Results of a Phase I Clinical Study Using Dendritic Cell Vaccinations for Thyroid Cancer

Koichiro Kuwabara,1 Toshihide Nishishita,1 Mariko Morishita,6 Naoki Oyaizu,3 Shunichi Yamashita,6 Takashi Kanematsu,7 Takao Obara,8 Yoshikazu Mimura,9 Yusuke Inoue,4 Michio Kaminishi,10 Kimitaka Kaga,11 Nobuyuki Amino,14 Masafumi Kitaoka,12 Koichi Ito,13 Akira Miyauchi,14 Shiro Noguchi,15 Kaoru Uchimaru,2 Eiji Akagawa,5 Nobukazu Watanabe,5 Tsuneo A. Takahashi,5 Kaori Sato,1 Takeshi Inazawa,1 Takashi Nakaoka,1 and Naohide Yamashita1

Objective: We assessed the feasibility and efficacy of dendritic cell (DC) therapy for advanced thyroid papillary and follicular cancer. Design: Six Japanese patients (2 men and 4 women; aged 46–72 years, mean 60 years), who were diagnosed as advanced thyroid cancer with refractory distant metastases (papillary, n = 5; follicular, n = 1), were enrolled. Patients were first vaccinated weekly for 4 weeks with 10^7 autologous tumor lysate-pulsed monocyte-derived mature DCs followed by fortnightly vaccinations for 8 weeks (total = 8 vaccinations). Low-dose (350 KIU) interleukin-2 was also administered for 3 days at each vaccination. Clinical response, adverse effects, delayed-type hypersensitivity skin testing (DTH), and IFN-γ production by peripheral CD3+ lymphocytes were evaluated. Main Outcome: Of the 6 patients, disease was assessed as stable in 2 and as progressive in 4. No adverse events were observed. Results of DTH and IFN-γ production in peripheral lymphocytes did not correlate to the clinical response. Conclusions: DC immunotherapy could be administered to patients with thyroid papillary or follicular cancer without substantial side effects.

Introduction

Thyroid cancers are the most common malignancies among endocrine tumors and are histologically classified as papillary, follicular, medullary, or anaplastic. With the exception of anaplastic thyroid cancer (undifferentiated carcinoma), the remaining (differentiated) thyroid cancers can generally be controlled by surgery and/or internal radiation. However, in some cases with differentiated thyroid cancers, distant metastases to tissues such as bone or lung may be difficult to manage with conventional surgical and radiation therapies, resulting in impairment of patients’ quality of life and potentially reduced survival (1). Novel therapies for incurable thyroid cancers are accordingly desired. Recently, dendritic cells (DCs) have been demonstrated the most effective cells to present antigen (2), and immunotherapy using DCs pulsed with tumor-associated antigen has been attempted to target various malignancies, including B-cell lymphoma (3), metastatic melanoma (4–12), renal cell carcinoma (13–17), prostate cancer (18–26), hepatocellular carcinoma (27, 28), gastrointestinal cancer (29, 30), and other tumors (31). In general this therapy appears to be safer than chemotherapy or radiation therapy. However, no consensus exists regarding clinical response.

With regard to thyroid cancers, DC-based immunotherapy has also been adopted in advanced medullary thyroid cancer (MTC) and some clinical benefits are reported. Schott et al. describe the use of tumor lysate as source for antigen delivery to DC in parathyroid carcinoma (32). Stift et al. also report that vaccination with tumor-lysate pulsed DCs results in the induction of a specific immune response in patients with MTC (33). Similarly, Schott et al. have found that vaccination with calcitonin and/or CEA peptide-pulsed DC results in the induction of a cellular antigen-specific immune response in patients with MTC, leading to clinical response in some patients (34). The thyroid gland is known to be a target of common autoimmune diseases such as Graves' disease and Hashimoto's disease (chronic thyroiditis), in which thyroid tissues are

1Department of Advanced Medical Science, 2Department of Hematology and Oncology, 3Department of Laboratory Medicine, 4Department of Radiology and Division of 5Cell Processing, Institute of Medical Science, University of Tokyo, Tokyo, Japan, 6Department of Molecular Medicine and 7Department of Surgery, Nagasaki University Graduate School of Biomedical Sciences, Nagasaki, Japan, Department of Endocrine Surgery, 8Tokyo Women’s Medical College, Tokyo, Japan, 9Department of Surgery, and 10Department of Gastrointestinal Surgery, 11Department of Otorhinolaryngology, University of Tokyo School of Medicine, Tokyo, Japan, 12Showa General Hospital, Tokyo, Japan, 13Ito Hospital, Tokyo, Japan, 14Kuma Hospital, Kobe, Japan, Division of Endocrinology and Metabolism, and 15Noguchi Thyroid Clinic and Hospital Foundation, Oita, Japan.
infiltrated by T cells (35,36). From this standpoint thyroid cancer would appear a good candidate for immunotherapy. Papillary and follicular thyroid cancers are much more common than MTC. MTC originates from parafollicular calcitonin-secreting cells (neuroendocrine cells) in the thyroid gland, whereas papillary and follicular thyroid cancers originate from thyroid follicular cells. Although DC-based immunotherapy has been attempted in MTC, this therapy has not been reported in papillary and follicular cancers. In this study we accordingly trialed DC immunotherapy in papillary and follicular thyroid cancers, and investigated the safety of this therapy, clinical course, and relation of the clinical course to immunological response.

Materials and Methods

Patient selection

The study protocol was approved by the Institutional Review Board of the Institute of Medical Science, University of Tokyo, Japan. All eligible patients were histologically proven to have thyroid cancer with distant metastases. Six Japanese patients (2 men and 4 women; aged 46–72 years, mean 60 years) who had previously undergone thyroidectomy were enrolled (Table 1). The study period was August 2001–August 2003 and patients have been following up until April 2005. No other therapy was administered during the DC study protocol. All participants provided signed informed consent according to the Declaration of Helsinki before enrolling in the study.

In vitro generation and culture of immature DCs (iDCs) from thyroid cancer patients

Procedures for the generation of DCs and the quality control of DCs have already been reported (11). Peripheral blood mononuclear cells (PBMCs) were collected from leukaphereses from the patients using a COBE Spectra Apheresis System (Gambro BCT, Inc., Lakewood, CO, USA). The collected cells were then separated by density gradation using Ficoll-Hypaque (Amersham Biosciences UK Ltd., Buckinghamshire, England). The cells were then resuspended in cold phosphate-buffered saline (PBS) and allowed to adhere to plastic dishes (Primaria, Becton Dickinson Biosciences, Hampshire, England). The cells were then resuspended in Ficoll-Hypaque (Amersham Biosciences UK Ltd., Buckinghamshire, England). The cells were then resuspended in cold phosphate-buffered saline (PBS) and allowed to adhere to plastic dishes (Primaria, Becton Dickinson Biosciences, Franklin Lakes, NJ, USA), after which non-adherent cells were removed and the remaining adherent cells were collected for DC preparation.

To prepare the iDCs, the cells were cultured in plastic dishes (104 cells/dish) with 6 mL of complete medium (RPMI 1640, antibiotic-antimycotic and 5% human type AB serum) containing rhGM-CSF (final concentration 50 mg/mL) and rIL4 (50 mg/L) for 7 days at 37°C under a humidified atmosphere of 5% CO2 in air, after which the resultant iDCs were harvested.

Freezing and thawing procedures

iDCs were washed and diluted in the complete medium to be 4 x 107 cells/mL. 2 x 107 of the cells were distributed in 0.5 mL of dimethyl sulfoxide (DMSO) freezing medium (40% human type AB serum, 20% DMSO and 40% complete medium) into pre-cooled plastic vials and were stored at −135°C until used.

Tumor lysate extraction

Tumor lysates were used as a tumor antigen. To prepare the lysates, tumor mass was obtained by surgery from each patient. The isolated mass was then homogenized in PBS and filtered through BD Falcon cell strainer with 100 µm mesh (BD Biosciences, San Jose CA, USA), and tumor cell suspensions were obtained. Aliquots of the isolated tumor cells (107 cells/tube) were then lysed by putting them through 3 freeze (in liquid nitrogen) and thaw (in a 37°C water bath) cycles. The lysed cells were centrifuged at 12,000 rpm for 5 minutes at RT. The protein contents of the resultant cell-free lysates were determined using MicroBCA Protein Assay Kit (Pierce Biotechnology, Inc., Rockford, IL, USA). Aliquots (300 µg/tube) were then stored at −135°C until use.

Preparation of tumor lysate-pulsed mature DCs (mDCs) for vaccination

iDCs (>107 cells/dish) in plastic dishes were cultured for 24 h in 3 mL of medium containing 100 µg/mL of autologous tumor lysates. After 7 mL of the complete medium containing 50 ng/mL of rhTNFα was added to the medium, the loaded cells were subsequently cultured for another 4 days to develop into mDCs. Phenotypical analysis of the iDCs and mDCs from thyroid cancer patients was carried out as

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Age/Sex</th>
<th>Tumour type</th>
<th>Previous therapy</th>
<th>Sites of metastases</th>
<th>No. of vaccination</th>
<th>Clinical response</th>
<th>Response to DTH skin test</th>
<th>Survival days from first vaccination</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>54/F</td>
<td>follicular</td>
<td>surgery, irradiation</td>
<td>lung, bone</td>
<td>16</td>
<td>PD</td>
<td>– – –</td>
<td>&gt;1370</td>
</tr>
<tr>
<td>2</td>
<td>52/F</td>
<td>papillary</td>
<td>surgery, embolization, irradiation</td>
<td>lung, bone</td>
<td>5</td>
<td>PD</td>
<td>n.d. n.d.</td>
<td>151</td>
</tr>
<tr>
<td>3</td>
<td>72/M</td>
<td>papillary</td>
<td>surgery</td>
<td>LNs, lung, bone</td>
<td>16</td>
<td>PD</td>
<td>– – –</td>
<td>379</td>
</tr>
<tr>
<td>4</td>
<td>46/M</td>
<td>papillary</td>
<td>surgery, irradiation</td>
<td>LNs, lung</td>
<td>9</td>
<td>PD</td>
<td>n.d. n.d.</td>
<td>&gt;1313</td>
</tr>
<tr>
<td>5</td>
<td>68/F</td>
<td>papillary</td>
<td>surgery</td>
<td>LNs</td>
<td>16</td>
<td>SD</td>
<td>– – –</td>
<td>&gt;1174</td>
</tr>
<tr>
<td>6</td>
<td>67/F</td>
<td>papillary</td>
<td>surgery, irradiation, PEIT</td>
<td>LNs, lung</td>
<td>16</td>
<td>SD</td>
<td>– – – ±</td>
<td>&gt;1048</td>
</tr>
</tbody>
</table>

DTH, delayed-type hypersensitivity; PEIT, percutaneous ethanol injection therapy; LN, lymph node; PD, progressive disease; SD, stable disease; n.d., not done. DTH skin test was performed before vaccination, and after the first vaccination and after the second vaccination (if available) and judged negative (−) for erythemas <2 mm in diameter, and (±) for erythemas <10 mm in diameter, and positive (+) for erythemas >10 mm in diameter and/or induration.
DENDRITIC CELL THERAPY FOR THYROID CANCER

Table 2. Screening of adverse effects

<table>
<thead>
<tr>
<th>Patient no.</th>
<th>Blood/Bone marrow Platelets</th>
<th>Constitutional symptoms</th>
<th>Dermalogical</th>
<th>Immunological</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Fever</td>
<td>Weight gain</td>
<td>Weight loss</td>
<td>Injection site reaction</td>
</tr>
<tr>
<td>1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>2</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>4</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>5</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>6</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

ANA, antinuclear antibody; TgAb, antithyroglobulin antibody; TPOAb, antithyroid peroxidase antibody; n.d., not done.

The procedure for intracellular IFN-γ staining in peripheral CD3+ lymphocytes has already been reported (37). Briefly, peripheral blood mononuclear cells (1–2 x 10^6) were cultured with mDCs and anti-CD28 monoclonal antibody for 18 hours. After culture, cells were first stained with ethidium monooxazide bromide, which detects dead cells, and then with anti-CD4 or CD8 monoclonal antibodies. After addition of 4% formaldehyde, cells were permeabilized using dPBS/BSA/azide buffer (Sigma, St. Louis, MO) containing 0.5% saponin and stained by anti-IFN-γ monoclonal antibodies (Cosmo Bio, Tokyo, Japan). Flow cytometric analysis was done on a FACSCalibur flow cytometer (Becton Dickinson Immunocytometry Systems, BDIS, San Jose, CA, USA) using CELLQuest software (BDIS).

Results

Patient characteristics and clinical outcome

Five patients with advanced papillary thyroid cancer and 1 with follicular thyroid cancer were enrolled into our study. Their clinical status varied widely; previous therapies and clinical responses are summarized in Table 1 and Table 2. During the therapeutic protocol, no clinical adverse events were observed, including serological induction of autoimmunity, fever, or local skin inflammation at vaccination sites. No sign of NCI-CTC grade 3–4 toxicity was evident following DC-based vaccination. We therefore conclude that our phase I immunotherapy protocol using autologous, tumor lysate-pulsed, monocyte-derived mDCs plus rhIL2 can be applied repeatedly to patients with thyroid papillary or follicular cancers, without causing substantial side effects.

Following the 5–16 vaccinations, 4 patients exhibited PD response and 2 (both of them suffered from papillary thyroid cancer) showed SD response during the immunotherapy. Figure 1a shows the time course of a representative lung metastasis in patient 6, calculated from CT images (longest diameter of the largest metastasis in the section, Fig. 1b–d). Tumor growth rate appeared to be reduced during DC therapy. Similar analysis of patient 5 also revealed inhibition of tumor growth (Fig. 1f).

Immunological monitoring

For immunological monitoring delayed-type hypersensitivity (DTH) skin tests against tumor lysate (11) and IFN-γ production in peripheral CD3+ lymphocytes (37) were examined. The first DTH skin test was performed within 4 weeks before the first vaccination and the second, within 4 weeks after the last vaccination. Forty-eight hours after each injection, the diameter of the erythema and the induration were measured. Erythema <2 mm in diameter were considered negative (−), those 10 mm in diameter were considered equivocal (+), and those >10 mm in diameter accompanied by induration were judged positive (+).
Changes of the metastatic lesion during DC therapy.

FIG. 1.

(A) Time course of a representative lung metastasis in patient 6. Three metastatic lesions are observed in the section. The longest diameter of the largest lesion (arrow) in the section was plotted. The diameter just before DC therapy is represented by 100%. Day 0 was defined as the date of commencing DC therapy. \( g \text{-} \text{day} \) is the whole CT image from which the close-up B was taken. (B) Time course of a large mass in the mediastinum in patient 5.

on the second DTH skin test performed after the 8th vaccination, no significant DTH responses were observed in any of the patients.

For further evaluation of tumor-specific immunity, we assessed IFN-\( \gamma \) production in peripheral CD3\(^+\) lymphocytes. After co-culture with tumor-lysate pulsed mDCs, the expression of IFN-\( \gamma \) in CD3\(^+\) cells did not change significantly between before and after DC therapy (data not shown).

Discussion

We herein report a phase I clinical study using autologous tumor lysate-pulsed monocyte-derived mDC vaccinations plus low-dose rhIL-2 for advanced papillary and follicular thyroid cancer. During the therapeutic protocol, no serious clinical adverse events occurred including autoimmunity, and we safely completed the immunotherapy in all the enrolled patients. We used tumor lysate as tumor antigen because epitopes of HLA-restricted tumor antigen have not been determined in thyroid papillary and follicular cancers. One might think that thyroglobulin (Tg) could be used as a tumor-associated antigen in DC therapy for papillary and follicular thyroid cancers because this antigen is produced and secreted by thyroid cancers as well as the normal thyroid gland. Thyroglobulin, a macromolecular homodimeric glycoprotein, is secreted only from thyroid cells and provides a matrix for the synthesis of thyroid hormones and a vehicle for their subsequent storage (35). In normal healthy volunteers, Tg-pulsed monocyte-derived DC could induce Tg-specific CD4\(^+\) T cell activation. However, Tg-specific T cells were not activated by Tg-pulsed mDCs in advanced thyroid cancer patients (38). Although the exact reason for this is unknown, advanced thyroid cancer patients might have tolerance to Tg because their circulating blood Tg levels are persistently high. We therefore adopted thyroid tumor lysate as the antigen with which to pulse DCs. The use of tumor lysate also has the advantage of overcoming the heterogeneity of malignancies.

Among 6 patients, 2 with papillary carcinoma exhibited SD response, whereas the other patients showed PD response. In this study we could not use the serum Tg level as a surrogate marker of tumor growth because serum anti-Tg antibody, which interferes with Tg measurement, was positive in 1 patient before DC therapy. DTH tests were negative, with the exception of 1 patient with SD response in whom DTH reactivity became equivocal after DC immunotherapy. In this study, erythemas <2mm in diameter were considered negative because it was difficult to distinguish this degree of erythema from the needle reaction. Hence the possibility exists that we might have ignored slight DTH reactivity with these criteria. However, DTH tests in this study did not correlate with the clinical response, nor did the expression of IFN-\( \gamma \) in CD3\(^+\) cells after DC therapy.

For functional analysis of cancer immunotherapy, ELISPOT assay, tetramer analysis, and CTL assay have been used in addition to DTH testing and IFN-\( \gamma \) production. Although there may be some problem in our assay procedures, the correlation between clinical response to DC therapy and immunological assays is controversial; some studies report a positive correlation (12), while others do not (7,9–11,33,39). Although further research may yield better analyses, it appears that the immunological analyses available at present are not useful surrogate endpoints of DC therapy. The tumor responses to DC therapy in this clinical study were classified as negative according to criteria. However, 2 patients exhibited SD response during immunotherapy. We have been continuing to follow the surviving patients after DC therapy. In 2 patients with SD response, after cessation of DC therapy, tumors resumed growing when measured by CT scan, suggesting that DC therapy affected thyroid cancer growth in these patients.

With regard to advanced medullary thyroid carcinoma, Stift et al. performed tumor lysate-pulsed DC therapy in a group of patients with this condition (33). Seven of 10 enrolled patients showed at least a transient decrease of tumor marker levels (calcitonin and CEA), 4 patients exhibited radiological or clinical reduction in measurable lesions, and 2 patients demonstrated prolonged stable disease. Schott et al. have reported CEA- and calcitonin-pulsed DC therapy in advanced medullary thyroid carcinoma (39); clinical response was observed in 3 of 7 patients. One of the patients who
responded showed a significant regression of liver and pulmonary lesions and other 2 patients achieved SD. Although response rates in medullary thyroid cancer differ from those seen in the present study, it appears that some patients with advanced thyroid cancers can obtain clinical benefit from DC therapy irrespective of histological type. However, more studies are needed to establish this concept because the number of treated patients is small.

Many problems remain in current DC therapy, such as standardization of ex vivo cultures, vaccination against multiple tumor antigens as well as tumor-associated antigens expressed by stromal or endothelial cells in the tumor, and prevention of regulatory T cells that may inhibit immune responses in cancer patients (13,31). If these issues were addressed, DC therapy would likely become a more effective agent against malignant tumors, including those of the thyroid.

References


Address reprint requests to:
Naohide Yamashita
Department of Advanced Medical Science
Institute of Medical Science
University of Tokyo
4–6–1 Shirokanedai
Minato-Ku
108-8639 Tokyo
Japan

E-mail: yama-nao@ims.u-tokyo.ac.jp