Vaccination Of Pediatric Solid Tumor Patients with Tumor Lysate-pulsed Dendritic Cells Can Expand Specific T Cells and Mediate Tumor Regression

James D. Geiger, Raymond J. Hutchinson, Lyndon F. Hohenkirk, Elizabeth A. McKenna, Gregory A. Yanik, John E. Levine, Alfred E. Chang, Thomas M. Braun, and James J. Mulé

Department of Surgery, Section of Pediatric Surgery, Section of General Surgery, the Tumor Immunology and Immunotherapy Program [J. D. G., L. F. H., E. A. M., G. A. Y., J. E. L., A. E. C., J. J. M.], Department of Pediatrics [R. J. H.], and Biostatistics the University of Michigan Medical School [T. M. B.], Ann Arbor, Michigan 48109-0245

ABSTRACT

Dendritic cells (DCs) have been shown to be a promising adjuvant for inducing immunity to cancer. We evaluated tumor lysate-pulsed DC in a Phase I trial of pediatric patients with solid tumors. Children with relapsed solid malignancies who had failed standard therapies were eligible. The vaccine used immature DC (CD14-, CD80+, CD86+, CD83-, and HLA-DR+) generated from peripheral blood monocytes in the presence of granulocyte/monocyte colony-stimulating factor and interleukin-4. These DC were then pulsed separately with tumor cell lysates and the immunogen protein keyhole limpet hemocyanin (KLH) for 24 h and then combined. A total of 1 × 10^8 to 1 × 10^9 DC are administered intradermally every 2 weeks for a total of three vaccinations. Fifteen patients (ages 3–17 years) were enrolled with 10 patients completing all vaccinations. Leukapheresis yields averaged 2.8 × 10^8 peripheral blood mononuclear cells (PBMC)/kg, and DC yields averaged 10.9% of starting PBMC. Patients with neuroblastoma, sarcoma, and renal malignancies were treated without obvious toxicity. Delayed-type hypersensitivity (DTH) response was detected in 7 of 10 patients for KLH and 3 of 6 patients for tumor lysates. Priming of T cells to KLH was seen in 6 of 10 patients and to tumor in 3 of 7 patients as demonstrated by specific IFN-γ-secreting T cells in unstimulated PBMC. Significant regression of multiple metastatic sites was seen in 1 patient. Five patients showed stable disease, including 3 who had minimal disease at time of vaccine therapy and remain free of tumor with 16–30 months follow-up. Our results demonstrate that it is feasible to generate large numbers of functional DC from pediatric patients even in those highly pretreated and with a large tumor burden. The DC can be administered in an outpatient setting without any observable toxicity. Most importantly, we have demonstrated the ability of the tumor lysate/KLH-pulsed DC to generate specific T-cell responses and to elicit regression of metastatic disease.

INTRODUCTION

Although there have been a number of major advances made in the treatment of childhood malignancies, cancer remains the second most common cause of death for children >1 year of age in the United States (1). In addition, many of the successful cancer therapies are associated with significant toxicity leading to long-term morbidity and an increasing second malignancy rate (2, 3). New therapies that offer less toxicity and greater potential for cure are clearly needed. With the rapid progress in our understanding of the immune system, immunotherapy is moving closer to becoming an additional modality of anticancer therapy. By using a patient’s own immune system to generate antitumor responses, immunotherapy should have fewer side effects and, more importantly, the opportunity to generate long-term immunity.

A number of significant advancements in our understanding of the immune response to cancer have brought us closer to realizing the potential of cancer immunotherapy: (a) it has been recognized that tumors express unique gene products, such as melanoma antigen E-1 (4), carcinoembryonic antigen (5), and HER-2/neu (6), which can be presented to in the context of the MHC complex leading to the generation of specific effector T cells (7); and (b) there is a growing body of information from both preclinical and clinical studies to indicate that DCs are the cells that initiate and direct the immune response leading to tumor regression (8–10).

Hsu et al. (11) reported that DC pulsed with a tumor antigen could elicit specific tumor-reactive T cells and have clinical efficacy in patients with lymphoma. A number of other Phase I and early Phase II clinical trials support the idea that DCs presenting TAAAs can lead to partial or complete regression of tumors (12–15). To date, all of the initial clinical trials of DC-based vaccines have been completed in adults. We have completed the first Phase I trial of tumor lysate-pulsed DCs in the therapy of several different pediatric solid tumors; the early clinical results of the trial were reported previously (16). Here we report details of the clinical results for all patients enrolled, as well as describe the immune analysis of the vaccinated patients.

MATERIALS AND METHODS

Eligibility Criteria. Patients with relapsed solid tumors who had failed standard therapies were eligible for the study. Patients were required to have a source of tumor, a lymphocyte count >1000, and a good performance status. Anergy was assessed by measuring DTH to the common recall antigens candida albicans and tetanus; however, patients were not excluded if found to be anergic. Patients who had undergone an allogeneic bone marrow transplant previously were excluded.

Clinical Protocol

Tumor Cell Culture. Pediatric solid tumor samples were received at the Mott Children’s Hospital, Ann Arbor, MI. Fresh sterile tumor was transported on ice to the laboratory where a combination of mechanical and chemical dissociation was used to create a single cell suspension. Chemical digestion was completed by incubation in 50–ml RPMI (BioWhittaker, Walkersville, MD), 0.00044% DNase I (Sigma Chemical Co., St. Louis MO), 150 units/ml collagenase (Sigma Chemical Co.), and 750 units/ml hyaluronidase (Sigma Chemical Co.) with constant stirring for 3–12 h. The resulting cell suspension was passed through a 70-μm cell strainer. The flow-through was pelleted, resuspended in DMEM (Life Technologies, Inc., Grand Island, NY/10% heat-inactivated fetal bovine serum (BioWhittaker)/1 × Transferrin-Insulin-Selenium A (Life Technologies, Inc.), and cultured in a tissue culture flask at

Received 6/27/01; accepted 10/1/01.

The costs of publication of this article were defrayed in part by the payment of page charges. This article must therefore be hereby marked advertisement in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

1 Presented in part at the 2001 American Association of Cancer Research Meeting in New Orleans, LA. Supported by NIH/National Cancer Institute Grants 1-R29-CA-77471-01 and PO1 CA 93927, NIH Training Grant T32-CA-09672, and the University of Michigan General Clinical Research Center Grant M01-RR00042 and gifts from C. J. and E. C. Aschner and Abbott Laboratories and the Dorothy and Herman Miller Research Endowment.

2 To whom requests for reprints should be addressed, at Section of Pediatric Surgery, F3970 C. S. Mott Children’s Hospital, 1500 East Medical Center Drive, Ann Arbor, MI 48109-0245. Phone: (734) 936-1539; Fax: (734) 936-9784; E-mail: jgeiger@umich.edu

3 The abbreviations used are: DC, dendritic cell; KLH, keyhole limpet hemocyanin; IL, interleukin; PBMC, peripheral blood mononuclear cell; DTH, delayed-type hypersensitivity; FACS, fluorescence-activated cell sorter; TAA, tumor-associated antigen; PBSC, peripheral blood stem cell; ELISPOT, enzyme-linked immunospot assay; SI, stimulation index; i.d., intradermal; DPPE, Dibalucco’s phosphate buffered saline.

DENDRITIC CELL VACCINATION OF CHILDREN WITH SOLID TUMORS

37°C, 5% CO2. Trypsin-EDTA (Life Technologies, Inc.) was used to passage tumor cell lines. For long-term storage, tumor cells were frozen in 90% human AB serum (BioWhittaker) and 10% DMSO.

Preparation of Tumor Lysates. Tumor cells (fresh or short-term cultured cell lines) were suspended in PBS. The cell suspension was frozen in liquid nitrogen for 1.5 min, then thawed in a 37°C water bath for 4 min. The freeze-thaw cycle was repeated three times in rapid succession; cells were irradiated at 10,000 cGy and stored in liquid nitrogen for later use.

Leukapheresis Protocol. Using the COBE Spectra apheresis machine, we selected either a WBC or an Auto PBSC tubing set to collect the cells. Children <20 kg required a blood prime to maintain hemodynamic stability; for these patients, we used a WBC set to perform a “manual” cell collection. Children >20 kg can tolerate the procedural fluid shifts, and, therefore, “automated” cell collection is performed. Flow rates are determined by the child’s size as the machine has an extensive algorithm to determine anticoagulant tolerance. We processed two to three times the patient’s blood volume, which averaged 3–4 h/procedure.

Venous access was accomplished by either two large peripheral arm veins, if the patient was cooperative, or, as in most cases, by a rigid dialysis catheter placed in the femoral vein and then removed postleukapheresis. Product volumes varied depending on the type of collection performed. WBC/manual collection yielded a product of ~100–250 ml, whereas auto PBSC was more concentrated yielding 40–100 ml. We routinely measured the patient’s ionized calcium level and titrated a calcium gluconate drip to maintain the ionized calcium at 1–1.3 mm.

DC Generation and Vaccine Preparation. The generation of DC and preparation of the vaccine was performed according to IND #BB6958 filed with and approved by the Food and Drug Administration. Cell cultures and antigen pulsing were performed in the Human Applications Laboratory of the University of Michigan’s General Clinical Research Center, which is a facility that operates under good manufacturing procedures. The PBMCs were recovered from leukapheresis product by density gradient centrifugation over Accudyn Prep Lymphocytes (Accurate Chemical & Scientific Corp., Westbury, NY). The PBMCs were resuspended in X-VIVO 15 medium (BioWhittaker) at 1 × 10^6 cells/ml, and 30 ml of cell suspension was added to each 225 cm² tissue culture flask (Costar). The flasks were incubated in 5% CO2, 37°C, and after the nonadherent cells were gently removed. The adherent cells were cultured in X-VIVO 15 medium containing recombinant human granulocyte/macrophage colony-stimulating factor (100 ng/ml; Schering-Plough, Kenilworth, NJ) and recombinant human IL-4 (50 ng/ml; Schering-Plough) for 6 days. After 6 days, the DCs were harvested by adding 10 ml of 3% EDTA-PBS. A total of 1 × 10^7 DC was transferred to 75 cm² flasks (Costar) and either pulsed at a 1:1 ratio with autologous tumor lysates or 300 μl of KLH stock solution (50 μg/ml; Calbiochem, San Diego, CA).

Pulsed DCs were incubated for 18 h, harvested by incubation in PBS, washed twice with PBS, and then harvested and combined for vaccine administration. Autologous DCs (1 × 10^7) in 400 μl of PBS were prepared for i.d. administration.

Study Design. The primary end points for the Phase I study were to assess feasibility and toxicity of the DC vaccination regimen. The National Cancer Institute Common Toxicity scale was used. Secondary end points were to assess immune responses induced by the vaccination procedure to the marker antigen KLH and to autologous tumor. The patients were given a total of three vaccinations i.d. near the inguinal lymph nodes. The first 3 patients received 1 × 10^7 cells; the dose was then escalated to 1 × 10^8 cells in subsequent patients. All vaccinations were administered in an outpatient clinic of the General Clinical Research Center. One month after the last vaccination, patients underwent follow-up imaging studies, repeat leukapheresis for immune monitoring, and DTH testing to KLH and autologous tumor. Standard criteria for tumor response were used. Patients with partial responses or stable disease were eligible for a second set of vaccinations if adequate tumor was available. This protocol was approved by the University of Michigan’s Institutional Review Board (96-213). Informed consent was obtained from each patient’s legal guardian before enrollment.

Immune Response

Skin Tests. Tumor lysates were diluted in DPBS and administered in the following doses in 0.5 ml of volume: 1 × 10^7, 1 × 10^6, and 1 × 10^5 tumor equivalents. For KLH skin tests, 200, 20, and 2 μg of KLH were suspended in 200 μl of DPBS and administered i.d. Induration was measured 48 h later in two perpendicular diameters. Positive DTH reactions were judged as positive if the average perpendicular measurements exceeded 5 mm.

ELISPOT. ELISPOT plates (Polytronics) were coated with 100 μl/well α-IFN-γ monoclonal antibody (Endogen, Woburn MA) at 4 μg/ml in sterile PBS (BioWhittaker) wrapped in Saran wrap and incubated overnight at 4°C. Plates were washed twice with 200 μl of PBS and blocked with 200 μl of X-VIVO/10% heat-inactivated human AB serum (BioWhittaker) for 1 h at room temperature. Blocking solution was removed from the plate. Unstimulated PBMC samples were plated in triplicate as follows: each well contained 1 × 10^7 PBMC in 200 μl of X-VIVO/1% human AB serum with or without 8 μg of KLH (Sigma Chemical Co.). Plates were incubated for 18–24 h at 37°C, 5% CO2. Cells were removed, and plates were washed three times with PBS and three times with PBS/1% Tween-20 (Sigma Chemical Co.). Biotinylated anti-IFN-γ monoclonal antibody (100 μl; Endogen) at 4 μg/ml in PBS/1% Tween-20/10% heat-inactivated human AB serum was added to each well. Plates were incubated 2 h at room temperature or overnight at 4°C. Plates were then washed six times (≥2 min each) with 200 μl PBS/1% Tween-20. Streptavidin-horseradish peroxidase (Dako, Carpenteria, CA) was diluted 1:2000 in PBS/1% Tween-20/10% heat-inactivated human AB serum, and 100 μl were added to each well. Plates were incubated 90 min at room temperature, then washed six times (1 min each) with PBS/1% Tween-20 and washed twice with PBS.

3-Amino-9-ethyl-carbazole solution [100 mg of 3-amino-9-ethyl-carbazole (Pierce) in 10 ml of N,N-Dimethyl Formamide] was diluted 1:30 in 0.1 M acetate buffer. Then 30% H2O2 was added 1:2000 to this solution, and 200 μl were immediately added to each well of the ELISPOT plate. After development of spots, the reaction was stopped with distilled water. Plates were inverted and allowed to dry overnight protected from light. Spots were counted using the ImmunoSpot Series 1 Analyzer (Cellular Technology, Ltd., Cleveland, OH).

Proliferation Assay

PBMCs were thawed, washed, and suspended in X-VIVO 15 medium supplemented with 1% human AB serum (BioWhittaker), 2 mM glutamine, 10 units of penicillin, 10 mg/ml streptomycin, and 100 μM 2-mercaptoethanol (Sigma Chemical Co.). Viability was assessed by trypan blue exclusion, and cell concentration was adjusted to 6 × 10^5/ml. Cells were added to 96-well, round-bottomed plates in 100-μl volumes and incubated in a final volume of 200 μl with either medium alone, KLH (40 μg/ml), or tumor lysate (preprepared to deplete autologous tumor cell equivalence) for a total of 6 days at 37°C, 5% CO2. Phytomenadione (Sigma Chemical Co.) was added to some of the wells as a positive control on day 3. The cultures were pulsed with 1 μCi/well of [3H]thymidine (ICN, Costa Mesa, CA) on day 5 and incubated overnight before harvest onto glass fiber filter plates (Millipore, Bedford, MA). Data were collected on a TopCount NXT scintillation counter (Meriden, CT). An SI was calculated: SI = average cpm of antigen-stimulated culture/average cpm of unstimulated culture.

FACS Staining. Cells (5 × 10^5) were suspended in 50 μl of diluted FITC-conjugated monoclonal antibody (CD3, CD4, CD8, CD25, CD28, CD45RA, CD45RO, CD57, and CD86; PharMingen). They were incubated for 30 min at 4°C in the dark and then washed twice with FACS buffer (DPBS/2% fetal bovine serum/0.1% NaN3). They were resuspended in 500 μl of 1% paraformaldehyde in PBS. Data were analyzed using the Becton Dickinson FACSCalibur and CellQuest v3.2.1f1.

RESULTS

Patient Characteristics. A total of 15 patients were entered into the trial, and 10 of these completed all three vaccinations and were evaluated for toxicity, immune response, and tumor response. Five other patients had from one to three vaccinations but, because of progressive disease, were not able to be fully evaluated. All of the patients enrolled in the study were heavily pretreated for relapsed disease, and 7 of the patients had undergone some form of hematopoietic transplant (autologous PBSCT or bone marrow transplant)
before vaccination. The characteristics of the 15 enrolled patients are summarized in Table 1. A number of different extra-cranial solid tumors were treated, including primitive neuroectodermal tumor and Ewing’s sarcoma (n = 4), neuroblastoma (n = 3), other sarcomas (n = 6), and 1 patient with renal cell cancer and another with Wilms’ tumor.

Vaccine Preparation and Characterization. Leukapheresis averaged 2.8 × 10^6 PBMC/kg, and DC yields averaged 10.9% of starting PBMC. Thus, it was feasible to administer three DC vaccinations at 1 × 10^7 cells in all children. The DCs generated by this technique demonstrated characteristic morphology by light microscopy, were functional in all patients tested in an allogeneic-mixed lymphocyte reaction (data not shown), and demonstrated an immature DC phenotype by FACS analysis (CD14−, CD80%, CD86+, CD83−, and HLA-DR+). The level of CD83 expression did increase with pulsing and replating of the DC, as shown in a representative sample in Fig. 1, but remained relatively low.

Toxicity. There were no significant toxicities associated with the DC vaccine administrations, and all patients received their vaccinations in an outpatient setting. Mild local erythema was seen after vaccine administration in the majority of the patients and usually resolved within 5 min. Four patients had an increased local reaction, with induration always <10 mm with each subsequent vaccination. None of the local reactions progressed to ulceration or required pain medicine.

A panel of autoimmune serologies was drawn at baseline and 1 month after the third vaccination. In 1 patient (patient 1) the anti-dsDNA titer turned positive, and in another (patient 2), the nuclear antibody titer turned positive to 1:160 after vaccination. In both patients, the abnormal titers later returned to normal. None of the patients in the study demonstrated any clinical signs of autoimmune disease.

Tumor Response. The primary end point of this Phase I trial was toxicity; however, patients were evaluated for clinical responses as well. The clinical results are summarized in Table 1. One patient with a fibrosarcoma with numerous metastatic nodules to the lungs and a thoracic spine mass had a significant partial response. Many of the pulmonary metastases, including one that measured 2 cm, regressed

---

Table 1  Patient characteristics and clinical results

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Treatments before vaccination</th>
<th>Relapsed tumor sites before vaccination</th>
<th>DC dose (No. of vaccinations)</th>
<th>DTH response K (KLH) L (lysat)e</th>
<th>Tumor response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>F</td>
<td>Fibrosarcoma</td>
<td>Surgery Chemotherapy (CCG-7921)</td>
<td>Lung spine-thoracic</td>
<td>1 × 10^7 (3)</td>
<td>K ⊘</td>
<td>PR</td>
</tr>
<tr>
<td>2</td>
<td>12.5</td>
<td>F</td>
<td>PNET</td>
<td>Surgery Chemotherapy PBSCT Radiation</td>
<td>Lung-microscopic</td>
<td>1 × 10^7 (3)</td>
<td>K + + +</td>
<td>SD</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>F</td>
<td>PNET</td>
<td>Surgery Chemotherapy (CCG-7942B)</td>
<td>Thoracic-microscopic</td>
<td>1 × 10^7 (3)</td>
<td>K ⊘</td>
<td>SD</td>
</tr>
<tr>
<td>4</td>
<td>4.5</td>
<td>F</td>
<td>Neuroblastoma</td>
<td>Surgery PBSCT Radiation Anti-G22, antibody</td>
<td>Bone marrow-microscopic</td>
<td>1 × 10^7 (3)</td>
<td>K +</td>
<td>SD</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>M</td>
<td>Neuroblastoma</td>
<td>Surgery BMTg</td>
<td>Lung</td>
<td>1 × 10^8 (3)</td>
<td>K ⊘</td>
<td>PD</td>
</tr>
<tr>
<td>6</td>
<td>4.5</td>
<td>F</td>
<td>Neuroblastoma</td>
<td>Surgery Radiation BMT</td>
<td>Adrenal bed</td>
<td>1 × 10^6 (3)</td>
<td>K + + +</td>
<td>SD</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>M</td>
<td>Renal cell cancer</td>
<td>Surgery IL-2</td>
<td>Retroperitoneum-lymphatic</td>
<td>1 × 10^7 (3)</td>
<td>K +</td>
<td>PD</td>
</tr>
<tr>
<td>8</td>
<td>15.5</td>
<td>M</td>
<td>Osteosarcoma</td>
<td>Surgery Chemotherapy Steroids</td>
<td>Lungs</td>
<td>1 × 10^7 (3)</td>
<td>T +</td>
<td>PD</td>
</tr>
<tr>
<td>9</td>
<td>15</td>
<td>M</td>
<td>Inflammatory myofibroblastic sarcoma</td>
<td>Surgery Chemotherapy</td>
<td>Abdomen-mesenteric lymph nodes</td>
<td>1 × 10^7 (3)</td>
<td>K + + +</td>
<td>SD</td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>M</td>
<td>Hepatic sarcoma</td>
<td>Surgery Chemotherapy</td>
<td>Abdomen thoracic</td>
<td>1 × 10^6 (3)</td>
<td>K +</td>
<td>PD</td>
</tr>
<tr>
<td>11</td>
<td>14</td>
<td>M</td>
<td>Desmoplastic round cell</td>
<td>Surgery Chemotherapy</td>
<td>Abdominal chest wall</td>
<td>1 × 10^7 (2)</td>
<td>K</td>
<td>PD</td>
</tr>
<tr>
<td>12</td>
<td>13</td>
<td>M</td>
<td>Ewing’s sarcoma</td>
<td>Surgery Chemotherapy (CCG-7931)</td>
<td>Lung</td>
<td>1 × 10^7 (3)</td>
<td>K</td>
<td>PD</td>
</tr>
<tr>
<td>13</td>
<td>3</td>
<td>M</td>
<td>Clear cell sarcoma</td>
<td>Surgery Radiation BMT</td>
<td>Brain</td>
<td>1 × 10^7 (3)</td>
<td>K</td>
<td>PD</td>
</tr>
<tr>
<td>14</td>
<td>14.5</td>
<td>M</td>
<td>Ewing’s sarcoma</td>
<td>Surgery Chemotherapy PBSCT Radiation</td>
<td>Chest wall</td>
<td>1 × 10^7 (2)</td>
<td>K</td>
<td>PD</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>F</td>
<td>Wilms’ sarcoma</td>
<td>Surgery Chemotherapy (CCG-4941)</td>
<td>Abdomen Lung</td>
<td>1 × 10^6 (2)</td>
<td>K</td>
<td>PD</td>
</tr>
</tbody>
</table>

* Patients in shaded area did not complete the study protocol and, thus, were not able to be evaluated.
+ + +, a positive DTH response for KLH (K) at 2 μg or tumor lysate (T) at 10^7 tumor cell equivalents. +, a positive DTH response for KLH (K) at 20 μg or tumor lysate (T) at 10^6 tumor cell equivalents.
Children’s Cancer Group.
* Bone marrow transplant.

<https://cancerres.aacrjournals.org/content/61/19/8515.full.pdf>
DENDRITIC CELL VACCINATION OF CHILDREN WITH SOLID TUMORS

Immune Response to Tumor Lysate. Responses to tumor lysate were also evaluated in fresh unstimulated PBMCs in an ELISPOT assay for 6 patients that had adequate clinical material (Fig. 4). After vaccination, 3 of 6 patients demonstrated a >10-fold increase in the number of IFN-γ spots in response to tumor antigen. These 3 patients included patient 1, who had significant tumor regression, and patients 2 and 3, who had microscopic disease and were alive without evidence of disease at writing. Once again, the mean of each subject’s response before vaccine was compared with each subject’s response after vaccine via a paired t test. The response after vaccine demonstrated, on average, 68 more IFN-γ spots than before vaccine.

In patient 3, who had neuroblastoma, we evaluated the specificity of the antigen response in an ELISPOT assay. As shown in Fig. 5, PBMCs from the patient postvaccine responded to autologous tumor, as well as an HLA-matched neuroblastoma tumor from another patient, but not to a different histological (Wilms’) tumor.

DISCUSSION

There have been a number of early clinical trials evaluating DC-based vaccines in the therapy of cancer in adult patients with melanoma, renal cell cancer, and prostate cancer (12–15). We have now shown in pediatric malignancies that it is not only feasible to generate DC in children, including those as small as 13 kg, but that DC-based vaccines can be administered in an outpatient setting without significant toxicity. Of importance, tumor lysate-pulsed DC were able to generate specific T-cell reactivity and mediate regression of established metastatic tumors in heavily pretreated patients.

In most of the DC-based immunotherapy trials to date, the DCs were generated from adherent PBMCs (CD14+ monocytes) cultured with granulocyte/macrophage colony-stimulating factor and IL-4, which predominantly expressed an immature phenotype. The phenotype of the DCs generated in our study was also found to be consistent with that of immature DCs (i.e., no CD83), but after replating and pulsing with tumor lysates and KLH, the level of expression of CD83 tended to increase slightly. DC maturation has been shown to be accompanied by increased potency in stimulating T cells (perhaps mediated by tumor necrosis factor-α or cross-linking of CD40 by CD40 ligand) but a decreased ability to acquire and process antigen (17). We did not actively attempt to mature the DCs in our trial; however, tumor lysate, KLH, or perhaps the production of inflammatory cytokines because of replating may have led to the relative maturation observed in our cultures (18, 19). It is also possible that additional maturation of the...
injected DCs may occur in vivo after vaccine administration (20). The average DC yield of 10.9% of the starting PBMC was adequate for at least three vaccinations of 1 × 10^7 cells in each child. Although CD34^+ stem cells can also be used as a source of DC, the phenotype and functional characteristics of these DCs are more variable and less studied (21, 22). The optimal source and degree of maturation of the DCs used in immunotherapy will continue to be an important area of investigation.

We initially included KLH in our vaccine strategy as a tracer antigen to evaluate vaccine efficacy as originally described by Hsu et al. (11). However, the role and importance of KLH to enhance the immunogenicity of vaccines have since become elucidated (23). Recently, we have shown that KLH can both increase the degree of IFN-\(\gamma\) secretion by tumor-specific T cells and augment the efficacy of tumor lysate or peptide-pulsed DC immunization in mediating tumor rejection in mice (24). The effect was found to be CD4^+ T-cell dependent and could be enhanced by IL-2. Thus, the use of KLH may be beneficial in skewing the balance of the T-cell response toward a type 1 helper response.

We have elected to use tumor lysates as the source of antigen for this study for a number of reasons, which we et al. (25, 26) have discussed previously. Secondly, there are only a few described TAAs, and most of these have been identified in adult malignancies, particularly, melanoma. For most pediatric malignancies, there is a paucity of well-defined TAAs, and even less clear is whether there exists actual tumor-rejection antigens. In addition, lysate as a source of antigen should lessen the potential for tumor escape because of the generation of a larger repertoire of tumor-rejection antigens (27). One potential disadvantage of using tumor lysates as a source of TAA is the need for an adequate source of autologous tumor, and in fact, this did limit our ability to treat three of the patients in this study. However, we were able to overcome insufficient amounts of tumor in some patients by the successful generation of short-term tumor cul-

![Fig. 3.](image1) KLH-reactive T cells quantified by an ELISPOT assay. Unstimulated PBMCs (1 × 10^6) are cultured with or without 8 μg of KLH. Data are shown as the number of spots or reactive T cells per 1 × 10^6 PBMC at baseline and 4 weeks after the third vaccination. b, color photomicrograph of representative wells of an ELISPOT plate. Each red spot represents IFN-\(\gamma\) secretion by individual KLH-reactive T cells at baseline and 4 weeks after the third vaccination.

![Fig. 4.](image2) Tumor lysate-reactive T cells were quantified using an ELISPOT assay. Unstimulated PBMCs (1 × 10^6) were cultured with or without autologous tumor lysate. Data are shown as the number of spots or reactive T cells per 1 × 10^6 PBMC at baseline and 4 weeks after the third vaccination.

![Fig. 5.](image3) Tumor lysate-reactive T cells were quantified using an ELISPOT assay. Unstimulated PBMCs (1 × 10^6) were cultured with or without autologous tumor lysate. Autologous tumor lysate is compared with allogenic tumor lysate from the same or different tumor type. Data are shown as the number of spots or reactive T cells per 1 × 10^6 PBMC at baseline and 4 weeks after the third vaccination.
tures. It might also be possible in future studies to use allogeneic tumor lines as a source of TAA (28), dependent on the discovery that shared, MHC-restricted antigens exist among pediatric tumors. Indeed, T cells from 1 patient with neuroblastoma responded postvaccination to stimulation with an allogeneic neuroblastoma tumor cell line, which may indicate shared tumor antigen(s).

One potential disadvantage of the use of tumor lysates would be the inclusion of other “self antigens” that could lead to the generation of autoimmune sequelae. Although 2 of the patients in this study had transient conversion of autoimmune serologies, both subsequently reverted back to normal. In addition, we observed no clinical autoimmune disease and no other systemic toxicity to the vaccines. Although our experience is consistent with that in other DC immunotherapy trials, monitoring for autoimmunity will continue to be important in future studies.

The optimal route of administration of antigen-pulsed DCs remains controversial. Intranodal, i.v., s.c., and i.d. routes of administration have been used in different clinical trials (12, 29). In theory, for optimal T-cell activation, it would be expected that intradermally administered antigen-pulsed DCs would migrate to the regional draining lymph nodes, where contact with T cells would be maximal. It has been shown that immature DCs have the ability to migrate rapidly to lymph nodes and that this route of inoculation is superior for DC migration to the lymph nodes than the s.c. or i.v. route (30). Recently, intranodal DC delivery was shown to be superior to the i.d. route in a murine model for generating T-cell responses (31). Nestle et al. (12) have shown that intranodal delivery of DCs was feasible in adults and could lead to measurable tumor regression in patients with advanced melanoma. Future trials are designed to directly evaluate different routes of DC vaccine administration.

A distinguishing feature of our study compared with DC-based vaccine trials in adult patients is how extensively pretreated the children were enrolled in our trial. Most of the patients had multimodal therapy, the majority of patients in our study demonstrated DTH to KLH, and half demonstrated DTH to autologous tumor. Notwithstanding, it remains controversial whether or not DTH to autologous tumor can be a reliable correlate of clinical responses.

Quantification of active specific immune responses with immunological assays is critical to determining vaccine efficacy. The secretion of IFN-γ by antigen-stimulated T cells was shown to correlate with tumor regressions in vivo (34, 35). The ELISPOT assay has been found to be highly sensitive for detecting cytokine secretion by individual T cells. Thus, we used an ELISPOT assay to quantify IFN-γ secretion in response to KLH and to tumor lysates before and after DC vaccination. Of importance, the assays were performed on PBMC that had undergone no in vitro stimulation. The 4 patients who had better clinical responses also had significant increases in their KLH-reactive T cells, and 3 of 4 patients did not have adequate tumor for the assay) had significant responses to autologous tumor lysate as well. Although MHC-peptide tetramer analysis is another sensitive method of quantifying antigen-specific responses (36), it was not used because of the lack of defined TAA epitopes in the tumors studied. Also, the correlation of MHC-peptide tetramer-identified epitopes with tumor-rejection antigens is in question (37). Despite these dramatically improved assays, there is still a need for better assays to quantify active specific immune responses and find correlation with clinical responses.

The results of our initial experience with tumor lysate-pulsed DC vaccination in children with solid tumors indicate that this approach is feasible, and based on our clinical and immune responses, future studies seem warranted. Because of its low toxicity, indication of immune responses, and early clinical results, the use of DC-based tumor vaccines in children should be explored in the minimal disease setting, where they are likely to have more benefit.

ACKNOWLEDGMENTS

We thank Cindy Bower, Frank Marliotti, Adam Takake, Joel Whitfield, and Sheryl Woloskie for their expertise and assistance in completing the clinical trial. We also thank the patients and families who participated in the trial, without their courage, our study would not have been possible.

REFERENCES


Announcements

(Requests for announcements must be received at least three months before publication.)

FUTURE ANNUAL MEETINGS OF THE AMERICAN ASSOCIATION FOR CANCER RESEARCH

2002 April 6–10, San Francisco, CA
2003 April 3–9, Toronto, Ontario, Canada
2004 March 27–31, Orlando, FL

AACR SPECIAL CONFERENCES IN CANCER RESEARCH

A number of meetings are now being organized in the AACR’s series of smaller scientific meetings. Following are the topics, dates, locations, and program committees for these meetings. When full details of each meeting are available, AACR members will be the first to receive complete brochures and program committees for these meetings. Nonmembers may receive this information by sending their names and addresses to Meetings Mailing List, American Association for Cancer Research, Public Ledger Building, 150 South Independence Mall West, Suite 826, Philadelphia, PA 19106-3483. Up-to-date program information is also available via the Internet at the AACR’s website (http://www.aacr.org).

APOTOPSIS AND CANCER: BASIC MECHANISMS AND THERAPEUTIC OPPORTUNITIES IN THE POST-GENOMIC ERA

February 13–17, 2002
Hilton Waikolou Resort, Waikolou, Hawaii

Chairpersons
John C. Reed, La Jolla, CA
Scott W. Lowe, Cold Spring Harbor, NY

THE MOLECULAR GENETICS OF COLON CANCER

March 7–10, 2002
Loew’s Philadelphia Hotel, Philadelphia, PA

Chairpersons
Anil K. Rustgi, Philadelphia, PA
Raju Kucherlapati, Bronx, NY

ONCOGENOMICS 2002: DISSECTING CANCER THROUGH GENOME RESEARCH

May 1–5, 2002
Burlington Hotel, Dublin, Ireland

Chairpersons
Jeffrey M. Trent, Bethesda, MD
Bette Phimister, New York, NY

PROTEASES, EXTRACELLULAR MATRIX, AND CANCER

October 9–13, 2002
The Westin Resort, Hilton Head Island, SC

Chairpersons
Ruth J. Muschel, Philadelphia, PA
Yves A. DeClerck, Los Angeles, CA

UBIQUITINATION IN NORMAL AND CANCER CELLS

October 30–November 3, 2002
Westin Bayshore Hotel, Vancouver, British Columbia, Canada

Chairpersons
James M. Roberts, Seattle, WA
Raymond J. Deshaies, Pasadena, CA

CALENDAR OF EVENTS

13th Annual Colorectal Disease Symposium: An International Exchange of Medical and Surgical Concepts, February 14–16, 2002, Marriot’s Harbor Beach Resort and Spa, Fort Lauderdale, FL. 25.5 Category I CME credit hours. Contact: Cleveland Clinic Florida, Department of Continuing Education, 2950 Cleveland Clinic Boulevard, Weston, FL 33331. Phone: 954.659.5490; Fax: 954.659.5491; E-mail: cme@ccf.org; Website: www.cmeccf.com.

7th Annual NCCN Conference: Practice Guidelines and Outcomes Data in Oncology, February 28–March 3, 2002, Westin Diplomat Resort and Spa, Hollywood, FL. Contact: Tricia Wilson, National Comprehensive Cancer Network, 50 Huntington Pk, Suite 200, Rockledge, PA 19046. Phone: 215.728.2577; Fax: 215.728.3877; E-mail: information@nccn.org; Website: www.nccn.org.

International Symposium on Antimutagenesis and Anticarcinogenesis, April 26, 2002, New York Medical College, Valhalla, New York. Contact: Kathy Woodley, Basic Science Building, New York Medical College, Valhalla, NY 10595. Phone: 914.594.3084; Fax: 914.594.4163; E-mail: Kathy_Woodley@nymc.edu.


3rd International Conference on Osteopontin, May 10–12, 2002, The Menger Hotel, San Antonio, TX. Contact: David Denhardt, Rutgers University, Nelson Laboratories, 604 Allison Rd, Piscataway, NJ 08854. Phone: 732.445.4569; Fax: 732.445.0104; E-mail: denhardt@biology.rutgers.edu; Website: www.osteopontin.net.

15th International Symposium of the Journal of Steroid Biochemistry and Molecular Biology: Recent Advances in Steroid Biochemistry and Molecular Biology, May 17–20, 2002, Munich, Germany. Contact: Prof. J. R. Pasqualini, Institut de Patriculture, 26 Boulevard Brune, 75014 Paris, France. Phone: 33-1.45.39.91.09; Fax: 33-1.45.42.61.21; E-mail: Jorge.Pasqualini@wanadoo.fr.


3rd Annual Hermelin Brain Tumor Symposium: Cancer of the Central Nervous System: Clinical Practice, Clinical Trials, June 8–9, 2002, The Westin Hotel, Southfield, MI. Contact: Office of Continuing Medical Education, Henry Ford Health System, 2799 West Grand Boulevard, Room B-063, Detroit, MI 48202. Phone: 313.916.8354; Fax: 313.916.8315.

8th European Workshop on Cytogenetics and Molecular Genetics of Human Solid Tumors, September 12–15, 2002, Auditorium World Trade Center Barcelona, Barcelona, Spain. Contact: Rosa Miró, Institut de Biomedicina I de Biomedicina, Campus de la Universitat Autònoma de Barcelona, 08193 Bellaterra, Spain. Phone: 34.93.581.12.33; Fax: 34.581.20.11; E-mail: Solid.Tumours@uab.es; Website: http://ewst.uab.es.


9th International Congress of the Metastasis Research Society, September 20–22, 2002, Renaissance Hotel Chicago, Chicago, IL. Contact: Dr. Danny R. Welch, Penn State University College of Medicine, 500 University Drive,
Correction

In the article by J. D. Geiger et al., entitled "Vaccination of Pediatric Solid Tumor Patients with Tumor Lysate-pulsed Dendritic Cells Can Expand Specific T Cells and Mediate Tumor Regression," which appeared in the December 1, 2001 issue of Cancer Research (pp. 8513–8519), Table 1 appeared incorrectly. The correct Table 1 appears below.

<table>
<thead>
<tr>
<th>No.</th>
<th>Age</th>
<th>Sex</th>
<th>Diagnosis</th>
<th>Treatments prior to vaccination</th>
<th>Relapsed Tumor sites prior to vaccination</th>
<th>DC dose (# of vaccinations)</th>
<th>DTH response (KLH+lysate)</th>
<th>Tumor response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>16</td>
<td>F</td>
<td>Fibrosarcoma</td>
<td>surgery chemotherapy</td>
<td>Lung - pleuropulmonary</td>
<td>1x10^5(2)</td>
<td>KØ TØ</td>
<td>PR</td>
</tr>
<tr>
<td>2</td>
<td>12.5</td>
<td>F</td>
<td>PNET</td>
<td>surgery chemotherapy PBSC irradiation</td>
<td>Lung - microscopic</td>
<td>1x10^5(2)</td>
<td>K+++ T++</td>
<td>SD</td>
</tr>
<tr>
<td>3</td>
<td>7</td>
<td>F</td>
<td>PNET</td>
<td>Surgery chemotherapy (CCG-7492B) irradiation</td>
<td>Thoracic - microscopic</td>
<td>1x10^5(3)</td>
<td>KØ TØ</td>
<td>SD</td>
</tr>
<tr>
<td>4</td>
<td>4.5</td>
<td>F</td>
<td>Neuroblastoma</td>
<td>surgery PBSC irradiation</td>
<td>Bone marrow - microscopic</td>
<td>1x10^5(3)</td>
<td>K++ TØ</td>
<td>SD</td>
</tr>
<tr>
<td>5</td>
<td>8</td>
<td>M</td>
<td>Neuroblastoma</td>
<td>surgery BMT irradiation</td>
<td>Lung</td>
<td>1x10^5(3)</td>
<td>KØ</td>
<td>PD</td>
</tr>
<tr>
<td>6</td>
<td>4.5</td>
<td>F</td>
<td>Neuroblastoma</td>
<td>surgery BMT irradiation</td>
<td>Adrenal bed</td>
<td>1x10^5(3) 1x10^5(2)</td>
<td>K++ T+</td>
<td>SD</td>
</tr>
<tr>
<td>7</td>
<td>15</td>
<td>M</td>
<td>Renal cell cancer</td>
<td>surgery metastasis-2</td>
<td>Retroperitoneal - lymphatic</td>
<td>1x10^5(3)</td>
<td>K+ T+</td>
<td>PD</td>
</tr>
<tr>
<td>8</td>
<td>15.5</td>
<td>M</td>
<td>Osteosarcoma</td>
<td>surgery chemotheraphy</td>
<td>Lung</td>
<td>1x10^5(3)</td>
<td>K+++ TØ</td>
<td>PD</td>
</tr>
<tr>
<td>9</td>
<td>15</td>
<td>M</td>
<td>Inflammatory myofibroblastic sarcoma surgery</td>
<td>Abdominal - mesenteric lymph nodes</td>
<td>1x10^5(3)</td>
<td>K+++</td>
<td>SD</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>4</td>
<td>M</td>
<td>Hepatic sarcoma</td>
<td>surgery chemotheraphy</td>
<td>Abdominal - thoracic</td>
<td>1x10^5(3)</td>
<td>K+ TØ</td>
<td>PD</td>
</tr>
<tr>
<td>11</td>
<td>14</td>
<td>M</td>
<td>Desmoplastic round cell</td>
<td>surgery chemotheraphy</td>
<td>Abdominal - thoracic</td>
<td>1x10^5(3)</td>
<td>K+ TØ</td>
<td>PD</td>
</tr>
<tr>
<td>12</td>
<td>13</td>
<td>M</td>
<td>Ewing's sarcoma</td>
<td>surgery chemotherapy (CCG-7585) PBSC irradiation</td>
<td>Lung - chest wall</td>
<td>1x10^5(3)</td>
<td>KØ</td>
<td>PD</td>
</tr>
<tr>
<td>13</td>
<td>3</td>
<td>M</td>
<td>Clear cell sarcoma</td>
<td>surgery chemotheraphy radiatation</td>
<td>Brain - chest wall</td>
<td>1x10^5(3)</td>
<td>KØ</td>
<td>PD</td>
</tr>
<tr>
<td>14</td>
<td>14.5</td>
<td>M</td>
<td>Ewing's sarcoma</td>
<td>surgery chemotheraphy PBSC</td>
<td>Chest wall - lung</td>
<td>1x10^5(2)</td>
<td>KØ</td>
<td>PD</td>
</tr>
<tr>
<td>15</td>
<td>5</td>
<td>F</td>
<td>Wilms</td>
<td>surgery chemotheraphy (CCG-4941) PBSC irradiation</td>
<td>Abdominal - lung</td>
<td>1x10^5(2)</td>
<td>KØ</td>
<td>PD</td>
</tr>
</tbody>
</table>

*Patients in shaded area did not complete the study protocol and, thus, were not able to be evaluated.
**+, a positive DTH response for KLH (K) at 2 μg or tumor lysate (T) at 10^6 tumor cell equivalents; +++, a positive DTH response for KLH (K) at 20 μg or tumor lysate (T) at 10^6 tumor cell equivalents; +, a positive DTH response for KLH (K) at 200 μg or tumor lysate (T) at 10^6 tumor cell equivalents.

*Children’s Cancer Group.

*Bone marrow transplant.

James D. Geiger
Raymond J. Hutchinson
Lyndon F. Hohenkirk
Elizabeth A. McKenna
Gregory A. Yanik

John E. Levine
Alfred E. Chang
Thomas M. Braun
James J. Mulé
Vaccination Of Pediatric Solid Tumor Patients with Tumor Lysate-pulsed Dendritic Cells Can Expand Specific T Cells and Mediate Tumor Regression


Updated version
Access the most recent version of this article at:
http://cancerres.aacrjournals.org/content/61/23/8513

Cited articles
This article cites 37 articles, 21 of which you can access for free at:
http://cancerres.aacrjournals.org/content/61/23/8513.full#ref-list-1

Citing articles
This article has been cited by 28 HighWire-hosted articles. Access the articles at:
http://cancerres.aacrjournals.org/content/61/23/8513.full#related-urls

E-mail alerts
Sign up to receive free email-alerts related to this article or journal.

Reprints and Subscriptions
To order reprints of this article or to subscribe to the journal, contact the AACR Publications Department at pubs@aacr.org.

Permissions
To request permission to re-use all or part of this article, contact the AACR Publications Department at permissions@aacr.org.