Fusion Cell Vaccination of Patients with Metastatic Breast and Renal Cancer Induces Immunological and Clinical Responses

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ABSTRACT

Purpose: Dendritic cells (DCs) are potent antigen-presenting cells that are uniquely capable of inducing tumor-specific immune responses. We have conducted a Phase I trial in which patients with metastatic breast and renal cancer were treated with a vaccine prepared by fusing autologous tumor and DCs.

Experimental Design: Accessible tumor tissue was disrupted into single cell suspensions. Autologous DCs were prepared from adherent peripheral blood mononuclear cells that were obtained by leukapheresis and cultured in granulocyte macrophage colony-stimulating factor, interleukin 4, and autologous plasma. Tumor cells and DCs were cocultured in the presence of polyethylene glycol to generate the fusions. Fusion cells were quantified by determining the percentage of cells that coexpress tumor and DC markers. Patients were vaccinated with fusion cells at 3-week intervals and assessed weekly for toxicity, and tumor response was assessed at 1, 3, and 6 months after completion of vaccination.

Results: The vaccine was generated for 32 patients. Twenty-three patients were vaccinated with 1 × 10⁵ to 4 × 10⁶ fusion cells. Fusion cells coexpressed tumor and DC antigens and stimulated allologeneic T-cell proliferation. There was no significant treatment-related toxicity and no clinical evidence of autoimmunity. In a subset of patients, vaccination resulted in an increased percentage of CD4 and CD8+ T cells expressing intracellular IFN-γ in response to in vitro exposure to tumor lysate. Two patients with breast cancer exhibited disease regressions, including a near complete response of a large chest wall mass. Five patients with renal carcinoma and one patient with breast cancer had disease stabilization.

Conclusions: Our findings demonstrate that fusion cell vaccination of patients with metastatic breast and renal cancer is a feasible, nontoxic approach associated with the induction of immunological and clinical antitumor responses.

INTRODUCTION

Despite advances in the treatment of cancer, most patients ultimately succumb to the emergence of resistant disease. The identification of tumor-associated antigens in breast and renal carcinomas, such as MUC1, Her2/neu, renal tumor antigen (RAGE), PRAME, and gp-100, and the capacity of the T-cell repertoire to recognize them have indicated that immune-based therapy may effectively target malignant cells (1, 2). A vaccine for the treatment of cancer thus represents a potential approach to amplify tumor-specific immune responses and thereby eradicate the malignant cells.

Dendritic cells (DCs) are potent antigen-presenting cells that are uniquely capable of inducing primary immune responses (3–6). Based on these properties, DC vaccines have been developed as an approach for inducing antitumor immunity (7). Strategies to introduce tumor antigens into DCs have included loading of individual tumor peptides or proteins and transfer of tumor-specific DNA or RNA through lipofection or viral vectors (8–11). Antigen-specific vaccines are potentially limited by the relatively few identified tumor antigens, their uncertain immunogenicity, and the potential evasion of immunological targeting through their down-regulation by the tumor cell. In this context, whole tumor cell approaches have included the pulsing of DCs with tumor lysate, apoptotic bodies, or tumor RNA (12–15).

Another strategy for the generation of DC vaccines is based on the fusion of autologous tumor cells with DCs (16–20). In this approach, the entire repertoire of tumor antigens, including those yet to be identified, is expressed with the immune-stimulating machinery of the DCs. The fusion cell vaccine allows for induction of helper T and CTL responses by class II presentation of exogenous protein and class I presentation of newly synthesized endogenous protein. Vaccination with fusion cells has eradicated established tumor in diverse animal models (16, 21, 22). In human MUC1 transgenic mice, vaccination with fusion cells reverses immunological unresponsiveness to MUC1 and results in the rejection of MUC1-positive tumors (23). Preclinical studies with patient-derived breast cancer cells and DCs have also demonstrated that fusion cells induce tumor-specific CTL responses and lysis of autologous tumor cells (24).

In the present study, patients with metastatic breast and renal cancer were vaccinated with fusion cells generated from...
patient-derived tumor cells and autologous DCs. The results demonstrate that fusion cell vaccination is feasible and nontoxic and induces immunological and clinical antitumor responses.

MATERIALS AND METHODS

Study Design. This study was conducted with approval of an Investigational New Drug Application by the Food and Drug Administration and clinical protocols by our Institutional Review Board. Patients with metastatic breast and renal cancer with tumor lesions that were accessible to biopsy or resection without invasive surgery were potentially eligible for enrollment. Patients were excluded if they had clinically significant autoimmune disease, clinical evidence of central nervous system metastases, or treatment with hormonal, chemotherapeutic, or immunotherapeutic agents within 1 month of vaccination. Successive cohorts of breast cancer patients were vaccinated with doses of $1 \times 10^5$, $3 \times 10^5$, or $1 \times 10^6$ fusion cells. Based on the lack of toxicity observed and cell availabilities, successive cohorts of renal cancer patients were treated with $1 \times 10^6$, $2 \times 10^6$, or $4 \times 10^6$ fusion cells. The fusion cells were administered s.c. at 3-week intervals. To assess their capacity to generate a primary cellular immune response to vaccination, patients also received a single s.c. vaccination of $1 \times 10^6$ DCs pulsed with keyhole limpet hemocyanin (KLH) protein that was given at a separate site at the time of the first fusion cell vaccination. Patients were monitored weekly during the vaccination period for treatment-related adverse events and evidence of autoimmunity. An increase in the antinuclear antibody titer of >1 dilution was defined as significant. Toxicities were graded according to National Cancer Institute Common Toxicity Criteria 2.0.

Isolation of Tumor Cells. Patient-derived tumor tissue was subjected to mechanical disruption and, when necessary, digestion with collagenase (GIBCO-BRL Life Technologies, Inc., Bethesda, MD) to generate a single cell suspension. Alternatively, tumor cells were isolated from malignant ascites or pleural effusions, and excess RBCs were lysed in the presence of ammonium chloride (Sigma, St. Louis, MO). Tumor cells were cultured in RPMI 1640 containing gentamicin (Baxter, Deerfield, IL), human insulin (Humulin R; Eli-Lilly, Indianapolis, IN), and 10% autologous plasma at 37°C. An aliquot of cells was subjected to immunohistochemical staining and/or fluorescence-activated cell-sorting analysis to assess expression of tumor-associated (MUC1, CAM, and cytokeratin) and DC (DR, CD86, CD80, CD40, CD54, and CD83) antigens. Before fusion, an aliquot of tumor cells was harvested to generate tumor lysate for in vitro testing.
Isolation of DCs. Patients underwent a single leukapheresis collection. Peripheral blood mononuclear cells (PBMCs) were isolated by Ficoll-Paque (Pharmacia Biotech, Piscataway, NJ) density centrifugation and cultured in RPMI 1640 with 1% autologous plasma for 1–2 h. Nonadherent cells were removed, and the adherent population was cultured in RPMI 1640 containing gentamicin, 1% autologous sera, 500 units/ml recombinant human interleukin (IL)-4 (Peprotech, Rocky Hill, NJ), and 1000 units/ml granulocyte macrophage colony-stimulating factor (GM-CSF; Immunex, Seattle, WA) for 7–10 days. After an aliquot was removed for immunocytochemical and functional analyses, the DCs were used for fusion or pulsed overnight with 50 μg/ml KLH protein (Biosyn, Carlsbad, CA), suspended in 10% DMSO (Sigma)/90% autologous plasma, and frozen in liquid nitrogen as a single dose. Before the administration of the DC/KLH preparation, the absence of contamination was confirmed by assays for sterility, endotoxin, and Mycoplasma.

Preparation of DC/Tumor Fusions. Tumor cells and DCs were combined at ratios of 1:3 to 1:10 (dependent on cell yields) and washed in serum-free medium. After low-speed centrifugation, the cell pellets were resuspended in 500 μl of 50% polyethylene glycol (Sigma) in Dulbecco’s PBS without Ca^{2+} and Mg^{2+} (GIBCO-BRL), washed free of polyethylene glycol, and cultured in RPMI 1640 with 10% autologous plasma and 500 IU/ml GM-CSF. The cells were frozen in liquid nitrogen in single dose aliquots. Based on preclinical testing of radiation sensitivity, the fusion cells were irradiated with 3,000 (breast cancer) or 10,000 (renal cancer) rads before vaccination. Doses were based on the number of viable fusion cells (as assessed by trypan blue exclusion) that coexpressed DCs and tumor-associated markers. An adequate yield of fusion cells and an absence of contamination as assessed by assays for sterility, endotoxin, and Mycoplasma were required to proceed with vaccination.

Immunocytochemical Analysis. Cells were stained with primary murine monoclonal antibodies against MUC1 (Phar-mingen, San Diego, CA); cytokeratin (Boehringer Mannheim, Indianapolis, IN); CAM (Becton Dickinson, San Jose, CA); HLA-DR, CD40, CD54, CD80, CD83, or CD86 (Pharmingen); and an isotype-matched negative control for 60 min. The cells were incubated with a biotinylated F(ab’)_2 fragment of horse antimouse IgG (Vector Laboratories) for 45 min, washed twice with PBS, and incubated for 30 min with avidin biotin complex reagent solutions followed by 3 amino-9-ethyl carbazole solution (Vector Laboratories). Detection of MUC1, CAM, or cytokeratin with the avidin biotin complex reagents was followed by staining for other markers with the avidin biotin complex.
alkaline phosphatase kit (Vector Laboratories). Membrane staining intensity was evaluated using a scale ranging from 0 to 4+ (0, completely negative; 1+, faint positivity; 2+, moderate positivity; 3+, strong positivity; 4+ very strong positivity). For a grade 2+ to 4+, staining had to be present in a majority (>50%) of the cells.

**Fluorescence-Activated Cell-Sorting Analysis.** Cells were incubated with the indicated primary monoclonal antibody or a matching isotype control for 30 min at 4°C. Bound primary monoclonal antibodies were detected with a secondary affinity-purified FITC-conjugated goat antimouse IgG (Chemicon International, Temecula, CA) followed by fixation in 2% paraformaldehyde. For bidimensional flow cytometry, cells were incubated with antibody directed against MUC1, CAM, or cytokeratin; FITC-conjugated secondary antibody; and then antibody directed against DR or CD86 conjugated with phycoerythrin. Analysis was performed on a FACScalibur flow cytometer (Becton Dickinson) using CellQuest software (Becton Dickinson).

**Mixed Lymphocyte Reactions.** Allogeneic T cells were isolated from nonadherent PBMCs by passage through a T-cell enrichment column (R&D Systems, Minneapolis, MN). Cultures were set up in triplicate in 200 μl of culture medium in 96-well U-bottomed culture plates (Costar, Cambridge, MA) for 5 days with T cells (1 × 10⁵) and autologous tumor cells, DCs, or fusion cell preparations at a ratio of 30:1, 100:1, 300:1, and 1000:1. T-cell proliferation was determined by measuring incorporation of [³H]thymidine after overnight pulsing (1 μCi/well; New England Nuclear, Boston, MA). Results were normalized over different time points and expressed as stimulation index. The stimulation index was determined by calculating the ratio of DC-, tumor-, or fusion cell-induced [³H] thymidine incorporation (mean of triplicates) over background [³H]thymidine incorporation (mean of triplicates) of the unstimulated T-cell population.

**In Vitro T-Cell Proliferative Responses to KLH.** Freshly isolated patient PBMCs (1 × 10⁵) were cocultured in 200 μl of medium in 96-well U-bottomed culture plates for 5 days with 100 μg/ml KLH. Proliferation was determined by measuring incorporation of [³H]thymidine after overnight pulsing of triplicate samples.

**Tumor Lysate-Induced IFN-γ Expression.** Tumor lysate was prepared by repeated freeze-thaw cycles of patient-derived tumor cells. PBMCs were harvested before each vaccination and at 1, 3, and 6 months after vaccination and cryopreserved in liquid nitrogen in the presence of 10% DMSO in human male AB serum.

After completion of the vaccinations and follow-up assessments, the PBMCs were thawed, washed, plated at 1 × 10⁶ cells/well in a 24-well plate (Becton Dickinson, Franklin Lakes, NJ), and pulsed with tumor lysate generated from 1 × 10⁵ autologous tumor cells, tetanus toxoid (10 μg/ml), or culture media alone for 5 days. On day 5, the cultures were restimulated for 6 h and cultured overnight with 1 μg/ml GolgiStop (Pharmingen) to inhibit cytokine secretion. The cells were then harvested, washed, and incubated with blocking buffer (10% human IgG; Sigma) for 30 min and stained for CD4 and CD8 by incubation with antibodies conjugated to FITC (Pharmingen) for 30 min. Cells were permeabilized by incubation in Cytofix/Cytoperm plus (containing form-

aldehyde and saponin; Pharmingen) for 30 min followed by two washes in Perm/Wash solution (Pharmingen). Cells were then incubated with phycoerythrin-conjugated antihuman IFN-γ (Caltag, Burlingame, CA) or a matched isotype control antibody for 30 min, washed twice in Perm/Wash solution, fixed in 2% paraformaldehyde, and analyzed by flow cytometry using FACSscan (Becton Dickinson).

**RESULTS**

**Patient Profile.** Fifty-eight patients were enrolled in Phase I studies for breast (32 patients) and renal cancer (26 patients) from July 1999 to March 2002. Vaccine generation was successful in 32 patients (16 breast cancer and 16 renal cancer patients). Vaccine production was not successful for 24 patients due to inadequate cell yields. In two patients with breast cancer, pleural effusions were found to not contain malignant cells, and no other accessible lesions were identified. Core needle biopsies of lung or liver lesions and resection of lesions of <1 cm did not yield sufficient tumor cells and were not pursued later in the study. Malignant effusions, accessible lymph nodes, and peripheral lung nodules resected via thoracoscopic approach were the most reliable source of tumor cells for vaccine generation (Table 1). One breast cancer patient who developed a paraneoplastic neurological syndrome and eight additional patients (five breast cancer and three renal cancer patients) with clinically significant disease progression were withdrawn from the study before receiving the vaccine. The mean age of the treated population was 54 years.

Ten breast cancer patients were treated with the fusion vaccine at a dose of 1 × 10⁵ (three patients) 3 × 10⁵ (five patients), or 1 × 10⁶ (two patients) fusion cells. Eight patients had tumors that expressed the estrogen receptor; four patients overexpressed the HER2/neu antigen. Breast cancer patients had received a mean of 2.8 prior chemotherapy and 2.6 prior hormonal regimens (in estrogen receptor-positive patients; Table 1).

Thirteen patients (10 men and 3 women) with renal cancer were treated with the fusion vaccine at a dose of 1 × 10⁶ (9 patients), 2 × 10⁶ (3 patients), or 4 × 10⁶ (1 patient) fusion cells. All but one patient had received prior therapy for metastatic disease, with the majority of patients having had disease progression after an IL-2-containing regimen. Prior treatment is listed in Table 1.

**Vaccine Generation.** After a single leukapheresis collection, DCs were generated from adherent PBMCs cultured for 7–10 days with GM-CSF and IL-4. DC yields were available for 42 breast and renal cancer patients, with a mean value of 9.2 × 10⁵ cells. Cell populations were assessed by immunocytochemical analysis and a grading of 2+ or greater was considered positive. In the treated patient population, DC preparations uniformly expressed DR, CD86, and CD54. CD83 expression was observed in 9 of 23 specimens. Expression of cytokeratin was absent, and MUC-1 staining was observed in only one sample (Figs. 1A and 2, A and B; Table 2). Mean viability of the DC preparations was 84%. As a measure of their potency as antigen-presenting cells, DCs were assayed for stimulation of allogeneic T-cell proliferation in a standard mixed lymphocyte
reaction assay. The median stimulation index was 85 for DCs generated from both breast and renal cancer patients.

Breast carcinoma cells were obtained from malignant pleural effusions (three patients), malignant ascites (one patient), superficial lymph nodes/s.c. lesions (four patients), chest wall/breast tissue (one patient), and bone marrow (one patient). Renal carcinoma cells were obtained from primary renal tumors (one patient), pulmonary nodules accessible by thoracoscopy (six patients), and nodal/s.c. masses (six patients). Mean viability of the tumor preparations was 80%. Tumor cells were maintained in single cell suspension cultures before fusion. Tumor cell preparations uniformly expressed cytokeratin. MUC-1 was observed in 9 of 10 breast cancer patient and 5 of 13 renal cancer patient samples, respectively (Figs. 1B and 2; Table 2). Of note, CD86 was detected in 2 specimens, and DR expression was seen in 12 of 13 and 3 of 10 of the renal and breast carcinoma preparations, respectively. Tumor cells were less effective than DCs in stimulating allogeneic T-cell proliferation. Median stimulation indices were 9, 26, and 12 for patients with breast cancer, patients with renal cancer, and the combined populations, respectively.

Fusion cells were generated by coculture of DCs and tumor cells in the presence of polyethylene glycol. The cells were then cultured for 2–3 days in the presence of GM-CSF before analysis. The percentage of fusion cells that coexpressed tumor-associated and DC markers was determined by immunohistochemical staining and/or fluorescence-activated cell-sorting analysis (Figs. 1 and 2; Table 2). For example, fusion of CAM(+/−)CD86(−) renal carcinoma cells with CAM(−)/CD86(+) DCs generated a cell population that coexpressed CAM and CD86 as shown by immunohistochemistry (Fig. 2). The mean percentage of the viable cells that coexpressed DCs and tumor markers was 45% in the fusion cell preparations (range, 18–71%; Table 2). Mean viability of the fusion cell preparations was 77% and did not differ between the breast and renal cancer fusions. Fusion cell preparations were also assessed for their capacity to induce allogeneic T-cell proliferation. Median stimulation indices were 15, 58, and 38 for the breast cancer, renal cancer, and combined populations, respectively.

**Vaccination.** Twelve patients underwent three vaccinations. Six patients received two doses because of limited cell yields (five patients) and disease progression after the second vaccine (one patient). One patient was withdrawn from the study due to disease progression after the first dose. Four patients...
received a fourth prepared dose as per protocol after demonstrating stable disease following the third vaccination.

**Adverse Events.** No dose-limiting toxicities were observed. Dose escalation proceeded to the highest intended dose level and was limited only by cell yields. No significant treatment-related toxicities were observed. Toxicities judged to be potentially treatment related were transient and assessed as grade I (National Cancer Institute Common Toxicity Criteria 2.0). These included pain at tumor sites approximately 24–48 h after receiving the vaccine (14 events), flu-like symptoms/myalgia (2 events), fever (2 events), fatigue (1 event), pruritus (2 events), injection site discomfort (12 events), and minimal pedal edema (1 event). Antinuclear antibodies were determined at serial time points to assess for evidence of autoimmunity. One

**Table 2** Vaccine characteristics

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>DC markers/viability</th>
<th>Tumor markers/viability</th>
<th>Fusion markers/viability</th>
<th>Fusion efficiency</th>
</tr>
</thead>
<tbody>
<tr>
<td>1B</td>
<td>DR, CD80, CD86, CD83/97%</td>
<td>Cytokeratin, MUC-1/100%</td>
<td>DR-Cytokeratin/87%</td>
<td>42%</td>
</tr>
<tr>
<td>2B</td>
<td>DR, CD86, CD83/90%</td>
<td>DR, cytokeratin, MUC-1/83%</td>
<td>CD86-MUC-1/68%</td>
<td>30%</td>
</tr>
<tr>
<td>3B</td>
<td>DR, CD80, CD86, CD54, CD83/79%</td>
<td>Cytokeratin, MUC-1/93%</td>
<td>DR-MUC-1/86%</td>
<td>40%</td>
</tr>
<tr>
<td>4B</td>
<td>DR, CD80, CD86, CD83/84%</td>
<td>Cytokeratin, MUC-1/84%</td>
<td>DR-MUC-1, CD86-MUC-1/88%</td>
<td>40%</td>
</tr>
<tr>
<td>5B</td>
<td>DR, CD86, CD54/87%</td>
<td>Cytokeratin, DR, MUC-1/77%</td>
<td>CD86-MUC-1/76%</td>
<td>60%</td>
</tr>
<tr>
<td>6B</td>
<td>DR, CD86, CD54, CD80/88%</td>
<td>Cytokeratin, DR, MUC-1/77%</td>
<td>CD86-cytokeratin/91%</td>
<td>70%</td>
</tr>
<tr>
<td>7B</td>
<td>DR, CD86, CD40/90%</td>
<td>Cytokeratin, MUC-1/88%</td>
<td>CD86-MUC-1/NR</td>
<td>60%</td>
</tr>
<tr>
<td>8B</td>
<td>DR, CD86, CD54/76%</td>
<td>Cytokeratin/46%</td>
<td>DR-cytokeratin/31%</td>
<td>28%</td>
</tr>
<tr>
<td>9B</td>
<td>DR, CD86, CD83/82%</td>
<td>Cytokeratin, DR, MUC-1/85%</td>
<td>DR-MUC-1/66%</td>
<td>29%</td>
</tr>
<tr>
<td>10B</td>
<td>DR, CD86/92%</td>
<td>DR, cytokeratin, MUC-1/82%</td>
<td>CD86-MUC-1/91%</td>
<td>52%</td>
</tr>
<tr>
<td>11B</td>
<td>DR, CD86, CD54/91%</td>
<td>DR, MUC-1, cytokeratin, CAM/NR</td>
<td>CD86-CAM/94%</td>
<td>55%</td>
</tr>
<tr>
<td>12B</td>
<td>DR, CD86, CD54/78%</td>
<td>DR, CD86, CAM/96%</td>
<td>CD80-CAM/80%</td>
<td>55%</td>
</tr>
<tr>
<td>13B</td>
<td>DR, CD86, CD54, CD40/85%</td>
<td>DR, cytokeratin, CAM, MUC-1/69%</td>
<td>CD86-CAM/70%</td>
<td>34%</td>
</tr>
<tr>
<td>14B</td>
<td>DR, CD86, CD54, CD83, MUC-1/85%</td>
<td>DR, MUC-1, CD54, CD83/55%</td>
<td>CD86-CAM/85%</td>
<td>38% and 30%</td>
</tr>
<tr>
<td>15R</td>
<td>DR, CD86, CD54/75%</td>
<td>CAM, DR/85%</td>
<td>CD86/cytokeratin/68%</td>
<td>63%</td>
</tr>
<tr>
<td>16R</td>
<td>DR, CD86/67%</td>
<td>CAM, DR/83%</td>
<td>CD86-CAM/80%</td>
<td>40%</td>
</tr>
<tr>
<td>17R</td>
<td>DR, CD86, CD54/86%</td>
<td>MUC-1, CAM, DR/89%</td>
<td>CD86-CAM, DR-CAM/84%</td>
<td>47% and 59%</td>
</tr>
<tr>
<td>18R</td>
<td>DR, CD86, CD54/80%</td>
<td>CAM/CD54/72%</td>
<td>CD86-CAM, DR-CAM/75%</td>
<td>71%</td>
</tr>
<tr>
<td>19R</td>
<td>DR, CD86, CD54, CD83/74%</td>
<td>CAM, DR, CD83/75%</td>
<td>CD86-CAM/75%</td>
<td>39%</td>
</tr>
<tr>
<td>20R</td>
<td>DR, CD86/95%</td>
<td>CAM, DR-CAM, MUC-1, cytokeratin</td>
<td>CD86-CAM/70%</td>
<td>52% and 54%</td>
</tr>
<tr>
<td>21R</td>
<td>DR, CD86, CD83, CD54, CD40/84%</td>
<td>CAM, DR/72%</td>
<td>CD86-cytokeratin/75%</td>
<td>36%</td>
</tr>
<tr>
<td>22R</td>
<td>DR, CD86/80%</td>
<td>CAM, cytokeratin/54%</td>
<td>CD86-CAM/67%</td>
<td>23%</td>
</tr>
<tr>
<td>23R</td>
<td>DR, CD86, CD83/88%</td>
<td>CAM, cytokeratin, DR/78</td>
<td>CD-86-CAM/49%</td>
<td>18%</td>
</tr>
</tbody>
</table>

* DC, dendritic cell; B, breast cancer; NR, not recorded; R, renal cancer.
Disease response was associated with the lesion had regressed by over 80% and 90%, respectively. At 1 month and 4 months after her third vaccination, an 8 increase in the percentage of CD8 cells expressing IFN-γ (Fig. 4). To assess the effects of fusion cell vaccination, PBMCs were isolated at serial time points, and pulsed overnight with [3H]thymidine. Results are presented as the T-cell stimulation index (mean ± SE of triplicates) for baseline (□) and peak values (■) in patients who demonstrated at least a 2-fold increase after vaccination.

Fig. 3 Effect of vaccination on keyhole limpet hemocyanin-induced T-cell proliferation. Peripheral blood mononuclear cells were isolated before each vaccination and at 1, 3, and 6 months after completion of therapy (in those patients remaining on study), cocultured with 100 µg/ml keyhole limpet hemocyanin for 5 days, and pulsed overnight with [3H]thymidine. Results are presented as the T-cell stimulation index (mean ± SE of triplicates) for baseline (□) and peak values (■) in patients who demonstrated at least a 2-fold increase after vaccination.

patient developed a transient elevation in the ANA titer (1:320) without associated clinical evidence of autoimmunity. On subsequent testing, the titer returned to baseline. No clinical evidence of autoimmunity was observed in the patient population.

Effect of Vaccination on KLH-Induced T-Cell Proliferation. All patients underwent a single vaccination with KLH-pulsed DCs to assess their capacity to exhibit a primary immune response. PBMCs were isolated at serial time points, and proliferation after in vitro exposure to KLH was measured (Fig. 3). Of 21 evaluable patients, 8 patients demonstrated a significant increase in the KLH-induced T-cell proliferation index after vaccination. For patients demonstrating a response, the median peak postvaccination stimulation index was 3.5 as compared with 0.4 before vaccination.

Effect of Vaccination on Tumor-Induced T-Cell Expression of IFN-γ. To assess the effects of fusion cell vaccination on the capacity of patients to mount a tumor-specific CTL response, PBMCs were isolated at serial time points, cocultured with autologous tumor lysate, and analyzed for intracellular expression of IFN-γ by CD4+ and CD8+ T-cell populations. Of 18 patients with sufficient tumor cells to perform the assay, 10 patients demonstrated at least a 2-fold increase in the percentage of CD4+ cells expressing IFN-γ after tumor lysate exposure, with a mean prevaccine level of 1.4% and a mean peak postvaccine level of 4.0% (Fig. 4A). Seven patients demonstrated at least a 2-fold increase in the percentage of CD8+ T cells expressing IFN-γ (Fig. 4B). The mean prevaccine level was 1.4% for this cohort, and the mean peak postvaccine level was 4.0%.

Clinical Responses. After vaccination, a breast cancer patient (patient 6) responded with near complete regression of an 8 × 6-cm chest wall mass and no evidence of new disease at other sites. At 1 month and 4 months after her third vaccination, the lesion had regressed by over 80% and 90%, respectively. Disease response was associated with a >50% decline (171 to 78) in serum CA-15-3 (MUC1). Approximately 3 months after completion of vaccine therapy (after disease regression had been observed), the patient elected to resume leuprolide and exemestane. Her disease status remained stable with no evidence of progression until 24 months after vaccination. Of note, this patient had a steady rise over time in the tumor-induced percentage of CD4+ and CD8+ T cells expressing intracellular IFN-γ (Fig. 5). Expression of IFN-γ by CD4+ cells peaked 3 months after completion of vaccine therapy at a level that was 6.2-fold of that found before vaccination. The percentage of CD8+ cells expressing IFN-γ peaked at 6 months postvaccination with a level 2.8-fold of that measured prevaccination.

A second breast cancer patient (patient 5) demonstrated 50% and 44% regression of an adrenal mass and pulmonary nodule, respectively, and stable disease at other sites. Evidence of disease progression was detected at 6 months after completion of vaccination. No other therapy was administered during this time. Six patients (five patients with renal cancer and one patient with breast cancer) had stabilization of disease with progression noted at 3–9 months after the completion of vaccination.

DISCUSSION

Clinical trials of DC vaccines have demonstrated minimal toxicity, evidence for the induction of tumor-specific cellular immunity, and, in certain patients, clinical response (25–30). Use of single antigen-based vaccines provides a defined target for immunological monitoring. However, peptide-based vaccinations are restricted to a particular HLA subtype, immunodominant antigens have not been defined for most tumors, and tumor cells may evade host effector cells through the down-regulation of expression of a single antigen. In contrast, use of whole cell approaches such as DC/tumor fusion allows for the presentation of multiple antigens, including those yet to be identified, in the context of DC-mediated costimulation. The fusion cell approach allows for the presentation of internalized and newly synthesized tumor antigen by the class II and I pathways, respectively. The manipulation of whole tumor cells for vaccine generation, however, is associated with certain technical challenges.

In the present study, patients with metastatic breast and renal cancer were vaccinated with autologous tumor cells fused to autologous DCs. A concern was the feasibility of producing adequate fusion cell yields for vaccination from patient-derived specimens. DCs were generated in sufficient quantities from a single leukapheresis collection and exhibited phenotypic and functional characteristics of partially mature DCs. The cells uniformly expressed class II and costimulatory molecules, but CD83 expression was modest and seen only in a subset of specimens. The major challenge for vaccine generation was obtaining an adequate yield of tumor cells. Pleural effusions, ascites, and superficial nodal and soft tissue lesions provided the most reliable source of tumor cells. In most cases, tumor cells did not expand ex vivo, and core needle biopsies, fibrotic skin nodules, and tumor tissue less than 1 cm did not provide adequate yields of cells. With adequate DC and tumor cell yields, fusion cell generation proved to be feasible with a mean efficiency of 45%.

Induction of an immunological response after peptide-based vaccination has been assessed by measuring T-cell binding to HLA/p peptide tetramers or antigen-induced IFN-γ expression by Elispot, ELISA, or intracellular fluorescence-activated...
cell-sorting analysis. Assessment of response to vaccination with tumor lysate or whole tumor cells has also involved quantification of the antitumor immunity in the absence of a defined antigen. In the present study, a subset of patients demonstrated evidence of immunological response to fusion cell vaccination by an increased percentage of T cells expressing intracellular IFN-γ after ex vivo exposure to tumor lysate. The correlation between in vitro immunological assays and clinical response is not well defined. In one study, immunological response to at least two antigens after vaccination with DCs loaded with multiple peptides was associated with clinical outcome (27). In our study, disease regression in response to vaccination was associated with increased tumor lysate-induced IFN-γ expression. In one patient with an immune response, vaccination resulted in the near complete regression of a large chest wall mass. Hormonal therapy was initiated 3 months postvaccination (after disease response was observed), and the patient remained without evidence of disease progression for 2 years. Of note, autoimmunity is a potential concern when using whole cell vaccine approaches in which tumor-associated antigens are introduced with shared self-antigens. In the present study, vaccination was well tolerated, and there was no evidence of clinically significant autoimmunity.

DC-based vaccine studies have demonstrated encouraging results; however, issues remain regarding the optimal approach. One issue is whether autologous or allogeneic DCs are more effective as fusion partners. DCs isolated from cancer patients can exhibit an impaired capacity to express costimulatory molecules (31). However, functional deficiencies observed in DCs isolated from cancer patients are not generally seen in DCs generated by ex vivo culture of progenitors with cytokines (32, 33). Fusions generated with allogeneic DCs are dependent on tumor cell expression of class I molecules for antigen presentation. In this regard, loss of class I expression has been demonstrated in tumor cells as a potential mechanism for evasion of host immunity (34). Immune response after DC vaccination is
also determined, in part, by the stage of DC maturation (35). Immature DCs secrete IL-10 and bias responding T cells toward a T helper 2 phenotype (36, 37). By contrast, mature DCs secrete IL-12 and are potent inducers of T helper 1-mediated cytotoxicity (38, 39). Moreover, with fusion cells, production of IL-10 by the tumor could potentially inhibit DC maturation and function (40–42). The use of cytokine adjuvants may thus augment effectiveness of the fusion cell vaccine. IL-12 is a heterodimeric cytokine that up-regulates DC expression of costimulatory molecules, stimulates T helper 1 reactivity, expands antigen-specific CD8+ T cells (43, 44), and enhances the effectiveness of DC-based antitumor vaccines (45–47). Moreover, administration of IL-12 with DC fusion cells has substantially improved induction of antitumor immunity in animal tumor models of intracranial glioma and multiple myeloma (21, 22). Thus, administration of IL-12 could potentiate the effectiveness of the fusion cell vaccine in patients.

Based on the present results, which demonstrate the induction of immunological and clinical antitumor activity, and the potential for improving these responses, clinical trials are under way to identify a more effective strategy for fusion cell vaccination. These trials will define the toxicity, immunological responses, and clinical efficacy of fusions with mature autologous or allogeneic DCs and vaccination in the context of adjuvant IL-12 or GM-CSF.

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Fusion Cell Vaccination of Patients with Metastatic Breast and Renal Cancer Induces Immunological and Clinical Responses

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