td>
<td>2.1</td>
<td>4.7</td>
<td>3.4</td>
<td>4.8</td>
<td>1.0</td>
<td>4.8</td>
<td>11.0</td>
</tr>
<tr>
<td>No. of TCGF-expanded lymphocytes infused × 10^9 (Day = 8 of each cycle)</td>
<td>—</td>
<td>0.5</td>
<td>0.8</td>
<td>0.9</td>
<td>2.3</td>
<td>3.0</td>
<td>—</td>
<td>3.7</td>
<td>15.0</td>
</tr>
<tr>
<td>Total no. of cells infused × 10^9</td>
<td>0.5</td>
<td>1.0</td>
<td>2.9</td>
<td>5.6</td>
<td>13.5</td>
<td>35.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Numbers in parentheses denote cycle in those patients receiving multiple cycles.

MLC: mixed lymphocyte culture; TCGF: T-cell growth factor.

TABLE 2. Cyclophosphamide Dose and Cell Infusion Numbers for Each Patient

MLC cells. Inadequate cell recovery prevented the infusion of TCGF-expanded cells for Patient cycle F(1). The cell dose of each infusion, as well as the total number of cells infused, progressively increased with each patient. Patient F received a total of 35.5 × 10^9 cells with single infusions of 11.0 × 10^9 and 15.0 × 10^9 MLC-activated and TCGF-expanded cells, respectively, in the third cycle.

Toxicity

The anticipated nausea and vomiting for 24 to 36 hours following the administration of CPM was experienced by all patients. Delayed toxicities from CPM included declines in both leukocyte and platelet counts. In no case did the absolute neutrophil count drop below 500/mm^3, and no infectious complications were seen. Platelet count depression was minimal, and nadirs were never below 100,000/mm^3. Two patients, Patients C and E, complained of minimal hair loss.

No immediate toxicities accompanied the administration of either alloactivated or TCGF-expanded lymphocytes. Blood pressure and pulse, checked frequently during each infusion, showed no appreciable changes. Ear oximetry done before and 4 hours after each of the cell infusions showed a mean decrease of 1.3% in O_2 saturation (range, 0%–5%). No patient reported dyspnea, cough, or chest pain during or after the infusion. Chest roentgenograms of the first two patients 24 hours after infusions showed no evidence of pulmonary infiltrates.

A transient febrile response (temperature (T) > 38°C) was noted following 3 of the 16 cell infusions. This was seen following the MLC-activated cell infusion in Patient C and after both the MLC-activated and TCGF-expanded cell infusions for Patient F(3). The febrile reaction was accompanied by chills in Patient F. Microbiologic culturing of blood and urine were negative, and there was no evidence of hemolytic reaction. In both cases the patients were afebrile within 4 to 6 hours using acetaminophen as the only treatment.

Of the five patients receiving cell infusions through peripheral lines (Patient B had a Hickman catheter), four developed erythema and induration along the venous track of the intravenous injection site 12 to 24 hours after lymphocyte infusions. Gamma camera scanning of the involved area revealed marked ^111^In-oxine activity corresponding to the venous path and the area of erythema (Fig. 1). This suggested a delayed-type hypersensitivity reaction to the endothelial cells of the vein by the alloactivated haploididentical cells entering in a concentrated suspension at that site. Warm packs were used to treat affected patients, with one patient requiring additional topical steroid cream. No permanent scarring or desquamation was noted.

No evidence of graft versus host (GVH)-related complications were noted in any of the patients. A single patient (Patient E) had watery, nonbloody diarrhea as the only symptom 4 hours after the infusion of both MLC-activated and TCGF-expanded cells. Diarrhea resolved within 2 hours without treatment, and subsequent bowel movements were normal. Liver function tests for all patients showed minimal variation with no consistent or significant change.

Distribution of Lymphocytes

Sequential gamma camera scans were done 1 to 2 hours after the completion of each infusion and at approximately 24 and 48 hours postinfusion. Representative scans from a single patient following an MLC-activated cell infusion are shown (Figs. 2A–2D). The mean percent radioactivity (Fig. 3) shows a marked immediate uptake by the lungs of the ^111^In-oxine. Over a period of
FIG. 1. Delayed hypersensitivity reaction caused by alloactivated lymphocytes. Gamma camera scan at 24 hours of left arm of Patient D showing increased uptake of $^{111}$In-oxine-labeled lymphocytes along peripheral vein. The arrow indicates the intravenous site. L: left lung; S: spleen.

FIGS. 2A–2D. Sequential gamma camera scans from Patient D taken following the MLC infusion. Scans A, B, and C are posterior views of thorax and upper abdomen. (A) Scan is 1 hour after infusion and shows the majority of radioactivity localized in both lungs. (B) At 19 hours, the activity in the lungs has decreased, and increased activity is seen in both the liver and spleen. (C) By 46 hours, a further decrease in lung activity is noted. (D) An anterior view of pelvis at 19 hours shows $^{111}$In-oxine activity corresponding to the bone marrow.

24 hours, a decrease in pulmonary activity is accompanied by a progressive rise in hepatic activity. Smaller but easily detectable increases in activity over the 48 hours were seen in the marrow and spleen. No difference in the patterns of distribution between MLC-activated or TCGF-expanded cells could be demonstrated.

In Vitro Testing

Primary mixed leukocyte culture (Table 3) demonstrated good proliferative responses by lymphocytes from all but one of the donors tested. Lymphocytes from the donor for Patient D showed poor in vitro responses, which may have been an artifact of that day's assay, since a good recovery (260%) of donor lymphocytes followed bulk culture priming.
Table 3. Proliferative Responses of Donor Lymphocytes in Primary MLC

<table>
<thead>
<tr>
<th>Donor lymphocytes for patient infusion</th>
<th>A</th>
<th>B</th>
<th>C</th>
<th>D</th>
<th>E(1)</th>
<th>E(2)</th>
<th>F(1)</th>
<th>F(2)</th>
<th>F(3)</th>
</tr>
</thead>
<tbody>
<tr>
<td>cpm ³H-thymidine</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Donor</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>---</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
<td>------</td>
</tr>
<tr>
<td>Media</td>
<td>967</td>
<td>1073</td>
<td>1094</td>
<td>205</td>
<td>139</td>
<td>144</td>
<td>162</td>
<td>2881</td>
<td>1269</td>
</tr>
<tr>
<td>Donor, Donor</td>
<td>720</td>
<td>1385</td>
<td>978</td>
<td>NT</td>
<td>298</td>
<td>196</td>
<td>165</td>
<td>758</td>
<td>NT</td>
</tr>
<tr>
<td>Pool</td>
<td>39,075</td>
<td>78,997</td>
<td>46,886</td>
<td>166</td>
<td>64,088</td>
<td>100,767</td>
<td>15,645</td>
<td>80,246</td>
<td>23,848</td>
</tr>
<tr>
<td>X</td>
<td>2118</td>
<td>135,244</td>
<td>490</td>
<td>270</td>
<td>27,739</td>
<td>36,802</td>
<td>25,774</td>
<td>39,655</td>
<td>19,987</td>
</tr>
<tr>
<td>Patient</td>
<td>21,169</td>
<td>19,568</td>
<td>40,683</td>
<td>NT</td>
<td>23,519</td>
<td>26,267</td>
<td>31,757</td>
<td>12,075</td>
<td>NT</td>
</tr>
</tbody>
</table>

Donor lymphocytes for each patient were cultured as responding cells for 7 days with media, irradiated autologous lymphocytes (Donor), pooled allogeneic lymphocytes (Pool), unrelated lymphocytes (X), or the patient's lymphocytes (Patient). Responders and stimulators were both

The cytotoxic activity of the MLC-activated and TCGF-expanded lymphocytes is shown in Table 4. For each infusion, cytotoxic activity by either MLC-activated or TCGF-expanded donor lymphocytes could be demonstrated to either the patient's lymphocytes, the patient's tumor cells, or to lymphocytes from an unrelated person. Low-level cytotoxicity was seen in the MLC-generated lymphocytes for Patient D. This also was most likely the result of technical problems with that day's assay, as lymphocytes from this same culture expanded in growth factor showed a good cytotoxic response.

Clinical Responses

Before entering this Phase I study all patients had received conventional, and in some cases experimental, therapies, with progression of their disease. One patient in this study had a disappearance of all evidence of disease after the first cycle of COPA and MLC-alloactivated haploidentical lymphocytes. Of the remaining five patients, there was no evidence of any therapeutic response, and two patients (Patients A and B) have died of progressive disease.

Case Report

Patient F is a 58-year-old man who presented to his local physician with a superficial swelling over the dorsum of his left wrist in July 1980. After an unsuccessful trial of antibiotics and the development of multiple other skin nodules, a biopsy of the wrist mass was performed. Interpretation of the biopsy at the Armed Forces Institute of Pathology and the University of Wisconsin Hospital and Clinics (UWH) was consistent with lymphoma.

Initial evaluation at UWH revealed multiple nodules involving the back and thigh as well as prominent axillary and cervical adenopathy. Fever, night sweats, and weight loss were denied, and hepatosplenomegaly was not detected on physical examination. Bilateral lower extremity lymphangiogram and bone marrow biopsy specimen were normal. A repeat biopsy of a superficial nodular lesion showed diffuse histiocytic lymphoma (DHL) Stage IVA.

Initial chemotherapy was instituted with 6 monthly cycles of COPA (cyclophosphamide 1100 mg intravenously [IV], vincristine 2 mg IV, prednisone 200 mg orally × 5 days, doxorubicin 90 mg IV). At the conclusion of the sixth cycle (January

Table 4. Cytotoxic Responses of Pool Primed Donor Lymphocytes After MLC Activation and TCGF Expansion

<table>
<thead>
<tr>
<th>Effector</th>
<th>Patient</th>
<th>Tumor</th>
<th>X</th>
</tr>
</thead>
<tbody>
<tr>
<td>Donor Pool, ± Tu,</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>A. *MLC Day = 1</td>
<td>19.1 ± 1.7</td>
<td>0.2 ± 0.3</td>
<td>NT</td>
</tr>
<tr>
<td>B. *MLC Day = 1</td>
<td>11.3 ± 1.5</td>
<td>4.4 ± 0.4</td>
<td>35.0 ± 0.6</td>
</tr>
<tr>
<td>C. *MLC Day = 1</td>
<td>8.4 ± 2.1</td>
<td>10.9 ± 2.0</td>
<td>NT</td>
</tr>
<tr>
<td>TCGF Day = 8</td>
<td>NT</td>
<td>12.4 ± 2.5</td>
<td>NT</td>
</tr>
<tr>
<td>D. *MLC Day = 1</td>
<td>0.0 ± 1.3</td>
<td>NT</td>
<td>15.2 ± 1.0</td>
</tr>
<tr>
<td>TCGF Day = 8</td>
<td>NT</td>
<td>NT</td>
<td>11.6 ± 2.9</td>
</tr>
<tr>
<td>E. (1) MLC Day = 1</td>
<td>22.9 ± 2.2</td>
<td>NT</td>
<td>25.6 ± 0.9</td>
</tr>
<tr>
<td>TCGF Day = 8</td>
<td>4.7 ± 1.2</td>
<td>NT</td>
<td>25.1 ± 4.1</td>
</tr>
<tr>
<td>E. (2) MLC Day = 29</td>
<td>18.6 ± 1.6</td>
<td>NT</td>
<td>25.2 ± 2.6</td>
</tr>
<tr>
<td>TCGF Day = 36</td>
<td>26.2 ± 3.9</td>
<td>NT</td>
<td>27.4 ± 1.5</td>
</tr>
<tr>
<td>F. (1) MLC Day = 1</td>
<td>NT</td>
<td>NT</td>
<td>38.2 ± 4.3</td>
</tr>
<tr>
<td>TCGF Day = 8</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
<tr>
<td>(2) MLC Day = 29</td>
<td>3.5 ± 1.2</td>
<td>NT</td>
<td>2.9 ± 0.6</td>
</tr>
<tr>
<td>TCGF Day = 36</td>
<td>46.4 ± 8.5</td>
<td>NT</td>
<td>12.0 ± 0.6</td>
</tr>
<tr>
<td>(3) MLC Day = 57</td>
<td>18.4 ± 1.2</td>
<td>NT</td>
<td>26.8 ± 3.7</td>
</tr>
<tr>
<td>TCGF Day = 64</td>
<td>NT</td>
<td>NT</td>
<td>39.3 ± 2.3</td>
</tr>
</tbody>
</table>

Peripheral blood lymphocytes from each donor were cultured in MLC with irradiated allogeneic pooled lymphocytes (Pool), and where available (*) irradiated cyropreserved patient tumor cells (Tu). After 7 days of culturing, the cells were harvested and tested for cytotoxic function using a 4-hour ³Cr-release assay. Targets included lymphocytes from the patient, tumor, and an unrelated individual. MLC-activated lymphocytes were continued in culture with TCGF for an additional 7 days. Cytotoxicity was tested again using the same target cell populations; the effector to target ratio shown is 30:1 in all cases (NT: not tested). The "Day" indicated for MLC and TCGF cells reflects the day of ³Cr-release testing relative to the initial dose of intravenous cyclophosphamide, and in all cases corresponds to the day of infusion of the indicated cell populations. Donors A through F correspond to those in Tables 1 to 3.

MLC: mixed lymphocyte culture; TCGF: T-cell growth factor.
all skin lesions had resolved. Axillary adenopathy was, however, still present. A specimen from a repeat bone marrow biopsy, chest and abdominal roentgenograms, and left axillary node biopsy specimen showed no evidence of lymphoma. Three months after chemotherapy, new skin lesions and cervical adenopathy were noted. Biopsy specimen of a skin nodule showed recurrent DHL. Repeat lymphangiogram, chest roentgenogram, and bone marrow biopsy specimen were normal. Combination chemotherapy was begun with cyclophosphamide (1500 mg IV) vincristine (2 mg IV), and prednisone (200 mg orally), with the disappearance of both skin lesions and cervical adenopathy by the third cycle.

Six weeks after the completion of this second course of chemotherapy, new skin lesions developed that were identical in appearance to those previously documented by biopsy as being lymphoma. Over the next 8-month period, November 1981 through July 1982, the patient received cyclophosphamide (1500 mg IV every 3 weeks X three cycles, 600 mg orally X 5 days every 3 weeks X eight cycles) and prednisone (200 mg orally X 5 days every 3 weeks). Again there was rapid clearing of the lymphomatous skin lesions. On several occasions new skin lesions appeared between cycles; these would resolve following the next cycle of chemotherapy.

From August 1982 through October 1982, multiple new skin lesions and adenopathy appeared despite cyclophosphamide (600 mg orally X 5 days), prednisone, bleomycin (10 mg intramuscularly), and procarbazine (200 mg orally X 10 days) every 3 weeks. The addition of doxorubicin to the above regimen provided an excellent response, but after four cycles of doxorubicin the maximum dose (765 mg) was reached. A radionuclide-determined ejection fraction revealed evidence of cardiac toxicity. Cyclophosphamide, procarbazine, and bleomycin, at the same doses, were continued, but multiple new skin lesions appeared, as did cervical adenopathy.

At the time of this patient's entry to this study, multiple skin lesions were noted on his scalp and face. Also present were painful, visible right cervical and left inguinal adenopathy. The patient received 1500 mg of cyclophosphamide on February 21, 1983 followed in 24 hours by 1.0 X 10^6 alloactivated lymphocytes (F[1]). Within 7 days, evidence of a tumor response was noted. Before the beginning of the second cycle (4 weeks after the first) there was no adenopathy nor any detectable skin lesions. Two subsequent cycles (F[2] and F[3]) beginning on March 17, 1983 and April 21, 1983 were given. During these 3 months, no adenopathy or skin lesions were detected on weekly examinations. Relapse with multiple skin lesions was noted on May 19, 1983. To determine whether this patient's complete response to the three cycles of adoptive chemotherapy was solely due to the chemotherapy, cyclophosphamide (1500 mg IV) was given without subsequent cell infusions. Over the next 2 weeks, several of the skin lesions did show evidence of resolution. However, of the lesions noted before treatment, some had progressed and new lesions were noted within 1 week. This was in marked contrast to the complete response seen after cyclophosphamide combined with the immune cells. Two additional monthly cycles of cyclophosphamide alone were given, with no complete resolution of cutaneous lymphoma. A single antecubital lesion grew progressively during these 3 months. Finally, the next monthly dose of cyclophosphamide was given with another course of in vitro activated lymphocytes from the patient's son (5 X 10^9 MLC cells on day 1 and 1 X 10^9 TCGF cells on day 8). By day 8, a complete response of all cutaneous tumor resulted, including resolution of the large antecubital lesion. However, this response lasted only 2 weeks, since new cutaneous lesions were noted on day 22 of this cycle, 3 weeks after the cyclophosphamide with MLC cell infusion, and 2 weeks after the TCGF cell infusion.

**Discussion**

Appropriately sensitized allogeneic lymphocytes have been shown to have therapeutic activity against established tumors in several animal systems with a demonstrated graft versus tumor effect, separate from GVH. Bortin and Truitt14 were able to improve survival with a minimal increase in GVH in leukemic AKR mice treated with alloimmunized haploidentical lymphocytes. Meredith and Okunewick15 have recently demonstrated that both major and minor histocompatibility differences are important in obtaining a "graft versus leukemia" (GVL) effect and that the magnitude of this GVL effect does not necessarily parallel that of the GVH reaction. The existence of an immune response by HLA-identical lymphocytes to leukemic cells directed at minor histocompatibility antigen has also been shown in humans.16

A graft versus leukemia effect is suggested in survival analyses of bone marrow transplantation (BMT) patients.17,18 Following an allogeneic BMT for acute leukemia those patients whose course was complicated by moderate to severe GVH had a lower relapse rate and prolonged survival compared to those patients without GVH. In addition, relapse rates in syngeneic transplants are higher than those seen in allogeneic transplant recipients. Odom et al. reported two cases of ALL that relapsed following allogeneic bone marrow transplant but went into remission with the onset of GVH and no other antileukemic therapy.19 These results suggest that transplanted allogeneic human lymphocytes may have an antineoplastic effect.

The application of adoptive immunotherapy in patients has been limited.20-23 Thomas et al.20 treated eight patients suffering from acute nonlymphocytic leukemia with their own remission lymphocytes, which were cultured in vitro with autologous blasts for 96 hours. No difference in the mean duration of complete response was noted. Nadler and Moore21,22 paired unrelated patients with malignant melanoma and sensitized each to the other's tumor using subcutaneous melanoma im-
plants. Following graft rejection, leukocytes collected by pheresis were reciprocally infused. Of the 53 evaluable patients, 13 responses were reported. One of the two patients believed to have a complete response remained disease-free for longer than 3 years.

The six patients in this study received alloactivated lymphocytes from a haploidentical relative following immunosuppressive pretreatment with cyclophosphamide. Although HLA-identical donors have a lesser chance of causing GVH, they could share postulated immune response genes that prevent recognition of tumor-associated antigens.24 Such major histocompatibility complex (MHC)-associated immune response genes have been well-described in animals.25 This MHC restriction has been shown for T-cell responses to viral, bacterial, and synthetic antigens in human and murine systems, as well as in the recognition of murine tumor cells.26-27

Using the methods described here, we were able to generate in vitro up to $1.5 \times 10^8$ primed lymphocytes for a single infusion. These alloactivated cells were immunologically active in vitro and in vivo. No significant toxicities, including GVH reactions, were noted following CPM plus MLC-generated or TCGF-expanded lymphocytes despite proven in vitro immunologic activity. The absence of a GVH reaction may be due to incomplete suppression of the patient's immune system, which may still have allowed a host-mediated immune rejection of the donor's lymphocytes.

The distribution studies using $^{111}$In-oxine labeling of the allogeneic haploidentical lymphocytes expanded in TCGF are in accord with those using small numbers of autologous lymphocytes expanded in TCGF.28 Following initial uptake in the lungs, these haploidentical cells moved to areas of the reticuloendothelial system (bone marrow, spleen, and liver). Despite the immune reactivity of these cells against patient's normal lymphocytes in vitro, the movement of these cells through the pulmonary circulation was not accompanied by any detectable compromise of respiratory function. No difference could be seen between the MLC-generated or TCGF-expanded cells with regard to their distribution. Similar results have been reported for IL-2-dependent T-cell clones in the mouse.29 Although we did not attempt to show retention of $^{111}$In-oxine within the lymphocytes, this has been shown in animal studies.30

Two of our patients received more than $10^9$ total cells, which may approximate the magnitude needed to achieve therapeutic responses based on extrapolation from murine models. One of these patients did, in fact, have a complete response to this combined therapy. This complete response was not reproduced by 3 monthly cycles of the same dose of cyclophosphamide used alone, but a transient complete response was then reproduced by a subsequent cycle of cyclophosphamide followed by immune cells.

The biologic mechanism generating this response remains uncertain. Priming with alloantigens has enabled in vitro destruction of autologous tumor cells,11 and preliminary results document a partial clinical response by lymphoblastic lymphoma patients to in vivo immunization with pooled allogeneic leukocytes.31 This suggests that a pool of alloantigens may stimulate the patient's own (or a relative's in the current study) lymphocytes to recognize an antigen on the lymphoma cells that is immunologically cross-reactive with the stimulating alloantigens. Alternatively, alloactivation might nonspecifically activate a separate population of antitumor effector cells, as has been shown in vitro with mitogens and lymphokines.32 In the AKR mouse, alloactivated allogeneic lymphocytes can produce an antitumor response that cannot be generated by syngeneic lymphocytes.14 This latter may reflect an inability of the tumor-bearing strain to immunologically recognize the tumor antigens.33 Another possible mechanism would be the immune recognition of genetically controlled major and minor histocompatibility antigens by the allogeneic lymphocytes, with preferential (but not specific) destruction of the neoplastic cells.24

Adoptive chemoimmunotherapy using CPM and more than $10^{10}$ alloactivated haploidentical lymphocytes activated in MLC and expanded in TCGF has been given to these patients without significant toxicity. In addition, evidence presented here suggests that a therapeutic effect has been seen in a patient with a non-Hodgkin's lymphoma. Treatment of more patients with similar tumors is needed to verify this finding; the in vitro dissection of the mechanism underlying this effect may provide a more specific clinical approach.

REFERENCES