Bioactivity of Autologous Irradiated Renal Cell Carcinoma Vaccines Generated by 
ex Vivo Granulocyte-Macrophage Colony-stimulating Factor Gene Transfer

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ABSTRACT

Granulocyte-macrophage colony-stimulating factor (GM-CSF) gene-transduced, irradiated tumor vaccines induce potent, T-cell-mediated antitumor immune responses in preclinical models. We report the initial results of a Phase I trial evaluating this strategy for safety and the induction of immune responses in patients with metastatic renal cell carcinoma (RCC). Patients were treated in a randomized, double-blind dose-escalation study with equivalent doses of autologous, irradiated RCC vaccine cells with or without ex vivo human GM-CSF gene transfer. The replication-defective retroviral vector MFG was used for GM-CSF gene transfer. No dose-limiting toxicities were encountered in 16 fully evaluable patients. GM-CSF gene-transduced vaccines were equivalent in toxicity to nontransduced vaccines up to the feasible limits of autologous tumor vaccine yield. No evidence of autoimmunec disease was observed. Biopsies of intradermal sites of injection with GM-CSF gene-transduced vaccines contained distinctive macrophage, dendritic cell, eosinophil, neutrophil, and T-cell infiltrates similar to those observed in preclinical models of efficacy. Histological analysis of delayed-type hypersensitivity responses in patients vaccinated with GM-CSF-transduced vaccines demonstrated an intense eosinophil infiltrate that was not observed in patients who received nontransduced vaccines. An objective partial response was observed in a patient treated with GM-CSF gene-transduced vaccine who displayed the largest delayed-type hypersensitivity conversion. No replication-competent retrovirus was detected in vaccinated patients. This Phase I study demonstrated the feasibility, safety, and bioactivity of an autologous GM-CSF gene-transduced tumor vaccine for RCC patients.

INTRODUCTION

Cancer cell vaccines modified to secrete cytokines by ex vivo gene transfer generate antitumor immunity in preclinical models (1–13). A comparison involving multiple cytokine genes found that GM-CSF gene-transduced vaccines were the most potent inducers of long-lasting, specific tumor immunity even in poorly immunogenic tumor models (7). The efficacy of GM-CSF-transduced vaccines has been shown in preclinical models of melanoma, colon cancer, renal cancer, lung cancer, lymphoma, and prostate cancer (8–15). The therapeutic activity of GM-CSF involves the paracrine (local) action of the cytokine at the vaccine site in activating APCs. These GM-CSF-activated APCs include dendritic cells (the most potent APCs for T cells known) and macrophages (8–15). Systemic antitumor immunity is mediated subsequently by APC priming of CD4+ and CD8+ T cells, which recognize tumor-associated antigens at metastatic sites (8–17). The word “vaccine” is used in this context as the genetically engineered antigen source (cancer cell) for activation of immune responses against established metastatic cancer, as opposed to prophylactic immunization.

Clinical translation of this approach involves tumor resection, culture of the cancer cells, and ex vivo GM-CSF gene transfer, followed by patient vaccination with the genetically modified, lethally irradiated autologous cancer cells (18, 19). RCC was chosen to evaluate this strategy of ex vivo gene therapy, given large yields of tumor cells at surgery and the potential responsiveness of RCC to immunotherapies (18). The retroviral vector MFG permitted high efficiency GM-CSF gene transfer in primary RCC cultures without drug selection (18–20). With this vector, successful establishment of a permanent tumor cell line is not required for each patient, and primary cancer potentially maintains a greater diversity of RCC antigens (18, 19).

Patients were randomized to receive escalating vaccine cell doses of lethally irradiated, autologous RCC cells expanded in short-term culture and transduced with the human GM-CSF gene or equivalent doses of expanded, irradiated, nontransduced RCC cells. Nontransduced, cultured RCC secretes low levels of GM-CSF; therefore, randomization was performed to distinguish toxicities related to vaccine cell expansion from toxicities due to higher levels of GM-CSF secretion from human GM-CSF gene transfer. Randomization was double-blind until the conclusion of both toxicity and immunological evaluation to objectively measure biological effects in patients without bias toward the gene therapy arm. The primary objectives were: (a) to evaluate the safety and distinguish toxicities of injections of cultured, lethally irradiated, autologous RCC cells from similarly prepared RCC cells transduced with the human GM-CSF gene, secreting the cytokine at greater than 40 ng/106 vaccine cells/24 h; and (b) to assay both in vitro and in vivo the additional contribution, if any, of GM-CSF gene transduction to induction of tumor immunity induced by irradiated, autologous RCC vaccines.

PATIENTS AND METHODS

Selection of Patients. The protocol has been published previously (19). Patients with stage III T4b (inferior vena cava tumor thrombus above the level of the diaphragm), or stage IV (metastatic) RCC (histologically confirmed after surgery) were eligible. Systemic IL-2-based regimens or other investigational agents were also offered as treatment options for these patients. Eligibility criteria included primary RCC in place; Eastern Cooperative Oncology Group performance status of 0 or 1; appropriate surgical candidate (21) and estimated life expectancy of at least 6 months; no major surgery, radiotherapy, chemo-
Autologous irradiated RCC vaccines with or without human GM-CSF gene transfer were administered at the assigned dose level; 50% of total dose was given intradermally and 50% s.c. to compare patient tolerability of the two routes of administration.

<table>
<thead>
<tr>
<th>Dose level</th>
<th>Autologous cell dose</th>
<th>Intradermal injections</th>
<th>Cell dose and volume</th>
<th>s.c. injections</th>
<th>Cell dose and volume</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>4 × 10^6 cells</td>
<td>2</td>
<td>1 × 10^6 cells/0.5 cm³</td>
<td>2</td>
<td>1 × 10^6 cells/0.5 cm³</td>
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<td>2</td>
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<td>1 × 10^6 cells/0.5 cm³</td>
</tr>
<tr>
<td>3</td>
<td>4 × 10^6 cells</td>
<td>16</td>
<td>1.2 × 10^6 cells/0.6 cm³</td>
<td>5</td>
<td>4 × 10^6 cells/2 cm³</td>
</tr>
</tbody>
</table>


d Patients fully evaluable for toxicity and immunological effects.

Table 2: Autologous vaccine GM-CSF secretion by dose level

Transduction for patient 2 fell below the specifications of the trial (17 ng/10⁶ cells), and patient 27 was treated on dose level 2 on a compassionate basis, because of failure to achieve dose level 3 yields. Patient 28 did not have enough vaccine for one fully evaluable treatment at dose level 2 and was treated with 2 × 10^⁶ cells. These patients were treated as if specifications were met and were evaluated for all safety and toxicity endpoints but did not occupy a full evaluable patient treatment position for the dose escalation rules. Patient 25 did not have confirmed metastatic disease following surgery and was not treated.

Table 3: Characteristics of treated patients

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<table>
<thead>
<tr>
<th>Characteristic</th>
<th>No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. treated (fully evaluable)</td>
<td>18 (16)</td>
</tr>
<tr>
<td>Median age</td>
<td>59</td>
</tr>
<tr>
<td>Range</td>
<td>44-79</td>
</tr>
<tr>
<td>Male/female</td>
<td>10/8</td>
</tr>
<tr>
<td>Performance status (ECOG) = 0</td>
<td>18</td>
</tr>
<tr>
<td>Prior therapy</td>
<td>IL-2</td>
</tr>
<tr>
<td>Radiotherapy</td>
<td>1/18</td>
</tr>
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</table>

* Eastern Cooperative Oncology Group.
indirect immunofluorescent assay, described previously, using a polyclonal antibody to eosinophil MBP, was used to semiquantify and localize these granule proteins to intracellular or extracellular locations (29, 30). A species-matched, immunoglobulin subtype-matched protein was used as a negative control for each antibody; nasal polyposis tissue served as positive control tissue.

All EG2-stained specimens were scored by two independent investigators (L. A. B. and S. D.) in a blinded fashion and presented as number of positively staining cells/mm (2). The MBP staining was quantified using a scoring system from 0 to 3+ based on the extent and intensity of the staining (31). Slides were evaluated under code with the pathologist blinded to patient and transduction status. Variables ranked were number of cells stained with the above-mentioned antibodies per high-powered field. Only vaccine sites with tumor cells present (cytokeratin-positive cells) were ranked. For each cell type studied, the slides were ranked from lowest to highest by repeatedly comparing slides until the ranking was achieved.

**Statistical Features of the Trial Design.** This was a dose-finding trial intended to evaluate the maximum safely tolerated dose of vaccine cells prepared with or without GM-CSF gene transfer. A standard type of dose escalation was used, treating three patients on each treatment arm at each dose level, and escalating to the next higher dose if fewer than two patients experienced dose-limiting toxicity (19). This clinical trial also used an unusual design. To increase objectivity, investigators and patients remained blinded with respect to gene transfer until toxicity and immunological evaluation was completed. Patients were randomly assigned to treatment arms consisting of vaccine cells alone or with MFG-GM-CSF-transduced cells. The structure of this trial, although it was formally a double-blind, randomized trial, was not intended to make efficacy comparisons with high statistical power. Prior to lifting the blind, all clinical, immunohistological, and immunological data acquired were compiled.

**RESULTS**

**Autologous Vaccine Yield and Gene Transfer.** In this trial, primary RCC cultures were generated from large, advanced cancers with significant areas of necrosis. The rate of successful vaccine cell expansion at dose level 1 was 70%, (7 of 10), dose level 2 was 88% (8 of 9), and dose level 3 was 20% (2 of 10). If vaccine yield was insufficient to treat at the dose level assigned, patients were treated at the next lower dose level (19). A single transduction with MFG-GM-CSF generated GM-CSF secretion greater than 40 ng/10⁶ cells/24 h in eight of nine cases. Human GM-CSF cDNA gene copy number after a single transduction with MFG in transduced vaccines ranged from 0.1 copy/haploid genome to 1 copy/haploid genome (mean, 0.5 copy/haploid genome) by quantitative Southern blot analysis. The GM-CSF secretion by nontransduced vaccines ranged from 0 to 19 ng/10⁶ cells/24 h, compared to 42-149 ng/10⁶ cells/24 h after human GM-CSF gene transfer (Table 2). In preclinical models, the expression of paracrine GM-CSF greater than 35 ng/10⁶ cells/24 h by vaccine cells induced antitumor immunity (8, 10-15).

**Safety of Administration and Systemic Toxicities.** Patient characteristics are described in Table 3. The cohort was not heavily pretreated. Of the 33 patients enrolled, 18 ultimately received vaccine therapy. Of 28 patients with confirmed RCC at surgery, one patient did not have metastatic RCC, seven patients’ primary culture failed to meet vaccine cell yield specifications even for dose level 1, and two patients had progressive disease requiring radiotherapy and steroids for palliation prior to treatment, making them ineligible. No surgical complications were encountered precluding subsequent vaccination. Vaccine yield and clinical status permitting, multiple vaccinations were allowed for the study of cumulative side effects. Three patients received one vaccination, six received two vaccinations, seven received three vaccinations, and two received four vaccinations. Sixteen patients received 39 fully evaluable, 28-day treatment cycles through a dose escalation of 100-fold for vaccine cell dose. A fully evaluable patient was vaccinated at the assigned dose level with a 28-day period of toxicity and immunological assessment (19). However, progression of disease limited study of multiple vaccinations. In vitro expansion of vaccine cells would have permitted five of seven fully evaluable patients at dose level 1 to receive three vaccinations, six of seven patients at dose level 2, and two of two patients at dose level 3. The study was stopped before full dose level 3 accrual was completed, because dose level 3 vaccine yields were not regularly attainable.
No dose limiting systemic or cutaneous toxicities were observed in either arm of the trial (Table 4). The two most concerning potential toxicities, vaccine site-specific ulceration and development of acute autoimmune disease (specifically, nephritis in uninephric patients), were not observed. In addition, this was the first human clinical trial using the MFG retroviral vector. No RCR was detected in patients receiving MFG-GM-CSF gene-transduced vaccines.

The apparent lack of acute, systemic toxicities in this trial was also paralleled by the lack of plasma elevations of GM-CSF in pharmacokinetic studies following treatment at each dose level, independent of gene transfer (data not shown). Long-term toxicity and immunological follow-up was limited by short survival; 13 of 18 treated patients died of RCC progression within 12 months of their initial vaccination, despite further therapy with IL-2, other investigational agents, or the best supportive care.
Cutaneous Reactions at Vaccination Sites. Cutaneous reactions at vaccination sites were clearly cell dose dependent. At dose level 1 (1 \times 10^6 \text{ cells at four vaccination sites}), little erythema, pruritus, or induration was noted at either the s.c. or intradermal injection sites, irrespective of GM-CSF gene transfer. Moderate to beefy red erythema occurred at dose levels 2 and 3 at both the untransduced and transduced intradermal vaccine sites (1.0 \times 10^7 to 1.2 \times 10^7 \text{ cells per vaccination site}), with the maximum measure of erythema and induration noted between 24 and 48 h. At dose levels 2 and 3, vaccine site edema resolved in 2 days without medical intervention. One patient (patient 24), who experienced a partial regression of metastases (see below), experienced unique symptoms at the vaccine sites after treatment with transduced vaccine. In addition to edema encompassing the anterior thigh, draining inguinal lymph nodes were tender to palpation for 7 days after each vaccine cycle. Within minutes of receiving the third cycle of GM-CSF gene-transduced vaccine cells, this patient had intense vaccine site pruritus and generalized pruritus on the scalp, neck, and abdomen, but not generalized urticaria, bronchospasm, hypotension, or eosinophilia. Resolution occurred within 20 min without medical management. Up to 6 months after completion of vaccination, this patient experienced episodic pruritus at former vaccine sites.

Histopathology of the Vaccine Site. The histopathology of the vaccine sites was evaluated blind to treatment assignment. Cytokeratin staining of biopsies for RCC vaccine cells at 3 and 7 days following first vaccination, compared to the pretreatment control biopsy, showed that intradermal vaccine sites were more informative than s.c. sites. Thirty-two of 33 (98%) intradermal site biopsies had tumor vaccine cells present, whereas only 7 of 33 (21%) s.c. site biopsies had identifiable tumor vaccine cells; these cells were more dispersed than those of the intradermal site biopsies. Individual tumor cells, however, were clearly identifiable with cytokeratin staining as single-spindled to ovoid cells with enlarged nuclei. At all dose levels, regardless of GM-CSF gene transfer, reactive fibroblasts in a loose basophilic matrix regularly surrounded the tumor cells. At dose level 1, independent of gene transfer, and with untransduced tumor vaccines at dose levels 2 and 3, the fibroblastic response predominated over inflammatory cell infiltration compared to GM-CSF-transduced vaccine biopsies.

The intensity of cellular infiltration at the intradermal vaccine sites correlated with increasing total vaccine dose, and the phenotypes of the infiltrating cells were affected by GM-CSF transduction and time of biopsy. Compared to the minimal inflammatory cell infiltrates at dose level 1, dose level 2 biopsies were highly informative. At dose level 2, 3 days postvaccination, the GM-CSF-transduced tumor vaccine sites had more abundant macrophages (Ham56+) and granulocytes (H&E and Leder staining) compared to untransduced sites (Fig. 1, C versus F and A versus D). Most granulocytes were neutrophils by H&E and Leder staining (Fig. 1D), but eosinophils were evident by H&E and MBP staining. Few CD3+ T lymphocytes were present on day 3 (Fig. 1, B and E). Peritumoral S100+ cells, consistent with dendritic cells, were rare. B lymphocytes were not detected in either transduced or nontransduced vaccine sites (data not shown). By 7 days after vaccination, granulocyte and macrophage infiltration lessened (Fig. 1, I and L), and the predominant infiltrating cell type was CD3+ T lymphocytes. GM-CSF-transduced vaccine sites had distinctly more CD3+ T infiltrating cells than nontransduced sites (Fig. 1, H versus K). Peritumoral dendritic cells increased at day 7 following vaccination in the GM-CSF-transduced vaccines relative to untransduced biopsies (data not shown).

Induction of Systemic Immune Responses. As in previous tumor vaccine studies, DTH tests in this trial served as a qualitative measurement of T-cell response (22–27). DTH was measured as bidimensional induration at 48 h at the site of test antigen administration (23). Patients were tested 48 h prevaccination for T-cell anergy to 7 common recall antigens using the Multitest CMI (Connaught Laboratories). With the exception of two anergic patients enrolled at dose level 1, all subjects had CMI DTH scores within the range of normal volunteers or patients with localized cancer (24).

With the exception of two patients, significant DTH reactivity (≥5 mm) to unpassaged, irradiated autologous tumor cells (RCC) was not observed in patients prior to treatment (Fig. 2). Significant DTH reactivity was not noted at dose level 1. However, at dose level 2, DTH conversions to RCC were observed in patients receiving both transduced and nontransduced vaccine cells (Fig. 2). A trend toward increased DTH reactions was observed in GM-CSF-transduced vaccines at dose level 2, but the study was too small to estimate a statistical difference. In addition to induration, cutaneous reactions surrounding the indurated areas of DTH sites were noted in some patients. Twenty-eight days after first treatment with GM-CSF-transduced vaccine, patient 24 had an 80 \times 80-mm area of patchy erythema surrounding the largest indurated RCC cell DTH conversions recorded in the trial. Concomitantly, this patient experienced regression of multiple pulmonary metastases on CT scan (see below). After three vaccinations, edematous, macular erythematous skin reactions surrounding RCC autologous cell DTH sites were noted in patients 23, 24 (GM-CSF-transduced arm, dose level 2), and 26 (nontransduced arm, dose level 3). DTH responses were also seen against autologous normal kidney cells, and to a much lesser extent against peripheral blood lymphocytes; however, the possibility that some of the reaction is against residual contaminating collagenase or fetal bovine serum precludes drawing conclusions about antigen specificity.
Mouse

Fig. 3. Eosinophils predominate in the DTH reaction following vaccination with MFG-GM-CSF-transduced vaccines. Representative H&E-stained sections of posttreatment DTH biopsies of mice vaccinated with untransduced irradiated B16 melanoma vaccine (A) are compared with those transfected with MFG-GM-CSF vector secreting GM-CSF at 360 ng/10^6 cells/24 h (C), autologous irradiated human RCC (B), and irradiated RCC transduced with MFG-GM-CSF (D). For both murine and human DTH tests, the cell preparations, including freezing in fetal bovine serum, irradiation, and cell innocula, were equivalent. Eosinophils were quantitated as described in “Patients and Methods” for intradermal biopsies. Three eosinophils/200X hpf were seen in the DTH site of patient vaccinated with nontransduced RCC and 588 eosinophils/200X hpf in the DTH site of patient vaccinated with GM-CSF-transduced RCC. ×400.

Man

Histological Evaluation of DTH Sites. DTH biopsies manifested a cellular infiltration pattern similar to that generated by autologous irradiated tumor cell DTH testing in mice treated with GM-CSF-transduced vaccines (Fig. 3). In all patients, the characteristic DTH response consisted of mononuclear cell infiltration and perivascular cuffing by lymphocytes. Notably, an intense eosinophil infiltration was present at the reactive DTH sites of patients treated with GM-CSF-transduced vaccines at dose level 2, which was not observed in DTH biopsies of patients receiving nontransduced vaccines (Figs. 3, B and D, and 4). This eosinophil infiltration closely mirrors a difference observed between the DTH response of GM-CSF-transduced versus nontransduced tumor vaccines in murine tumor models (Fig. 3, A and C; Refs. 8 and 9). Staining for EG2 and MBP, a major secretory protein specific to eosinophil granules, was intense in the DTH biopsies from patients vaccinated with GM-CSF-transduced vaccines. Furthermore, much of the MBP had been released into the interstitial tissues, indicating significant degranulation characteristic of activated eosinophils (Fig. 4).

Objective Antitumor Responses. Assessment of efficacy was not a primary objective of this Phase I study. One patient (patient 24) had regression of multiple pulmonary metastases following treatment at dose level 2 (Fig. 5). Of note, this patient had progression of multiple pulmonary metastases during the 2 months between nephrectomy and first vaccination. This patient received three vaccinations with MFG-GM-CSF gene-transduced vaccines secreting 149 ng/10^6 cells/24 h. The patient received no prior systemic therapy, and the objective partial remission endured for 7 months. No other patient treated in the trial had objective evidence of treatment related tumor responses.

DISCUSSION

This trial was conducted to evaluate and compare the safety of escalating doses of irradiated autologous RCC vaccines with and without ex vivo GM-CSF gene transfer. Only minor toxicities were encountered from the effects of primary culture alone or the addition of human GM-CSF gene transfer (Table 4). The continued clinical
evaluation of MFG-GM-CSF gene-transduced tumor cell vaccines in outpatients thus appears justified.

A major limitation to the vaccine cell dose administered was not adverse reactions, but rather the vaccine yields from in vitro cell expansion. Achievable tumor vaccine cell yields from stage II and III RCC patients are apparently higher than those observed in this trial. (18, 19) Possibly, the more necrotic tissue encountered in larger primary RCC tumors of stage IV patients do not grow in vitro as well under the culture conditions used for vaccine cell propagation. Technical improvements in primary tumor culture conditions may permit improved yields in the future. Nevertheless, multiple vaccinations with $4 \times 10^7$ RCC vaccine cells appear technically achievable for stage IV patients.

In contrast to technical limits in autologous vaccine cell expansion, transfer of the GM-CSF gene and increased secretion of GM-CSF above 40 ng/10^6 cells/24 h was readily accomplished with a single transduction in nearly all cases (eight of nine cases). Variability in GM-CSF secretion was observed from tumor to tumor (Table 3). Heterogeneity in biological properties of tumor cells was anticipated in a vaccine strategy exploiting potential autologous tumor antigens. Preclinical studies indicate that heterogeneity in GM-CSF secretion does not interfere with vaccination efficacy, because equivalent antitumor immunity was produced over a wide range of GM-CSF secretion rates above a threshold of 35 ng/10^6 cells/24 h (8, 10–15). Nevertheless, it is unclear from this study whether or not the threshold of GM-CSF secretion necessary for optimum induction of systemic antitumor immunity in humans was achieved. For many RCC patients, autologous vaccine cells secreting bioactive levels of GM-CSF can be generated using retroviral gene transfer. Efficacy studies using a vaccine preparation of $4 \times 10^7$ autologous RCC cells secreting GM-CSF at >40 ng/10^6 cells/24 h, an immunologically active dose, appear safe and technically feasible.

In the context of sufficient tumor vaccine cell dose, however, paracrine GM-CSF following gene transfer produced specific immunological effects. Blind analysis of intradermal vaccine biopsy specimens at dose level 2 identified a stark contrast between the intensity of infiltration of APCs at GM-CSF-transduced vaccine site biopsies compared to nontransduced vaccine sites. Dense infiltrates of APCs predominate on day 3 at the intradermal vaccine site of GM-CSF gene-transduced vaccines. By day 7, the macrophage infiltration appears to be largely replaced by T-lymphocytes. In mouse models, this particular histological feature is one of the most distinct indicators of immunological priming by GM-CSF-transduced tumor vaccines at the vaccine site (8). Although the kinetics of cellular infiltrates was similar at the intradermal biopsy sites of nontransduced vaccines, fewer infiltrating APCs (day 3) and lymphocytes (day 7) were observed at nontransduced vaccine sites, even biopsies as dose level 3 compared to GM-CSF-transduced vaccine site biopsies at dose level 2.

Measurement of DTH responses using dissociated autologous RCC showed a trend toward greater responses at dose level 2 in patients treated with GM-CSF-transduced vaccines. The small number of patients in each group precludes statistical analysis. Of note, because the DTH cells were prepared by collagenase digestion and stored in fetal bovine serum, it is impossible to distinguish responses against
Fig. 5. Regression of metastases in patient 24. This patient was treated with $4 \times 10^7$ autologous MFG-GM-CSF-transduced RCC cells secreting GM-CSF at 149 ng/10^6 cells/24 h for three doses, consuming all vaccine. The patient’s CT scan is presented at four time points: at surgery, before treatment (2 months postnephrectomy), 28 days after the first vaccination, and after 3 treatments (6 months postvaccination).

these contaminating foreign proteins versus true RCC antigens. Ongoing analysis using T-cell clones from vaccinated patients will ultimately define the antigen specificity of induced immune responses (32–43). Nonetheless, because all DTH specimens were prepared identically, quantitative differences between patients vaccinated with GM-CSF-transduced versus nontransduced cells suggests that the paracrine production of GM-CSF at the vaccine site contributes to systemic immune activation.

The most clear-cut difference between patients vaccinated with GM-CSF-transduced versus untransduced vaccines was reflected in the histological analysis of the DTH sites. The identification of eosinophils at the reactive RCC DTH biopsy sites in patients treated with GM-CSF-transduced vaccines is provocative (Figs. 3 and 4). In preclinical studies, eosinophils were observed at vaccine sites of IL-4 and GM-CSF-transduced tumor vaccines (4, 8–9). Although GM-CSF-stimulated eosinophils are weak APCs in vitro, they are more conventionally understood to act as effector cells (44–48). Eosinophils are involved in both allergic reactions and cytotoxic responses to parasite infections (47–50). The predominance of eosinophils at the reactive RCC DTH challenge sites appears to be a hallmark of...
treatment with GM-CSF gene-transduced tumor vaccines. Eosinophils may act as effector cells following vaccination. Indeed, the finding of large amounts of interstitial MBP from degranulated eosinophils is indicative of eosinophil activation. Of note, urticaria and pruritus at DTH sites followed treatment of patient 24 with the GM-CSF-transduced vaccine. These allergic symptoms were experienced after an objective tumor response, which was measurable by CT scan.

Our recent studies in murine models of GM-CSF gene-transduced vaccines have shed some light on the basis and relevance of the eosinophil infiltrate. Analyses of systemic immunity induced by the objective tumor response, which was measurable by CT scan. Reduced vaccine. These allergic symptoms were experienced after an objective tumor response, which was measurable by CT scan.

Finally, reduction in pulmonary metastases followed treatment of patient 24 with MFG-GM-CSF gene-transduced vaccines secreting GM-CSF at 149 ng/10^6 cells/24 h. A spontaneous regression cannot be formally excluded in this patient. However, over 20 new pulmonary metastases appeared on CT scan after nephrectomy and before vaccination (Fig. 5). If a spontaneous regression occurred, it took place around the start of vaccine treatment. A nonreactive DTH to RCC cells measured prior to vaccination was followed 28 days later by the largest postvaccination DTH conversions measured in the trial. Observation of the potential efficacy of GM-CSF-transduced cancer vaccines in this toxicity study was unexpected for two reasons. First, the sample size was small. Second, the patients treated had established tumor burdens greater than 10^10 cells, in excess of the established tumor burdens in which GM-CSF-transduced tumor vaccines have efficacy in animal models (8–15). Prospective validation is required in trials statistically powered to estimate efficacy. Potentially important parameters for further evaluation include absolute amount of GM-CSF secreted/vaccine cells, optimum cell dose, frequency of vaccination, and duration of treatment. The low toxicity and bioactivity of GM-CSF gene-transduced tumor vaccines make efficacy evaluation compelling in patients with minimal residual cancer following surgery.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the significant contributions of Drs. Suzanne Topalian, Anton Berns, Gordon Parry, Donald S. Coffey, Hayden Braine, Thomas R. Hendrix, Michael Amey, David Blake, Alan W. Partin, Joel B. Nelson, and James Zabora and of Sujatha Ayyagari, Barbara Starklauf, Devon Young, Jo Foster, and Kimberly Cordwell.

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