Clinical Results and Characterization of Tumor-infiltrating Lymphocytes with or without Recombinant Interleukin 2 in Human Metastatic Renal Cell Carcinoma

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ABSTRACT

A Phase I trial of tumor-infiltrating lymphocytes (TIL) expanded in vitro and administered on Days 1 and 8, with or without continuous infusion recombinant interleukin 2 (rIL-2) in 25 patients with metastatic renal cell carcinoma, was conducted. Eighteen of the 25 eligible patients were treated with TIL and escalating doses of rIL-2 (0.0, 3.0, 4.5 x 10^4 units/m^2) on Days 1 to 5 and 8 to 12. Dose-limiting toxicity was pulmonary, and the maximum tolerated dose of rIL-2 was 3.0 x 10^4 units/m^2. No clinical responses were observed. Immunological monitoring of peripheral blood lymphocytes demonstrated significant increases in CD3+ and CD56+ cells, including the activated T-cell subsets. Phenotypic analysis of cultured TILs demonstrated significant heterogeneity and the presence of CD3+CD4+ and CD3+CD8+ T-cells, with CD3+CD56+ and CD3+CD56+ populations also present. The majority of cultured TILs expressed HLA-DR and CD45RO, with a variable number expressing CD25. The rIL-2-expanded TILs possessed cytotoxicity against allogeneic and autologous tumor, with cytolytic activity against only autologous tumor seen in one patient. Results demonstrate that in vitro expansion of TILs is possible, but further studies are needed to define the biology of TILs in renal cancer and to isolate and expand tumor-specific T-cells.

INTRODUCTION

Early reports have demonstrated that highly sensitized lymphoid cells when adoptively transferred can mediate tumor regression in animal systems (1, 2). LAK cells in combination with rIL-2 have also been extensively studied in animals (3-5) and were demonstrated to mediate regression of established metastases (3-5). Studies of the LAK effector cells have demonstrated that they are heterogeneous and capable of killing both allogeneic and autologous tumor (6). The majority of LAK activity was mediated primarily by NK cells and secondarily by MHC unrestricted T-cells (7, 8). Furthermore, these studies suggested that treatment efficacy may be related to tumor burden and the intensity of treatment with rIL-2 (3-5). While the clinical studies (9, 10) utilizing high-dose rIL-2 and LAK cells have demonstrated definite responses in RCC, the overall response rate was less than 25%, and toxicity was substantial. Therefore, new approaches to adoptive immunotherapy having increased activity and less toxicity are needed.

TIL represent part of the host immune response to human malignancy and contain an enriched population of cells with both cytotoxic and helper function that are reactive to the autologous tumor (11). The majority of TIL expanded by rIL-2 are composed of both CD3+CD4+ and CD3+CD8+ T-cells (12-14). In addition, TIL have been demonstrated to contain antigen-specific as well as non-specific cytotoxic lymphocytes (12-14). Finally, preclinical studies have demonstrated that rIL-2-activated TIL are 50 to 100 times more effective in their therapeutic potency than LAK cells (15). Finally, in several reports, TIL administered to patients with metastatic malignant melanoma, RCC, or lung cancer (16, 17) have produced tumor regressions.

The present trial was initiated to further evaluate the toxicity of this approach and characterize the TILs from patients with RCC. The present trial utilized CI rIL-2 with TILs given on Days 1 and 8. This report summarizes the results of this Phase I trial and presents data that further characterize cultured renal carcinoma TIL in terms of their phenotype and cytolytic properties.

MATERIALS AND METHODS

Patient Population. Patients (≥18 yr) with metastatic RCC, which was surgically incurable, who had tumor tissue (primary tumor or metastatic lesion) accessible for surgical removal, and had adequate numbers of autologous TIL (≥1 x 10^6) produced from their tumors were eligible. Objectively measurable or evaluable disease was required. Patients were also required to have a PS of ≤1 (Eastern Cooperative Oncology Group), a life expectancy of ≥3 mo, and to be fully recovered (≥14 days) from any recent surgical procedures or any infection (≥21 days). Minimal tumor burden was required and defined as having had a previous nephrectomy, ≤30% liver involvement by tumor, absence of central nervous system metastases, and a normal serum calcium without therapy in the presence of osseous metastases. Prior radiotherapy (≥8 days) was allowed for control of pain, but previous chemotherapy, immunotherapy, or hormonal therapy was not permitted. Exclusion criteria included any of the following: (a) a history of serious cardiac disease (New York Heart Association Class III or IV) or a need for antiarrhythmic therapy; (b) requirement for corticosteroids or nonsteroidal antiinflammatory agents; (c) history of malignant hyperthermia; (d) history of another curatively treated malignancy, except for basal cell or carcinoma of the cervix in situ; (e) female patients who were pregnant or lactating; (f) positive studies for HIV or HBsAg; (g) a contraindication for the use of vasopressors; and (h) the presence of significant effusion and/or ascites. Adequate organ function was also required and was defined as WBC > 4,000/μl, platelets ≥ 100,000/μl, hemoglobin ≥ 10.0 g/dl (without transfusions), serum bilirubin (total) ≤ 1.5 mg/dl, serum creatinine ≤ 1.5 mg/dl, creatinine clearance ≥ 60
Patients with a clinical or pathological diagnosis of metastatic RCC who met the previous eligibility criteria underwent surgery for removal of their primary or metastatic tumor. Specimens were then processed for TIL preparation as outlined subsequently. When adequate TIL growth (generally 4 to 6 wk) was seen, therapy was initiated. Biochemical and hematological baseline studies were repeated, and if present, the status of their measurable disease was determined.

**Treatmenyt and Study Design.** The rIL-2 (NSC 60064) used in this trial was produced by Hoffman LaRoche, Inc., and supplied by the NCI. It was produced using a gene cloned from human DNA that was expressed in *Escherichia coli*. The rIL-2 had specific activity between 5 and 1 x 10^6 units/mg of protein, was reconstituted with sterile water, and was administered as a constant infusion via the Pharmacia CADD I amnulatory infusion pump in 100 ml of a 5% solution of dextrose in water. Patients eligible for treatment received either TIL alone (Dose Level I) or TIL in combination with escalating doses of rIL-2. The dose escalation scheme and the patients evaluated and treated at each dose level are illustrated in Table 1. The TIL were administered without a filter, via a central i.v. catheter on Days 1 and 8, over approximately 30 min. The rIL-2 was administered as a continuous i.v. infusion on Days 1 to 5 and 8 to 12. At Dose Level IV, four patients also received cyclophosphamide 48 h prior to therapy. The rationale for this is the possible role of cyclophosphamide as an inhibitor of immune suppression (18). Three to six patients were treated at each dose level and observed for toxicity. No dose escalation within individual patients was permitted. The MTD was defined as the dose below which three or more patients experienced dose-limiting toxicity. Most patients received acetaminophen (650 mg p.o. every 6 h), ranitidine (150 mg p.o. every 12 h) as prophylaxis for gastrointestinal bleeding, and meperidine (10 mg i.v. every 4 to 6 h) to treat nausea and vomiting, and diphenoxylate hydrochloride with atropine sulfate (2.5 mg/ml) hypotension refractory to phenylephrine hydrochloride, altered mental status or coma, and dyspnea at rest or a requirement for mechanical ventilatory support. In the presence of these side effects or other Grade III or IV toxicity, therapy was held until there was a return to a Grade I level. The rIL-2 was then restarted with a 50% dose reduction. Development of any Grade IV nonhematological toxicity required the discontinuation of therapy.

**Human Renal Cell Carcinoma TIL Isolation and Expansion in Vitro.** The kidneys obtained from nephrectomies were perfused with 500 ml of Hanks’ balanced salt solution (Whittaker Bioproducts, Walkersville, MD) to remove extraneous blood. The primary and metastatic tumors were then processed as previously described (19).

The digested tumor material was placed in gas-permeable bags containing either X Vivo 10 media (Whittaker Bioproducts, Walkersburg, MD), or AIM V media (Gibco Laboratories) at a total cell concentration of 2.5 x 10^6/ml supplemented with 1000 units/ml of rIL-2 and incubated at 37°C in 5% CO2 and 95% humidity. TIL cultures were supplemented on Day 10 with fresh medium and rIL-2, and then as needed to maintain the cell concentration at 2 to 5 x 10^6/ml. TILs used for adoptive therapy were harvested using the SteriCell system (E. Dupont De Nemours & Co.).

**Isolation of Tumor Targets.** After a 4-h incubation period in collagenase and DNase, 1 to 2 x 10^6 tumor cells were placed in Primaria grade tissue culture flasks (Becton Dickinson, Oxnard, CA) and cultured short term as previously described (14). These short-term cultures represented RCC as demonstrated by morphological examination and by immunostaining with antibodies to epithelial tumor cells in general (AE1 and 3) and renal cell carcinoma in particular (Uro 2 and RCC). When sufficient cells were available, growth in nude mice was demonstrated for short-term cultured tumors. This was attempted in six instances, and in three tumor growth was noted with 5 to 10,000,000 cells per mouse. When examined histologically, the tumor resembled renal-cell carcinoma.

Daudi cells and the renal cancer line RC2 (20) were maintained in complete RPMI and also used as targets for cytotoxicity studies. Cell lines were periodically tested for and found to be free of *Mycoplasma* infection.

**Cytolytic Activity.** Detection of lytic activity was performed using a 4-h ^51^Cr release assay (19). Various concentrations of cultured cells were added to U-bottomed 96-well plates to achieve effector:target cell ratios of 25:1, down to 0.78:1 (6 dilutions). In all 4-h ^51^Cr release assays, spontaneous release never exceeded 20%.

Cytolytic activity was expressed as lytic units per 10^6 mononuclear cells tested as determined from linear regression analysis of dose-response curves, in which the natural log (ln) of the number of effectors is plotted against the percentage of specific lysis. One lytic unit was defined as the number of effector cells required to produce 15% specific lysis of 5.0 x 10^5 target cells in a 4-h period.

**Three-Color Immunocytochemistry.** Immunocytochemical analysis of PBL was performed using a modification of a previously described method (21). FITC, PE, and biotin-conjugated monoclonal antibodies were used to phenotypically identify and quantitative lymphocytic subsets and...
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included the following: FITC-anti-CD4, PE-anti-CD8, biotin-anti-CD3, FITC-anti-CD3, PE-anti-CD25, biotin-anti-HLA-Dr, PE-anti-CD56, biotin-anti-CD8, FITC-anti-CD11a, and PE-anti-CD16c (Becton Dickinson, Gentrac, and Coulter). Antibody titers were adjusted according to the fluorescence sensitivity of the flow cytometer (FACScan/BD) used. Isotypic controls for each particular subclass of immunoglobulin and system used were utilized to allow for the most accurate delineation of positive and autofluorescent populations and to control for nonspecific binding by a particular subclass of immunoglobulin as compared with autofluorescent background.

Analyses on the FACScan were performed utilizing an argon ion laser (Cyontics) with 15 mW of 488-nm excitation. Live gating of the forward versus orthogonal scatter channels, as determined by fluorescence (CD45+CD14—), was used to this same end and to selectively acquire events for lymphocytes. Polymorphonuclear leukocytes and monocytes were quantitated as nongated events derived from the gating tube (FITC-anti-CD45, PE-anti-CD14, DC-anti-CD2). Individual fluorescence populations were determined through the use of acquisition and contouring/quadrant analysis software ([FACScan Research Software] LYSIS and LYSYS II (Becton Dickenson)) as well as simultaneous multiparameter analysis software ([Paint-a-Gate/Becton Dickenson]) run on a HP310 work station (Hewlett-Packard).

Results for selected subpopulations were reported as a percentage and absolute number of total lymphocytes, corrected for nonspecific binding by isotypic controls.

Biopsy Immunohistology. Baseline pretreatment and posttreatment biopsy tissue was rapidly frozen in isopentane precooled to —130°C in liquid nitrogen and stored at —70°C until sectioning. Six-μm sections were fixed in reagent-grade acetone (Fisher) for 10 min, air dried, and immunostained with the avidin-biotinylated peroxidase complex method (22). Mouse monoclonal antibodies to the following determinants were incubated with the sections at dilutions optimized for tonsillar sections (30 min, ambient temperature): CD3; CD4; CD8; CD56; CD16b; HLA-Dr; HLA-A+B+C; and CD22. Sequential incubation was done with biotinylated, affinity-purified, horse anti-mouse immunoglobulin G (Vector), preformed avidin-biotinylated peroxidase complex (Elite ABC, Vector), and the chromogenic substrate 3-amino-9-ethylcarbazole/0.003% H2O2. Results were expressed using a semi-quantitative scoring system. Additionally, tumor tissue obtained for culturing TIL was examined for HLA-Dr expression.

Localization Studies. In selected patients, TILs were labeled with 111In, and their in vivo distribution was determined. Approximately 106 TIL were labeled with 1 mCi of 111Inoxine and suspended in 10 ml of autologous plasma. The labeling efficiency was then calculated, and cells were resuspended in plasma. Activity is then measured in a dose calibrator, and 500 μCi were injected into the patient. Images were obtained at 6 and 24 h and areas of uptake compared with known sites of metastatic disease. These studies were performed in three patients 24 to 48 h prior to initiation of treatment.

Statistical Analysis. Multiple determinations for the various immunological parameters in peripheral blood were performed before therapy (two to three) and during treatment. Data analysis was performed in a manner similar to our previous publications (23, 24). The two or three pretreatment studies and those obtained during treatment were averaged separately to obtain pretreatment and on treatment mean values. The differences between baseline values and the treatment means were calculated and examined for overall trends and for differences among dose levels. The fold increase of the cultured TIL was examined for site and medium effects as was the percentage of lymphocytes (subsets) in the cultured TIL. All comparisons were made using nonparametric statistical techniques, including the Kruskal-Wallis (25) and the Wilcoxon rank sum and signed rank tests (26). Absolute lymphocyte subset values were plotted throughout the follow-up period to examine their longitudinal behavior.

RESULTS

Clinical. Twenty-six patients were entered into the study, with one patient declared ineligible because of prior therapy with rIL-2 and α-interferon. This patient was entered at Dose Level II and was excluded from analysis. Twenty-five patients were eligible, and 18 (72%) were eventually treated. Reasons for nontreatment included inadequate TIL growth in two patients, contamination of cultures in three patients, and rapid disease progression before therapy could be started in two patients. Of the patients treated, there were 17 males and one female, with a median age of 56.5 yr (range, 30 to 73 yr). Fourteen patients had a PS of 1 and four of 0. Five of these patients presented with metastatic disease after a previous nephrectomy, and two had received prior radiotherapy.

The toxic effects associated with therapy in the 18 patients treated are summarized in Table 2. There were no previously unrecognized or fatal toxic effects secondary to treatment, and all side effects resolved completely after rIL-2 was discontinued. Three patients failed to complete 2 wk of rIL-2 therapy because of toxicity. Fever and/or chills were the only toxic effects associated with TIL infusion. Most of the toxicities were secondary to rIL-2 and appeared to increase in severity with continued treatment. Moderate gastrointestinal toxicity in the form of nausea and/or vomiting was seen in all patients. Renal toxicity in the form of reversible serum creatinine elevations was also common and seen in 83% of patients. Dose-limiting toxicity occurred at Dose Level IV (rIL-2, 4.5 × 106 units/m2) and consisted of pulmonary toxicity in three patients. This developed after 13, 15, and 17 days of rIL-2, respectively, and was not related to TIL infusion. It was characterized by dyspnea and hypoxia, but ventilatory support was not required. This toxicity disappeared in 24 to 48 h following discontinuation of rIL-2. Four patients at this level also received cyclophosphamide, and no differences in toxicity in this small subset were noted. In view of this, the entire cohort of six patients treated with 4.5 × 106 units/m2 of rIL-2 were considered a single group. The MTD was therefore identified as Dose Level III (rIL-2, 3.0 × 106 units/m2). No treatment-related deaths occurred, and the toxicity seen resembled that reported with CI rIL-2 (27, 28).

Table 2 Toxicity of therapy among eligible patients

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose level</th>
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<tbody>
<tr>
<td></td>
<td>I</td>
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<tr>
<td>TIL (≥1 × 106)</td>
<td>+</td>
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<tr>
<td>rIL-2 × 106 units/m2 CI, Days 1 to 5, 8 to 12</td>
<td>0</td>
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</table>

Toxicity parameter

Nausea/vomiting 3/3 4/4 5/5 6/6
Diarrhea 0/3 2/4 3/5 6/6
Mucositis 0/3 2/4 3/5 3/6
Creatinine 1.6—4.5 mg/dl 2/3 2/4 5/5 6/6
Bilirubin (total) 1.6—2.25 mg/dl 0/3 0/4 2/5 1/6
>2.25 mg/dl 0/3 1/4 0/5 1/6
Hypotension
Grade 1 or 2 1/3 1/4 1/5 3/6
Grade 3 (required pressor agents) 0/3 0/4 1/5 1/6
Pulmonary
Grade 4 (refractory) 0/3 0/4 0/5 1/6
Wt gain
5—10% of body wt 0/3 0/4 1/5 3/6
Neurological
Neutropenia 0/3 1/4 0/5 1/6
Neutropenia 1,000—2,000/μl 1/3 1/4 0/5 2/6
Thrombocytopenia
50—100,000/μl 0/3 0/4 2/5 5/6

° Unrelated to therapy.
* One instance unrelated to therapy.
Eighteen patients were evaluable for response. No objective tumor regression was seen; however, a single patient at Dose Level IV demonstrated a minor decrease in the size of pulmonary nodules. Of the 17 remaining patients, 8 had stable disease, and 9 had progressive disease at the end of wk.

Growth of TIL in rIL-2. Growth characteristics of cultured TIL are shown in Table 3. In the 18 patients who were treated with TILs from the primary tumor or metastatic sites, the TILs were expanded to sufficient numbers for infusion (≥1 x 10^9 cells). The level of TIL expansion in vitro was not significantly associated with tumor site, metastatic versus primary, the degree of initial lymphoid infiltrate, or the phenotypes of the TILs (data not shown). The total number of TIL infused per patient ranged from 1.2 x 10^9 to 3 x 10^12, and in 8 of 18 patients > 1 x 10^11 cells were infused.

Phenotypes of Cultured TILs. Phenotypes of the cultured TILs in the 18 eligible patients are presented in Table 4. In 14 of 18 instances >70% of TIL were T-cells with a CD3+ phenotype. The number of CD3+CD4+ and CD3+CD8+ subsets within the cultured TIL varied considerably from one patient to the next. In 4 of 18 patients, cells were predominantly CD56+ (Leu19+) and, in two of these cases, cells with the phenotype of NK cells (CD3-CD56+CD8- or CD3-CD16a+CD56+) predominated. Therefore, analysis demonstrated that cultured TIL from renal tumors were mostly T-cells, but were extremely heterogenous in their phenotypes. NK cells rarely predominated in culture, although some preparations did contain a significant number of these cells. The effects of media on TIL growth and phenotypes were also examined, since AIM V medium was used in 10 patients (Patients 9 to 24), and X Vivo was used in 8 (Patients 1 to 8). No differences in growth or expansion were noted, but TIL cultured in AIM V did have a significantly greater proportion of CD3+ cells that were activated (CD25+HLA-DR+) when compared with X Vivo medium (P = 0.02). Additionally, TIL with an NK phenotype (CD16a+CD56+CD3-) were more frequent in cultures containing AIM V (data not shown).

The relationship between phenotypes of TIL infused on Day 1 and HLA-DR expression in tumor tissue was also examined (Table 5). Based on this preliminary analysis, HLA-DR expression (>50% tumor cells) appeared to be associated with an increase in growth of the CD3+CD4+ and CD8- subset of TIL (P = 0.02). The frequency of CD4+ TIL in these cultures did not appear to be explained by their numbers within the tumor infiltrates. In a small subset of five TIL patients, the initial T-cell infiltrate was examined by flow cytometry. The CD3+CD8+ population (44%) was twice as frequent as the CD3+CD4+ subset (19%). In a separate study, tumors of 13 patients were analyzed, and similar results were found with TIL containing 29% CD3+CD8+ cells and 14% CD3+CD4+ cells.

Cytolytic Activity of Cultured TIL. The cytolytic activity of the cultured TIL is outlined in Table 6. The majority of cultures displayed non-MHC-restricted lytic activity. In 12 of 14 instances, TIL were cytotoxic to autologous tumor targets, and in one of 14 instances, cytotoxicity was detected against autologous tumor only. Non-MHC-restricted cytotoxicity was found against the Daudi cell line in 14 of 17 TIL cultures and against the allogeneic renal carcinoma cell line RC-2 in 9 of 17 cultures. In all TIL cultures except one (Patient 19), cytolytic activity against autologous tumor cells or allogeneic cell lines was present.

Changes in the Peripheral Blood of TIL + Interleukin 2-treated Patients. Lymphocyte subsets in the peripheral blood of 14 treated patients were analyzed using three-color flow cytometry. At Dose Level I no significant changes from baseline were observed; however, at the other dose levels, significant increases in the CD3+ (P = 0.0009), CD56+ (P = 0.0012), CD3+CD56+CD8- (P = 0.04), CD3-CD56+CD8- (P = 0.001), CD16a+ (P = 0.05), and CD16a+CD56+CD3- (P = 0.02), CD8+CD3+CD4+ (P = 0.0002), and CD8-CD3+CD4+ (P = 0.01) subsets with time were seen. The activated T-cell subsets (CD3+CD25+HLA-DR+ or CD3+CD25+HLA-DR+) also increased significantly with time (P = 0.0005). In contrast, no changes were seen in the CD3+CD56+ and CD3+CD56+CD8+ subsets.

Immunohistochemical and Localization Studies. These studies were performed to examine for possible changes in tumor infiltrate and or localization of TIL in metastatic lesions. Biopsies were attempted in 10 patients, and in 5 instances, sufficient material was obtained for analysis. In the majority of patients, the predominant mononuclear cell-infiltrating tumors were a
Cytolytic activity of cultured TIL

The assay was performed on TIL infused on Day 1.

Table 6 Cytolytic activity of cultured TIL

<table>
<thead>
<tr>
<th>Patient</th>
<th>Lytic units/10⁶ TIL*</th>
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<tbody>
<tr>
<td></td>
<td>Autologous</td>
</tr>
<tr>
<td>1</td>
<td>ND*</td>
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<tr>
<td>2</td>
<td>20.9</td>
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<tr>
<td>3</td>
<td>75.2</td>
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<td>4</td>
<td>ND</td>
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<td>ND</td>
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<tr>
<td>7</td>
<td>ND</td>
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<tr>
<td>8</td>
<td>42.5</td>
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<tr>
<td>9</td>
<td>44.2</td>
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<tr>
<td>10</td>
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<tr>
<td>11</td>
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<td>12</td>
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<tr>
<td>13</td>
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<tr>
<td>14</td>
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<tr>
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<tr>
<td>16</td>
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<tr>
<td>17</td>
<td>26.7</td>
</tr>
<tr>
<td>18</td>
<td>42.3</td>
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* Based on 15% lysis.
ND, not done.

CD3+ lymphocyte, with few CD56+ cells detected. No changes in the infiltrate following the administration of TIL and rIL-2 could be detected. Additionally, three patients received 111In-labeled TIL. Follow-up scans at 6 and 24 h failed to demonstrate localization in tumors, and the patterns seen represented uptake by lungs, liver, and spleen. In two cases the majority of TIL were T-cell (71 and 79% CD3+), whereas in the third, the predominant population (80%) were NK cells (CD3-CD56+).

The phenotypes of the labeled TIL populations are illustrated in Table 4 (Patients 22, 23, and 24).

DISCUSSION

The present trial demonstrates the feasibility of large-scale culture of TIL from primary tumors and metastatic lesions obtained from patients with RCC. The toxicity of TIL alone was minimal and, when administered with rIL-2, was similar to that seen with continuous infusion of this cytokine (27, 28).

Several other reports (16, 17) utilizing TIL grown from patients with RCC have appeared. The Phase I trial reported by Topalian et al. (16) included four patients with renal cancer. High-dose intermittent rIL-2 and cyclophosphamide were utilized in this study, and one of four patients responded. In contrast the study of Kradin et al. (17) used more frequent TIL administration (3 times weekly) and continuous infusion of 1.0 to 3.0 x 10⁶ units/m² of rIL-2 over 13 days. In this report, two of seven patients had partial responses. In contrast to renal cancer, larger numbers of patients with malignant melanoma have been treated with TIL. In a Phase I/II trial, Rosenberg et al. (29) reported on 20 patients, 10 of whom had partial tumor regressions, and in the paper by Kradin et al. (17), 3 of 13 patients responded. The toxicities reported in these studies have reflected the dose and schedule of rIL-2 utilized.

In our study, 18 patients were treated, and only one minor tumor regression was observed. It is difficult to compare our results with previously reported studies because of the following differences: the use of various schedules of rIL-2 and TIL administration; variations in TIL activations and growth; and the use of cyclophosphamide.

Additionally, the extreme heterogeneity of the cultured TIL infused makes comparisons difficult. In our study, this was demonstrated by the detailed three-color flow cytometry analysis. Thus, the heterogeneity of cells may be a major contributing factor in abrogating or diluting activity of any particular subset in the bulk population of TIL. Additionally, the influence of the medium used and culture conditions may be an important variable influencing the nature of cultured TIL. Finally, the Phase I nature of the present trial makes conclusions preliminary regarding the efficacy of TILs prepared in this manner in patients with RCC.
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Fig. 1. Phenotypic analysis of peripheral blood lymphocytes from patients treated at Dose Level III (rIL-2, 3.0 × 10⁶ units/m² CI i.v., Days 1 to 5, 8 to 12). Changes in absolute numbers of CD3+, CD56+, CD3+CD25−HLA-DR+, and CD3+CD25−HLA-DR+ subsets with time on therapy are illustrated.

Fig. 2. Cytolytic activity of peripheral blood lymphocytes against either Daudi or autologous tumor in patients receiving TIL plus 3 × 10⁶ units/m² of rIL-2 (Dose Level III). Each line represents lytic activity of PBL from individual patients.

Phenotypic analysis demonstrated that a significant portion of the TIL are activated based on their expression of HLA-DR (Table 4) and their expression of more permanent markers (32) associated with an activated state (CD45RO+ and CDW29, data not shown). Expression of the p55 (CD25) molecule of the interleukin 2 receptor on cultured TIL was either low or moderate, which agrees with previous reports (16, 33). The inability to detect the CD25 molecule on many of the TIL may indicate that proliferation is mediated through the p70 molecules of interleukin 2 receptor as has been reported for some TIL (34). It is also possible that, because TAC is transiently expressed, we analyzed the cells at a time when the protein was not expressed at very low levels. A previous report by Kradin et al. (33) with TIL from primary lung cancer has demonstrated that CD25 peaked at 2 to 5 days after stimulation with phytohemagglutinin and then decayed rapidly.

Functional analysis of TIL from renal tumors demonstrates that they display non-MHC-restricted cytolytic activity (35–38). Previously we (35) and others (36) have shown that a significant portion of the lytic activity of rIL-2-cultured TIL from renal carcinomas was mediated by CD56+ lymphocytes, particularly NK cells. These results are in agreement with reports characterizing TIL from head/neck and ovarian tumors, where it was found that most of the lytic activity of the cultured TIL was attributable to LAK cells (38, 39). Thus, it appears that most of the TIL that expanded in high-dose rIL-2 from certain tumors are CD3+CD56− and are not generally cytotoxic. However, we have reported that some long-term cultured TIL which expressed the CD3+CD56− phenotype were cytotoxic for the autologous tumor, although they were not specific (40). It appears that, in contrast to melanoma, where specific MHC-restricted CTL (14, 36) derived from tumor can be readily demonstrated, autologous tumor-specific CTL from RCC have been difficult to document and may represent an infrequent population. Beldegrun et al. (41), however, have recently characterized a CD4+ tumor-specific CTL clone derived from the ascitic fluid of a patient with RCC.

In conclusion, the lack of efficacy noted in this trial is not surprising and may be related to several factors. These include the heterogeneity of the cultured TIL and the absence of specific cytolytic activity for autologous tumor in most cases. Strategies alone (28, 30). In patients receiving TIL only, no changes in lymphocyte phenotypes or cytolytic activity were detected. Localization of TIL could not be demonstrated by either immunohistochemical or radiolabeling techniques as in the report by Kradin et al. (17). This is in contrast to studies (29) using labeled TIL in patients with malignant melanoma and resembles the distribution in patients of labeled LAK cells (31).
for activating and expanding of T-cell subsets with specific helper of lytic activity are now being investigated.

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Clinical Results and Characterization of Tumor-infiltrating Lymphocytes with or without Recombinant Interleukin 2 in Human Metastatic Renal Cell Carcinoma


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