Marked clinical and immunological response of MAGE-A3 peptides vaccination in a patient with adenocarcinoma of the lung case report: A case report

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SUMMARY

A 56-year patient was diagnosed adenocarcinoma of the right lung and underwent lower lobectomy. Pleural dissemination was observed, and the pathological stage was T4N0M0. Systemic chemotherapy was performed with paclitaxel and vinorelbine after the surgery. Because multiple pulmonary and liver metastases were appeared 1 year after the surgery, the second line chemotherapy (CDDP and 5FU) was performed. The response of the chemotherapy was not observed, and thus the patient was enrolled in the clinical trial of MAGE-A3 peptide vaccine with OK-432. The peptide (300 mg) was injected subcutaneously at the upper thigh or upper arm a total of 6 times over 2 months (days 1, 8, 15, 22, 36, and 50) with OK432. During two courses of the vaccinations, the tumor markers (CA19-9 and CEA) decreased markedly. Furthermore, the tumor size of the lung and liver metastases decreased to 30% of their pre-treatment size. The delayed type hypersensitive reaction became positive after the first course of vaccinations. The precursor CTL for the peptide in the peripheral blood had increased after the vaccination. MAGE-A3 peptide vaccination with OK-432 has a potential to induce immunological response and clinical response in advanced lung cancer patients.

Key words: *Lung Neoplasms; Adenocarcinoma; T-Lymphocytes, Cytotoxic; Antigens, Neoplasm; Cancer Vaccines; Picibanil*

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INTRODUCTION

Lung cancer is the most common cause of cancer-related mortality worldwide (1). After a surgical resection as the first line therapy for non-small cell lung cancer (NSCLC), the overall 5-year survival is approximately 50% (2). The poor prognosis of lung cancer patients after surgery is attributed to minimal residual tumor cells, which develop a loco-regional recurrence and distant metastases (3,4). Although platinum-based chemotherapy alone or in combination with radiation is often effective, quality of life can be compromised due to the severe adverse effect of the treatment. Owing to these circumstances, it is important to develop a more efficient, less toxic, and clinically applicable treatment strategy. Identification of human tumor antigen in 1991 by Boon *et al.* has triggered an interest in antigen-based immunotherapy, mainly in patients with malignant melanoma (5). Since then, both cellular and humoral immune responses against cancer have been elucidated at the molecular level. The MAGE gene family is composed of several subgroups such as the MAGE-A, -B, -C, and -D genes, which map to chromosome X (6). It has been reported that most MAGE family genes are detected in 20-45% of lung cancers (7,8). In normal tissues, MAGE genes are expressed in spermatogonia, oogonia, and trophoblast (9,10). Therefore, they are called "cancer/germline antigens" (cancer/testis antigen). MAGE-A3 has been reported to bear several epitope peptides in the context of different HLA molecules, and they have been applied in several clinical vaccine trials (11-13). It has been administered by the subcutaneous injection with 100 or 300 μg of peptide and no severe toxicity has been reported in the clinical trials (14,15). The present study was a pilot study to investigate the safety and toxicity of the MAGE-A3 peptide vaccine in combination with OK-432 and to evaluate the clinical and immunological response in lung cancer patients vaccinated with MAGE peptides. We report here clinical and immunological response of MAGE-A3 peptides vaccine with OK-432 in a patient with adenocarcinoma of the lung.

A CASE REPORT

Patients provided informed consent to participate in the experimental vaccination study and to donate blood for immunological monitoring. A signed consent form was also obtained from each patient before obtaining the tissue samples of lung cancer used in this study. The study protocol was approved by the Human and Animal Ethics Review Committee of the University of Occupational and Environmental Health, Japan. The peptides used as the vaccine and those for the *in vitro* assays were synthesized by Multiple Peptide Systems (San Diego, California, USA), and all were clinical-grade peptides as assessed by HPLC and mass-spectrometric analysis.

A 56-year patient was diagnosed adenocarcinoma of the right lung and underwent lower lobectomy with mediastinal lymph node dissection. Pleural dissemination was observed, and therefore, the pathological stage was diagnosed as T4N0M0. MAGE-A3 expression of the primary lung cancer tissue by RT-PCR was positive. The patient was treated with two courses of systemic chemotherapy with paclitaxel and vinorelbine after the surgery. Because multiple pulmonary and liver metastases were appeared 1 year after surgery, the second line chemotherapy (CDDP and 5FU) was performed. However, the response of the chemotherapy was not observed, and the patient was enrolled to the clinical trial of MAGE-A3 peptide vaccine with OK-432. The 300 μg of MAGE-A3 peptide (IMPKAGLLI) was injected subcutaneously at the upper thigh or upper arm a total of 6 times in 2 months (days 1, 8, 15, 22, 36, and 50) in combination with OK432 (0.5 KE) as an adjuvant. OK-432 is a penicillin-killed and lyophilized preparation of a low-virulence strain (Su) of *Streptococcus pyogenes* (16). Because the tumor had stabilized or regressed after the first course (6 vaccinations), additional course of vaccination was started after 2-week intervals.

Before and after each 8 week cycle of vaccination, chest roentgenography, computed tomography (CT) of the brain, chest, and upper abdomen, and bone scintigraphy were employed to assess the treatment response. The clinical responses were assessed by the Response Evaluation Criteria in Solid Tumors (RECIST). All adverse effects were recorded on the appropriate adverse event reporting sheet and evaluated according to the NCI-CTC / Common Toxicity Criteria, version 2.

The vaccination was mostly accompanied by redness of the skin at the injection site. The local pain at the injection site was slight and evaluated as lower than grade 1 according to the common toxicity criteria. Another adverse effect was fever (grade 1), but it was not required any treatment. The tumor markers (CA19-9 and CEA) decreased markedly by one course of the vaccination (Figure 1).

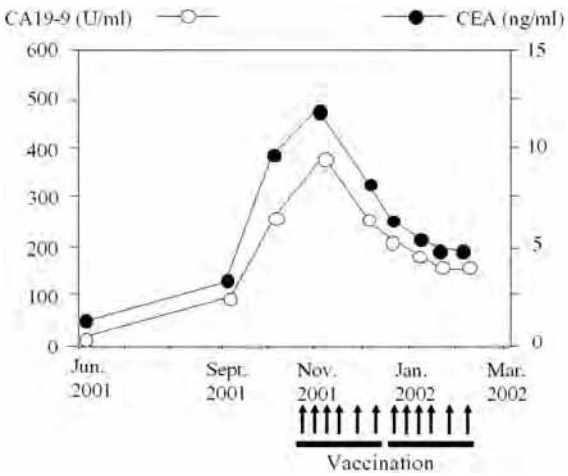
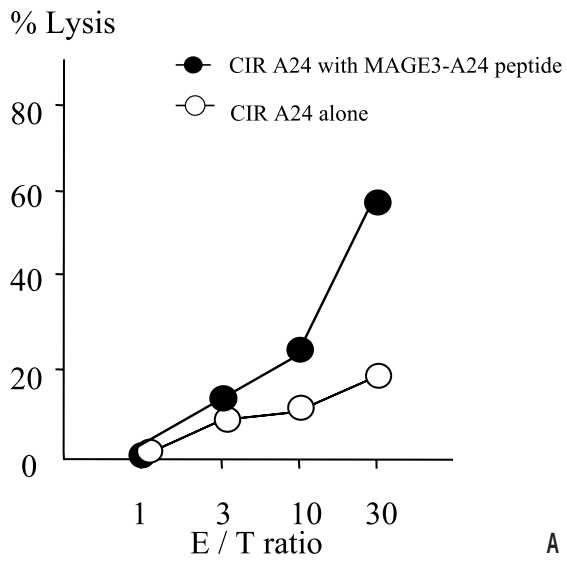
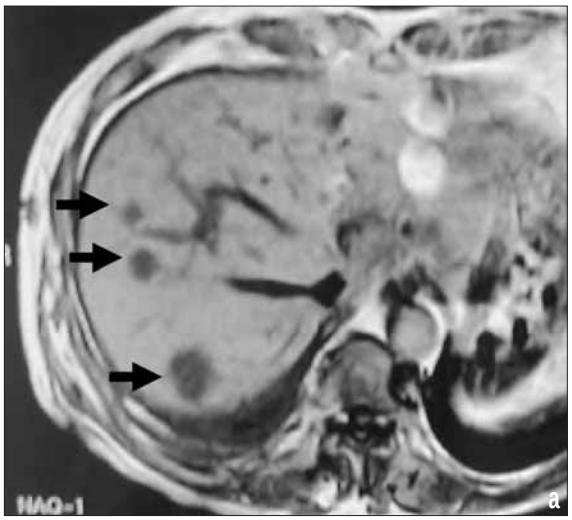


Figure 1. Change of tumor markers in patient 4

The tumor markers (CA19-9 and CEA) decreased remarkably during the 2 courses of the vaccination.





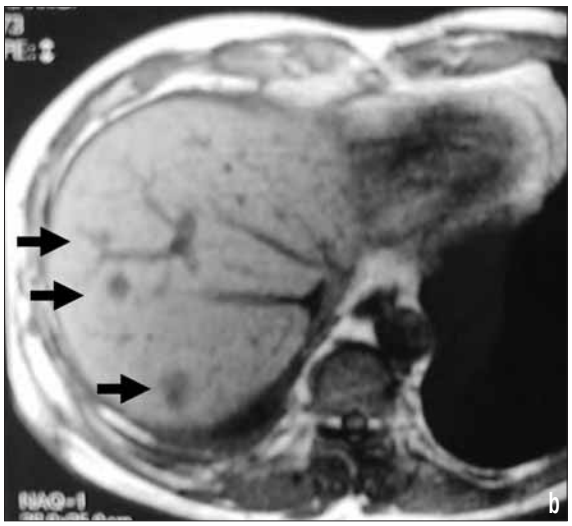


Figure 2. Clinical response of liver metastases in patient 4; a. November, 2001 (Before vaccination); b. March, 2002 (After vaccination)

The tumor size of liver metastases decrased up to 41% after the second course of the vaccination as shown in the magnetic resonance imaging findings.

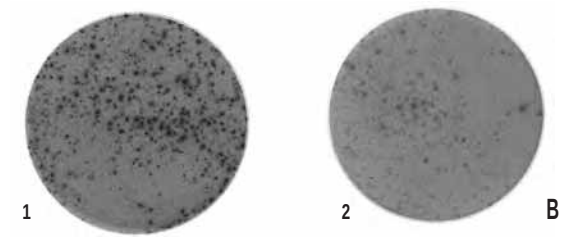


Figure 3. Immunological responses after vaccination; A) ^{51}Cr-release assay; B) Elispot assay: 1) CIR A24 pulsed with MAGE-3 peptide, number of spots: 340; 2) CIR A24 alone, number of spots: 114

Peripheral blood mononuclear cells (PBMCs) were evaluated after 2 times of *in vitro* peptide-stimulation. A) The cytotoxicity of MAGE3 peptide specific CTL was assessed by a standard ^{51}Cr release assay. The CTL activity was compared between the CIR A24 cell line alone and the CIR A24 cell line pulsed with MAGE3 peptide. B) The Elispot assay with MAGE-A3 peptide specific CTL. For the Elispot assay, 96-well nitrocellulose plates were coated with anti-IFN-γ mAb. Presensitized PBL (1×10^5) and 5.0×10^4 target (CIR-A24 cells) were added to each well and incubated for 20 h. After washing the plate, biotinylated anti-IFN-γ mAb was added to each well and the plate was incubated for 2 h at 37°C. After a chromogenic reaction, the dark brownish-red spots were counted. Positivity was considered when the spots of MAGE-A3 peptide, which pulsed C1R-A24, were three times as much as that of C1R A24 alone. Peptide specific CTLs were detected in 4 out of 17 wells.

The tumor size of the lung and liver metastases were decreased by up to 30 % (Figure 2). The clinical response of the patient was diagnosed as partial response by RECIST criteria. The delayed type hypersensitive (DTH) reaction to the peptide became positive after the first course of the vaccination. After the 3 courses of vaccination, liver metastases showed re-growth. After that, the patient underwent treatment by epidermal growth factor receptor - tyrosine kinase inhibitor (EGFR-TKI) (gefitinib), resulting partial response (PR), because an exon 19 deletion of EGFR gene was detected in the primary lesion of his lung cancer. After 6.3 months with PR status, re-growth of the tumor was shown, and the gefitinib-resistant tumor showed T790M acquired mutation, as shown in previous report (17). The patient died from tumor progression 3 years after MAGE-A3 peptide vaccination.

The detection of precursor CTL was performed by ^{51}Cr-release assay and Elispot (enzyme-linked immunospot) assay after *in vitro* stimulation of the PBMCs (peripheral blood mononuclear cells). The *in vitro* stimulation of the PBMCs

was performed as described previously (18,19). Briefly, the PBMCs were suspended at 10^7 cells/ml in RPMI medium supplemented with 1% human serum. The PBMCs were incubated for 60 min at room temperature with the peptide MAGE-A3 (20 μM). These peptide-pulsed PBMCs were washed and suspended at 1×10^6 cells / 2 ml in each well, with RPMI medium in the presence of 10% human serum, IL-2 (20 U/ml), IL-4 (10 ng/ml), and IL-7 (10 ng/ml). On day 7, half of the medium was replaced by fresh medium containing IL-2, IL-4, IL-7, and MAGE-A3 peptide (20 μM). As a target for the ^{51}Cr release assay and Elispot assay, HLA-A24-transfected Epstein-Barr virus transformed lymphoblastoid cells (CIR-A24 cells) were used with or without peptide pulse.

After the 2nd course of the vaccination, the peptide specific CTLs in the peripheral blood were detected in 4 out of 17 wells, both in the ^{51}Cr release assay and in the Elispot assay, although precursor CTL could not be detected at all before the vaccination. The representative data are shown in Figure 3. These $CD8^+$ T cells did not recognize C1R-A24 cells without peptide pulse, while they revealed cytotoxic activity against C1R-A24 pulsed with MAGE-A3 peptide. The IFN-μ producing T cells were detected up to 340 spots per 10^5 PBMC in the stimulation with peptide pulsed C1R-A24 in the ELISpot assay, in comparison to 114 spots without the peptide pulse.

DISCUSSION

Cancer-germline antigens are encoded by genes that are completely silent in most normal tissues, but are expressed in various tumors (7-9). Therefore, they may represent attractive targets for cancer specific immunotherapy in different fields of clinical oncology. Activation of cancer-germline genes in tumor cells has been reported to be associated with demethylation of their promoters in the process of the acquisition of malignant characteristics (20-22). MAGE proteins have been reported to lack signal sequences and contain a potential transmembrane domain that may function such as anti-apoptotic property (23). However, a recent investigation indicates that the expression of MAGE genes in cancer cells contributes directly to the malignant phenotype and the response to various therapies, and that the sensitivity to tumor-necrosis factor (TNF) are correlated with the expression of *MAGE-A1, MAGE-A2,* or *MAGE-A3* in human cancer cell lines (24). The overexpression of the *MAGE-A2* or *MAGE-A6* genes also leads to the acquisition of resistance to the widely used chemotherapeutic drugs such as paclitaxel and doxorubicin (25). Because of their strict tumor-specificity, cancer-germline genes are very promising targets for anticancer vaccines.

Atanackovic et al. reported that MAGE-A3 protein vaccination was performed in patients with MAGE-A3-expressing NSCLC at stage I or II in the post-operative adjuvant setting (26). The MAGE-A3 protein vaccination in combination with adjuvant AS02B (Adjuvant System 2B; GlaxoSmithKline) induced remarkable $CD4^+$ T cell responses that correlated with antibody production (27). Furthermore, another MAGE-A3 protein immunotherapy as post-operative adjuvant therapy was performed in stage IB/II NSCLC patients in a randomized Phase II study (27). The results were excellent compliance with treatment and feasibility with minimal toxicity. The recombinant MAGE-A3 protein vaccine revealed a 27% reduction in the relative risk of cancer recurrence following surgery, in comparison to the placebo group. These Phase II trial with MAGE-A3 protein suggested that patients with microscopic residual disease are considered to be good candidates for immunotherapy. Based on these promising results, a further Phase III study is now in progress (28). MAGE-$A3_{195\text{-}203}$ peptide IMPKAGLLI was identified using peptide stimulating approach (reverse immunology method) (29). It was previously reported that MAGE-$A3_{195\text{-}203}$ peptide was poorly processed in melanoma cell line and anti-MAGE-A3 CTL clone could not lyse melanoma cell line which was both positive for MAGE-A3 and HLA-A24 (30). However, several clinical trial used the MAGE-$A3_{195\text{-}203}$ peptide and clinical responses have been reported (31,32). The present case report also suggested that anti-MAGE-A3 CTL play a role in the tumor regression. One possible explanation of this discrepancy is change of CTL cytolytic activity during *in vitro* culture. Demottie et al. reported that human CTL clones lose their specific cytolytic activity and cytokine production under certain stimulation conditions and that tumor specific CTL clones often lose their specific effector function when they were restimulated with EBV-transformed B cells presenting the peptide instead of stimulation with tumor cells (33).

OK-432 is also well known to elicit local immune responses *in vivo* and to augment the cytotoxic activity of various effector cells such as lymphocytes, macrophages, and natural killer cells, and to induce the production of multiple cytokines (34,35). Recent reports demonstrated that Toll-like receptor (TLR) 4 is involved in anticancer immunity induced by OK-432 (36). OK-432-induced cytokine production by dendritic cells (DCs) is reported to be dependent on TLR4 - MD2 signaling pathway (37). On the basis of these findings, OK-432 is a useful immune adjuvant, which serves to induce a cancer specific immune response through various Th1 type cytokines and dendritic cell maturation. The present clinical study showed that immunization with MAGE-A3 peptides with OK-432 was feasible even in advanced NSCLC patients. No severe adverse effects were observed. The clinical responses generated by the vaccine were limited, but a significant decrease in the tumor marker and the tumor size were achieved during 2 courses of the vaccination. The clinical responses were accompanied with cellular immune responses such as CTL induction and DTH reaction.

A recent study of MAGE-A3 protein vaccination reported the importance of appropriate antigen priming using an adjuvant for generating persistent B and T cell memory, which allows typical booster responses with re-immunization (38). The present study suggested that MAGE-A3 peptide vaccination with OK-432 was also a safe treatment for advanced lung cancer patients, and that the vaccination has a potential to induce immunological response and clinical response in advanced lung cancer patients. There are still many hurdles to be overcome, however, the accumulated knowledge and improved techniques encourage us to believe that cancer vaccine strategies will become a hopeful and effective weapon against lung cancer in the future.

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Conflict of interest

We declare no conflicts of interest.

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