Dendritic Cell-Based Immunotherapy for Prostate Cancer

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Introduction

Prostate cancer is the most common non-skin malignancy currently diagnosed in American men, second only to lung cancer as the leading cause of cancer death among males. Although the majority of prostate cancer cases are localized to the prostate, nearly a third of newly diagnosed patients has locally advanced or metastatic prostate cancer. Available treatments for metastatic prostate cancer, including hormonal, chemotherapeutic, and radiation strategies, have failed to demonstrate curative potential in these patients.

Current standard therapies for early-stage, localized prostate cancers—prostatectomy and radiation therapy—are associated with failure rates of nearly 20%. As a result, increasing numbers of treated-patients develop metastatic disease or remain at very high risk for the development of such disease. Because the options for these primary treatment failures, as with the incident metastatic cases, are few in number and severely limited in terms of efficacy, there is great demand for new and improved treatments for prostate cancer. Immunotherapeutic approaches to cancer treatment have shown great promise in experimental studies, and a number of immunotherapies are now being tested in clinical settings.

Cancer Immunotherapy Strategies

Tumor suppression phenomena ostensibly mediated by host immune systems have been reported by a number of investigators. These phenomena include spontaneous regression of various tumors; the observation of a direct association between immunosuppression and increased incidence of cancer; and the presence of leukocytes surrounding and infiltrating tumor tissues. Expanding knowledge about the specific mechanisms involved in the immune response has been utilized to elicit or amplify anti-tumor immune responses.

T-CELL ACTIVATION

T cell immune responses, for example, are triggered by the interaction of the T cell receptor (TCR) and antigenic peptides bound to major histocompatibility complex (MHC) antigens expressed on the surface of specialized antigen presenting cells (APC). If this interaction is accompanied by binding of costimulatory receptors (e.g., CD28) to their ligands (CD80 and CD86), then an intracellular cascade of biochemical events is initiated that results in T cell activation and proliferation. Activated T cells, in turn, are able to lyse cells that express the stimulating antigen and the appropriate MHC antigen, in this case, tumor cells.
Cytokines
Additionally, the presence or absence of cytokines, such as interleukins 2, 4, 10, and 12 can have a profound effect on the activation and proliferation of T cells and consequently on the potency and character of the immune response. Cytokines also affect, both positively and negatively, the function of APC.

Based on these observations, interleukin 2 (IL-2), interferons, and granulocyte-macrophage colony stimulating factor (GM-CSF) have been administered systemically to stimulate anti-tumor immune reactivity. IL-2 has also been used ex vivo to generate lymphokine activated killer (LAK) cells and, later, to grow tumor infiltrating lymphocytes (TIL) for reinfusion into patients. These forms of adoptive immunotherapy have had limited success in patients with metastatic melanoma, and continue to be refined by methods that increase the proportion of tumor-specific lymphocytes.

Vaccines
An alternative to systemic cytokines and adoptive immunotherapy is vaccination of patients to elicit in vivo activation of tumor-specific T cells. Irradiated tumor cells derived from the patient (autologous) or from other individuals (allogeneic) have been utilized to inoculate cancer patients with the aim of generating a therapeutic immune reaction. Some procedures have introduced genes coding for cytokine or costimulatory molecules into the tumor cells prior to inoculation to enhance recognition by the immune system. Others have isolated specific tumor antigens to focus the immune response on proteins overexpressed by or unique to the tumor.

The most recent advance in cancer vaccines has been the use of autologous APC to present tumor antigens to patients’ T cells. The rationale for this technique is to use the cells that are specialized for antigen presentation, so that all factors necessary for initiation of the immune response, including those not yet defined, will be present.

Dendritic Cell-Based Immunotherapy
Dendritic cells (DC) (Fig. 1) are considered the most potent APC of the immune system, and are unique in their ability to stimulate naive T cells. DC are adapted to capture proteins, proteolytically digest them, and present the resulting peptides on their cell membranes bound to MHC antigens. Formation of this MHC-peptide complex is crucial to the activation of T cells. In addition, DC express high levels of the costimulatory molecules, CD80 and CD86, which are required for full T cell activation. DC are found in the epidermal layer of the skin, the respiratory and gastrointestinal systems, and the interstitial regions of several solid organs where they function as sentinels, capturing invading microorganisms for presentation to immune cells. Until recently, the study of DC was limited because few cells could be isolated from tissues or peripheral blood. With improvements in DC isolation and culture techniques, much larger numbers of DC are available and immunotherapy using DC is now feasible. DC can be derived from peripheral blood using cytokines, such as GM-CSF, IL-4, and tumor necrosis factor-alpha (TNFα).

DC-based cancer vaccines have recently been tested with some success in clinical trials with several types of cancers, such as follicular-B cell lymphoma and melanoma. Our group has been developing a prostate cancer vaccine using autologous DC as a vehicle to present prostate antigens to T cells in vivo. Our phase I and II clinical trials in prostate cancer with DC pulsed with HLA-A0201-specific prostate-specific membrane antigen (PSMA) peptides will be discussed in this report.

Prostate Cancer Vaccine
Two major components comprise our new prostate cancer vaccine. The first component is DC, which are isolated and cultured from patient peripheral blood.
The second component is a specific antigen used to target prostate cancer tissues. Few antigens specific to prostate tissue have been characterized for use as target antigens for immunotherapy. This short list includes prostate-specific antigen (PSA) and prostate-specific membrane antigen (PSMA).²⁶, ²⁷

PSMA is a 750 amino-acid membrane-bound protein expressed by prostate epithelial cells.²⁷ It has been utilized diagnostically as part of a prostate cancer imaging method (ProstaScint®, Cytogen Corp., Princeton, NJ), which uses a monoclonal antibody specific for PSMA (7E11.C5.3).²⁸ Enhanced expression of PSMA was detected in hormone refractory prostatic carcinoma.²⁹ In addition, levels of PSMA are elevated in the sera of hormone refractory advanced prostate cancer patients.³⁰ Our immunotherapy protocols use PSMA as target antigen for T cell attack in vivo.

T cell recognition requires binding of the TCR molecules to antigen-derived peptides and MHC proteins. Previous studies have identified specific sequence motifs of MHC class I-binding peptides, making it possible to predict potential T cell epitopes from proteins of known amino acid sequences.³¹ Using these specific motifs, we identified the PSMA peptides with a high affinity for an MHC class I protein (HLA-A0201) expressed by a large fraction of the population to be used as antigens in our phase I and II clinical trials.³², ³³

Phase I Clinical Trial

The phase I study,³⁴ conducted at Northwest Hospital in Seattle in December 1995, examined the safety of administering HLA-A0201-specific PSMA peptides (PSM-P1 and PSM-P2), autologous DC, and PSM-P1 and -P2 pulsed autologous DC to 51 patients with advanced hormone refractory prostate cancer. The majority of these patients (39/51) were classified as stage D₂ (T₄N₁-3M₁a-c). Many of them were anemic and had undergone
various treatments that resulted in impaired immune competency. Fewer than 25% of these patients were considered fully immunocompetent at the start of the study as assessed by delayed-type hypersensitivity skin tests.

At the completion of four infusion cycles, the maximum tolerated dose had not yet been achieved. Neither significant acute nor chronic toxicity was observed at all doses of test substances, except for mild to moderate infusion-related hypotension. In addition, no significant increase in serum TNF-α or interferon γ (IFN-γ) was noted during the course of the study.34

Patients were monitored for cellular immune modulation to the appropriate PSMA peptides (PSM-P1 or -P2). An enhanced cellular response was observed within the HLA-A2 positive subjects who were infused with DC pulsed with PSM-P1 or -P2.34 Patient clinical responses were analyzed based on National Prostate Cancer Project (NPCP) criteria plus measurement of PSA levels. Seven patients exhibited partial responses.34,35 Average PSA levels increased in the non-responder group, both among those with low (0-19) and high (>19) pre-infusion values, while a decrease was observed in the seven partial responders (Fig. 2). All patients who were on hormone therapy before the trial continued their treatments during the trial. Patients who stopped anti-androgen therapy before the trial were not eligible to enter the study until three months had elapsed and the PSA measurement was at an acceptable level.

Phase II Clinical Trial

In January 1997, we started a phase II study involving 107 patients, 66 of whom had hormone refractory, clinically progressive prostate cancer (group A), and 41 of whom had locally recurrent prostate cancer (group B). Half of group A patients (33/66), participants in the phase I study, requested enrollment in the phase II trial. We anticipate that the results of this phase II trial will provide information on the efficacy of our DC-based prostate cancer vaccine in both disseminated and localized prostate cancer.

As in the phase I study, group A subjects continued any hormone therapy that had been initiated before the trial.

All phase II participants received a total of six infusions of autologous DC pulsed with a PSM-P1 and -P2 cocktail at six-week intervals. A subset of the participants in each group also received subcutaneous injection of GM-CSF as a systemic adjuvant with each DC infusion (Table 1). GM-CSF is a potent enhancer of differentiation for various hematopoietic cells, including DC.16 Moreover, administration of systemic GM-CSF promotes the outgrowth and antigen-presenting capabilities of DC.

All study participants were followed before, during, and after treatment with periodic measurements of PSA, free PSA, PSMA, complete blood counts, CHEM-22, and bone alkaline phosphatase, as well as with chest x-ray, bone scans, and ProstaScint scans. All testing was conducted on an outpatient basis at Northwest Hospital. Patients were also evaluated every infusion cycle by one of the study physicians. After the conclusion of the scheduled infusions and follow-up observations, clinical status was evaluated according to NPCP criteria and 50% reduction of PSA levels.

Results

Thirty-three patients with metastatic prostate cancer in group A-I who had also participated in the phase I study (Table 2) were the first to complete the phase II study. Nine of the 33 subjects (27.3%) were identified as partial responders;36 11 patients (33.3%) exhibited no significant change during the phase II trial; and 13 patients experienced disease progression. Seven patients died during the study. Among the partial responders, four were also responders in the phase I study, with a total response period of over
Each group is divided into low (initial PSA values 0-19) and high PSA (initial PSA values >19) categories. Normal ranges for PSA are 0-4 ng/ml. Mean and standard error of the mean (SEM) values were obtained from patient sera drawn pre-infusion and seven days post-infusion. There were 24 patients in the non-responder and two patients in the responder low PSA groups. There were 20 patients in the non-responder and five patients in the responder high PSA groups. In the latter, the mean PSA pre-infusion level for the non-responder group was 175.0 ng/ml, and at the end of four infusions, it was 583.4 ng/ml. For the responder group, the mean PSA values were 60.0 ng/ml at the pre-infusion level and 24.4 ng/ml at completion. In both groups, significant differences in pre- versus post-infusion values (p value < 0.05) were observed. Adapted from Tjoa et al.35 with permission.
370 days. Five additional responders in this phase II study had been non-responders in the phase I study. Their average partial response period was 196 days (Fig. 3). The total duration of this phase I/II study was 613 days. Twelve of 19 subjects (63%) with hormone refractory metastatic prostate cancer survived for more than 600 days. We believe this is a significant observation because comparable patients with hormone refractory metastatic prostate cancer historically survive a median of only 6 months.37

All scheduled infusions were completed by September 1998. A total of 95 evaluable subjects were assessed for response to treatment using NPCP criteria and 50% reduction in PSA levels. The NPCP response criteria included comparisons of pre- versus post-study bone scans and ProstaScint scans, which allowed us to evaluate both bony and nodal disease status.

Table 3 summarizes the clinical status of patients in groups A-II and B. In group A-II, 32% of subjects experienced disease regression: Two patients (8%) were complete responders and six patients (24%) were partial responders. One patient exhibited no significant change, while 16 patients (64%) showed disease progression.

Ten group B participants (27%) were partial responders and one subject (3%) was a complete responder. Eight patients (22%) showed no significant change, and 18 patients (49%) showed disease progression.

**IMMUNE RESPONSE ASSAYS**

Several assays are being utilized to characterize the immune response to infused, peptide-pulsed DC. The frequency of antigen specific cells is expected to be quite low, on the order of 1 in 100,000 to 1 in 1,000,000 T lymphocytes, prior to immunization. Following infusion of peptide-pulsed DC, the frequency of antigen specific T cells may increase tenfold. De-
tection and quantitation of these low frequency cells requires very sensitive assays of T cell activity. The number of peptide specific T cells is quantitated in an ELISPOT assay based upon their secretion of IFN$\gamma$ following in vitro stimulation with peptides. The amount of IFN$\gamma$ secreted after stimulation with peptides is also measured, as is secretion of IL-10. Secretion of IFN$\gamma$ indicates development of type 1 T cells, which are thought to represent the type of immune response required for anti-tumor effects. IL-10 is considered a regulatory cytokine produced by type 2 T cells. IL-10 has been shown to suppress antigen presentation by DC and to inhibit secretion of cytokines by type 1 T cells. Therefore, IL-10 secretion is monitored to detect immune responses that might inhibit anti-tumor effects. Cytokine secretion assays are performed on primary cultures of peripheral blood lymphocytes directly following isolation from peripheral blood and also after several rounds of in vitro stimulation. Repeated in vitro stimulation will increase the number of antigen

**Table 2**

Clinical Status of Phase II Subjects
(Based on NPCP Criteria, Plus 50% Reduction in PSA Levels)

| Clinical Status     | No. of Patients (%) | HLA-A2+(-) | $D_0$ | $D_1$ | $D_2$ |
|---------------------|---------------------|------------|-------|-------|-------|
| Progression         | 13* (39.3%)         | 12 (1)     | 2     | 1     | 10    |
| No change           | 11 (33.3%)          | 5 (6)      | 5     | 2     | 4     |
| Partial response    | 9** (27.3%)         | 6 (3)      | 3     | 1     | 5     |
| Total               | 33 (100%)           | 23 (10)    | 10    | 4     | 19    |

* Six patients from this group are deceased
** One patient from this group is deceased

Adapted from Tjoa et al., with permission.

**Table 3**

Clinical Responses of Study Participants in Groups A-II and B*

| Group | Response | Total | %  |
|-------|----------|-------|----|
|       | CR       | 2     | 8  |
|       | PR       | 6     | 24 |
| A-II  | NC       | 1     | 4  |
|       | P        | 16    | 64 |
|       | Total    | 25    |    |
|       | CR       | 1     | 3  |
|       | PR       | 10    | 27 |
| B     | NC       | 8     | 22 |
|       | P        | 18    | 49 |
|       | Total    | 37    |    |

* As of 9/17/98. CR=complete response; PR=partial response; NC=no change; and P=disease progression
reactive cells to the level where they can be more readily detected.

Using these assays, responses to PSMA peptides were measured in patients participating in this clinical trial. In most patients analyzed, IFNγ production is detected and the response to peptides is transient. Two examples of this type of reactivity, measured by the ELISPOT assay, are depicted in Figure 4. T cells reactive to PSM-P2 were detected in the peripheral blood of patient 55 (Panel A) after two infusions of DC, but no reactivity was detected at later time points. This patient also experienced a decrease in serum PSA during the time that peptide reactive cells were detected in the blood. Similar data collected on patient 61 (Panel B) show a response to both PSMA peptides after two infusions of DC that declines thereafter. Similar patterns are observed when total IFNγ secretion is measured. Secretion of IL-10 has also been observed in a minority of patients by two different assay systems.

Primary cultures of lymphocytes from patients 46 and 54 produce IL-10 when stimulated with PSMA peptides (Fig. 5). In both patients, this reactivity appeared to peak after four infusions and then decrease. As might be expected, given the role of IL-10 in the immune response, patient 54 has experienced disease progression. In the case of patient 46, serum PSA decreased during the period of DC infusions but rose after the final DC infusion. In testing lymphocyte cultures after three rounds of in vitro stimulation, two other patients have been identified as producing IL-10 in response to
Detection of peptide-reactive peripheral blood mononuclear cells (PBMC) by IFNγ ELISPOT. PBMC were isolated at the indicated times and tested by IFNγ ELISPOT. Data are presented as the number of antigen reactive cells per 250,000 PBMC. M1 peptide is derived from the matrix protein of the influenza A virus and is included as a recall antigen. Panel A (top) depicts results of testing PBMC from patient 55. Reactivity to M1 is present at all time points while significant reactivity to PSM-P2 is only observed after two infusions of DC. Panel B depicts results of testing in patient 61. Reactivity to PSM-P1 is observed after the second infusion of DC, while reactivity to PSM-P2 is detected after both the second and fourth infusions.
Production of IL-10 by peripheral blood mononuclear cells (PBMC) following in vivo immunization with peptide-pulsed DC and in vitro stimulation with peptide-pulsed T2 cells. PBMC were isolated at the indicated times and stimulated by co-culture with peptide-pulsed T2 cells. After 48 hours, culture medium was collected and assayed for the presence of IL-10 by sandwich ELISA. Panel A depicts data from patient 54, panel B depicts data from patient 46.
peptide stimulation. As more data are collected, the possible significance of these observations will be clarified.

**Future Directions in Prostate Cancer Vaccine Development**

The current studies demonstrate the potential of autologous DC as a vehicle to deliver specific target antigen, a crucial issue in prostate cancer vaccine development. In our future clinical trials, we hope to expand the antigen repertoire of our vaccine study from two PSMA peptides (each of which represents nine amino acids) to a recombinant PSMA protein consisting of the native sequence without the transmembrane domain. This change will multiply the number of targets for T cell attack, and thus produce a potentially more effective vaccine.

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