Recently, dendritic cells (DC) have been shown to play a crucial role in the induction of the primary T-cell-dependent immune responses. DC-based cancer immunotherapy has been shown to induce significant immunological responses and some clinical improvement in patients with different malignant disorders, including gastric carcinoma [14], prostate cancer [15], and the adenocarcinoma of colon [16]. The most effective route of administration of the tumor antigen-pulsed DC to induce effective anti-tumor responses remains to be determined. The migration and selective homing of DC, after the uptake of antigen from peripheral tissues and its delivery to the regional draining lymph nodes, is considered to be a distinct immunological property. In human studies, peripheral blood DCs administered in the upper thigh have been observed to migrate to the regional inguinal lymph nodes [17]. However, the migration patterns of DC from the upper respiratory tract have not been studied extensively in humans.

Most head and neck tumors arise from upper respiratory or gastrointestinal mucosa, and the lymph nodes in the neck region are the principal draining sites for these tissues. Thus, intramucosally administered DC may be expected to migrate to the regional lymph nodes and effectively induce mucosal anti-tumor responses locally.

In the present studies, we examined the migration of autologous, tumor antigen-pulsed DC, after administration into the nasal submucosa, direct inculcation into the nasopharyngeal tonsils, or after intravenous injection in a small number of patients with different carcinomas of head and neck region.

Materials and Methods

Eleven patients with squamous cell carcinomas of head and neck region were enrolled in this study, as shown in Table I. All patients were positive for human leukocyte antigen (HLA) A2402 and included four subjects with maxillary cancer and seven subjects with pharyngeal carcinoma. The study protocol was approved by the institutional ethics committee, and written informed consent was obtained from each patient.

SART-1 peptides

SART-1 is a carcinoma rejection antigen identified by using a cytotoxic T lymphocyte (CTL) clone developed from a patient with esophageal cancer. The clinical grade SART-1 peptides (SART-1 690–698) recognized by HLA-A2402 restricted tumor-specific cytolytic T lymphocytes [18] were prepared by NeoMPS. Inc., San Diego, CA. HLA-matched HIV-specific peptides (HIV Env 584–592) were used as controls [19].

Preparation of DCs

Peripheral blood mononuclear cells (PBMC) were prepared from 100 ml of peripheral blood from each patient. The cells were incubated for 2 h in a six-well plate under 5% CO_2 at 37°C. After the removal of non-adherent cells, the adherent cells were cultured in AIM V medium (Gibco, Rockville, MD) containing granulocyte macrophage colony stimulating factor (GM-CSF; 1,000 U/ml) and IL-4 (600 U/ml; Primmune K.K, Osaka, Japan) for 7 days to generate a DC-rich cell population. The cultured cells were harvested by vigorous washing. The enriched DCs were then cultured overnight in a medium containing 2 μg/ml squamous cell carcinoma specific SART-1 peptide. The DCs were then washed and resuspended in normal saline containing 5% autologous serum from a given patient. The phenotypic markers of the DC were determined by flow cytometry using monoclonal antibodies to HLA-DR, CD83, CD80, and CD86 (BD Pharmingen Fujisawa, Tokyo, Japan).

Table I Patients Profiles

Patient	Age/gender	Tumor lesion	Stage	Labeled DCs	Un-labeled DCs
1	68/M	maxilla	T4N1M0	ns	
2	72/M	maxilla	T4N0M0	ns	
3	67/M	maxilla	T4N0M0	ns	
4	47/M	pharynx	T3N1M1	ton	
5	56/M	pharynx	T2N1M0	ton	
6	70/F	maxilla	T3N0M0	iv	
7	56/M	pharynx	T3N1M0	iv	
8	73/M	pharynx	T2N3M0		ns
9	56/M	pharynx	T4N2M0		ns
10	71/F	pharynx	T4N2M0		ns
11	66/M	pharynx	T4N0M0		ns

DCs were administered into *ns* nasal submucosa, *ton* intratonsil, *iv* intravenous.

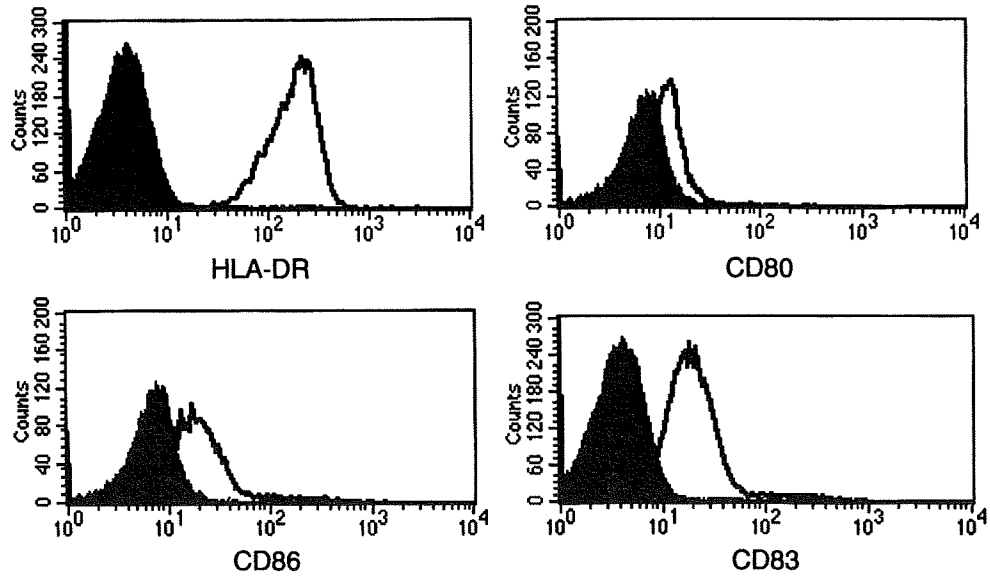

Fig. 1 The SART-1 pulsed DC possess an activated phenotype, including an upregulation of HLR-DR, CD80, CD86, and CD83, shown as *white*. The isotype match control is shown as *black*.

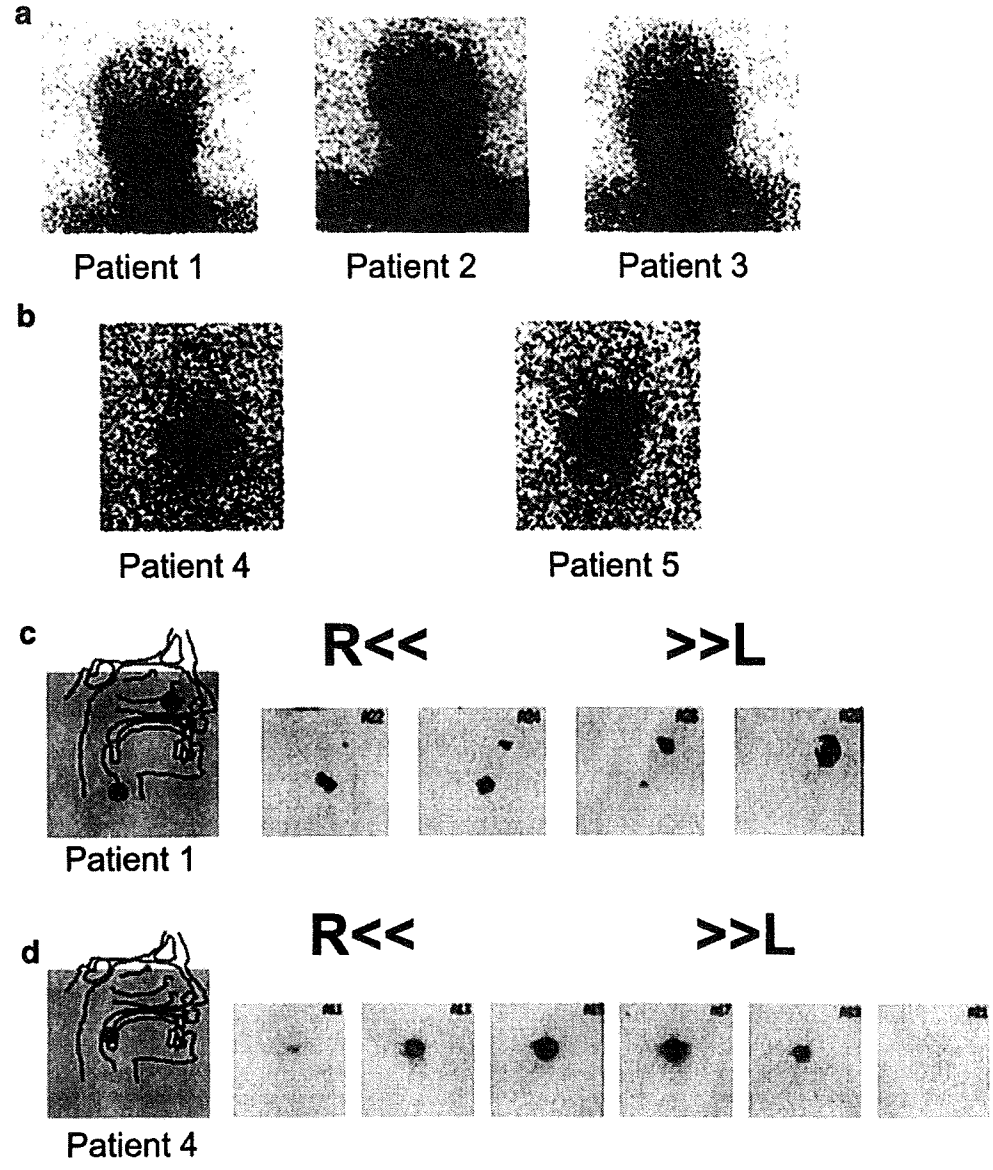

Fig. 2 An anterior image of the head and neck region recorded 48 h after injection. These patients were administered with DC into the left nasal inferior turbinate or tonsil. The DCs were intense in the neck as in the nose (**a**) or into the left palatine tonsil (**b**). A sagittal SPECT image of the head and neck region 48 h after injection. A series images were made at 2-cm intervals. The intense spots were observed in the injection site of nose and the regional lymph nodes (**c**). In contrast, when DCs were injected into the left palatine tonsil, the intense spots were only observed in the injected tonsil (**d**).

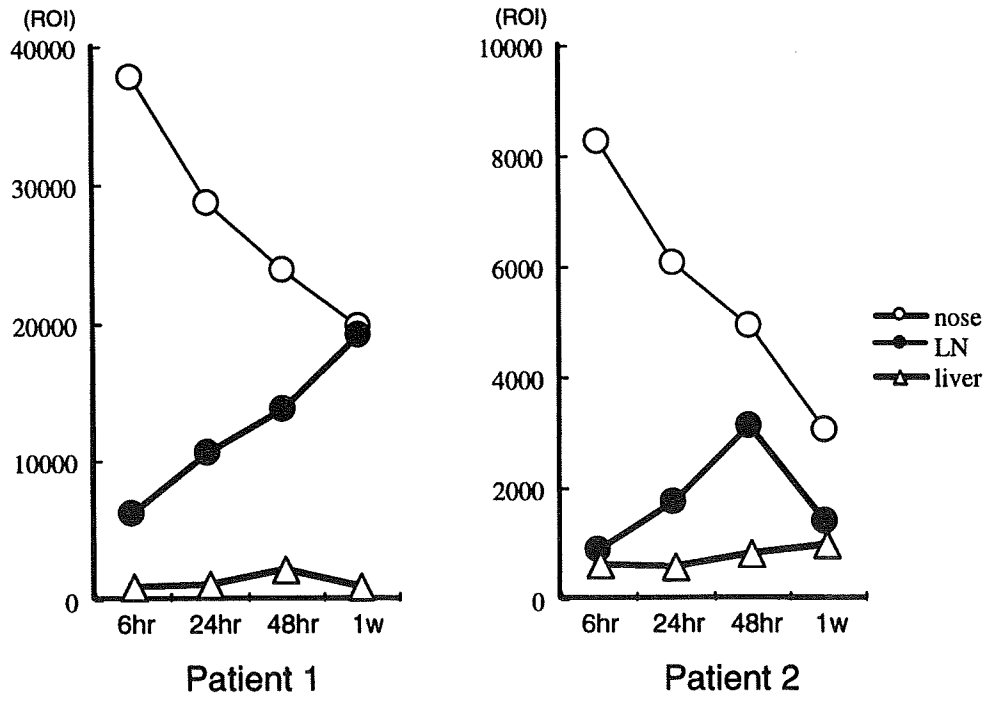

Fig. 3 The isotope counts in the ROI analysis of the neck, nose, and liver after the labeled DCs injection into the nasal mucosa. Although the isotope counts in the nose rapidly decreased, the peak count in the neck was observed 48 h or after. The count in the liver was lower.

In-111 Labeling of DC

Indium-111-Oxine(^{111}in) is used widely as a diagnostic radiopharmaceutical for radiolabeling autologous leukocytes (white blood cells). DC labeled with ^{111}In are routinely used to detect immunological events associated with antigen presentation. The labeling of DC with ^{111}In was done according to the manufacturer's protocol (Amersham Co. Ltd., UK). The SART-1 peptide-loaded DCs were resuspended in 10 ml phosphate-buffered saline (PBS) containing 37 Mbq ^{111}In ($T_{1/2}$=2.8005 days). After incubation for 15 min, the DCs were washed with normal saline containing 5% autologous serum. The labeling efficacy was determined by measuring the radioactivity of the cellular fraction in comparison to that of the supernatant, and it was usually found to be more than 80%.

Administration of Labeled DC

Three patients received an injection of 10×10^6 ^{111}In-labeled DCs in 200 μl of PBS submucosally into the inferior turbinate. Two patients received the same number of the DC via injection into the palatine tonsil. The remaining two patients received the DC intravenously (Table I). Total body image and single photon emission computed tomography (SPECT) images were performed 6, 24, 48 h and 7 days after the administration of the labeled DC to determine the kinetics of the DC migration. An image analysis was performed using a region-of-interest (ROI) analysis of the regional lymph nodes, using the liver as a control, to obtain decay-corrected counts and geometric means for the anterior and posterior views.

Detection of Tumor Antigen Specific Cytotoxic T Cells in the Neck Lymph Nodes

Four patients received the unlabeled DCs by nasal mucosal injection, and 7 to 10 days later, specimens of neck nodes in the neck were obtained after surgical dissection. The tumor antigen-specific activating IFN-γ secreting cells were identified by an enzyme-linked immunosorbent spot (ELISPOT) assay using the surgically obtained lymph nodes. After cutting the nodes into small pieces, lymphocytes were obtained with a cell strainer using the Ficoll–Hypaque technique. The lymphocytes were then washed and transferred 1×10^5 cells to an ELISPOT assay kit (BD Pharmingen) with 96-well filtration plates coated with anti-IFN-γ capture antibody (Mabtech Co. Ltd., Stockholm, Sweden) for 16 h. The stimulation conditions were 100 ng/ml of SART peptides in AIM-V. After extensive washing with PBS, biotinylated anti-human IFN-γ antibody was added. Two hours later, the spots were detected by an avidin biotin-peroxidase complex and aminoethyl carbazole solution. The mean values of the spots in three wells were utilized for subsequent analysis. The statistical analysis was done using a Wilcoxon-signed rank test for paired and unpaired data.

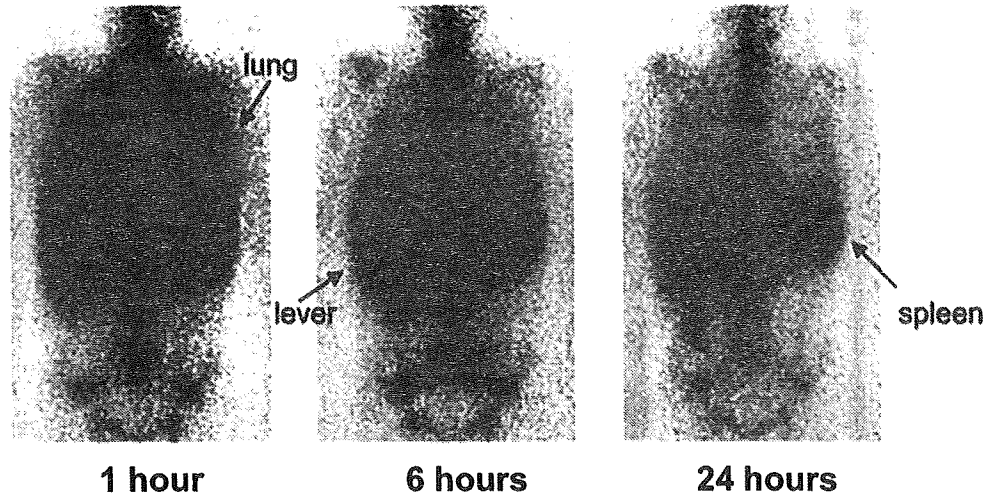

Fig. 4 An anterior image of chest recorded 1, 6, and 24 h after the intravenous injection of labeled DCs. The intense DC spots were observed in the lung and liver 1 h after injection, but the spots in the lung were very weak 6 h thereafter and then were not detected 24 h later.

Results

Using flow cytometry, the DCs were characterized by the expression of HLA-DR and by the lack of expression of CD3, CD14, CD19, and CD56 markers (data not shown). The DC represented an activated phenotype, as reflected by their increased expression of major histocompatibility complex class II (MHC II) molecules and costimulatory CD80 and CD86 molecules and by the presence of activation marker CD83 (Fig. 1).

After the administration of antigen-pulsed DCs, two patients developed transient mild fever, but no other symptoms or major physical abnormalities were detected. No major toxicity (above Common Terminology Criteria for Adverse Event-grade 2) was observed in any of the patients studied.

In the three patients who received labeled DCs via an injection into the nasal mucosa of the inferior turbinate, DC spots were observed at the primary injection sites, ipsilateral to the upper neck lymph nodes. An anterior image (Fig. 2a) and a SPECT image of a sagittal section (Fig. 2c) were recorded for 48 h after the injection of DCs into the left nasal inferior turbinate. The DCs spots were detected with high intensity in the neck region. The administered DCs migrated to the neck but did not spread within the nasal mucosa. An anterior image (Fig. 2b) of the head and neck area 48 h after injection and a SPECT sagittal section image (Fig. 2d) of the two patients who received labeled DCs through injection into the palatine tonsils are shown. The DCs remained in the injected tonsils and did not migrate to the neck lymph nodes or to the nasal mucosa.

The radioactivity counts in the neck, nose, and liver for the two patients who received labeled DC via nasal mucosal injection are shown in Fig. 3. Peak radioactivity was observed at 48 h. Although the isotope counts in the nose decreased rapidly, some activity was observed in the neck lymph nodes even 6 h after administration of labeled DC. The counts in the liver were lower and the peak obscure.

When the labeled DC were administered intravenously, the DC spots were observed in the lung, liver, and spleen 1 h after injection but were detected only rarely 6 h later. The DC spots were detected regularly in the liver and spleen 24 h later (Fig. 4). No DC spots were observed in the neck or in the upper respiratory mucosa.

Figure 5 shows the IFN-g secreting cells in the upper neck lymph nodes 5 days after the administration of the SART-peptides-pulsed DCs into the nasal submucosa.

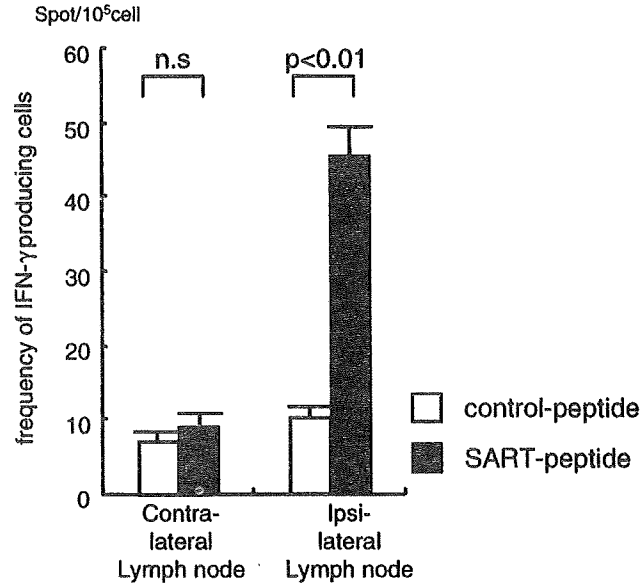

Fig. 5 IFN-g-producing cells in the upper neck lymph nodes after the administration of the SART peptides pulsed DCs into the nasal submucosa. SART-specific IFN-g-producing cells were detected in the ipsilateral lymph nodes of the administered DCs but not in the contralateral lymph nodes 5 days after administration. The data was expressed as mean±SD of the detected spots in each two lymph nodes of ipsilateral and contralateral sides from four patients.

SART peptides specific IFN-γ secreting cells induced by the DCs were detected only in the ipsilateral neck lymph nodes after the DCs administration but not in the contra lateral lymph nodes after 5 days.

Discussion

In this study, we examined the migration of isotope-labeled tumor antigen-specific peptide-pulsed DC after administration in the upper respiratory mucosa. The DCs administered to the palatine tonsils remained at the injection site in the tonsils and did not migrate to the neck lymph nodes. These observations suggest that human tonsils serve as an inductive site of mucosal immunity. An earlier study on the intratonsillar immunization with cholera and tetanus toxoids has shown the induction of the antibody secreting cells in the tonsils and specific IgA and IgG antibody response in the nasal wash and in the serum [20]. These observations support the possibility that human tonsils support the development of immunologic memory and serve as an inductive site for mucosal immune responses.

In the present studies, it was observed that the DCs administered into the nasal submucosa quickly migrated to the regional neck lymph nodes of ipsilateral sides of the administration, and this migration increased over time, but spreading within the nasal mucosa was not observed. These DCs also tended to remain in the neck and, after migration, induced the tumor-antigen-specific IFN-γ secreting cells in the neck lymph nodes in the ipsilateral sides of the DCs administration but not on the contra lateral sides where labeled DCs spots were not observed.

The migration of DCs, upon the uptake of antigens, from peripheral tissues to the regional draining lymph nodes is considered to be an important immunological function of such cells. However, the migration of DCs in the upper respiratory tract has not been studied extensively in man. The observations reported in this study suggest that the major area of antigen presentation and of induction of memory cells or effector cells may be in the regional neck lymph nodes in humans and not in the nasal mucosa. Most head and neck cancers arise from the upper respiratory or digestive mucosa, and neck lymph nodes tend to normally be defined as regional. The submucosally administered DCs are thus expected to migrate to the regional lymph nodes, thereby effectively inducing mucosal anti-tumor responses. The actual induction of tumor antigen-specific IFN-γ secreting cells in the neck lymph nodes after tumor antigen-pulsed DCs administration into the nasal submucosa observed in this study may have therapeutic implications in the specific immunologic treatment of some head and neck cancers. However, DC migration is known to be affected by various proinflammatory and anti-inflammatory mediators [21, 22]. In addition, cancer cells could produce factors, which would affect DC migration [23]. The influence of these mediators and of pulsed tumor antigen on DC migration need to be studied in further detail.

Conclusions

In summary, the tumor specific-antigen-pulsed DCs administered into the nasal submucosa of the patients with head and neck cancer quickly migrated to the regional neck lymph nodes and induced the tumor antigen-specific IFN-γ secreting cells. Further studies should be carried out to examine the antigen-processing phenomenon in the nasal mucosa and the distribution of the CTLs or effector cells in the nasal mucosa and tonsils and the cancer lesions after the administration of antigen-pulsed DCs.

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ORIGINAL ARTICLE

Phase I study of α-galactosylceramide-pulsed antigen presenting cells administration to the nasal submucosa in unresectable or recurrent head and neck cancer

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Abstract
Background Human Vα24 natural killer T (NKT) cells are activated by the specific ligand, α-galactosylceramide (α-GalCer), in a CD1d-dependent manner. Potent anti-tumor activity of activated NKT cells has been previously demonstrated.
Methods We conducted a phase I study with α-GalCer-pulsed antigen presenting cells (APCs) administered in the nasal submucosa of patients with head and neck cancer, and evaluated the safety and feasibility of such a treatment. Nine patients with unresectable or recurrent head and neck cancer received two treatments 1 week apart, of 1×10^8 of α-GalCer-pulsed autologous APCs into the nasal submucosa.
Results During the clinical study period, no serious adverse events (Common Terminology Criteria for Adverse Events version 3.0 greater than grade 3) were observed. After the first and the second administration of α-GalCer-pulsed APCs, an increased number of NKT cells was observed in four patients and enhanced natural killer activity was detected in the peripheral blood of eight patients.
Conclusion The administration of α-GalCer-pulsed APCs into the nasal submucosa was found to be safe and induce anti-tumor activity in some patients.

Keywords NKT cell · α-Galactosylceramide · Nasal submucosa · Clinical trial · Immunotherapy

Abbreviations

α-GalCer	α-Galactosylceramide
APCs	Antigen presenting cells
CT	Chemotherapy
CTCAE	Common Terminology Criteria for Adverse Events
DCs	Dendritic cells
NK	Natural killer
NKT	Natural killer T
OP	Operation
PBMCs	Peripheral blood mononuclear cells
PD	Progressive disease
PR	Partial response
PS	Performance status
QOL	Quality of life
RT	Radiation therapy
SD	Stable disease

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Introduction

Head and neck cancer has been estimated to be the sixth most common malignancy worldwide, with about 500,000 patients diagnosed annually. The management of head and neck cancer has generally involved the combined-modalities of chemotherapy, radiation therapy and surgery. In recent years, cisplatin-based chemotherapy combined with radiation has been widely employed in the treatment of

advanced head and neck cancer with the intent of preserving speech and swallowing or in the post-operative setting for patients at high risk for recurrence [1, 2]. However, the increased toxicity and extensive functional morbidity induced by these combined therapies can severely impair the quality of life (QOL), and, at the same time, the prognoses remains poor [3]. The improved survival rate by intensive combined therapy has only been shown to be 0~10% more effective than conventional radiotherapy. In order to improve the prognosis and QOL of patients with head and neck cancer, the development of new treatment strategies is therefore of critical importance [4–6].

Natural killer T (NKT) cells represent a unique lymphocyte subpopulation that is characterized by the co-expression of T cell and natural killer (NK) receptors [7–10]. NKT cells exert strong anti-tumor activity both in vivo and in vitro settings [8, 11, 12]. NKT cells are activated by a specific glycolipid antigen; α-galactosylceramide (α-GalCer) in a CD1d-dependent manner [13, 14]. CD1d is a HLA class 1b antigen priming molecule that does not exhibit any type of allelic polymorphism. Therefore, the anti-tumor activity of NKT cells is not restricted to MHC antigens. Several clinical trials aimed at NKT cell activation using α-GalCer have been carried out [15–17]. Recently, we have demonstrated the safety and effectiveness of induction of NKT cell activation by the administration of 1×10^9 antigen presenting cells (APCs) pulsed with α-GalCer, in patients with lung cancer [18].

The present studies were designed to evaluate the safety, immunological responses and possible anti-tumor effects of a smaller number (1×10^8) of α-GalCer-pulsed APCs, administered in the nasal submucosa in patients with unresectable or recurrent head and neck cancer.

Patients and methods

Patient eligibility criteria

A group of patients between 20 and 80 years of age, with a histological diagnosis of squamous cell carcinoma of the head and neck, for which no standard treatment was available, were enrolled in the study. Other criteria for enrollment included a performance status (PS) of 0, 1, or 2; an expected survival of 6 months or more; and normal or near-normal renal, hepatic, and hematopoietic functions. The patients had not received chemotherapy or radiotherapy for at least 6 weeks prior to enrollment. The exclusion criteria for exclusion from these studies included positive antibody activity against HIV, hepatitis C virus, or human T-cell lymphotrophic virus; presence of hepatitis B surface antigen; active inflammatory disease or active autoimmune disease; a history of hepatitis; pregnancy or infections; concurrent corticosteroid therapy; and existence of another malignant neoplasm. The histological types and the anti-tumor effects of the treatment were classified according to the general rules for the clinical and pathologic recording of cancer as described by the Japanese Head and Neck Cancer Society.

Preparation of APCs containing dendritic cells from peripheral blood

All procedures were conducted according to Good Manufacturing Practice standards. Peripheral blood mononuclear cells (PBMCs) were collected from peripheral blood samples from nine patients and separated by density gradient centrifugation (OptiPrep, Nycomed Amersham, Oslo, Norway). PBMCs were washed three times and resuspended in AIM-V (Invitrogen Corp., Carlsbad, CA, USA) with 800 units/ml of human granulocyte macrophage colony-stimulating factor (GeneTech Co., Ltd., China) and 100 Japanese reference units per milliliter of recombinant human IL-2 (Imunace, Shionogi, Osaka, Japan) as described [18]. After 7 days of cultivation, the cells were harvested and washed three times and then resuspended in 0.2 ml of 2.5% albumin in saline. The cultured cells were pulsed with 100 ng/ml of α-GalCer (KRN7000; Kirin Brewery, Gunma, Japan) on the day before administration. The cultured cells were then administered into the nasal submucosa of each patient. The criteria for α-GalCer-pulsed APCs administration included a negative bacterial culture 48 h prior to APCs administration, cell viability >60%, and an endotoxin test with <0.7 Ehrlich units/ml, 48 h before APCs administration.

Clinical protocol and study design

This study was conducted at the Department of Head and Neck Surgery, Chiba University Hospital, Japan. Written informed consent was obtained from each of the patients before undergoing a screening evaluation to determine their eligibility. The protocol was approved by the Institutional Ethics Committee. On days 0 and 7, 150 ml aliquots of peripheral blood were collected from each patient. The PMC were processed and pulsed with the antigen as described. The cells were resuspended to a final volume of 0.2 ml and injected into the nasal submucosa. Each patient received two injections of 1×10^8 α-GalCer-pulsed APCs. The injection site was the anterior portion of the bilateral inferior turbinate. We used a 27 G needle and a 1-mL syringe for the nasal submucosal injection. Extensive clinical and laboratory assessments were conducted weekly and included a complete physical examination and determination of standard laboratory values for 5 weeks. All patients underwent hematological and tumor status assessments by computed tomography both at baseline, 4 and 8 weeks after

the first administration ofα-GalCer-pulsed APCs (namely, 9 weeks after study entry).

APCs phenotype evaluation

The APC phenotypes were determined using a FacsCaliber flow cytometer (BD biosciences). The monoclonal antibodies (mAb) employed included FITC-labeled anti-HLA-DR, PE-labeled anti-CD86, and APC-labeled anti-CD11c (Becton Dickinson, San Diego, CA, USA). Isotype-matched mAbs were used as negative controls. The phenotypes of the cultured cells containing DCs were analyzed by flow cytometry prior to each administration. The DC-rich population (large, granular lymphocyte fraction) was electronically gated by forward and side scatter parameters (FSChigh SSChigh).

Immunological monitoring

The PBMC samples were obtained by extracting 30 ml of blood at least twice before the administration of APCs and weekly up to 3 weeks after the final treatment. The frequencies of $V\alpha24^+V\beta11^+$ NKT cells in PBMCs were assessed by flow cytometry. Mononuclear cells were stained with FITC-conjugated anti-T-cell receptor (TCR) Vα24 mAb (C15; Immunotech, Marseilles, France), PE-conjugated anti-TCR Vβ11 mAb (C21, Immunotech), and APC-conjugated anti-CD3ε mAb (UCTH1; PharMingen) as previously described [19]. The stained cells were subjected to flow cytometry and the percentages of $V\alpha24^+V\beta11^+CD3^+$ cells among mononuclear cells were calculated. The peripheral NKT cell numbers (counts/ml) were subsequently estimated based on the PBMCs cell counts. The remaining PBMCs were suspended to 5×10^5/ml in Cell Banker 2, and stored at −80°C.

ELISPOT assay of IFN-γ producing cells in PBMCs

Frozen PBMCs from each patient were thawed and cultured for 6 h in AIM-V. The cultured cells were washed and transferred to an ELISPOT assay kit (BD Pharmingen) in 96-well filtration plates coated with anti-IFN-γcapture antibody for 16 h [20]. The stimulation conditions were 100 ng/ml of α-GalCer in AIM-V. After extensive washing with PBS, biotinylated anti-human IFN-γ antibody was added. Two hours later, spots were detected by an avidin–biotin–peroxidase complex and aminoethyl carbazole solution. The mean values of the spots in three wells were utilized for the subsequent analysis. According to our ELISPOT assay protocol, the majority of the IFN-γ producing cells detected after α-GalCer stimulation for 16 h were determined to be $CD56^+$ NK and NKT cells.

Evaluation of antitumor effect

The disease progression was defined as an increase of target lesions of malignancy by >20% and the improvement of the disease was defined as a decrease in the target lesions by 30% or more. Any adverse events and changes in laboratory values were graded according to the Common Terminology Criteria for Adverse Events version 3.0.

Results

Patient characteristics

In accordance with the protocol, a total of nine patients who satisfied all entry criteria were enrolled into this study from January 2005 to March 2006. All patients completed this study without dropping out. The patient characteristics are summarized in Table 1. The median age was 59.2 years, and PS0/1 was 6/3, respectively. Five patients had previously undergone a surgical resection, while four patients had only undergone chemo-radiotherapy. Cases 001, 006, and 007 still had unresolved cancer lesions despite prior treatments, whereas the remaining cases had recurred after initial remission after previous treatment. As for the cancer lesions themselves, five cases had evidence of disease

Table 1 Patient profiles

Case	Age/gender	Primary lesion	Present tumor lesion	Pretreatment	History of bilateral neck dissection
Case 001	54/male	Hypopharynx	Primary lesion	CT, RT	–
Case 002	48/female	Maxillary sinus	Primary lesion	CT, RT	–
Case 003	65/male	Hypopharynx	Cervical lymphnode	CT, RT, OP	–[a]
Case 004	57/male	Maxillary sinus	Lung	CT, RT, OP	–[a]
Case 005	60/male	Hypopharynx	Lung	CT, RT, OP	+
Case 006	71/male	Hypopharynx	Lung	CT, RT	–
Case 007	63/male	Maxillary sinus	Primary lesion	CT, RT	–
Case 008	61/male	Mesopharynx	Primary lesion	CT, RT, OP	–[a]
Case 009	54/male	Larynx	Skin	CT, RT, OP	+

CT chemotherapy, *RT* radiation therapy, *OP* operation

[a] Unilateral neck dissection

limited to the local site, and four cases exhibited distal metastasis.

Adverse events

No major toxicity (above CTCAE-grade 2) or severe side effects were observed in any of the patients (Table 2). One patient (case 003) exhibited temporary anemia (CTCAE-grade 2) 1 week after the first injection, but recovered the following week without any treatment.

Clinical outcome

All nine cases were evaluated at the end of the clinical trial period. According to the findings of computed tomographic examinations with enhancement, one patient demonstrated a partial response with some clinical improvement (case 003). In this patient, the tumor diameter decreased from 22 to 7 mm. The computed tomography findings and the photographic view of the neck tumor mass before and after cell therapy in this patient (case 3) are shown in Fig. 1.

In other five cases no change was noticed in the existing diseases status, and three remaining patients cases exhibited continued progression of the disease (Table 2).

Phenotypes of APCs containing dendritic cells

The APC profiles of three representative cases are shown in Fig. 2. In all preparations, the FSChigh, SSChigh cells exhibited a mature monocyte-derived DC phenotype, expressing HLA-DR, CD11c, CD80, and CD86 (Fig. 2a). The proportion of DCs in the APCs were observed to vary from case to case (Fig. 2b).

Immunological monitoring

Specific immunological testing was conducted in each of the nine patients who completed this study (summarized in Table 3). As shown in Fig. 3, one patient (case 001) exhibited a dramatic increase in the number of circulating NKT cells after the first administration of α-GalCer-pulsed APCs. The absolute number of Vα24 NKT cells increased in four patients (cases 001, 002, 007 and 008). The increased levels were sustained for at least 1 week. However, no such increase was detected after the second administration of α-GalCer-pulsed APCs in cases 001, 002, and 007. The NKT cell numbers decreased slightly in five patients (cases 003, 004, 005, 006 and 009). In two patients who exhibited decreased NKT cells (cases 004 and 009), the number of NKT cells returned to pretreatment baseline values, while the cell numbers in the remaining cases continued to remain low. Only case 003 exhibited increased peripheral blood NK counts (data not shown), however, the remaining cases did not show any significant change in the number of NK cells in PBMCs.

ELISPOT assays revealed IFN-γ spot forming cells, thus reflecting the NK and NKT activity, and which increased substantially in eight cases (except for case 005) (Fig. 4, three representative cases are shown). Even after the second administration of α-GalCer-pulsed APCs, three patients (case 003,004, and 008) exhibited a further increase in the number of IFN-γ spot-forming cells. Case 005 exhibited a decrease in the number of IFN-γ spots after the second administration.

Table 2 Clinical effects

Case	Clinical outcome	Adverse event
Case 001	SD	–
Case 002	SD	–
Case 003	PR	Anemia (grade 2)
Case 004	PD	–
Case 005	PD	–
Case 006	SD	–
Case 007	SD	–
Case 008	SD	–
Case 009	PD	–

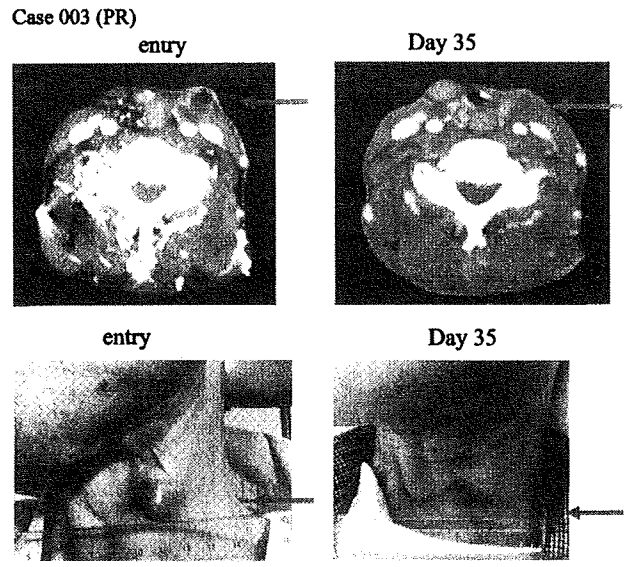
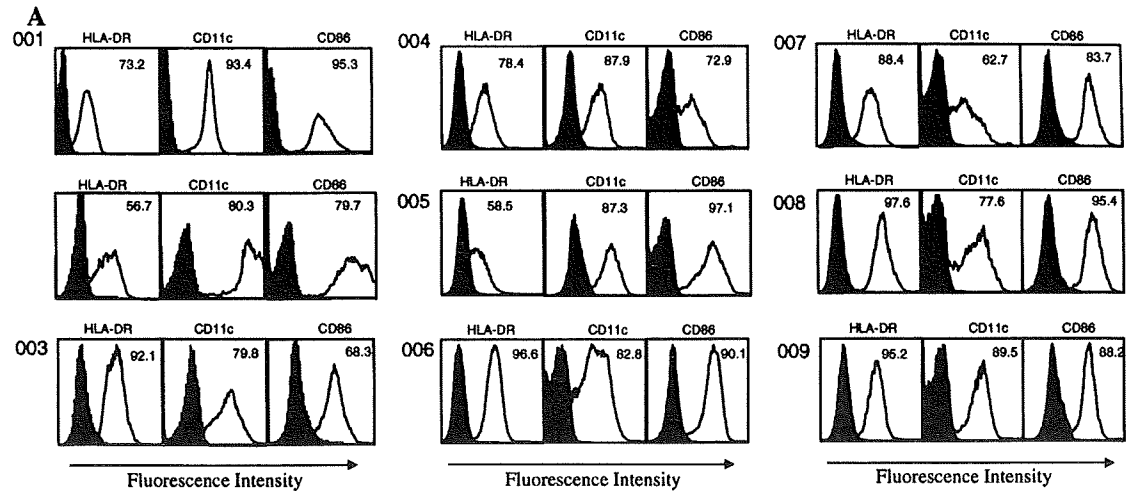


Fig. 1 Computed tomography findings and photographs of case 003 before and after treatment. The *arrows* indicate the tumor lesion

Discussion

The relative frequency of NKT cells in the peripheral blood are generally quite low (usually less than 0.1% of PBMCs),



B

CASE #	001	002	003	004	005	006	007	008	009
Number of DCs	$3.0x10^7$	$4.3x10^6$	$3.4x10^7$	$4.3x10^7$	$4.4x10^7$	$5.2x10^7$	$3.9x10^7$	$5.5x10^7$	$5.0x10^7$

Fig. 2 A flow cytometric analysis of APCs (GM/IL-2 cultured) in all cases. **a** The expression levels of HLA-DR, CD11c, CD86 assessed by flow cytometry. The *shaded areas*: background staining with an isotype control. The *solid lines*: staining profiles of the indicated molecules. The values represent the percentages of the positive cells. **b** A summary of the contents of the APCs administered for the first time. The percentages of the indicated fraction in whole APCs are shown. The number of cells with a DC phenotype ($CD11c^+HLA\text{-}DR^+CD86^+$) in the APCs ($1 \times 10^8$) is shown

Table 3 Immunological profiles of PBMCs

Case	Primary NKT cells frequency (%)	Primary NKT cell counts/ml	NKT cell increase	Enhanced NK activity
Case 001	0.14	1,021	+	+
Case 002	0.014	150	+	+
Case 003	0.0025	20	–	+
Case 004	0.04	664	–	+
Case 005	0.05	767	–	–
Case 006	0.007	67	–	+
Case 007	0.004	15	+	+
Case 008	0.022	150	+	+
Case 009	0.02	231	–	+

The frequencies and numbers of NKT cells in PBMCs at primary points were shown

these cells play a very important roles in the development of both innate and acquired immune responses [8, 9]. The anti-tumor activity of NKT cells has been well documented in several recent publications [11, 12]. NKT cells exhibit cytotoxic effects through direct killing. These cells have also been shown to activate NK and specific cytotoxic T cells. [21–24]. In our previous studies employing intravenous administration of α-GalCer-pulsed APCs [18] or activated NKT cells [20] in patients with lung cancer, an increased number of NKT cells and a significant enhancement of NK cell activity was observed in 30 and 80% of the patients, respectively.

Most head and neck cancers arise from the upper respiratory or digestive mucosa. In a study with mice, the proliferation of NKT cells was observed in cervical lymph nodes following the intranasal administration of α-GalCer [25], and when Salmonella toxin was administrated orally to sheep, the migration of antigen presenting DCs was observed from the oral mucosa to the regional lymph nodes [26]. In our previous study, when autologous indium-labeled DCs that had been pulsed with specific tumor antigen, were administered into the submucosa, the labeled DCs migrated quickly and selectively to the regional lymph nodes where they then remained for more than 1 week

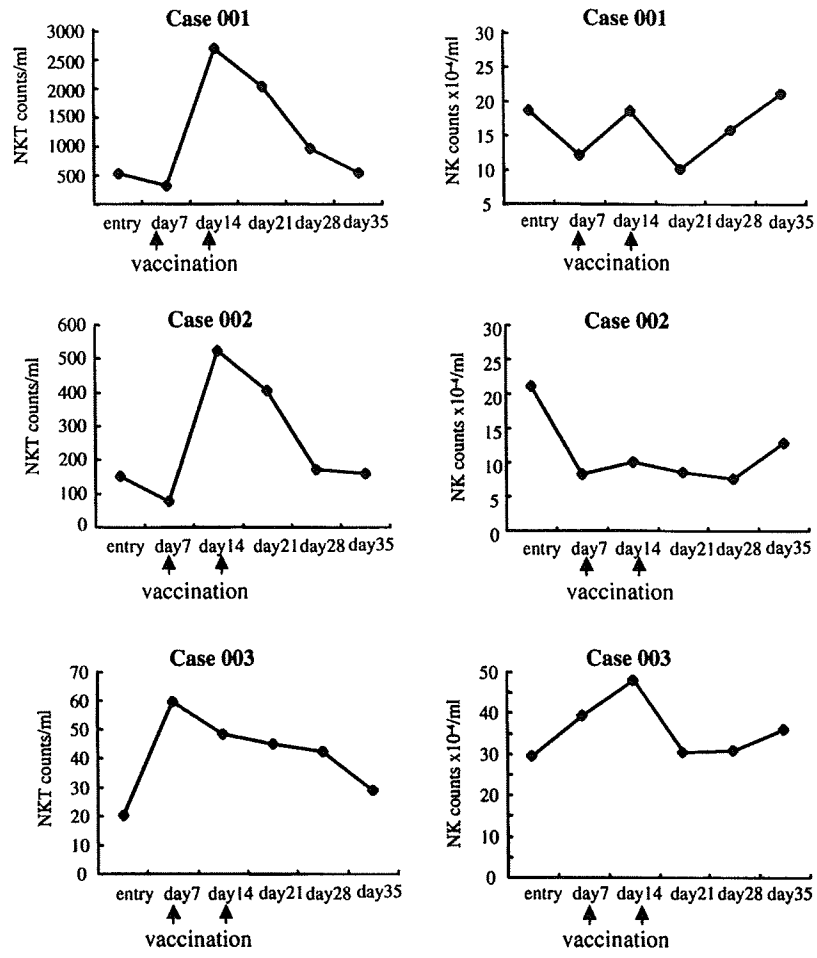


Fig. 3 The number of Vα24 NKT cells and NK cells in the peripheral blood during the course of treatment in three representative cases. The number of peripheral blood NKT cells ($V\alpha24^+V\beta11^+$ cells) and NK cells ($CD3^-CD56^+$ cells) are shown. Peripheral blood samples were taken before intranasal injection on days 7 and 14. The primary frequencies of the NKT cells in PBMCs of each case were as follows; case 001, 0.14%; case 002, 0.014%; case 003, 0.0025%

(unpublished observation). We therefore chose the submucosal administration of α-GalCer-pulsed APCs, which is expected to effectively induce mucosal anti-tumor responses. In fact, in some patients, we detected an increased number of NKT cells in the PBMCs and an increase in the number of IFN-γ producing NK cells in PBMCs (Table 3, Figs. 3 ,4). This is a striking observation because we injected only 1×10^8 APCs into the nasal submucosa, however, substantial changes in the systemic NKT/NK activity were observed. These results indicate that the regional immune system in the upper respiratory and digestive organs is quite unique in its ability to initiate and modulate local immunologic functions. The observations reported here suggest that submucosal administration of antigen or antigen-pulsed APC is an effective and useful route for the modulation of immune system.

In this study, we prepared APCs by culturing whole PBMCs with IL-2 and GM-CSF, but not by the use of either adherent or CD14-positive cells. When the proliferative activity of NKT cells was examined, a stronger response was observed with APCs derived from whole PBMCs than with those derived from adherent cells [27], thus suggesting the existence of a difference in the effective antigen presentation between glycolipid and protein antigens.

The patients tolerated well the treatment with 0.2 ml saline containing 1×10^8 of α-GalCer-pulsed APCs twice with a 1-week interval. The treatment did not induce any pain and could be administered to outpatients easily without any anodyne. No severe adverse events were observed in the present investigation. Temporally associated anemia (CTCAE-grade2) was observed in one case (case 003). This patient had gastric and esophageal cancer, and had already undergone a total gastrectomy and irradiation therapy 5 years previously, and hypopharyngeal cancer had later developed as a new malignancy. Despite surgery and chemotherapy, the patient experienced local recurrence, and therefore he was registered for this study. Even before entry into the study, an aggravation of the anemia was occasionally observed in this patient. Therefore, the causality of anemia as a result of immunotherapy employed in this study could not be clearly established.

In an animal model, some hepatic disorders were observed after α-GalCer administration [28–30]. In our previous clinical study with lung cancer patients, some mild adverse events, including hot flashes, headache, hyperkal-

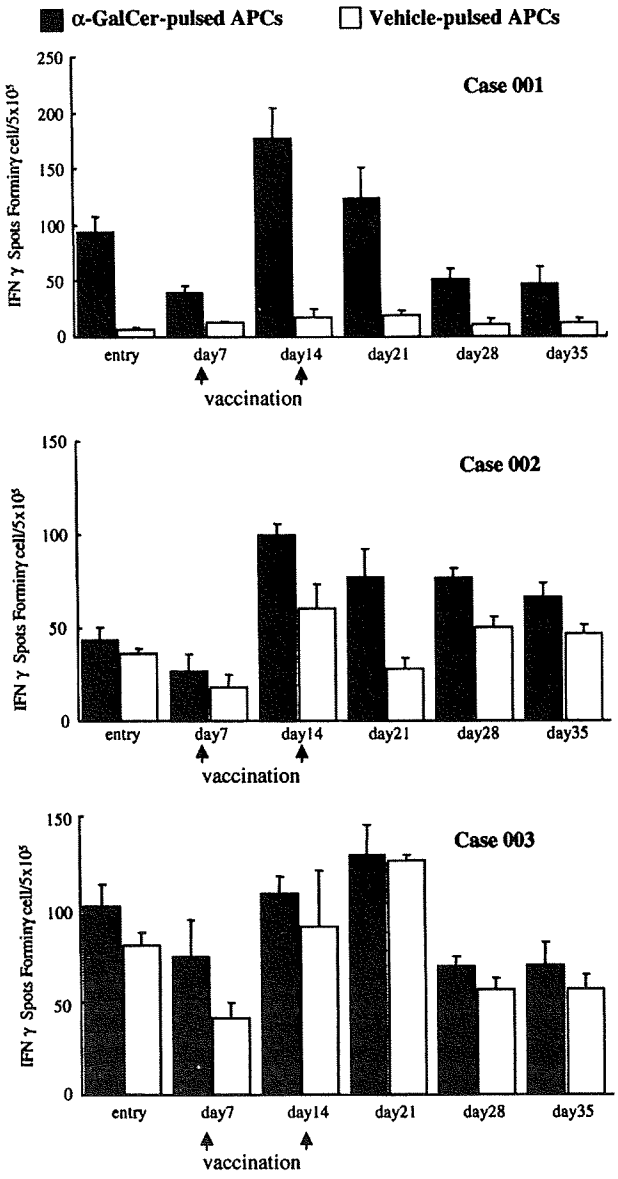


Fig. 4 The increased number of IFN-γ spot forming cells induced by in vitro stimulation with α-GalCer during the course of treatment in three representative cases. Peripheral blood samples were taken before the intranasal injection on days 7 and 14. Freeze-stocked PBMCs were thawed, and cultured for 6 h in AIM-V at 37°C. Next, the cells were stimulated with either α-GalCer (100 ng/ml) or vehicle in 5×10^5/well in a 96-well flat bottom plate for 16 h. All spots were counted, and the mean values are shown with SDs

emia, and general fatigue (CTCAE-grade 1 and 2) were observed [18]. The reason why fewer adverse events were observed in the current study may be related to the fact that a smaller number of α-GalCer-pulsed APCs were administered.

The observed immunological responses were comparable with those obtained in the previous study with lung cancer patients [18], into whom $1 \times 10^9/m^2$ of α-GalCer-pulsed APCs were administered intravenously. Even the smaller number of α-GalCer-pulsed APCs was sufficient to induce a significant activation of NKT cells and NK cells when administered directly into the sub-mucosa. The four patients who exhibited increased NKT cells (Table 3) had not undergone a bilateral neck dissection (cases 001, 002, 007, and 008). The presence of regional lymph nodes might therefore be indispensable for the effective increase of NKT cells. Nasal mucosal administration is easy for patients, as only 150 ml of peripheral blood extract is sufficient to prepare 1×10^8 APCs and no apheresis is necessary.

Following the intranasal mucosal administration of α-GalCer-pulsed APCs, the IFN-γ producing cells, which reflected the NK and NKT activities, were significantly enhanced in 89% (8/9 patients). It is difficult to determine the frequency of IFN-γ producing cells are NKT cells, because the expression of Vα24 TCR tends to be very quickly down-modulated after activation [31, 32] and the specific activation of NKT cells has been reported to lead to a rapid induction of extensive NK cell proliferation and cytotoxcity, partially depending on the IFN-γ production by NKT cells [21, 22, 33]. Case 005 alone did not exhibit any augmentation of the activities. This case had previously undergone an extensive bilateral neck dissection, which may therefore have impaired the development of increased NK and NKT activities. After the activation of NKT cells, the anti-tumor activities are considered to be mediated by NK cells as well as NKT cells, as the absolute number of NKT cells is quite few in comparison to that of NK cells. Therefore, the enhanced NK activities observed in most of the patients enrolled in this study are thus considered to support the effectiveness of this treatment. On the other hand, in five of nine patients who received intranasal α-GalCer-pulsed APCs to the submucosa, the number of NKT cells did not increase in the peripheral blood. The reason for this was not clear. The increased number of NKT cells might accumulate in the cancer lesions from the peripheral blood. Chang et al. reported the sustained expansion of NKT cells after two intravenous injections of α-GalCer-pulsed mature dendritic cells [34], however, the number of local infiltrating NKT cells around cancer lesions still needs to be determined.

Regarding the clinical outcome, one patient, five patients and three patients exhibited PR, SD, and PD, respectively (Table 2). This outcome does not rule out the clinical efficacy of the treatment, because the status of the patients enrolled in this study, who had either unresectable or recurrent cancer, must be categorized as very serious. The results therefore most likely reflect the poor condition of these patients. Case 003 exhibited a PR response. This patient had developed three separate cancers and had undergone combined therapies as described above. The recurrent local tumor reduced in size, from 22 to 7 mm in diameter, after

two treatments with α-GalCer-pulsed APCs and the pain was also alleviated for several weeks.

In summary, the submucosal administration of α-GalCer-pulsed APCs for patients with head and neck cancer was safe and a small number of these APCs without inconvenience of apheresis with no adverse effects could exhibit significant immune responses and some positive clinical effects. Further studies should therefore be conducted to examine NKT cells in the cancer tissue, as well as the regional neck lymph nodes, following submucosal administration.

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Current Article

上気道の粘膜免疫：基礎から臨床へ
鼻アレルギー・頭頸部癌の免疫治療への展開

岡本 美孝*

I はじめに

全身の免疫とは異なったさまざまな特徴を有する粘膜の免疫，なかでも外界からのさまざまな抗原，微生物の曝露を常時受けている上気道粘膜の免疫システムが注目を集めるようになって約40年が経過した。筆者も耳鼻咽喉科医を目指しながらこの上気道の粘膜免疫に興味をもって研究に従事してきた。一介の耳鼻咽喉科医としてその寄与は微々たるものでしかなかったが，教室を主宰するようになって上気道粘膜免疫の臨床への展開を図ることに教室を挙げて精力的に取り組んでいる。以下，特にアレルギー，癌に対する免疫治療の研究結果を報告する。

II アレルギー性鼻炎に対する粘膜免疫療法：スギ花粉症への意義と作用機序

アレルギー性鼻炎は現在も患者数の増加が目立っている。昨年の全国調査の報告では罹患率が全国民の40%近くに達し，特にスギ花粉症の増加が目立っており，有病率26.5%と10年前の調査に比較して10%以上の増加が報告されている[1]。アレルギー性鼻炎は直接死に至る疾患ではないが，このような患者数の増加と，睡眠，就業，学業への影響から患者のQOL障害が強いこと，さらに青壮年では自然改善が非常に少ないことから根本的な対応が望まれている[2]。

抗原特異的免疫療法（減感作療法）は唯一，この自然経過を改善させる方法として知られている。確かに欧米でのプラセボを対照とした比較試験での症状の改善効果は認められ，かつ臨床効果は投与終了後も少なくても数年は持続することが確認されていて，小児期のアレルギー性鼻炎に対する免疫治療はその後の喘息発症の抑制，ほかの抗原感作の獲得も抑制することが報告されている[3,4]。ただ，従来の皮下注射による免疫治療の意義は評価されているものの，一方で2年以上の治療期間が必要で注射での投与のためにその間50回以上の通院をする必要がある。また，頻度が低いとはいえ副作用がみられ，喘息発作は1,000～2,000回に1回，重篤な致命的にもなる全身アナフィラキシーは200万回の注射で1回生ずるとされている[5]。実際の投与に当たっては，注射後30分は医師の監視下に置き，ショックなどの反応出現に備えておく準備が必要である。この患者負担の大きさから，免疫療法は有効性が示され，かつ国内外のガイドラインで推奨されているのにもかかわらず，実際には実施する医療機関や受ける患者は減り続けている。

従来の抗原の皮下注射による抗原特異的免疫療法に替わる方法として抗原の粘膜投与が期待されている。経口，経鼻免疫といった方法も検討されてきたが，最も注目されているのは舌下免疫療法である。舌裏面に抗原の保存を図り，口腔底粘膜を利用した粘膜投与であり，医師の指導下ではあるが，自宅での投与が可能であり，重篤な副作用の減少から患者の負担が著しく軽減されるものとして注目されている。舌下減感作療法に関する研

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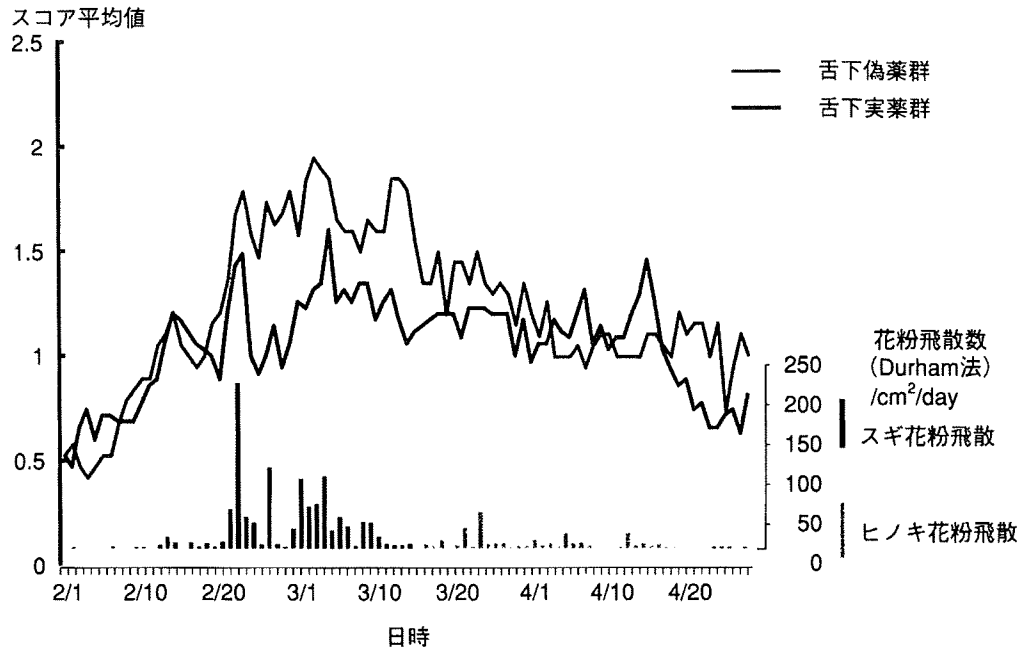

図 1　スギ花粉症患者を対象にして舌下免疫療法のランダム化試験の結果
横軸に日時，縦軸に症状スコア，花粉飛散量を示す。〔文献 8 より引用〕

究は，これまで主にヨーロッパで行われ，100 を超える臨床試験の報告があり，特に南ヨーロッパでは既に広く治療として認められている[6,7]。

ただ，舌下減感作療法についてはさまざまな抗原濃度，投与回数，投与期間，投与形態で検討が行われているが，抗原の違い，試験内容の違いからこれらの試験のメタ解析が容易ではない。一方，作用機序についてもさまざまな検討が行われているが，十分なコンセンサスは得られていない。同時に舌下免疫療法の有効性を示すバイオマーカー，治療効果の有無の予測因子の確立が求められている。

わが国特有といえるスギ花粉症に対しても舌下免疫療法の検討は奥田，大久保らによって 1990 年代後半から検討された。筆者らもランダム化試験に不可欠な concealment を徹底していくことで有効性についてのエビデンスの確立とさらにその作用機序，バイオマーカー，効果予測因子の解明の確立を目指して研究に加わった。

現在，スギ花粉症に対しては，皮下注射で用いられているスギ花粉エキス（トリイ®）しかないが，まずわれわれ自身で安全性を確認するため，2004〜2005 年にオープン試験を行い，その後，本エキスを用いて，スギ花粉症に対する舌下免疫療法の有効性を検討するため，プラセボ対照にランダム化比較試験を 2005〜2006 年に行った[8]。花粉曝露量をほぼ一定にする必要があり，対象は千葉市近郊に在住する成人スギ花粉症ボランティア 67 名で，2005 年 10 月よりスギ花粉エキス（実薬群）あるいは inactive placebo（偽薬群）を投与した。実薬，偽薬を 2：1 で振り分けた盲検法により，投与は 2 分間舌下に保持して吐き出す spit 法を用いた。Spit 法を用いたのはこれまでスギ花粉症に対する舌下免疫療法の安全性が確認されていなかったためであり，1 JAU/回の投与より開始して 1 か月で維持量 1,000 JAU/回として以後週 1 回で 4 月末まで投与を行い，アレルギー日記よりスギ花粉飛散時期の症状および薬物スコアを調査した。従来の皮下注射法では 200 JAU 0.2 ml を維持量としていたが，維持期の月当たりのエキスの投与量として当科での従来の皮下注射法に比較して約 300 倍に設定した。また，試験開始直前，花粉飛散直前（2006 年 1 月），投与終了後（2006 年 5 月）に採血を行い，スギ，ヒノキ花粉特異的 IgE 抗体，特異的 IgG4 抗体，総 Th1/Th2 細胞，Cry j 特異的 Th2（IL-4）メモリー細胞を測定した。

4 名が個人的理由で脱落したが，重篤な有害事象は認められなかった。2006 年の千葉市でのスギ，

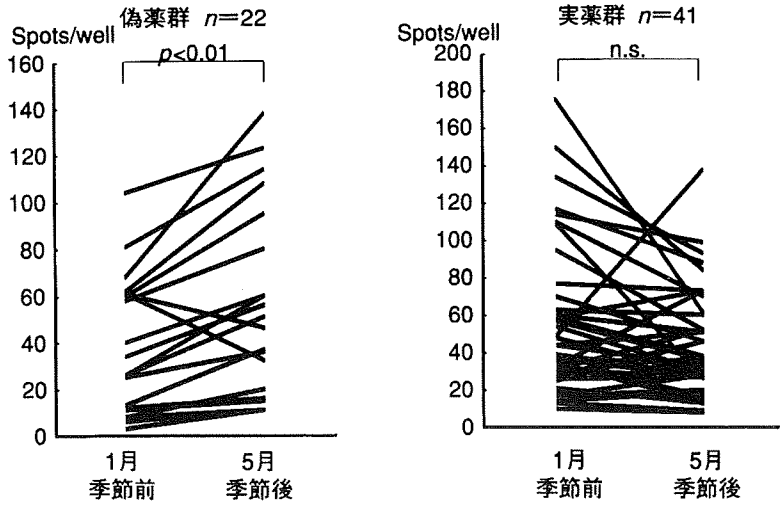

図 2　スギ花粉特異的 Th2 メモリー細胞のクローン数の変化
〔文献 8 より引用〕

ヒノキ花粉飛散数を**図 1** に示すが，スギ花粉飛散は 2 月中旬に始まり，3 月中旬に終息，以後はヒノキ花粉飛散が始まり 4 月中旬まで続いた。症状・薬物スコアの検討からスギ花粉飛散ピーク時には有意に実薬群でスコア値が低値を示した。これらの結果はスギ花粉症に対しても，舌下減感作療法の安全性と有効性を示唆するものであった。一方，血中 IgE 値，総 Th1/Th2 細胞では 2 群間に差はみられなかったが，血中の Cry j 特異的 IgG4 抗体は実薬群のみで上昇がみられた。特に興味深かったのは，enzyme-linked immunosorbent spot（ELISPOT）で測定した Cry j 特異的 Th2（IL-4 産生）細胞クローンで，この数は季節性変動を示すことを以前確認しており[9]，偽薬群では確かにスギ花粉飛散後に飛散前と比較して増加がみられた。しかし，実薬群では増加は認められず（**図 2**），免疫療法の作用機序との関連が考えられた。

この結果を基に 120 名の成人スギ花粉症患者を対象に 2006 年秋～2008 年 4 月まで 1 年半に及ぶ同様の投与法によるプラセボ対照比較試験を行った。一定の期待した効果が得られている（投稿中）。ただ，スギ花粉症に対する免疫治療の問題点は抗原エキスの濃度が WHO やこれまでの欧米での臨床試験から推奨されている濃度に比べて 100 分の 1 以下と低い点である。この背景には欧米で花粉症の中心である草本花粉に比較してスギ花粉の抗原抽出は非常に難しいこと，皮下注射では現在の標準化されたスギ花粉エキス低濃度でも強い痛みの誘発と免疫応答の誘導は確認されていることから，花粉の違いにより至適投与抗原濃度，投与プロトコールの設定は独自に必要である。昨年からはスギ花粉エキスの連日投与による比較試験を行っており，現在この試験についてもキーオープンに向けて解析を進めているところである。また，作用機序の解明から，有効性を示すバイオマーカーの検討，抗原特異的免疫療法の効果を有する症例の予測因子の検討はこの治療法の普及に欠かせない。前述した最初のランダム化試験結果から抗原特異的 Th2 細胞の増加抑制がみられていることから，2007～2008 年にまず少数例ではあるがスギ花粉症患者対象にオープン試験を行い，バイオマーカーの検討を行った。その結果，末梢血液中の Cry j 1 特異的 FoxP3 陽性，IL-10 陽性，CD4 陽性，CD25 陽性細胞が，抗原特異的 Th2 細胞，さらに臨床効果と高い関連を示すことが見出され(**図 3**)[10]，現在二重盲検試験での多数症例でその結果を追試している。

前述した抗原特異的 Th2 メモリー細胞クローンとこの抗原特異的調節性 T 細胞，さらに発症に直接かかわる肥満細胞のシグナル伝達へのこれらの細胞の関与，関連を明らかにしていくことが舌下免疫療法の機序としての key になるものと思われる。

一方，舌下免疫療法の有効性の向上，特に経済

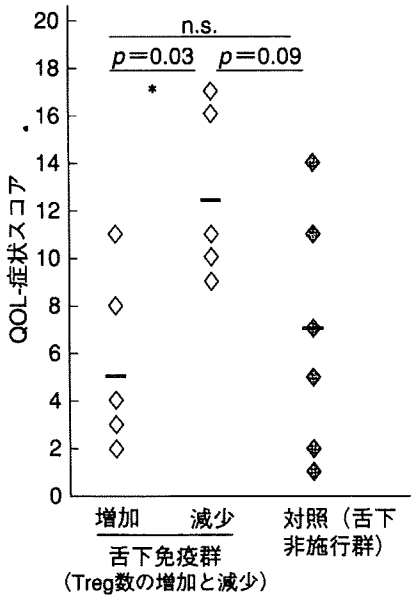

図 3　Cry j 1 特異的調節性 T 細胞数の変動
〔文献 10 より引用〕

性向上，投与期間の短縮には有効なアジュバントの開発が不可欠である。現在，世界でさまざまなアジュバントの開発が進んでいるが，われわれは乳酸菌に注目して検討を進めている。抗原とともに乳酸菌を取り込んだ未熟樹状細胞は CCR-7 というケモカインの発現を亢進して migration 能が増強すること，PD-L2 という副刺激シグナルの発現増強を介して選択的に Th2 細胞からのサイトカイン産生の抑制が生ずることがヒト樹状細胞を用いた *in vitro* の検討，ならびにマウスでの *in vivo* での検討で明らかになった（投稿中）。スギ花粉症患者を対象にランダム化試験に取り組んでいる。投与量や投与スケジュールなどの確立が不可欠であるが，高い安全性もあり，臨床展開が期待される。

III 頭頸部癌に対する細胞免疫治療

進行頭頸部癌の治療成績は向上したとはいえ，拡大切除を行っても 5 年生存率の多くは 40～50% 以下であり，また，化学療法併用放射線の治療効果も認められるとはいえ，さらなる治療成績の向上，患者負担の軽減には新規治療法の開発が不可欠である。癌の成長因子をターゲットにしたさまざまな抗体治療については，欧米ですでにいくつも臨床試験が先行している。ただヒト化抗体とはいえ，安全性の検討も含めて膨大なコスト，時間は必要で，製薬メーカーの強力な back up が不可欠で容易にできるものではない。また，魅力はあるが遺伝子治療の開発はまだまだハードルが高い。

細胞免疫治療は患者の細胞を取り出して，これに『加工』（抗腫瘍活性の付加）を加えて体内に戻す治療であり，安全性の確認，『加工』の内容によっては高額なコストが必要であるが，患者自身の細胞を用いるためそのハードルは比較的低い。代表が養子免疫療法で，いわゆる LAK（lymphokine activated killer）細胞を用いた LAK 療法が以前より臨床導入され，数多くの施設で試みられた。ただ，悪性黒色腫，腎細胞癌などの腫瘍にのみある程度の効果があるとされている。この数年は，ヒト腫瘍において腫瘍関連抗原の同定がなされ，その自己腫瘍細胞の抗原を特異的に認識する細胞傷害性 T リンパ球（CTL）の存在が注目され，*in vitro* においては LAK 細胞に比べて約 100 倍の抗腫瘍効果が報告されている。実際に，癌患者由来のリンパ球から *in vitro* で CTL の誘導が可能となっている。

われわれも転移リンパ節浸潤リンパ球（TIL）を用いた免疫療法が患者の非担癌生存期間に及ぼす効果を明らかにすることを目的に，高率に遠隔転移が出現することが知られている下咽頭癌 N2b, N2c, N3 症例を対象に非担癌生存期間延長をエンドポイントにした歴史対照・単アームコホート試験を行った。すなわち根治切除手術を 2002 年 10 月～2003 年 8 月に受け，その後，化学療法併用（シスプラチン）術後照射療法からなる 3 者併用療法を受けた新鮮下咽頭癌 N2b, N2c あるいは N3 症例と，2003 年 9 月～2004 年の 9 月までの手術時に採取した移転リンパ節から分離した TIL を術後 3 週間目に放射線治療開始前に投与した，同様な新鮮下咽頭癌症例を対象とした。手術は，全例両側頸部郭清，咽頭・喉頭・頸部食道切除，遊離空腸（結腸を含む）再建で，同一術者が行い，術後化学療法（シスプラチン），放射線治療（60 Gy）の内容も同一であった。無菌的に採取した移転リンパ節から腫瘍浸潤リンパ球（TIL），リンパ節由来リンパ球（RLNL）を，不連続比重遠心法を用いて分離した。これらのリンパ球を無血清培地（AIM-V培地）にヒト ricombinant IL-2（rIL-2）を

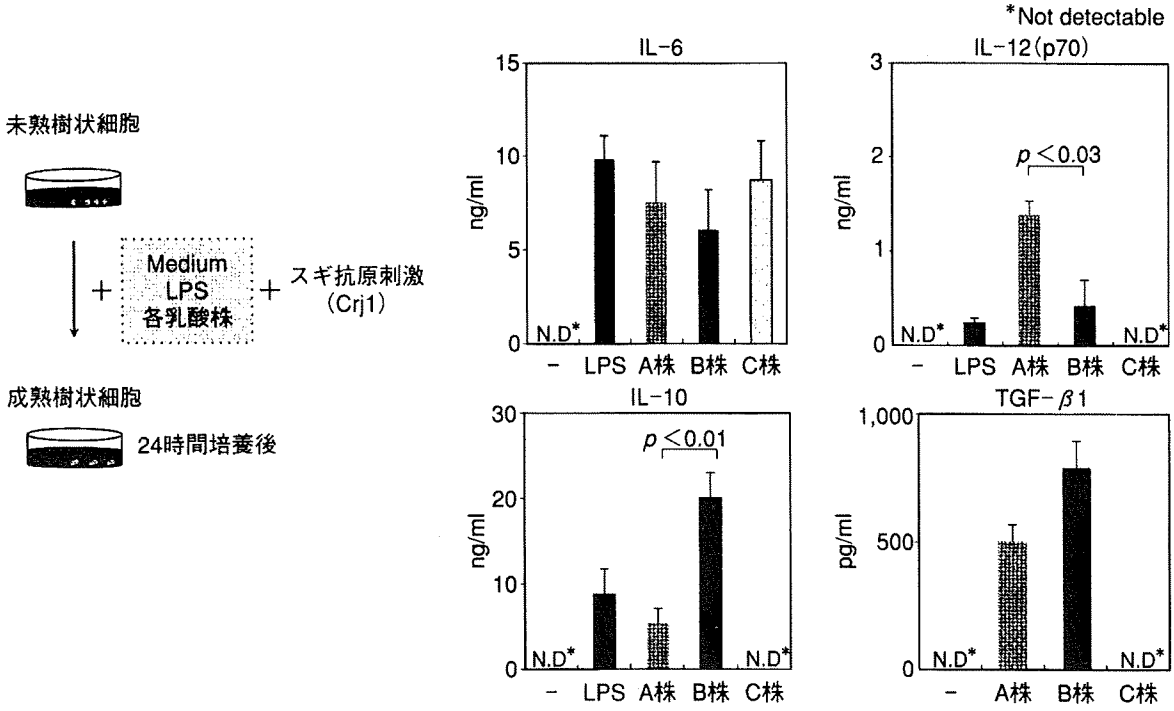

図 4　乳酸菌による樹状細胞の成熟化への影響（投稿中）

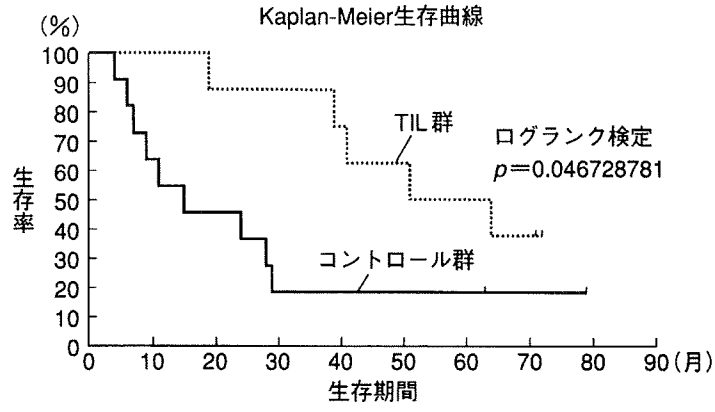

図 5　腫瘍浸潤 T 細胞を用いた下咽頭進行癌（N2b，N2c，N3）症例への adjuvant 治療

5 加えて，*in vitro* で約 3 週間培養した。3 週間培養した 1×10^8個以上のリンパ球を同患者に経静脈的経路で投与した。その際，このリンパ球の特異性を検討すべく，*in vitro* で自己腫瘍細胞（凍結保存）と混合培養し，リンパ球より放出される TNF-α を測定することにより，腫瘍特異性を確認した。

5 年以上経過した 18 症例の無再発生存期間を図 5 に示すが，TIL を併用していない下咽頭癌 N2b，N2c，N3 症例に比較して，同一術者による手術，術後照射・化学療法でも TIL 併用群では明らかに生存率の有意な上昇が認められた。

このように確かに TIL には進行癌治療後の再発を防ぐ一定のアジュバント効果が頭頸部癌でも認められる可能性が高いが，細胞免疫治療により能動的に腫瘍に対する免疫反応を誘導することができれば，さらに高い治療効果が期待される。近年，末梢血単核球から比較的容易に樹状細胞（dendritic cell：DC）を分離，調整できることが明らかにされ，DC を抗原提示細胞（antigen presenting cell：APC）として用いた能動免疫療法が臨床導入できるようになっている[11]。

頭頸部扁平上皮癌における細胞免疫治療の導入