Induction of Peptide-Specific Immune Response in Patients with Primary Malignant Melanoma of the Esophagus after Immunotherapy Using Dendritic Cells Pulsed with MAGE Peptides

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Primary malignant melanoma of the esophagus (PMME) is a very rare disease with an extremely poor prognosis. Surgery is currently considered its best treatment, while any other measures are ineffective. We studied the effect of active specific immunotherapy using monocyte-derived dendritic cells (DCs) pulsed with the epitope peptides of melanoma-associated antigens (MAGE-1, MAGE-3) in patients with PMME after surgery, for the first time. The patient received passive immunotherapy with lymphokine-activated killer cells concomitantly.

Two HLA-A24-positive patients with PMME were treated. Both patients initially received radical esophagectomy with regional lymphadenectomy, followed by adjuvant chemotherapy with dacarbazine, nimustine, vincristine and interferon-α. In the case 1 patient, active specific immunotherapy was used to treat a large abdominal lymph node metastasis that became obvious 21 months after surgery. The disease remained stable for 5 months, and the patient survived for 12 months after the initiation of immunotherapy. In the case 2 patient, immunotherapy was tried as post-operative adjuvant treatment after adjuvant chemotherapy. There was no tumor recurrence for 16 months after the immunotherapy. As of 49 months after esophagectomy, the patient is still alive. In both patients, the ability of peripheral lymphocytes to produce IFN-γ in vitro in response to peptide stimulation was significantly enhanced and delayed-type hypersensitivity skin test response to MAGE-3 peptide was turned positive after immunotherapy. In conclusion, active specific immunotherapy for PMME with the use of DCs and MAGE peptides was safe and capable of inducing peptide-specific immune responses. This case report warrants further clinical evaluation of this immunotherapy for PMME.

Key words: esophageal malignant melanoma – cellular immunotherapy – dendritic cell – lymphokine-activated killer cell – MAGE peptide

INTRODUCTION

Primary malignant melanoma of the esophagus (PMME) is an uncommon tumor comprising only 0.1–0.2% of all esophageal malignancies (1,2). Only about 250 cases have been reported in the English-language literature to date (3,4). The prognosis of PMME is extremely poor, with a reported median overall survival of 10–14 months (1,2). Radical esophagectomy with regional lymphadenectomy is the mainstay of therapy for patients with resectable PMME; however, the 5-year survival rate after surgery is less than 5% (1). Various regimens have been tried as post-operative adjuvant treatment to prolong survival, including chemotherapy, radiotherapy, and immunotherapy using recombinant cytokines. However, because very few studies on PMME are available, potential benefits of these treatments remain uncertain.

Since the latter half of the 1990s, dendritic cell (DC)-based immunotherapy has been used to treat cancer,
especially immunogenic tumors such as malignant melanoma. Some promising results have been reported (5). We previously tried this approach to manage other refractory malignancies, such as gastro-intestinal and lung adenocarcinomas. Our results suggested that DC-based immunotherapy is a safe and feasible treatment clinically effective in some patients (6). DC-based immunotherapy may be an alternative treatment for PMME, capable of improving the currently disappointing outcome after surgery.

In this article, we describe two patients with PMME who underwent radical esophagectomy and received active specific immunotherapy using autologous monocyte-derived DCs pulsed with the epitope peptides of melanoma-associated antigens (MAGE-1, MAGE-3) (7,8). Both patients were concurrently given passive adjuvant immunotherapy with lymphokine-activated killer (LAK) cells.

CASE REPORT

CASE 1

A 68-year-old man with dysphagia and epigastric discomfort was found to have an esophageal tumor on esophagography and esophagoscopy performed at a nearby hospital. He was referred to us for further evaluation and treatment on 25 January 2000. Physical examination on admission revealed no evidence of pigmented lesions in the skin, eyes, or rectum. An esophagogram showed a polypoid tumor, 6.0 cm long, obstructing the lower thoracic esophagus (Fig. 1A). A grayish-black tumor was observed on esophagoscopy (Fig. 1B). A biopsy specimen was positive for S-100 protein on immunohistochemical staining; and thus malignant melanoma was suspected. Subtotal esophagectomy and extended lymphadenectomy were performed via a right thoracotomy and median laparotomy. Reconstruction was performed with a gastric tube, pulled up to the neck through a retrosternal route. The surgical specimens contained four nodular protruding tumors with partial black pigmentation (Fig. 1C).

Histopathologically, tumor cells containing melanin pigment were observed around the basal layer of the squamous epithelium (Fig. 2A, B). Immunohistochemical staining demonstrated that the tumor cells showed a positive reaction to HMB-45 and S-100 protein (Fig. 2C, D). These findings were consistent with the diagnosis of malignant melanoma. The tumor invaded the muscularis propria. Three of 19 resected lymph nodes were infiltrated with tumor cells. The post-operative course was uneventful. One course of adjuvant chemotherapy with dacarbazine (DITC, 200 mg/day on days 1–5), nimustine (ACNU, 100 mg on day 1), vincristine (VCR, 1 mg on day 1), and interferon (IFN)-α (2.0 × 10^6 U/day on days 1–5) was administered immediately after recovery from surgery.

Twenty one months after surgery, follow-up computed tomography (CT) revealed a large abdominal lymph node and a small subcutaneous tumor in the abdominal wall. The subcutaneous tumor was excised and immunohistochemically stained for MAGE-1 and MAGE-3 antibodies (a generous gift from Dr Giulio C. Spagnoli, University Hospital, Basel, Switzerland) (9). Because the tumor showed a strong reaction to MAGE-3 and a partial reaction to MAGE-1 (Fig. 2E, F), we applied both as tumor-specific antigens for active immunotherapy. The protocol for immunotherapy was approved by the Institutional Ethics Review Committee, Kyoto Prefectural University of Medicine, and written informed consent was obtained from the patient. The procedure for generation of peptide-pulsed DCs was the same as that described previously (6). Briefly, the patient underwent leukapheresis to obtain peripheral blood mononuclear cells (PBMCs). The leukapheresis products were separated by density gradient centrifugation using Ficoll-Paque Plus (Pharmacia Biotech, Stockholm, Sweden) and then cryopreserved in a liquid-nitrogen tank. The PBMCs were thawed at each treatment and cultured in complete medium (CM) consisting of RPMI 1640 (Nikkken, Kyoto, Japan) supplemented with 5% heat-inactivated pooled human AB serum and gentamicin (Fujisawa Pharmaceutical, Osaka, Japan) for 2 h at 37°C in a 5% CO₂ incubator. Non-adherent cells were
removed and subsequently used for LAK cell induction. Remnant adherent cells were further cultured in CM supplemented with 50 ng/ml each of recombinant human granulocyte-macrophage-colony stimulating factor (GM-CSF) (Peprotech, London, UK) and recombinant human interleukin-4 (IL-4) (Peprotech, London, UK). On day 7, DCs were harvested and suspended in phosphate-buffer saline (PBS) containing 1% human albumin (Fujisawa, Osaka, Japan). HLA-A2401-restricted peptides of MAGE-1 (a peptide with an amino acid sequence of NYKHCFPEI) and MAGE-3 (a peptide with an amino acid sequence of IMPKAGLLI) (40 μg/ml, respectively) (Takara, Shiga, Japan) were added to the DC suspension at room temperature for 4 h (7,8). Thereafter, the cell suspension was washed twice with PBS and used as a vaccine (MAGE-DC vaccine) in a final volume of 3 ml of PBS containing 1% human albumin.

The MAGE-DCs were administered (5.5 ± 0.4 × 10^7 cells into inguinal lymph nodes; 6.0 ± 1.2 × 10^7 cells intradermally) every 2 weeks for 6 months. In addition to MAGE-DC vaccination, the patient received passive non-specific immunotherapy with LAK cells as an adjuvant. Peripheral blood lymphocytes (PBLs), obtained as a non-adherent cell fraction from apheresis products, were activated in vitro with coated anti-CD3 mAb and 100 U/ml of recombinant human IL-2 (Shionogi, Osaka, Japan) for 10–14 days according to the method previously developed by us (10). The resulting LAK cells (2.8 ± 1.1 × 10^9 cells) were washed twice with PBS and transferred intravenously in a final volume of 50 ml of saline containing 1% human albumin immediately after the MAGE-DC vaccination every 2 weeks (Fig. 3).

To compare the peptide-specific T cell response before and after immunotherapy, we conducted an in vitro cytokine assay (11). PBMCs, obtained from the patient before starting immunotherapy and after three sessions of immunotherapy, were pulsed with MAGE-1 and MAGE-3 peptides (40 μg/ml, respectively) and activated in vitro with 20 U/ml of IL-2 for 11 days. The resulting PBMCs were then cultured with autologous PBMCs that had been pulsed in advance with MAGE-1 and MAGE-3 peptides. The cultures were incubated for 24 h at 37°C in 5% CO2. IFN-γ release into the supernatant was measured with a standard enzyme-linked immunosorbent assay (ELISA). The results demonstrated that IFN-γ secretion from PBMCs was prominently enhanced after immunotherapy (Fig. 4).

No severe toxicity directly attributed to the immunotherapy was observed. The disease did not progress for 5 months after the immunotherapy. The serum concentration and urinary excretion of 5-S-cysteinylidopa (5-S-CD), a tumor marker for malignant melanoma, were stabilized, falling to <1.0 ng/ml (cut-off value, 12 ng/ml) and 724 μg/day (cut-off value, 284 μg/day) after the immunotherapy (Fig. 5). However, the disease progressed gradually, and the patient died of widespread metastases 34 months after...
esophagectomy (12 months after immunotherapy began). No autopsy was performed.

CASE 2

A 57-year-old man with alcoholic hepatitis and dysphagia was suspected to have esophageal varices on esophagoscopy performed at a nearby hospital. He was referred to us for further evaluation and treatment on 25 April 2002. An esophagogram showed a well-defined tumor with an irregular surface, 5.0 cm long, in the abdominal portion of the esophagus and esophagoscopy revealed a large polypoid tumor with a pigmented surface beginning 33 cm from the incisors (pictures not shown). The histological diagnosis of the biopsy specimen was malignant melanoma. Subtotal esophagectomy with partial resection of the upper stomach and extended lymphadenectomy were performed via a right thoracotomy and median laparotomy. The esophagogastric anastomosis was made with a gastric tube, pulled up to the neck through a retrosternal route. Histopathologically, tumor cells containing melanin pigment were observed around the basal layer of the squamous epithelium. Immunohistochemically, the tumor cells showed a positive reaction to S-100 protein, while Melan-A, desmin, and HHF35 stains were negative (pictures not shown). The depth of tumor invasion was submucosal. The lymph nodes along the celiac axis and splenic artery were positive for metastasis. The post-operative course was uneventful. One course of the adjuvant chemotherapy, as that used for the case 1 patient, was administered immediately after recovery from surgery.

After immunohistochemically confirming that MAGE-1 and MAGE-3 were expressed as tumor-specific antigens in the surgical specimen, we employed active and passive immunotherapy as adjuvant treatments. The methods used to prepare the MAGE-DCs and LAK cells for immunotherapy were the same as those used for case 1. The MAGE-DCs (6.2 $\pm$ 0.9 x 10^7 cells) were injected into the inguinal lymph nodes and LAK cells (1.2 $\pm$ 0.1 x 10^9 cells) were transferred intravenously every 2 weeks for 6 months. In vitro cytokine assay revealed that IFN-$\gamma$ secretion from PBMCs was substantially enhanced after five sessions of immunotherapy (Fig. 4).

Our regimen for immunotherapy had no adverse effects and the patient remained free of relapse for 16 months after surgery. However, 9 months after the last session of immuno therapy, recurrence was detected in a cervical paratracheal lymph node on Fluorin-18 fluorodeoxyglucose positron emission tomography and CT scanning. The metastatic lymph node was completely excised. There has been no evidence of recurrence for 33 months, with no further treatment. The patient is still alive 49 months after esophagectomy.

DISCUSSION

The treatment of PMME remains challenging. The aggressive nature of this disease generally leads to extremely poor outcomes. To date, only seven patients with PMME have survived for longer than 5 years after diagnosis (12–18). All of these patients underwent esophagectomy, but only three received post-operative treatments, such as radiotherapy, immunochemotherapy, or hormone-based chemotherapy. Surgical resection is supposed to be the treatment of choice for PMME; however, the effectiveness of post-operative adjuvant therapies, including chemotherapy and radiotherapy, remains unclear. New treatment strategies for PMME are thus required not only for recurrent disease, but also for adjuvant therapy designed to prevent recurrence and prolong survival.

In this article, we described a combined cellular immunotherapy approach for PMME, using both active specific DC-based immunotherapy and non-specific passive immunotherapy with LAK cells. We used our regimen to treat two
patients in different situations. One had recurrent disease, while the other received immunotherapy as post-operative adjuvant treatment. Although the preparation of DCs as well as LAK cells is laborious, LAK cells are usually induced from non-adherent cells derived from PBMCs harvested as the cell source for DCs and are normally discarded with no clinical use. Our technique for cell preparation allows LAK cells to be efficiently generated for clinical use without waste (10). The MAGE-1 and MAGE-3 peptides used in this study are members of the melanoma-associated antigen gene family of cancer/testis antigens and have been frequently used for DC-based immunotherapy in patients with cutaneous malignant melanoma (5,19,20). These peptides have also been shown to be potential immunotherapeutic targets in PMME (21). In fact, the tumor in both of our patients showed a strong reaction to MAGE-3 Ab and a partial reaction to MAGE-1 Ab. We thus employed both of these peptides as tumor-specific antigens for DC-based active immunotherapy and performed adoptive transfer of LAK cells as an adjuvant treatment.

The primary goal of this study was to examine for the first time whether immunological response could be induced in patients with PMME by active specific immunotherapy with the use of DCs and MAGE peptides. We confirmed significant increases in IFN-γ secretion from PBMCs in both patients after immunotherapy (Fig. 4). In addition, delayed-type hypersensitivity skin test response to MAGE-3 peptide turned positive with skin erythema >10 mm in both patients (6). These results suggest that DC-based immunotherapy combined with MAGE peptides could be a rational approach for the treatment of PMME, even for patients with advanced and recurrent disease.

The second objective of our study was to determine whether anti-tumor response could be induced. In the case 1 patient with a large recurrent abdominal lymph node metastasis, the disease remained stable for 5 months and the patient survived for 12 months after immunotherapy, or 34 months after esophagectomy. Given the poor outcomes of PMME, including a reported median overall survival of 10–14 months (1,2), immunotherapy appears to have contributed to the relatively long survival. For the case 2 patient, immunotherapy was given in a post-operative adjuvant setting. The tumor did not recur for 14 months after immunotherapy began. Recurrence in a cervical paratracheal lymph node was then detected and the involved node was surgically resected. As of this writing, the patient is alive with no new recurrence, 49 months after esophagectomy. Because we have administered no further treatment after surgical resection of the paratracheal node, the effect of immunotherapy might be long lasting.

In 2005 Asakage et al. published (as a ‘letter to the editor’) a report of a PMME patient treated by esophagectomy and post-operative adjuvant DC therapy (22). They used DCs pulsed with autologous tumor lysates. The patient received four intradermal injections of DCs weekly after esophagectomy and survived for 2.5 years after esophagectomy. Because it was a short letter, the results of immunological evaluations and other clinical details were not included.

In summary, we described two cases of patients with PMME treated by a combined cellular immunotherapy. We believe that this is the first report to document the use of this approach for the treatment of PMME. Active specific immunotherapy with DCs and MAGE peptides was safe and capable of inducing peptide-specific immune responses in both patients. Further clinical evaluation of this immunotherapy for PMME is warranted.

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Conflict of interest statement
None declared.

References


