Induction of Immunity to Prostate Cancer Antigens: Results of a Clinical Trial of Vaccination with Irradiated Autologous Prostate Tumor Cells Engineered to Secrete Granulocyte-Macrophage Colony-stimulating Factor Using ex Vivo Gene Transfer


INTRODUCTION

New PCA treatments are needed urgently for the more than 40,000 men in the United States who die from metastatic PCA annually. Several new PCA treatment approaches aim to eradicate PCA cells by inducing systemic immunity to antigens expressed by PCA cells (1–11). Because many such antigens may also be present in normal prostate epithelial cells as well as PCA cells, one major therapeutic challenge for induction of anti-PCA immune responses may be the need to overcome immune tolerance against normal prostate antigens.

GM-CSF-secreting cancer cell vaccines, generated from cancer cells by ex vivo gene transfer, have been shown to elicit tumoricidal antitumor immune responses in a variety of animal tumor models (12–23), including preclinical models of PCA (24, 25), and in human clinical trials (26, 27). Irradiated GM-CSF-secreting cancer cell vaccines induce antitumor immune responses by recruiting antigen-presenting cells, such as DCs, to immunization sites. DCs, the most potent immunostimulatory antigen-presenting cells known, activate antigen-specific CD4+ and CD8+ T-cells by priming them with oligopeptides processed from the dying cancer cells (28). Recent preclinical studies have suggested that CD4+ T-cells activated by GM-CSF-secreting cancer cell vaccines do not merely facilitate cancer cell destruction by CD8+ T-cell cells (28). Rather, vaccination simultaneously elicits both Th1 and Th2 CD4+ T-cell responses, leading to cancer cell killing by a variety of mechanisms (28). In a clinical trial of this treatment approach for malignant melanoma, both T-cell and B-cell immune responses against melanoma antigens were detected (26). Experience from previous melanoma vaccine clinical trials, using irradiated allogeneic melanoma cell lines as vaccines, has indicated that vaccination-associated B-cell responses elicited by vaccination include the generation of new antibodies recognizing polypeptides present in both normal and neoplastic cells (29).

To determine whether GM-CSF-secreting PCA vaccines might elicit T-cell and B-cell immune responses against normal and neoplastic prostate cells in men with PCA, we conducted a clinical trial of this treatment strategy. The clinical translation involved tumor resection by radical prostatectomy, establishment of primary PCA cultures, and ex vivo gene transfer. This was the first NIH Office of Recombinant DNA Activities-approved trial of human gene therapy for PCA. Eight men with PCA were treated by intradermal injections of irradiated PCA vaccine cells, and then monitored for treatment-associated side effects, for signs of PCA progression after vaccination, and for evidence of induction of T-cell and B-cell immune responses. Results indicated that this new PCA immunotherapy treatment approach, featuring vaccination with GM-CSF-secreting autologous prostate tumor cells, was feasible, safe, and capable of eliciting systemic immune responses against PCA antigens.

MATERIALS AND METHODS

Selection of Patients for Treatment with Genetically Modified PCA Vaccines. Candidates for enrollment into the Phase I clinical trial included men with adenocarcinoma of the prostate who had undergone a radical pros-

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4 The abbreviations used are: PCA, prostate cancer; DC, dendritic cell; DTH, delayed-type hypersensitivity; GM-CSF, granulocyte-macrophage colony-stimulating factor; PSA, prostate-specific antigen; PSMA, prostate-specific membrane antigen; RCR, replication-competent retrovirus.

Abstract

Vaccination with irradiated granulocyte-macrophage colony-stimulating factor (GM-CSF)-secreting gene-transduced cancer vaccines induces tumoricidal immune responses. In a Phase I human gene therapy trial, eight immunocompetent prostate cancer (PCA) patients were treated with autologous, GM-CSF-secreting, irradiated tumor vaccines prepared from ex vivo retroviral transduction of surgically harvested cells. Expansion of primary cultures of autologous vaccine cells was successful to meet trial specifications in 8 of 11 cases (73%); the yields of the primary culture cell limited the number of courses of vaccination. Side effects were pruritis, erythema, and swelling at vaccination sites. Vaccine site biopsies manifested infiltrates of dendritic cells and macrophages among prostate tumor vaccine cells. Vaccination activated new T-cell and B-cell immune responses against PCA antigens. T-cell responses, evaluated by assessing delayed-type hypersensitivity (DTH) reactions against untransduced autologous tumor cells, were evident in two of eight patients before vaccination and in seven of eight patients after treatment. Reactive DTH site biopsies manifested infiltrates of effector cells consisting of CD45RO+ T-cells, and degranulating eosinophils consistent with activation of both Th1 and Th2 T-cell responses. A distinctive eosinophilic vasculitis was evident near autologous tumor cells at vaccine sites, and at DTH sites. B-cell responses were also induced. Sera from three of eight vaccinated men contained new antibodies recognizing polypeptides of 26, 31, and 150 kDa in protein extracts from prostate cells. The 150-kDa polypeptide was expressed by LNCaP and PC-3 PCA cells, as well as by normal prostate epithelial cells, but not by prostate stromal cells. No antibodies against prostate-specific antigen were detected (26). Experience from previous melanoma vaccine clinical trials, using irradiated allogeneic melanoma cell lines as vaccines, has indicated that vaccination-associated B-cell responses elicited by vaccination include the generation of new antibodies recognizing polypeptides present in both normal and neoplastic cells (29).
tatectomy with curative intent and were found to have metastatic PCA at surgery despite careful preoperative staging. Before surgery, men who were considered for treatment provided informed consent to permit immediate postoperative establishment of primary autologous PCA cultures as a first step in PCA vaccine preparation. Further processing of the primary PCA cultures, including retroviral gene transfer, proceeded only after histopathological identifica-
tion of PCA lymph node metastasis, PCA seminal vesicle invasion, or both. Additional eligibility criteria for enrollment into the trial included the following: age, ≥18 years; Eastern Cooperative Oncology Group performance status of 0 or 1; suitable candidacy for radical prostatectomy; serum direct bilirubin, ≤2.0 mg/dl; serum creatinine, ≤2.0 mg/dl; WBC count, ≤3500/ mm³; platelet count, ≤100,000/mm³; no previous history of cancer; no previ-
ous history of autoimmune diseases; seronegativity for antibodies to HIV; no recent immunotherapy; and no recent immunosuppressive therapy (including oral or topical corticosteroids, cytotoxic chemotherapy, or radiation therapy).

Design of the Phase I Trial of Genetically Modified PCA Vaccine Therapy.
The three primary end points of the Phase I study were as follows: (a) to evaluate the safety of intradermal vaccination of cultured, lethally irradiated, autologous (PCA) vaccines transduced with the MFG-S-GM-CSF vector to secrete human GM-CSF at 150-1500 ng/million cells/24 h; (b) to describe and quantitate the acute and long term toxicities of treatment with ex vivo GM-CSF gene-transduced prostate vaccines; and (c) to assay in vitro and in vivo the contribution of effects of vaccines to the induction of antitumor immune responses to (PCA). Patients were treated at two vaccine dose levels: 1 × 10⁷ and 5 × 10⁷ autologous GM-CSF-transduced prostate vaccines. The trial was designed to evaluate three patients enrolled for dose level 1, and five patients were to be enrolled for dose level 2 if dose escalation was permitted by the safety rules of the trial. Patients were considered evaluable for safety and toxicity using National Cancer Institute Common Toxicity Criteria if they completed three full courses of vaccination. Vaccinations were administered every 21 days. Patients were vaccinated until exhaustion of the supply of vaccine, but no fractional doses were permitted. In the event that a patient accrued at dose level 2 had sufficient GM-CSF gene-transduced autologous vaccine yields for three full dose treatments at dose level 1, and three full dose treatments at dose level 2, the trial rules permitted treatment at dose level 1. Dose level 2 represented the estimated upper limit of vaccine cell yield in over 20 clinical trial simulations of stage T₃b tumors (2–4 g tumors) using radical prostatectomy specimens. If no dose-limiting toxicities were observed at dose level 2 after a total Phase I accrual of 8 patients, a 30-patient Phase II study was planned based on Phase I safety, review by the FDA, and Office of Recombinant DNA Activities review.

The Phase I trial proceeded in three stages. Stage I involved surgical harvest of prostate tumor cells, cultivation of primary tumor explants, ex vivo gene transfer with a replication-defective retrovirus containing cDNA encoding GM-CSF, lethal irradiation of the genetically modified prostate tumor cells, and formulation for intradermal administration as vaccines (see below and Refs. 24 and 27).

In stage II of the trial, men were subjected to treatment with the PCA vaccines at two dose levels. For dose level 1, men were treated with 1 × 10⁷ PCA vaccine cells, administered as two intradermal injections of 0.5 × 10⁷ cells/0.5 ml of HBSS, every 21 days until the vaccine cell supply was exhausted. A patient was evaluable for toxicity if he completed three vaccinations. Vaccine injections were given in the limbs, with at least 5 cm separating inoculation sites. Different limbs were used in different treatment cycles.

For stage III of the trial, vaccinated men were monitored for treatment-associated side effects, including the possible appearance of RCR in the peripheral blood, after each vaccine injection and then monthly for 3 months, every 3 months for 9 months, and yearly thereafter. A thorough evaluation for the appearance of autoimmune diseases, including autoimmune serology studies, was conducted every 6 months after completion of the vaccination course. All toxicities, including autoimmune toxicities, were graded using the National Cancer Institute Common Toxicity Criteria. At follow-up visits, vaccinated men were also assayed for (PCA) progression, using serum PSA determinations. Radiographic imaging studies were also used as needed. The plasma pharmacokinetics of absorbed GM-CSF following vaccine administration was monitored as described previously (27, 30). Vaccinated men were also mon-
itored for induction of T-cell and B-cell immune responses (see below).

The rationale for choosing dose level 1 at 1 × 10⁷ cells was based on four facts. First, it was based on preclinical studies showing efficacy against preestablished tumors in this vaccine cell dose range in the hormone-refractory Dunning rat (PCA) model (24, 25). Second, the cell dose range was found to be safe in renal cell carcinoma patients in a Phase I study using MFG-GM-CSF gene-transduced tumor vaccines (27). Third, in clinical trial simulations, autologous prostate cell vaccines could be generated consistently in the dose level 1 range following gene transfer with the MFG-GM-CSF vector in patients undergoing anatomical radical prostatectomy. (24). Fourth, this vaccine cell dose range secreted GM-CSF conferred an objective clinical re-

IRRADIATED GM-CSF GENE-TRANSUCED PCA VACCINES

RESULTS

Generation of GM-CSF-secreting Autologous Prostate Tumor Cell Vaccines Using ex Vivo Gene Transfer. After radical prostatectomy, 11 men with PCA were found to have metastatic cancer and were eligible for participation in the Phase I clinical trial. For each of the 11 men, autologous prostate tumor cells were placed in primary cultures using 2–5 grams of prostate tumor tissue dissected from radical prostatectomy resection specimens. The accrual of the Phase I trial was 8 patients evaluated for toxicities after a minimum of three vaccinations given every 2 weeks. Primary cultures were successfully established in 8 of 11 patients (73%; see Table 1). Prostate tumor cell expansion via propagation in vitro to cell yields adequate for treatment at the assigned dose level was somewhat less successful. For patients accrued in the trial at dose level 1 (1 × 10⁶ cells/vaccination), sufficient cells were expanded in three of four primary culture attempts (75%); one primary culture failed to be established at dose level 1. When dose level 1 was found to be clinically safe in three treated patients, a total of seven patients were accrued for dose level 2 (5 × 10⁶ cells/vaccination). Sufficient cells were recovered in only three of seven patients’ primary cultures to allow three vaccinations at dose level 2. Thus, only three patients were treated at dose level 2. Two patients had tumors that failed to be established in primary culture, and the remaining two patients accrued at dose level 2 had sufficient vaccine cell yields to specifications to be treated at dose level 1. As a result, primary prostate tumor cell culture yields in this trial permitted five patients to be treated at dose level 1 and three to be treated at dose level 2 (Table 2). On average, >90% of the entire prostate tumor tissue was placed into primary culture, making it unlikely that total cell yield could be significantly increased by procuring more primary tumor at surgery (Table 2).

In contrast to the suboptimal success rate in expanding prostate tumor cell numbers in primary cultures, genetic modification of PCA cells to achieve high level secretion (>140 ng/10⁶ cells over 24 h) of GM-CSF using ex vivo retroviral gene transfer was remarkably effective (eight of eight attempts, or 100%; see Table 1). Previous studies using preclinical models have suggested a minimum threshold of >35 ng of GM-CSF/10⁶ cells over 24 h secreted by vaccine cells might be required to elicit therapeutic antitumor immune responses (12). Transduction of the prostate tumor cells by the replication-defective retrovirus MFG-GM-CSF resulted in viral integration to a copy number of between 1 and 4 per haploid genome as assessed by quantitative Southern blot analysis (not shown; see Ref. 24). Resultant GM-CSF secretion by the prostate tumor cells increased from <0.2 ng/10⁶ cells over 24 h to between 143 and 1403 ng/10⁶ cells over 24 h (Table 2).

Vaccine Administration. Eight men were treated with irradiated GM-CSF-secreting prostate tumor cell vaccines at 2 dose levels (five at dose level 1 and three at dose level 2). Up to six intradermal vaccinations were administered every 2 weeks. Skin reactions (see Fig. 1), including discomfort, erythema, swelling, and pruritis, were common after vaccine treatment (Table 3). The maximum level of erythema and induration, up to 4 cm in diameter, typically appeared within 24–48 h of vaccination and resolved without intervention in 5–7 days. Mild low-grade fevers, chills, and malaise were noted by a few men as a consequence of vaccine inoculation (Table 3). No severe or dose-limiting cutaneous or systemic side effects were observed. No significant alterations in serum electrolyte and chemistry values or in hematology counts, including WBC counts, total eosinophil counts, and total neutrophil counts, were seen. Plasma GM-CSF pharmaco-kinetic analyses failed to show any vaccine-associated rise in plasma GM-CSF levels. No RCR was detected in any vaccine cell preparation or in blood from any of the vaccinated men at any time.

Assessment of PCA treatment efficacy was not a primary end point of the Phase I clinical trial of GM-CSF-secreting autologous prostate tumor cell vaccines. With eight fully evaluable patients enrolled and treated, the trial did not have adequate statistical power to estimate a treatment-associated response rate. The median serum PSA before radical prostatectomy was 28.85 ng/ml (with a range of 6.7–75 ng/ml), and the median serum PSA at first vaccination was 0.65 ng/ml (with a range of 0.1–30.4 ng/ml; see Table 1). Ultimately, all eight vaccinated patients manifested a rising serum PSA, showing PCA progression.

Recruitment of Immune Effector Cells to Vaccine Sites. Intra-dermal injection was chosen for the mode of vaccination because of the abundance of Langerhans cells, the skin DCs, which constitute the critical antigen-presenting cells for priming immune responses to irradiated GM-CSF-secreting tumor cells (28). Vaccine site biopsies were obtained 3 days after the first and final vaccine inoculations. Extensive histopathological and immunohistochemical analyses of these vaccine site biopsies were undertaken to characterize immune effector cell infiltrates appearing as a consequence of vaccination (26,
**Table 1 Characteristics of patients**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. enrolled in study (including those without metastatic PCA)</td>
<td>21</td>
</tr>
<tr>
<td>No. clinically eligible</td>
<td>11</td>
</tr>
<tr>
<td>No. treated with metastatic PCA</td>
<td>8</td>
</tr>
<tr>
<td>No. of successful PCA cell cultures (sufficient for vaccine preparation)/no. of culture attempts</td>
<td>8/11</td>
</tr>
<tr>
<td>No. of successful GM-CSF gene transductions/no. of transduction attempts</td>
<td>8/8</td>
</tr>
<tr>
<td>Median age of treated patients (range), yr</td>
<td>51 (46–64)</td>
</tr>
<tr>
<td>Mean age of treated patients, yr</td>
<td>53</td>
</tr>
<tr>
<td>Median PSA level presurgery for treated patients (range), ng/ml</td>
<td>28.85 (6.7–75)</td>
</tr>
<tr>
<td>Mean PSA level presurgery for treated patients, ng/ml</td>
<td>30.85</td>
</tr>
<tr>
<td>Median PSA level for treated patients at 1st vaccination (range), ng/ml</td>
<td>0.65 (0.1–30.4)</td>
</tr>
<tr>
<td>Mean PSA level for treated patients at 1st vaccination, ng/ml</td>
<td>6.08</td>
</tr>
</tbody>
</table>

*GM-CSF secretion before MFG-GM-CSF transduction, <0.2 ng/10^6 PCA vaccine cells/24 h; average GM-CSF secretion after transduction, 461.5 ng/10^6 PCA vaccine cells/24 h.*

**Table 2 GM-CSF secretion by PCA vaccine cells following MFG-GM-CSF gene transfer**

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Dose level</th>
<th>GM-CSF secretion ng/10^6 PCA vaccine cells/24 h</th>
<th>Total vaccinations</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>1</td>
<td>143</td>
<td>6</td>
</tr>
<tr>
<td>2</td>
<td>1</td>
<td>197</td>
<td>6</td>
</tr>
<tr>
<td>3</td>
<td>1</td>
<td>349</td>
<td>6</td>
</tr>
<tr>
<td>4</td>
<td>1</td>
<td>607</td>
<td>5</td>
</tr>
<tr>
<td>5</td>
<td>2</td>
<td>1403</td>
<td>3</td>
</tr>
<tr>
<td>6</td>
<td>1</td>
<td>240</td>
<td>3</td>
</tr>
<tr>
<td>7</td>
<td>2</td>
<td>520</td>
<td>6</td>
</tr>
<tr>
<td>8</td>
<td>2</td>
<td>233</td>
<td>3</td>
</tr>
</tbody>
</table>

**Table 3 Toxocities accompanying vaccination with irradiated with GM-CSF-secreting autologous PCA cells**

<table>
<thead>
<tr>
<th>Toxocities</th>
<th>Dose level 1 (5 patients)</th>
<th>Dose level 2 (3 patients)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Fully evaluable cycles</td>
<td>26</td>
<td>15</td>
</tr>
<tr>
<td>Systemic side effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Low-grade fever (grade 2 or less)</td>
<td>0/26</td>
<td>2/15</td>
</tr>
<tr>
<td>Chills</td>
<td>0/26</td>
<td>3/15</td>
</tr>
<tr>
<td>Malaise</td>
<td>1/26</td>
<td>4/15</td>
</tr>
<tr>
<td>Acute vaccine site effects</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Injection site erythema/swelling (grade 2 or less)</td>
<td>26/26</td>
<td>15/15</td>
</tr>
<tr>
<td>Site tenderness (1–3 days)</td>
<td>5/26</td>
<td>5/15</td>
</tr>
<tr>
<td>Pruritus (grade 2 or less)</td>
<td>3/26</td>
<td>10/15</td>
</tr>
<tr>
<td>Pain during injection</td>
<td>26/26</td>
<td>15/15</td>
</tr>
</tbody>
</table>

27). Autologous prostate tumor cells, characterized by positive immunohistochemical staining for cytokeratins, were present in all vaccine site biopsies evaluated (see Fig. 2B). Acanthosis, a thickening of the stratum spinosum of the epidermis often associated with autoimmune diseases or malignancy, was apparent in each of the vaccine site biopsies (Fig. 2A). The severity of acanthosis, graded by a pathologist (A. M. D.), appeared to increase from the first vaccine site biopsy to the last for each of the vaccinated men.

Inflammatory cell infiltrates were present in each of the vaccine site biopsies compared to pretreatment biopsies. The magnitude of the inflammatory cell response surrounding intradermal deposits of tumor vaccine appeared to be greater in dose level 2 patients compared to the dose level 1 cohort. Langerhans cells, the skin DCs, were evident at the junction of the epidermis and dermis and also in the dermis near vaccine cells in one vaccine site biopsy examined (Fig. 2C). Macrophages were also detected (Fig. 2D). Neutrophils and eosinophils, usually present near vaccine cells in the dermis, increased in abundance from <1 to 37.5% and from <0.5 to 15% of inflammatory cells, respectively, in skin biopsies from vaccine sites versus skin biopsies from similar locations obtained before vaccination. Eosinophils infiltrating the vaccine sites showed evidence of activation and massive levels of degranulation (Fig. 3). After repeated vaccinations, the fraction of eosinophils (assessed as the ratio of eosinophils to CD3+ T-cells) at vaccine sites increased. In areas of acanthosis, dermal blood vessels manifest a striking eosinophilic vasculitis, with characteristic subendothelial eosinophils (Fig. 2F). Small blood vessels near vaccine cells were also typically surrounded by eosinophils, often accompanied by other mononuclear inflammatory cells, including CD3+ T-cells (Fig. 2E). CD3+ T-cells also appeared near DCs and macrophages recruited to vaccine sites. Frequent CD8+ T-cells were consistently present in the dermis following vaccination.

**Induction of T-cell Immune Responses against Prostate Tumor Antigens.** The T-cell epitopes that were recognized by infiltrating CD-3+ T-cells on newly induced DTH sites following vaccination with GM-CSF gene-transduced autologous prostate vaccines are not known at this time. Several new reports suggest that PCA patients may even have preexisting CD-4 and CD-8 T-cells against many potential PCA associated antigens, including PSA and PSMA (8, 52). In previous tumor vaccine studies, in which defined peptide antigens were not known, tumor antigen-specific T-cell responses were assessed using cutaneous DTH testing against antigens present in autologous tumor cells (26, 27). For this study, irradiated autologous prostate tumor cells served as DTH target cells. These prostate tumor cells were propagated in serum-free media and cryopreserved in a buffer containing human serum albumin. Thus, the prostate tumor DTH cells were largely free of contaminating xenoproteins, such as collagenase or proteins present in bovine sera, which have been reported to confound interpretations of DTH skin tests in some other cancer vaccine trials (57). DTH reactivity was measured as the bidimensional extent of induration at the intradermal DTH injection site at 48 h.

Vaccinated men were subjected to prostate tumor DTH testing before vaccination, after three vaccine treatments, and at the end of the vaccine course. All vaccinated men were also subjected to DTH testing for reactivity against seven common recall antigens, as described previously (27). Each of the vaccinated men appeared immunocompetent, as evidenced by DTH reactivity against one or more of the recall antigens, before beginning treatment with PCA vaccines. In addition, all vaccinated men displayed normal levels of T-cell receptor ζ chains in circu-
lating CD4 T-cells (not shown; see Ref. 59). Reactive prostate tumor DTH tests were present before vaccination in two of eight men. Following vaccination, seven of eight patients manifested positive DTH tests to challenge with irradiated, untransduced autologous prostate cell targets range (5–23 mm). Of the six of eight patients with no positive DTH to challenge with autologous prostate cells prior to vaccination, five of six became positive after treatment. In the case of the two of eight patients with a pretreatment positive DTH reaction to their autologous tumor prior to treatment, patient 3 (dose level 1) showed a 2-fold increase in induration (6 to 12 mm) following vaccination, whereas patient 5 (dose level 2) manifested no appreciable increase in DTH from 8 mm following vaccination. The study was too small to ascertain any statistically significant differences in DTH induction sizes associated with vaccine treatment at dose level 1 versus dose level 2. The magnitude of reactivity of DTH could not be clearly ascribed to total number of vaccinations or GM-CSF levels. The largest DTH conversion (3 to 25 mm) was observed in patient 8, who received only three vaccinations at dose level 2 (Table 2). Interestingly, the largest DTH reactivity appeared in patient 8, who had a history of clinical prostatitis.

Histopathological and immunohistochemical analysis of prostate tumor DTH site biopsies taken from two patients after vaccination revealed abundant inflammatory infiltrates not evident in skin biopsies obtained before vaccination. Perivascular cuffing by infiltrating lymphocytes, characteristic of DTH reactions, was present in all reactive DTH site biopsies (Fig. 4A). DTH site biopsies also disclosed ingress of macrophages (Fig. 4B) and of natural killer cells (Fig. 4C). Extensive eosinophil infiltrates and a subendothelial eosinophilic vasculitis, reminiscent of the vaccine site biopsies, were present in reactive DTH site biopsies taken after vaccination. T-cells were also present at DTH sites. In the DTH site biopsies, some 80% of the CD3+ T-cells expressed CD45RO, indicative of T-cell activation after vaccination (Fig. 4D). Similar to the vaccine site biopsies, the prostate tumor DTH site biopsies displayed an increasing abundance of eosinophils relative to T-cells as the vaccine treatment course proceeded. Few B-cells were evident at the DTH sites.

Pre- and postvaccine peripheral blood samples were also tested for proliferation and cytokine release (IL-2, GM-CSF, and γ IFN) in response to recombinant PSA, using candida as a positive control. All patients demonstrated responsiveness to candida, but none showed evidence of PSA-specific T-cell recognition. Due to low yields of

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autologous tumor cells in early passage, autologous tumor cells were not available to assess the priming of responses to undefined tumor-associated antigens.

**Induction of B-Cell Immune Responses against Prostate Tumor Antigens.** Although few B-cells appeared to be present at prostate tumor vaccine sites or at prostate tumor DTH sites, immunoblot analyses using sera from the eight treated men, obtained before and after vaccination, disclosed the appearance of increased titers of antibodies recognizing prostate tumor antigens in sera from three of the men as a consequence of vaccine treatment (Fig. 5, patients 1, 6, and 7). New antibodies at 1:1000 titer were observed in patients 1 and 6 (dose level 1) and patient 7 (dose level 2), 2 weeks following final vaccination (Fig. 5). All three of these patients had negative DTH skin reactivity to challenge with autologous prostate cells prior to vaccination with GM-CSF gene-transduced PCA vaccines. The induced immunoglobulins recognized polypeptides of 26, 31, and 150 kDa in extracts from LNCaP PCA cells (Fig. 5). The 150-kDa polypeptide was expressed by both normal and neoplastic prostate epithelial cells and by prostate smooth muscle cells, but not by prostate stromal cells, lung fibroblasts, or WBCs (Fig. 6A). The 150-kDa polypeptide was also expressed by a number of human cancer cell lines (Fig. 6B). Characterization of the precise epitope(s) recognized by the induced antibodies is ongoing. Using the same immunoblot analysis approach, no antibodies against PSA were detected (not shown). Despite the induction of new antibodies in three of eight patients, none of the eight patients developed any clinically detectable lymphadenopathy.

**DISCUSSION**

The results of the Phase I clinical trial of irradiated GM-CSF-secreting autologous prostate tumor vaccine therapy indicated that such treatment was safe and induced both B-cell and T-cell immune responses against PCA cell-associated antigens. A major difficulty for future clinical development of this autologous treatment approach was the low yield of autologous PCA vaccine cells recovered (often $5 \times 10^7$ or less) using cell culture approaches to expand PCA cell numbers. This approach appears clinically impractical for large Phase II studies to assess efficacy given the cell culture technology of primary cultures used in this study. Retroviral transfer of GM-CSF cDNA to permit high level secretion of GM-CSF was not limiting. Nonetheless, with limited numbers of autologous PCA vaccine cells likely available from most men with localized PCA, and no PCA vaccine cells likely available from most men with advanced PCA, exploration of higher vaccine doses and different vaccination schedules in future clinical trials will not be possible. Allogeneic PCA vaccines, prepared from PCA cell lines genetically modified to secrete high levels of GM-CSF, may offer a solution to this clinical development problem. Preclinical studies have suggested that antigens from irradiated GM-CSF-secreting cancer vaccine cells are presented to T-cells on MHC molecules expressed by host antigen-presenting cells (see Refs. 60 and 61); such vaccine cells thus do not necessarily need to be MHC-matched with host T-cells to be effective at eliciting potent antitumor immunity. Rather, the vaccine cells need to express tumor antigens expressed by metastatic PCA clones in patients, and they need to secrete sufficient paracrine GM-CSF to recruit bone marrow-derived antigen-presenting cells (DCs and macrophages) to sites of vaccination (60, 61). The PCA...
cell lines LNCaP and PC-3, which contain a number of common PCAs, express the newly identified 150-kDa polypeptide recognized by sera from men treated with autologous PCA vaccines. Based on data from this Phase I trial, a Phase II clinical trial of irradiated GM-CSF-secreting LNCaP and PC-3 vaccines, using vaccine cell doses higher than that possible with autologous PCA vaccines, is currently under way. GM-CSF gene-transduced allogeneic PCA vaccines appear to permit long schedules of booster vaccinations, given the inexhaustible supply of the permanently established tumor cell lines.

Tumor-specific cytotoxic CD8+ T-cells recognize tumor antigens presented on class I MHC molecules to target cancer cells for destruction. For many men with advanced PCA, class I MHC low or negative PCA cells may be present (62–65). Such cells would likely be difficult to eradicate with tumor-specific cytotoxic CD8+ T-cells alone. Fortunately, Hung et al. (28) have recently reported that vaccination with irradiated GM-CSF-secreting cancer vaccines activate tumor-specific CD4+ T-cells to simultaneously produce Th1 and Th2 responses. Both CD8+ T-cell effectors and non-MHC-dependent immune effectors of antitumor immunity are elicited by GM-CSF gene-transduced vaccine treatment. Immune response data collected during the conduct of the Phase I trial of irradiated GM-CSF-secreting PCA vaccines supports in man the conclusions Hung et al. (28) derived in mice. Notably, DCs and macrophages were recruited for antigen processing by paracrine GM-CSF secretion at tumor vaccination sites (Figs. 2 and 3). Following PCA antigen presentation in vivo, postvaccination reactive DTH site biopsies indicated the participation of degranulating eosinophils as well as circulating T-cells in vaccination induced immune responses to autologous PCA cell DTH tar-

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gets (Fig. 4). Architecturally, the concerted immunological response appears to include eosinophil accumulation and degranulation in the subendothelial space of small blood vessels immediately adjacent to depositions of DTH tumor antigen. Identical pictures of postvaccination DTH response to autologous untransduced tumor target cells have been reported in clinical trials of renal cell carcinoma and melanoma using GM-CSF gene-transduced autologous tumor vaccines (26, 27).

GM-CSF gene-transduced PCA vaccines increased antibody titers against prostate tumor cell line-associated antigens. This suggests that B-cells participated in the immune response following treatment. Increasing titers of antibodies to PCA antigens were detected among three of the men treated with irradiated GM-CSF-secreting autologous PCA cell vaccines. These antibody responses are reminiscent of antibody responses reported for a clinical trial of irradiated GM-CSF-secreting autologous melanoma cell vaccines (26). To our knowledge, this is the first report of induction of new antibody responses to PCA antigens in patients using cytokine gene-modified tumor vaccines, peptide-pulsed DCs, or any other strategy of immunotherapy. One of the antigen epitopes, present in a 150-kDa polypeptide, was expressed in normal prostate epithelial cells, as well as in PCA cells. However, it is unclear why the majority of patients (5 of 8) did not elicit detectable antibodies following treatment. The lack of yield of autologous PCA cells has not permitted testing of these antibodies against detectable antibodies following treatment. The lack of yield of autologous PCA cells has not permitted testing of these antibodies against detectable antibodies following treatment. The lack of yield of autologous PCA cells has not permitted testing of these antibodies against detectable antibodies following treatment.

REFERENCES


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