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Neuroprotective Therapy Using Granulocyte Colony–Stimulating Factor for Patients With Worsening Symptoms of Thoracic Myelopathy

A Multicenter Prospective Controlled Trial

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Study Design. An open-labeled multicenter prospective controlled clinical trial.

Objective. To confirm the feasibility of granulocyte colony–stimulating factor (G-CSF) administration for patients with thoracic myelopathy.

Summary of Background Data. Although G-CSF is best known as an important cytokine commonly used to treat neutropenia, it also has nonhematopoietic functions. Previous experimental studies have shown that G-CSF can enhance tissue regeneration of several organs, such as the heart and the brain. We previously reported that G-CSF promotes functional recovery after spinal cord injury in rodents. On the basis of those findings, we started a clinical trial of neuroprotective therapy, using G-CSF for patients with worsening symptoms of thoracic myelopathy.

Methods. Patients whose Japanese Orthopaedic Association (JOA) score for thoracic myelopathy had decreased 2 points or more during a recent 1-month period were eligible for entry. After giving informed consent, patients were assigned to G-CSF and control groups. The G-CSF group (n = 10) received G-CSF 10 μg/kg per day intravenously for 5 consecutive days. The control group (n = 14) received similar treatments as the G-CSF group except for G-CSF administration. The primary outcome was JOA recovery rate at 1 month after G-CSF administration or initial treatment.

Results. There was greater improvement in neurological functioning between baseline and 1-month follow-up in the G-CSF group (JOA recovery rate: 29.1 ± 20.5%) than in the control group (JOA recovery rate: 1.1 ± 4.2%) ($P < 0.01$). No serious adverse events occurred during or after the G-CSF administration.

Conclusion. The results provide evidence that G-CSF administration caused neurological recovery in patients with worsening symptoms of thoracic compression myelopathy.

Key words: *neuroprotective therapy, granulocyte colony–stimulating factor, thoracic myelopathy, clinical trial.* **Spine 2012;37:1475–1478**

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Granulocyte colony–stimulating factor (G-CSF) is a 19.6 kDa glycoprotein. It is best known as a growth factor for hematopoietic progenitor cells and is commonly used to treat neutropenia and to mobilize peripheral blood-derived hematopoietic stem cells for transplantation.[1] Several experimental studies have indicated that G-CSF also has nonhematopoietic functions and can enhance the tissue regeneration of several organs such as the heart and the brain.[2,3] We previously reported that G-CSF promotes functional recovery after spinal cord injury in rodents.[4–6]

On the basis of the experimental results described earlier, we hypothesized that administration of G-CSF can effect neurological recovery in patients with progressive compression

myelopathy and started a phase I/IIa clinical trial of G-CSF neuroprotective therapy.[7] In this study, we conducted a multicenter prospective controlled clinical trial (phase IIb) to assess the feasibility of the G-CSF therapy for patients with worsening symptoms of thoracic compression myelopathy.

MATERIALS AND METHODS

This clinical trial was designed as an open-labeled multicenter prospective controlled study and was performed with the approval of the institutional review board of each participating institute. Since April 2010, we recruited patients 20 to 85 years of age, in whom the Japanese Orthopaedic Association (JOA) score (full score = 11 points) decreased 2 points or more during a recent 1-month period.[7]

We assigned patients to a G-CSF group and a control group. Patients in the G-CSF group were given G-CSF 10 μg/kg per day intravenously for 5 consecutive days. Patients in the control group were enrolled in similar treatments as the G-CSF group except for the G-CSF administration. To evaluate neurological improvement resulting from neuroprotective therapy with G-CSF, we planned to follow patients in both groups without surgical treatment for 1 month after G-CSF administration or initial treatment and to provide them with equivalent conservative treatment, such as bed rest. When patients were given informed consent documents, we explained our plans regarding the time of surgery, and we administered G-CSF only to those patients who agreed with the protocol.[7] The G-CSF therapy was performed only in the institute to which the corresponding author (MY) belonged. At the other institutes, patients were treated without G-CSF administration.

The primary outcome was the JOA recovery rate at 1 month after G-CSF administration or initial treatment. We evaluated the patients' severity of myelopathy using the JOA score.[7] Then, we evaluated their motor and sensory functions by determining scores for muscle power and pain sensation according to the American Spinal Injury Association score.[7] In this study, 2 orthopedic spine surgeons specializing in thoracic spine surgery evaluated patients' neurological status independently after G-CSF administration and then mean data were calculated. In addition, we analyzed hematological data from the treated patients.

Statistical analyses were performed using a Mann-Whitney *U* test and a Fisher exact probability test. A *P* value less than 0.05 was considered statistically significant. Results are presented as means ± standard deviation of the mean.

RESULTS

Patient Data

Between April 2010 and October 2010, 24 patients (10 patients in the G-CSF group and 14 patients in the control group) were enrolled and examined for 1 month. Patient data for both groups are summarized in Table 1. In the control group, many patients had the most stenotic level at the lower thoracic spine (T9–T12), although no statistical difference was observed in the distribution of the most stenotic level between the G-CSF and control groups. No statistical difference was observed between groups regarding the spinal canal occupation ratio by heterotopic ossification or vertebral spurs at the most stenotic level.

TABLE 1. G-CSF and Control Group Patient Data

	G-CSF	Control
No. of patients	10	14
Sex		
Male	9	11
Female	1	3
Age, *M* ± SD (range), yr	49.7 ± 8.9 (32–74)	53.1 ± 10.6 (22–72)
Diagnosis		
Thoracic OPLL	5	4
Thoracic OLF	2	6
Thoracic spondylotic myelopathy	3	4
Most stenotic level		
Upper thoracic (T1–T4)	4	4
Middle thoracic (T5–T8)	4	2
Lower thoracic (T9–T12)	2	8
Surgical procedure		
Posterior decompression	5	10
Posterior decompression with instrumented fusion	5	4

G-CSF indicates granulocyte colony–stimulating factor; OPLL, ossification of posterior longitudinal ligament; OLF, ossification of ligamentum flavum.

Neurological Recovery

The JOA score immediately before G-CSF administration or initial treatment was 3.8 ± 1.3 in the G-CSF group and 4.1 ± 1.4 in the control group, showing no statistical difference between groups (Table 2). There was greater improvement in neurological functioning between baseline and 1-month follow-up in the G-CSF group (JOA recovery rate: 29.1 ± 20.5%) than in the control group (JOA recovery rate: 1.1 ± 4.2%) ($P < 0.01$) (Table 2).

Regarding the muscle power score, greater improvement between baseline and 1-month follow-up was observed in the G-CSF group (improvement of muscle power score: 2.8 ± 2.8) than in the control group (improvement of muscle power score: 1.6 ± 5.3) ($P < 0.05$) (Table 2).

There was also greater improvement in the pain sensation score between baseline and 1-month follow-up in the G-CSF

TABLE 2. Neurological Recovery

	G-CSF $M \pm SD$ (range)	Control $M \pm SD$ (range)	*P*
JOA score			
Immediately before treatment	3.8 ± 1.3 (1–5.5)	4.1 ± 1.4 (1.5–6.0)	0.501
One month after treatment	5.7 ± 2.4 (1.0–9.0)	4.3 ± 1.3 (2.5–6.0)	0.061
Recovery rate	29.1 ± 20.5 (0.0–63.6)	1.1 ± 4.2 (0.0–15.8)	<0.01
Muscle power score			
Immediately before treatment	41.9 ± 7.8 (22–50)	37.0 ± 15.5 (0–50)	0.884
One month after treatment	44.7 ± 7.6 (25–50)	38.6 ± 12.6 (20–50)	0.241
Increase of muscle power score	2.8 ± 2.8 (0–9)	1.6 ± 5.3 (0–20)	<0.05
Pain sensation score			
Immediately before treatment	68.3 ± 9. 7 (59–78)	74.1 ± 9.8 (60–92)	0.364
One month after treatment	74. 7 ± 10.4 (62–88)	74.9 ± 8.9 (64–92)	0.578
Increase of pain sensation score	6.4 ± 5.5 (1–17)	1.0 ± 3.2 (0–12)	<0.01

Recovery rate = (postoperative score − preoperative score/full score − preoperative score) × 100 (%).

Muscle power score (motor: 0–50 points) and pain sensation (pin prick: 0–98 points) score were defined according to the American Spinal Injury Association score.

G-CSF indicates granulocyte colony–stimulating factor; JOA score, Japan Orthopaedic Association score (thoracic myelopathy: 0–11 points).

group (improvement of the pain sensation score: 6.4 ± 5.5) than in the control group (improvement of the pain sensation score: 1.0 ± 3.2) ($P < 0.01$) (Table 2).

Blood Data and Adverse Events

In the G-CSF group, white blood cell count immediately before G-CSF administration was 7.3 ± 1.6 (× 10^3/mm^3). During the administration, it increased up to 36.7 ± 9.4 (× 10^3/mm^3), ranging from 19.2 to 50.3 (× 10^3/mm^3) (Table 3). G-CSF mobilized cells of the neutrophil lineage, but lymphocytes were not affected (Table 3). G-CSF also caused an increase of monocytes. There was no significant change in inflammation during G-CSF administration, as indicated by C-reactive protein levels (Table 3).

In this series, there was no patient who showed bone pain or hepatic dysfunction after the G-CSF administration. No other severe adverse event occurred during or after the administration.

DISCUSSION

To date, 3 clinical trials of G-CSF administration for neurological disorders have been reported; 2 for amyotrophic lateral sclerosis[8,9] and 1 for cerebral infarction.[10] Zhang *et al*[8] reported that the progression of amyotrophic lateral sclerosis symptoms was inhibited by G-CSF administration, although they did not use controls. Neffussy *et al*[9] performed a controlled study, but they showed no significant difference in the progression of amyotrophic lateral sclerosis symptoms between their G-CSF-treated group and controls. A single clinical trial with G-CSF administration for cerebral infarction has been reported by Shyu *et al*.[10] They reported that neurological symptoms were significantly improved by G-CSF administration.

In this study, we conducted the first clinical trial using G-CSF for patients with worsening symptoms of thoracic

TABLE 3. Hematological Data Before and After G-CSF Administration

	Baseline $M \pm SD$ (range)	Peak Value After G-CSF Administration $M \pm SD$ (range)*	*P*
WBC, ×10^3/mm^3	7.3 ± 1.6 (5.0–10.3)	36.7 ± 9.4 (19.2–50.3)	<0.01
Neutrophils, ×10^3/mm^3	4.6 ± 1.4 (2.1–6.9)	30.6 ± 6.7 (16.6–40.5)	<0.01
Lymphocytes, ×10^3/mm^3	2.1 ± 0.4 (1.5–2.5)	2.4 ± 0.7 (1.5–3.2)	0.29
Monocytes, ×10^3/mm^3	0.4 ± 0.2 (0.2–0.8)	1.9 ± 0.9 (0.7–2.8)	<0.01
CRP, mg/dL	0.1 ± 0.1 (0.0–0.3)	0.3 ± 0.2 (0.1–0.6)	0.08

**Highest level between the first and seventh day after G-CSF administration.*

G-CSF indicates granulocyte colony–stimulating factor; WBC, white blood cell; CRP, C-reactive protein.

compression myelopathy. One month after G-CSF administration, mean recovery rate of JOA score was 29.1%. In contrast, it was 1.1% in the control group at 1 month after initial treatment. In addition, we observed that both motor power and pain sensation scores significantly increased in the G-CSF group compared with the control group at 1 month after treatment. No surgical treatment was performed in patients of either group during the month after G-CSF administration or initial treatment, and they were equally provided conservative treatment such as bed rest. Thus, the present results strongly suggest that G-CSF administration exhibited a neuroprotective effect for the injured spinal cord in patients with worsening symptoms of thoracic myelopathy and improved the myelopathy.

To the best of our knowledge, there has been no other medical treatment that has provided reliable evidence for improvement of thoracic myelopathy. This study provides evidence that G-CSF neuroprotective therapy may be useful as a medical treatment of patients with worsening symptoms of thoracic compression myelopathy. The G-CSF therapy may be especially useful for patients in whom the treatment of complications other than myelopathy needs to be given priority and thus requires a long waiting period before surgery.

In our present trial, no severe side effects occurred. Thus, we suggest that the dose (10 μg/kg per d), duration (5 consecutive days), and route (intravenous administration) of G-CSF administration used in this study are principally safe for the treatment of patients with thoracic myelopathy.

The biggest limitation of this study was that the trial was performed as an open-labeled study and the selection of patients to the G-CSF group and the control group was not randomized. We cannot deny the possibility that a placebo effect of injection may participate in the improvement of neurological symptoms. To increase the level of evidence, in the next stage the study design should be a randomized, double-blind placebo-controlled study. By conducting a phase IIb clinical trial in a large number of patients with the study design described earlier, we will be able to reach a better conclusion regarding the effectiveness of G-CSF neuroprotective therapy for patients with worsening symptoms of thoracic compression myelopathy.

➢ Key Points

- ❑ A multicenter prospective controlled clinical trial was performed to confirm the feasibility of G-CSF administration for patients with worsening symptoms of thoracic myelopathy.
- ❑ For 10 patients with progressive myelopathy, G-CSF (10 μg/kg per day) was intravenously administered for 5 consecutive days.
- ❑ The administration of G-CSF caused neurological recovery in the patients.

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Accumulation of Activated Invariant Natural Killer T Cells in the Tumor Microenvironment after α-Galactosylceramide-Pulsed Antigen Presenting Cells

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Abstract
Purpose The intravenous administration of α-Galactosylceramide (α-GalCer)-pulsed antigen presenting cells (APCs) is well tolerated and the increased IFN-γ producing cells in the peripheral blood after the treatment appeared to be associated with prolonged survival. An exploratory study protocol was designed with the preoperative administration of α-GalCer-pulsed APCs to clarify the mechanisms of these findings, while especially focusing on the precise tumor site.
Methods Patients with operable advanced lung cancer received an intravenous injection of α-GalCer-pulsed APCs before surgery. The resected lung and tumor infiltrating lymphocytes (TILs) as well as peripheral blood mononuclear cells were collected and the invariant NKT (iNKT) cell-specific immune responses were analyzed.
Results Four patients completed the study protocol. We observed a significant increase in iNKT cell numbers in the TILs and augmented IFN-γ production by the α-GalCer-stimulated TILs.
Conclusion The administration of α-GalCer-pulsed APCs successfully induced the dramatic infiltration and activation of iNKT cells in the tumor microenvironment.

Keywords Invariant NKT cell · antigen presenting cell · immunotherapy · tumor infiltrating lymphocyte · non-small cell lung cancer

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Introduction

Vα24 invariant natural killer T (Vα24 iNKT) cells are a unique innate lymphocyte subpopulation characterized by the expression of a canonical invariant T cell receptor with a specific α-chain gene rearrangement (Vα24-Jα18) and pairing mostly with a Vβ11 β-chain in human. Synthetic glycolipid, α-Galactosylceramide (α-GalCer) is a mouse and human iNKT cell ligand, presented by a monomorphic class I-like antigen presenting molecule CD1d [1–3]. Ligand activated iNKT cells exhibit both direct and indirect potent anti-tumor activity.

Patients with malignant diseases show either a decreased number or functionally impaired Vα24 iNKT cells in human peripheral blood mononuclear cells (PBMCs) [4–9]. Head and neck cancer patients with poor circulating iNKT cell number show significantly worse clinical outcomes, suggesting an important contribution of iNKT cells to anti-tumor responses [10]. In addition, the ability to produce IFN-γ from circulating iNKT cells in cancer patients is preserved even though the absolute number of iNKT cells decreases, and thus, residual iNKT cells might still have a good competence to exert anti-tumor responses. Therefore, the expansion and activation of these cells in vivo may be therapeutically meaningful in patients with severely decreased or functionally deficient Vα24 iNKT cells. Clinical studies of α-GalCer-pulsed antigen presenting cells (APC)s have been conducted to recover a functionally sufficient number of Vα24 iNKT cells [11–14]. A phase I/II study ofα-GalCer-pulsed APCs in patients with advanced or recurrent non-small cell lung cancer (NSCLC) found that the treatment elicits Vα24 iNKT cell-dependent immune responses, which are correlated with prolonged overall survival time [13]. The mechanisms that underlie this positive clinical outcome are still unclear.

The current clinical trial focused on the iNKT cell-specific immunological responses in the tumor microenvironments to investigate further anti-tumor mechanisms of Vα24 iNKT cells after α-GalCer-pulsed APC treatment. Therefore, in this exploratory study, the preoperative administration of α-GalCer-pulsed APCs was performed to clarify the iNKT cell specific immune responses at the tumor site more precisely. The results indicated that α-GalCer-pulsed APCs successfully induced the activation of tumor infiltrating Vα24 iNKT cells in the lung.

Material and Methods

Patient Eligibility Criteria

The study included patients between 20 and 80 years of age, with a diagnosis of clinical stage IIB or IIIA NSCLC that was to be treated surgically. Further inclusion criteria were a performance status of 0, 1, or 2; normal or near normal renal, hepatic and hematopoietic function; and no chemotherapy or radiotherapy received for at least 4 weeks before enrollment. $V\alpha24^{+}V\beta11^{+}$ iNKT cells were detected by flow cytometry in the enrolled patients at a level of >10 cells in 1 ml peripheral blood. The exclusion criteria were a positive response to HIV, hepatitis C virus, or human T-cell lymphotrophic virus antibodies; positive for hepatitis B antigen; the presence of active inflammatory disease or active autoimmune disease; a history of hepatitis; pregnancy or lactation; concurrent corticosteroid therapy and evidence for another active malignant neoplasm. The α-GalCer-pulsed APC non-treatment cases were investigated as a control group to elucidate the effects of α-GalCer-pulsed APC treatment. The inclusion and exclusion criteria of the control group were the same as for the treatment group. The histological type, tumor-node-metastasis classification and the anti-tumor effect of treatment were classified according to the general rules for the clinical and pathologic recording of lung cancer as described by the Japan Lung Cancer Society.

Clinical Protocol and Study Design

The study was carried out in the Department of Chest Surgery, Chiba University Hospital, Japan, according to the standards of Good Clinical Practice for Trials on Medicinal Products in Japan. The protocol was approved by the Institutional Ethics Committee (No. 1972). In addition, this trial underwent ad hoc reviews by the Chiba University Quality Assurance Committee on Cell Therapy.

The study design is illustrated in Fig. 1. Written informed consent was obtained from all of the patients before undergoing a screening evaluation to determine eligibility. Clinical and laboratory assessments were conducted once a week, including of a complete physical examination and standard laboratory values. Any adverse events and changes in laboratory values were graded according to the National Cancer Institute Common Toxicity Criteria version 4.0.

Preparation of APCs from Peripheral Blood

All procedures were carried out according to the Good Manufacturing Practice standards. Eligible patients underwent peripheral blood leukapheresis (COBE Spectra, Gambro BCT,

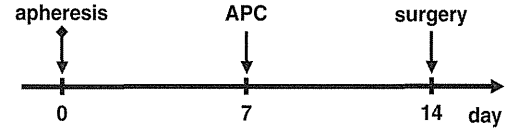


Fig. 1 Study design of α-GalCer-pulsed APC administration. The patients received α-GalCer-pulsed APCs. The timing for both apheresis and α-GalCer-pulsed APC administration are shown. APC, α-GalCer-pulsed APC administration

Inc., Lakewood, CO) and PBMCs were collected and further separated by density gradient centrifugation (OptiPrep, Nycomed Amersham, Oslo, Norway). Thereafter, whole PBMCs were cultured with GM-CSF and IL-2, as previously described [11, 15]. Briefly, PBMCs were washed three times and resuspended in AIM-V (Invitrogen Corp., Carlsbad, CA) 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). The cultured cells were pulsed with 100 ng/ml of specific ligand, α-GalCer (KRN7000; Kirin Brewery, Gunma, Japan) on the day before administration. Whole cells were harvested after 7 days of cultivation, washed 3 times and resuspended in 100 ml of 2.5 % albumin in saline. The patients received an intravenous injection of the cultured cells once (Fig. 1). The criteria for α-GalCer-pulsed APC administration included a negative bacterial culture 48 h before APC injection, cell viability >70 % and an endotoxin test 48 h before APC injection with a result <0.7 Ehrlich units/ml. The patients were injected with 1×10^9 cells/m^2/injection of APCs.

Phenotype Evaluation of APCs

The phenotypes of α-GalCer-pulsed APCs were determined using a FACSCalibur flow cytometer (BD biosciences). The monoclonal antibodies (mAb) used were FITC-labeled anti-HLA-DR, CD83, CD14; phycoerythrin-labeled anti-CD86, CD1d; and allophycocyanin-labeled anti-CD11c, CD40 (Becton Dickinson, San Diego, CA). Isotype-matched control mAbs were used as negative controls.

Preparation of Tumor Infiltrating Lymphocytes, Tumor Cells, Normal Lung Mononuclear Cells and Lymph Nodes Mononuclear Cells

Fresh tumor tissue specimens were obtained from the surgical specimens and the tissue was cut into small pieces with scissors. The tissue specimen was placed in a flask with a mixture of 0.1 mg/ml DNase type I, 1 mg/ml collagenase type IV and 0.5 mg/ml hyaluronidase type V (all from Sigma, St. Louis, MO) in RPMI 1640 and stirred at room temperature for 1 h. The resultant cell suspension was washed in HBSS and subjected to two-layered (75 and 100 %) Ficoll-Hypaque discontinuous density gradient centrifugation at 1200 g for 20 min. The cells from the 100 % interface and 75 % interface were used as tumor infiltrating lymphocytes (TILs) and tumor cells, respectively. Normal lung tissue and lymph nodes were excised from the surgical specimen, cut with scissors in RPMI 1640 containing enzymes and passed through a gauze filter. The resultant cell suspension was washed in HBSS and subjected to Ficoll-Hypaque gradient centrifugation. The interface was collected and used as either a normal lung or lymph node.

Immunological Monitoring

PBMC samples were obtained at least twice before APC administration and 1 week after APC injection.

Flow Cytometric Analysis of $V\alpha24^+V\beta11^+$ Inkt Cells in the Peripheral Blood and TILs

The cell concentrations of $V\alpha24^+V\beta11^+$ iNKT cells in PBMCs, TILs and mononuclear cells from normal lung tissue or lymph node were assessed by flow cytometry. Mononuclear cells were three-color stained with FITC-conjugated anti–T-cell receptor (TCR) Vα24 mAb (C15; Immunotech, Marseilles, France), phycoerythrin-conjugated anti-TCR Vβ11 mAb (C21, Immunotech) and APC-conjugated anti-CD3 mAb (UCTH1; BD Bioscience). The stained cells were subjected to flow cytometry and the percentages of $V\alpha24^+V\beta11^+CD3^+$ cells among mononuclear cells were calculated. Thereafter, the number of iNKT cells (counts/ml) was estimated based on the PBMC counts.

Single-Cell Enzyme-Linked Immunospot Assay

PBMCs, TILs and cells from normal lung tissue or lymph nodes were washed 3 times with PBS and then were stored in liquid nitrogen until use. IFN-γ-secreting cells were assayed in 96-well filtration plates (Millipore, Bedford, MA) coated with mouse anti-human IFN-γ (10 μg/ml; Mabtech, Nacka Strand, Sweden). The cells (5×10^5 per well) were incubated for 16 h with or without α-GalCer (100 ng/ml) in 10%FCS containing RPMI. Phorbol 12-myristate 13-acetate (10 μg/ml) plus ionomycin (10 nmol/l) was used as a positive control. After culture, the plates were washed and incubated with biotinylated anti-IFN-γ (1 μg/ml; Mabtech). Spot-forming cells were quantified by microscopy.

Quantitative Real Time PCR of Vα24 Invariant TCR and CD1d Expression

Total RNA was extracted from the tumors, normal lung tissue and lymph nodes using TRIzol Reagent (Sigma Aldrich) and reverse transcribed using Superscript II RT (Invitrogen Life Technologies) and oligo (dT12–18) primers (Invitrogen Life Technologies). The primers specific for the constant region of TCR α chain (Cα) (sense, CGCCTTCAA CAACAGCATTA; antisense, ACCAGCTTGACATCA CAGGA), TCR Vα24 (sense, GCAAAGCTCTCT GCACATCA; antisense, CCAGGGTTGAGCCTCTGTC), CD1d (sense,gtcagggaagtcggaactga; antisense, atcctgaga-catggcacacc) were used with 5 μg of sample cDNA and

amplified with *Taq* polymerase (Promega). Quantitative real-time PCR was performed using real-time Taq-Man technology and an ABI PRISM 7000 sequence detector (Applied Biosystems, Foster City, CA). The expression was normalized using the Cα signal for Vα24 and GAPDH for CD1d.

Statistical Methods

Statistical analyses were performed using Student's *t*-test.

Results

Patient Characteristics

A total of 4 patients met the inclusion criteria and were enrolled in the study. The patient characteristics are summarized in Table I. The study included one patient with adenocarcinoma and three patients with squamous cell carcinoma. Two patients were stage IIb and two were stage IIIa primary lung cancer. No patients had received any previous treatments.

In addition, a total of 6 patients who had not received α-GalCer-pulsed APC injection were enrolled as the control group. Fresh tumor tissue, normal lung tissue and lymph nodes were excised from the surgical specimens. The patient characteristics of the control group are also listed in Table I.

Phenotypes of αGalCer-Pulsed APCs

The phenotypes of αGalCer-pulsed APCs prepared for administration were analyzed by flow cytometry. All profiles for each patient are shown in Fig. 2. The percentages of HLA-DR$^+$, CD11c$^+$, CD86$^+$, CD40$^+$, CD83$^+$ and CD1d$^+$ cells were determined by the overtone subtraction test using the population comparison platform in the FlowJo software package. More than 50 % of the cultured cells were HLA-DR$^+$ cells, 10 % to 50 % were CD11c$^+$ cells and 50 % to 80 % were CD86$^+$ cells. Interestingly, the majority of the cultured cells were CD3$^+$ T cells or CD56$^+$CD3$^-$ NK cells, indicating the expression of HLA-DR$^+$, CD11c$^+$ or CD86$^+$ on human T cells or NK cells (data not shown). Some variations were observed in the expression of CD83 (23.9–55.4 %), CD40 (8.5–17.1 %) and CD1d (23.1–69.9 %; Fig. 2).

Adverse Events

No serous (grade >2) toxicity or severe side effects were observed in any patients.

Immunological Monitoring of PBMCs and Resected Specimens

Immunological assays were conducted for all patients. The frequency of peripheral blood Vα24 iNKT cells in all patients was measured by FACS. Figure 3 shows that two patients (cases 002 and 004) showed an increased number of circulating Vα24 iNKT cells after the α-GalCer-pulsed APC administration. No clear relationship was found between the number of circulating Vα24 iNKT cells and the α-GalCer-pulsed APC administration in the remaining two patients (cases 001 and 003).

Immunological assays were also performed for TILs and mononuclear cells (MNC)s from normal lung and lymph node tissues. TILs from all 4 cases in the α-GalCer-pulsed APC administration group contained a high percentage of Vα24 iNKT cells in comparison to the normal lung MNCs (TILs; 1.86 %, 0.32 %, 0.15 % and 0.39 % vs. lung MNCs; 0.031 %, 0.013 %, 0.003 % and 0.01 %, Fig. 4a). The frequency of Vα24 iNKT cells in the TILs in case 001 was 60 times higher than the normal lung MNCs. Though the content of Vα24 iNKT in the normal lung MNCs was extremely low in case 003, the Vα24 iNKT cells were found to have accumulated in the TILs. The average percentage of Vα24 iNKT cells in the TILs was 50 times higher than that

Table I Patient characteristics of α-GalCer-pulsed APC group and control group

Case	Treat[a]	Age/Sex	Histology	c-stage	Operation method
001	APC[b]	75/M	Ad[d]	T2N1M0 (stage IIB)	Lobectomy+LND[g]
002	APC	76/M	Sq[e]	T2N1M0 (stage IIB)	Lobectomy+LND
003	APC	74/M	Sq	T1N2M0 (stage IIIA)	Lobectomy+LND
004	APC	68/M	Sq	T3N1M0 (stage IIIA)	Pneumonectomy+LND
c-01	cont[c]	71 M	Sq	T2N1M0 (stage IIB)	Lobectomy+LND
c-02	cont	55 M	large[f]	T2N1M0 (stage IIB)	Lobectomy+LND
c-03	cont	70 M	Sq	T3N0M0 (stage IIB)	Lobectomy+LND
c-04	cont	72 M	Sq	T3N1M0 (stage IIIA)	Bilobectomy+LND
c-05	cont	56/M	Sq	T2N1M0 (stage IIB)	Lobectomy+LND
c-06	cont	63/M	Ad	T2N2M0 (stage IIIA)	Lobectomy+LND

[a]Treat, Treatment; [b]APC, α-GalCer-pulsed APC administration; [c] cont, control; [d]Ad, Adenocarcinoma; [e] Sq, Squamous cell carcinoma; [f] large, large cell carcinoma; [g] LND, Lymph Node dissection

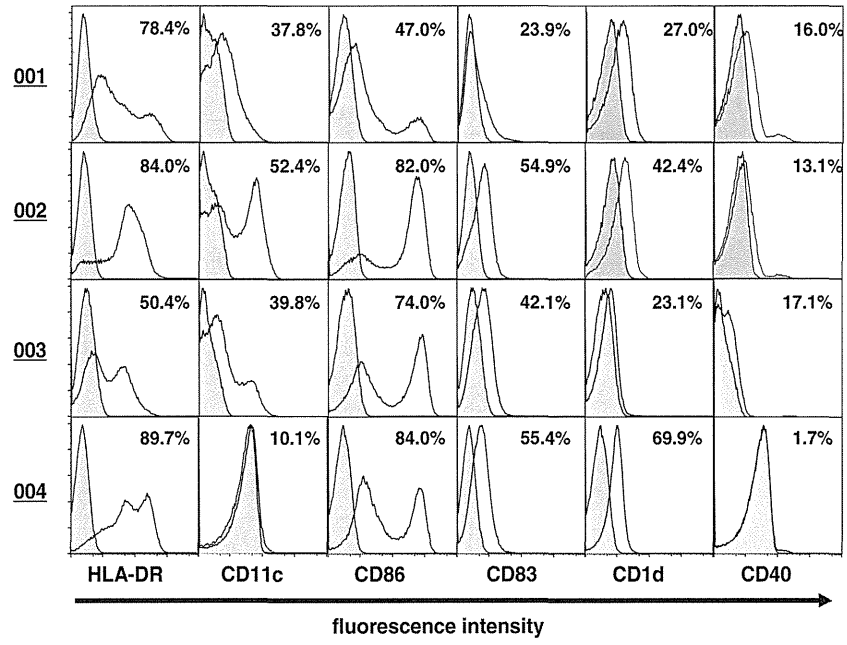


Fig. 2 Flow cytometric analysis of α-GalCer-pulsed APCs. The expression levels of HLA-DR, CD11c, CD86, CD83, CD1d and CD40 were assessed by flow cytometry. Shaded areas: background staining with an iso-type control. Solid lines: staining profiles of the indicated molecules. Values represent the percentages of positive cells

in normal lung MNCs. The Vα24 iNKT cell frequency in the draining lymph nodes of each case was almost the same as that in the normal lung MNCs (Fig. 4a).

The proportion of Vα24 iNKT cells in the control group showed a relatively high percentage of TILs in comparison to the normal lung MNCs (TILs; 0.031 %, 0.058 %, 0.13 %, 0.47 %, 0.18 % and 0.12 % vs. lung MNCs; 0.034 %, 0.011 %, 0.004 %, 0.039 %, 0.014 % and 0.02 %, Fig. 4b). The average percentage of Vα24 iNKT cells in the TILs was only 8 times higher than that in the normal lung MNCs.

Normal lung MNCs in the control group demonstrated a trend toward a higher Vα24 iNKT cell rate in comparison to the treatment group (Fig. 4c). On the other hand, the proportion of Vα24 iNKT cells in TILs tended to increase in the α-GalCer-pulsed APC injected group in comparison to the

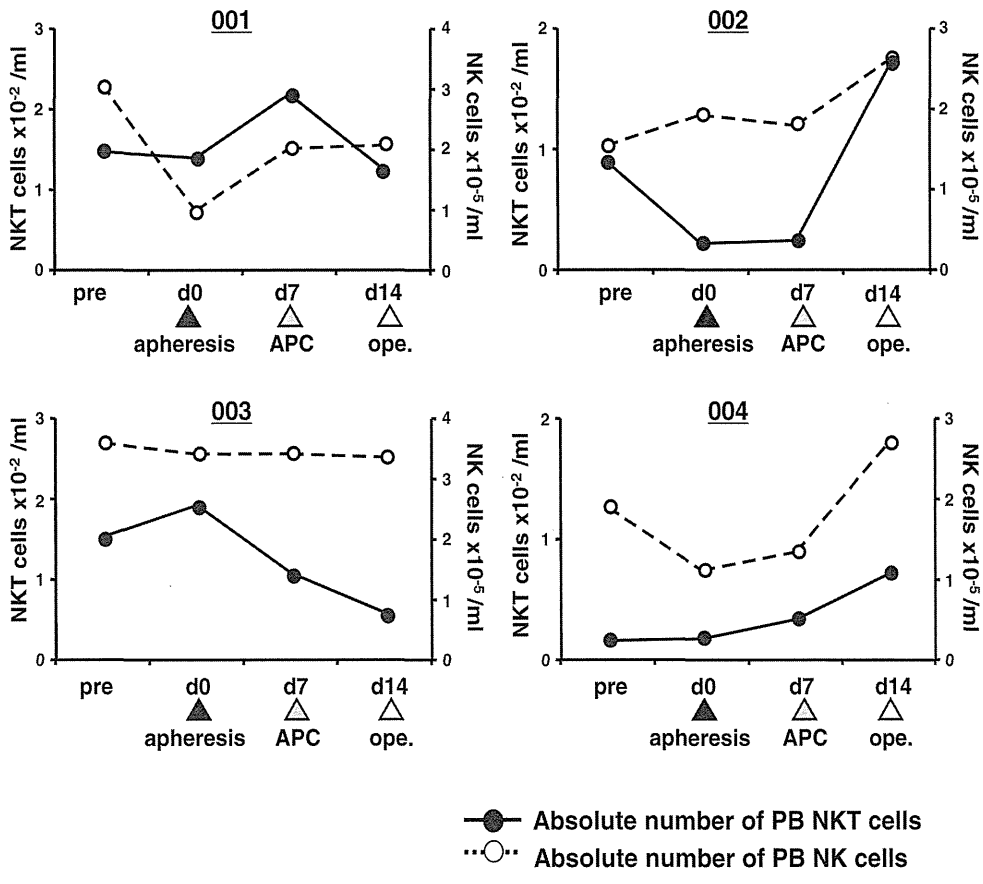
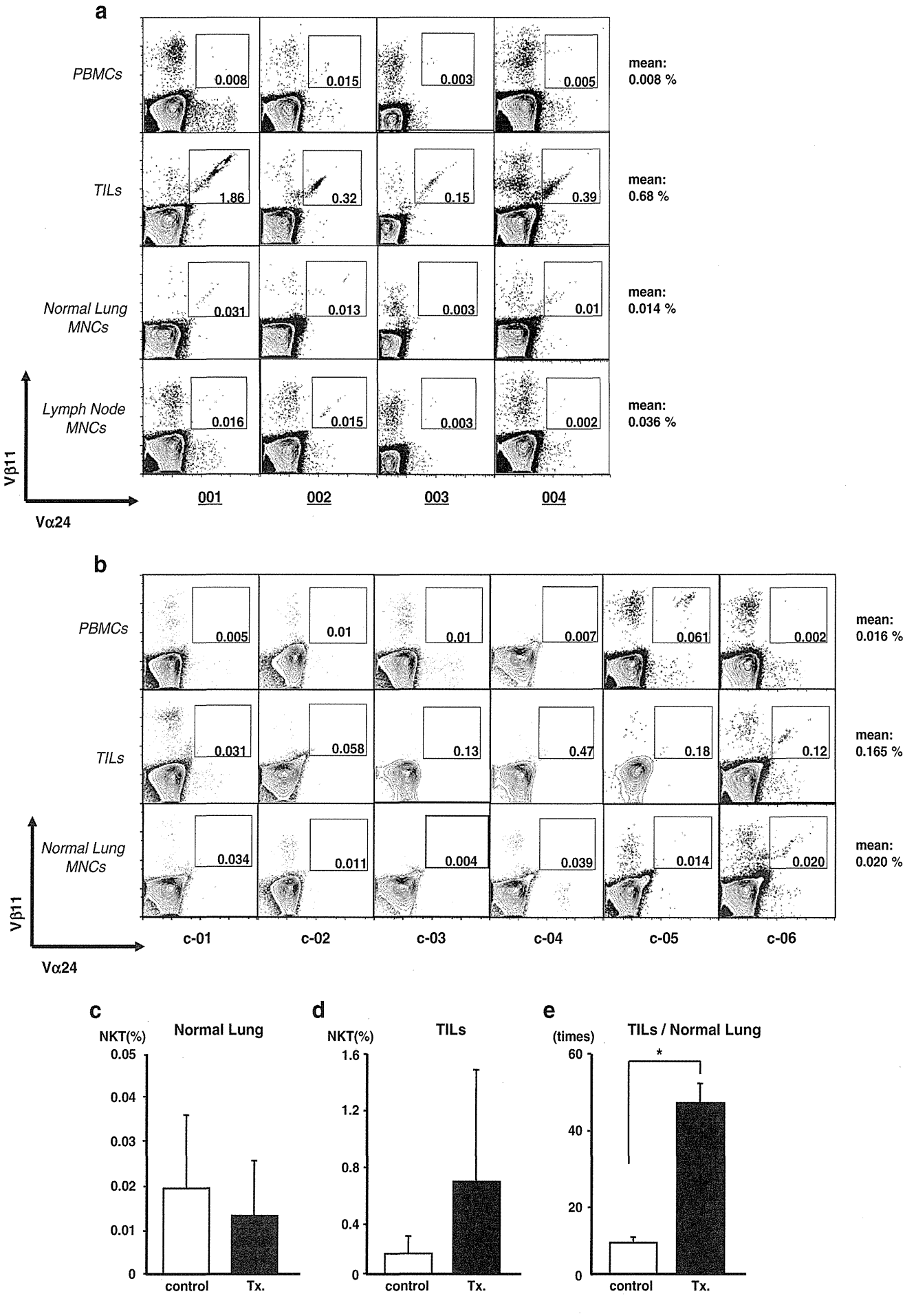


Fig. 3 Immunological monitoring of PBMCs of patients with α-GalCer-pulsed APC administration. The absolute number of peripheral blood iNKT cells ($V\alpha24^+V\beta11^+$ cells) and NK cells ($CD56^+CD3^-$ cells). Flow cytometric analysis and automated full blood counts (Chiba University Hospital) indicated the absolute number of Vα24 iNKT cells and NK cells. APC, α-GalCer-pulsed APC administration; ope., operation


a
PBMCs
TILs
Normal Lung MNCs
Lymph Node MNCs
0.008
0.015
0.003
0.005
mean: 0.008 %
1.86
0.32
0.15
0.39
mean: 0.68 %
0.031
0.013
0.003
0.01
mean: 0.014 %
0.016
0.015
0.003
0.002
mean: 0.036 %
Vβ11
Vα24
001
002
003
004
b
PBMCs
TILs
Normal Lung MNCs
0.005
0.01
0.01
0.007
0.061
0.002
mean: 0.016 %
0.031
0.058
0.13
0.47
0.18
0.12
mean: 0.165 %
0.034
0.011
0.004
0.039
0.014
0.020
mean: 0.020 %
Vβ11
Vα24
c-01
c-02
c-03
c-04
c-05
c-06
c
NKT(%)
Normal Lung
0.05
0.04
0.03
0.02
0.01
0
control
Tx.
d
NKT(%)
TILs
1.6
1.2
0.8
0.4
0
control
Tx.
e
(times)
TILs / Normal Lung
60
40
20
0
*
control
Tx.

◀ **Fig. 4** The frequencies of Vα24 iNKT cells in TILs and mononuclear cells from normal lung and lymph node tissues. **a**, The proportion of Vα24 iNKT cells ($Vα24^+Vβ11^+$ cells) in PBMCs on day 14, TILs, normal lung MNCs and lymph node MNCs in theα-GalCer-pulsed APC administration group were assessed by flow cytometry. The lymph node MNCs column depicts one representative MNC profile in the draining lymph nodes including hilar (#10, 11 and 12) and mediastinal (#1, 3, 4 and 7) nodes. **b**, The proportion of Vα24 iNKT cells in PBMCs, TILs and normal lung MNCs in the control group were assessed by flow cytometry. **c-d**, The comparison between the Vα24 iNKT cell contents in normal lung MNCs **c** and TILs **d** in the α-GalCer-pulsed APC treatment group and the control group. **e** The TIL/Normal Lung MNC ratio of Vα24 iNKT cell proportion. control, control group; Tx, α-GalCer-pulsed APC administration group; *p=0.0008

control group (mean percentage, 0.68 % and 0.165 %, Fig. 4d). The Vα24 iNKT cell ratio of TILs/normal lung in the α-GalCer-pulsed APC administration group was significantly higher than that of the control group (p=0.0008, Fig. 4e).

The number of IFN-γ-producing cells after restimulation with α-GalCer in vitro was concurrently monitored in PBMCs, TILs, normal lung MNCs and lymph node MNCs using an ELISPOT assay. An analysis of the PBMCs and resected specimen showed the highest value of α-GalCer-responsive IFN-γ-producing cell number in the TILs of the α-GalCer-pulsed APC treated group (Fig. 5a). The absolute number of α-GalCer-responsive IFN-γ-producing cells in the TILs was apparently high in cases 001 and 002, whereas a relatively low value was seen in cases 003 and 004. This observation was not detected with the use of control group specimens (Fig. 5b). Together with the results in Fig. 4, the administration of α-GalCer-pulsed APCs induced the mobilization of endogenous Vα24 iNKT cells into the primary site of the lung cancer and augmented the IFN-γ-producing ability of tumor infiltrating Vα24 iNKT cells.

In addition, the number of IFN-γ-producing cells in PBMCs was determined after restimulation with α-GalCer

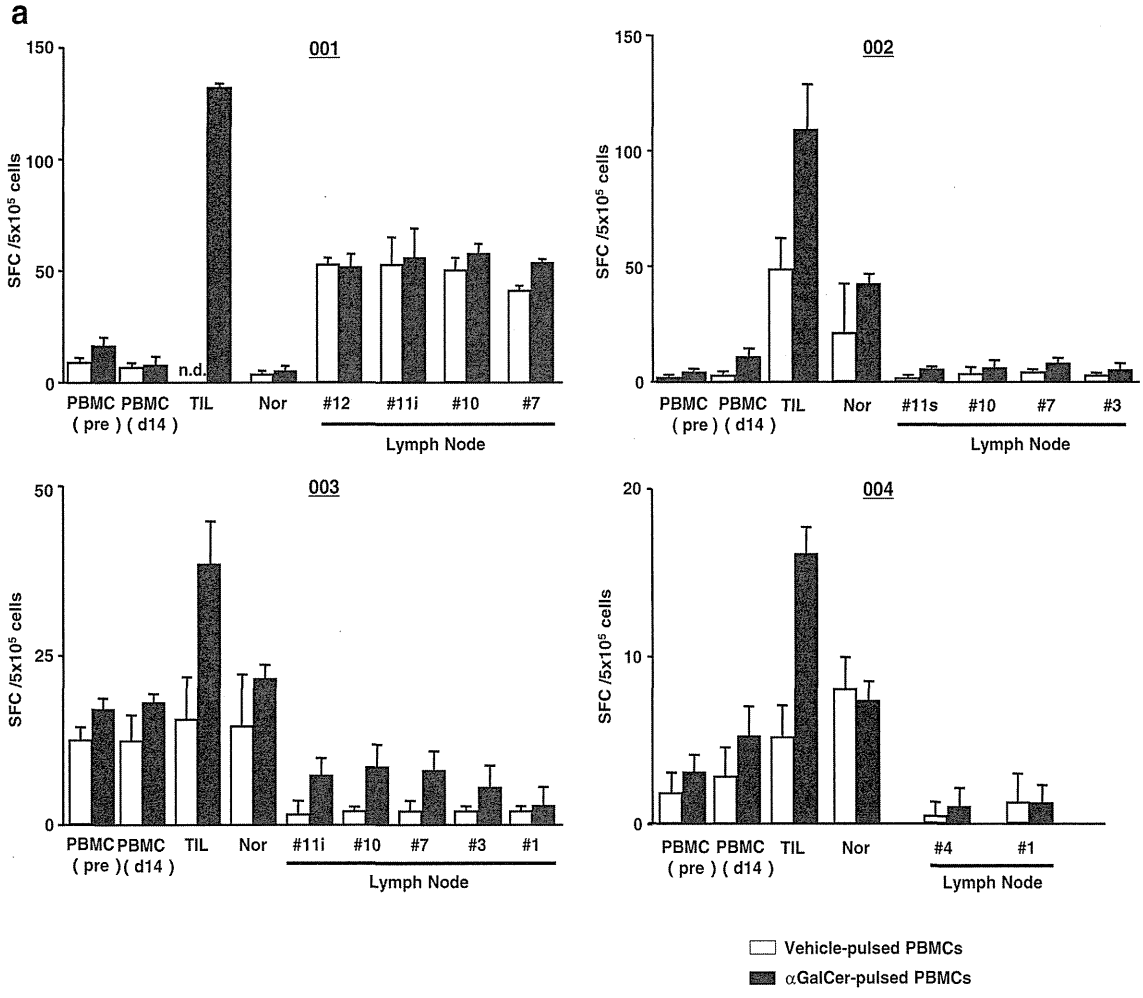


Fig. 5 Detection of α-GalCer-reactive IFN-γ-producing cells by enzyme-linked immunospot assay. **a** Cryopreserved PBMCs, TILs, normal lung MNCs and lymph node MNCs of the α-GalCer-pulsed APC treated group were thawed and cultured overnight with either α-GalCer or vehicle. The presence of IFN-γ-producing cells was quantified by an enzyme-linked immunospot assay. The resected draining lymph nodes including hilar (#10, 11 and 12) and mediastinal (#1, 3, 4 and 7) nodes are shown. Spot number of IFN-γ with standard deviation for triplicate culture of 4 cases are shown. **b** Spot-forming cell number in the control group. SFC, Spot Forming Cell; pre, pretreatment; d14, day 14; Nor, normal lung MNCs; n.d., not done; #11 s, lymph node #11 superior; #11i, lymph node #11 inferior

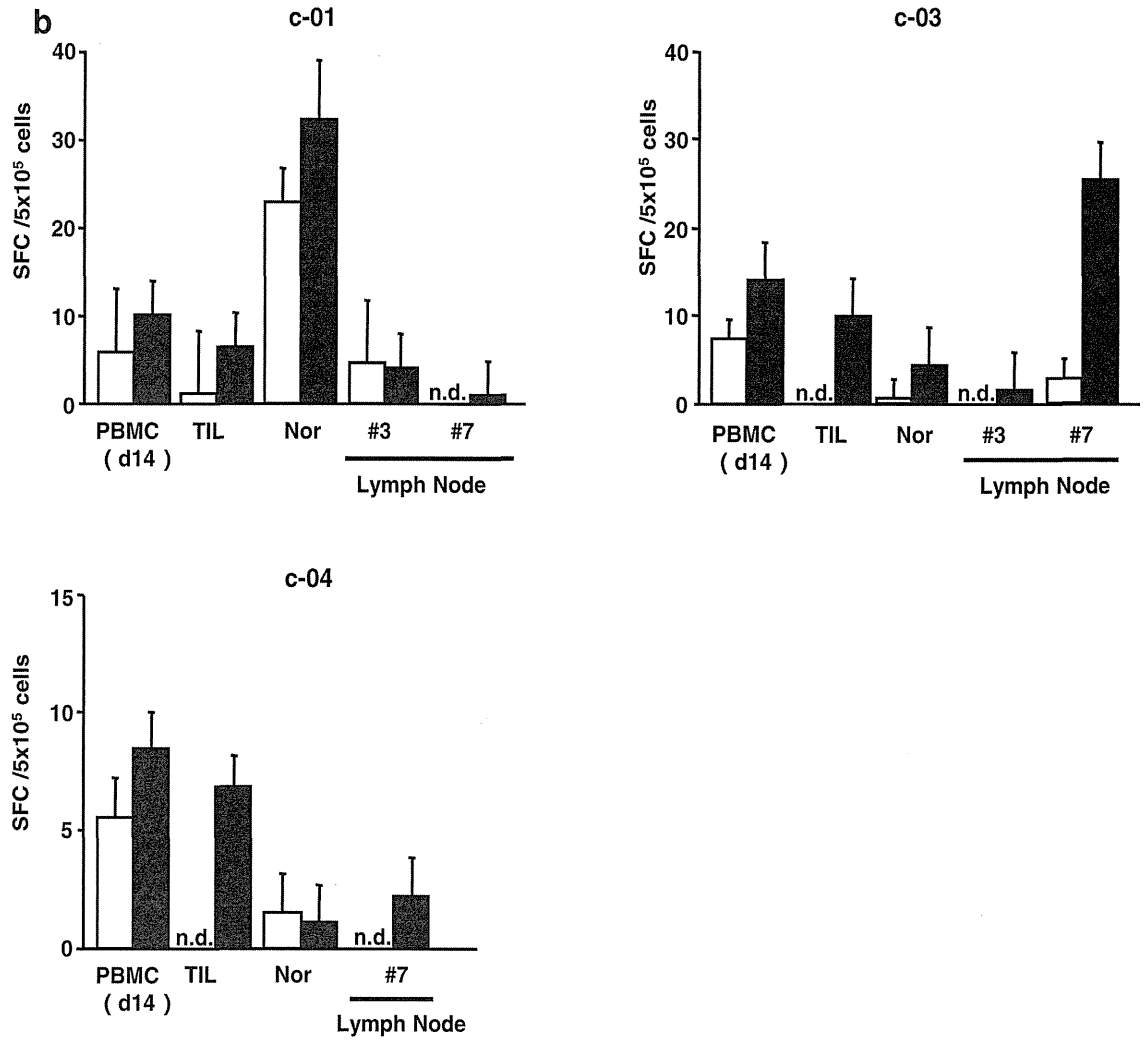


Fig. 5 (continued)

in vitro. In Fig. 5a, the number of IFN-γ producing cells in PMBC increased 14 days after treatment in case 002 and 004, indicating that global NKT cell activation in these patients.

The mRNA Expression Level of Vα24 Inkt Cell Receptor and CD1d

Quantitative RT-PCR was performed to further confirm the increase in the number of Vα24 iNKT cells. The mRNA from the primary tumor, normal lung tissue and lymph node samples was obtained from the α-GalCer-pulsed APC treated patients and the relative expression level of Vα24 invariant TCR and the constant region of TCR (Cα) mRNA were evaluated. The mRNA for Vα24 TCR was highly expressed in the tumor (Fig. 6a). The Vα24-Jα18 invariant TCR mRNA could not be detected in the normal lung tissue since the lung parenchyma included mainly alveolar epithelial cells and only a low copy number of Cα mRNA was detected. The relative gene expression of CD1d was also evaluated by quantitative RT-PCR. CD1d expression was ascertained both in normal lung tissue and tumor tissue in all 4 cases and the expression level appeared to be higher in normal lung tissue (Fig. 6b).

Discussion

The major aim of this study was to investigate the Vα24 iNKT cell-specific immune responses in the primary tumor site after the intravenous injection of α-GalCer-pulsed APCs in patients with advanced NSCLC. The development of these immunotherapeutic approaches with the administration of α-GalCer-pulsed APCs requires a thorough knowledge of local immune responses. Vα24 iNKT cells accumulate in lung cancer lesions [5], as observed in this report (Fig. 4e). A significant increase in the tumor infiltrating Vα24 iNKT cell population was detected after the administration of α-GalCer-pulsed APCs in comparison to the non-injected control group. This observation is quite reasonable for the iNKT cell-targeted therapy aimed at activation of Vα24 iNKT cells in the tumor located site in vivo. The activation status of CTLs, rather than just the existence

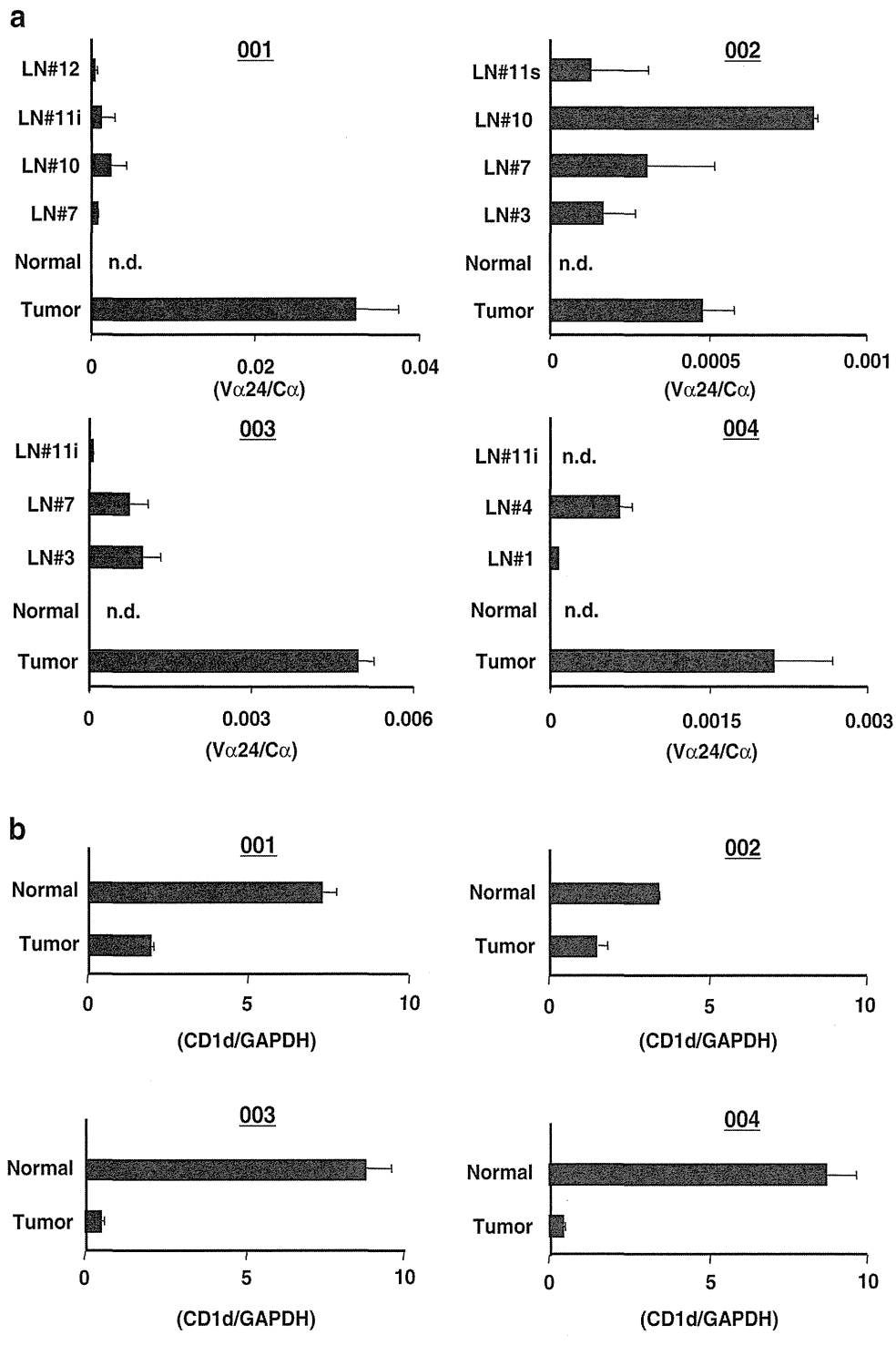


Fig. 6 The relative mRNA expression of the Vα24$^+$ TCR in tumor, normal lungs and lymph nodes. Cancer tissue, non-cancerous lung tissue and lymph nodes were obtained from α-GalCer-pulsed APC treated patients. **a** The expression level of Vα24 TCR mRNA in each sample was analyzed by quantitative RT-PCR. Each mRNA was quantified by the standard curve method and copy numbers of Vα24 TCR were normalized by the copy number of the constant region of the TCR α chain (Cα mRNA. **b** The expression level of CD1d mRNA in each sample was analyzed by quantitative RT-PCR. Each mRNA was quantified by the standard curve method and copy numbers of CD1d were normalized by the copy number of the GAPDH mRNA. LN, lymph node; Normal, normal lung tissue; Tumor, tumor tissue; n.d., not detected; #11 s, lymph node #11 superior; #11i, lymph node #11 inferior

of CTLs, has great prognostic significance [16–18]. Therefore, IFN-γ-producing cells were also monitored in PBMCs, TILs and MNCs from normal lung tissue and lymph nodes using an ELISPOT assay. After starting the protocol, standard preoperative chemo-radiotherapy was introduced to treat locally advanced NSCLC, and such a change of treatment strategy hindered the entry of patients into this protocol. In spite of the limited number of patients analyzed, the results obtained by ELISPOT assay indicated that tumor infiltrating mononuclear cells had augmented IFN-γ producing capacity, which may have a positive impact on the tumor microenvironment.

Tumor infiltrating lymphocytes are found in a variety of cancers and they are thought to be a result of a host immune response directed against tumor cells. Several reports have shown that the presence of large numbers of tumor-infiltrating CD8$^+$ T cells are associated with a favorable prognosis in esophageal carcinoma [18, 19], colorectal cancer [20, 21], ovarian cancer [22], and pancreatic carcinoma [23], while the infiltration of CD4$^+$ T cells that posses regulatory function,

such as Foxp3$^+$ regulatory T cells (Treg) are associated with the poor prognosis of ovarian cancer [24, 25]. The balance between CD8$^+$ CTLs and Tregs in tumors is critical for disease progression and survival [24, 26, 27]. These diverse results indicate that the functional roles of TILs are complicated and uncertain and the effects of TILs might vary with the type and stage of cancers. In addition to CD8$^+$ cytotoxic T cells, tumor infiltrating Vα24 iNKT cells have been reported to be a positive prognostic factor for colorectal carcinoma [28]. The current results indicated that the injection of α-GalCer-pulsed APCs could induce the accumulation of Vα24 iNKT cells in TILs, which would therefore lead to a good prognosis after a complete surgical resection.

Although a complete surgical resection is regarded as the optimal treatment for NSCLC, only around 25 % of NSCLC are suitable for potentially curative resection. Despite optimal surgical management, the 5-year survival rate of resected NSCLC ranges between 85.9 % for pathological stage Ia and 41 % for pathological stage IIIa [29]. Approximately 50 % or more of patients with NSCLC who undergo surgery experience relapse due to the existence of microscopic lesions that could not be detected by preoperative screening. Recently, adjuvant chemotherapy given after surgery has been shown to improve survival [30–32]. A meta-analysis suggested that cisplatin-based adjuvant chemotherapy could yield an absolute overall survival advantage of 5 % at 5 years [33]. At the same time, chemotherapeutic agents often show severe toxic effects and it was reported that in patients with early-stage disease have deleterious effects on long-term survival. This emphasizes the importance of development of less-invasive preoperative or postoperative therapy to suppress the growth of micrometastases. Therefore, immune cells for tumor surveillance, such as NK and iNKT cells, which possess anti-tumor activity, should be beneficial and post-surgical adjuvant immunotherapy by the use of these cells may be favorable since the residual tumor is quite small after a complete resection.

Conclusions

α-GalCer-pulsed APC administration successfully induced the dramatic infiltration and activation of Vα24 iNKT cells in the tumor lesion. This report is the first clinical trial of Vα24 iNKT cell targeted immunotherapy that shows a functional Vα24 iNKT cell accumulation in the tumor microenvironment. These results encourage the further development of immunotherapy aimed at the activation of endogenous Vα24 iNKT cells in the lung.

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Conflict of interest The authors declare that they have no conflict of interest.

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第11回CRCと臨床試験のあり方を考える会議2011 in 岡山／2011年9月25日・岡山コンベンションセンター

●パネルディスカッション3／
これからの時代を担うIRBの機能と責務－中央IRB（共同IRBを含む）の普及に向けて－／講演4

中央IRB等への移行過程で生じた課題とその解決に向けた取り組み

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大学病院臨床試験アライアンスではいわゆる中央IRBのあり方について検討を行うとともに，千葉大学，信州大学，新潟大学による中央IRBの審議を2010年7月より試験的に一試験について開始した。本稿では大学病院臨床試験アライアンスで実施している中央IRB検討ワーキンググループ（以下WG）およびその上部組織である推進室での議論，さらに欧州の中央IRBのあり方をふまえたIRBのあるべき姿と，実際の取り組みについて述べるものである。また，この内容については，筆者らの私見にもとづいており，各大学でのコンセンサスを得られた段階に至っていない。

はじめに

近年，治験の活性化の中で，中央IRBに向けた取り組みの必要性が謳われている。これは，多くの場合，治験を開始するにあたり，それぞれのIRBへの申請に関して多くの時間と労力を必要とするため，その業務に当たる費用が高額となり，治験依頼者への負担が増しているという事実がある。また，一方で，経験の乏しいIRBにおいて複雑な治験の審議をすることについて問題視する事実もある。このような状況のなか，GCPでは2度の改訂を経ているにもかかわらず，いまだ中央IRBの活動は活発ではない。平成23年6月には日本医師会治験促進センターによる治験等適正化作業班による提言がなされ，これを受ける形でさらなるGCPの改訂がなされた。作業班では，中央IRBの設置とIRB事務局と治験事務局の外部委託による効率化も検討された。

大学病院臨床試験アライアンスは，国際共同試験をわが国で主導することを目的として関東信越7大学において先進的な取り組みを治験において実施してきた。そのなかで中央IRBについては，はたして実施にあたりどのような課題があるか具体的に検討が必要との結論に至り，推進室の下部組織として信州大学を担当校とし各大学の担当者からなるWGを構成し議論を重ねてきた。

WGの検討内容について

そもそも中央IRBの設置の前提としていくつかの疑問がある。治験を実施する各施設個別の状況を知らない中央IRBにおいて，①迅速性と効率性と，科学性と倫理性は共に成り立つにはどのようにしたらいいのか，②被験者を保護するという原則はどのようにしたら確保されるのか，という2課題はWGの検討以前においてそもそも重要な問題であり，IRBの審議の根幹を成すものである。中央IRBで十分な議論ができる体制を維持すること，さらにそこでは，その施設固有の問題点を被験者保護という立場から話し合える状況にする必要があると考えている。

一方で，③審査手続きの共通化，④中央IRBの設置場所，⑤具体的な審議の方法，という3課題については，技術的な側面が強い課題であり，解決可能な課題とした。

欧米の中央IRBのあり方について

海外には中央IRBがすでに多く設置され，活用さ

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英国	3機関の承認が必要 1.NHS National Health Service 行政区ごとの local Research Ethical Committee (REC) 2.Main REC 3.MHRA (Medicines and healthcare products Regulatory Agency)
フランス	被験者保護委員会 CCP 被験者保護に関する事項 臨床試験のプロトコルデザインに関する事項 施設に関する事項 医療製品保険安全局 AFSSAPS 試験薬等の品質と安全性に関する事項 被験者の安全性に関する事項（適格基準、投与量、被験者のモニタリング，対照薬）
米国	All committees are registered with the Office for Human Research Protections (OHRP) . FDA, pharmaceutical companies, contract research organizations, as well as AAHRPP perform regular inspections of our processes. An FDA inspection in July 2009 resulted in no findings. CERTIFICATION: Staff and board members have earned certifications, such as Certified IRB Professional (CIP) , Certified Clinical Research Professional (CCRP) and Certified IRB Manager (CIM) .

図1　欧米の中央IRBに関する大学病院臨床試験アライアンスによる訪問調査

IRB審議資料

IRB開催日	千葉大	新潟大	信州大
2010/7/20	新規実施の適否	新規実施の適否 （治験参加カード）	新規実施の適否 （治験参加カード）
2010/9/21	実施計画書 2.0 版 同意説明文書	実施計画書 2.0 版	実施計画書 2.0 版
2010/10/18		同意説明文書	
2010/11/15	実施計画書 2.1 版 同意説明文書 監査手順書 2.0 版	実施計画書 2.1 版 同意説明文書 監査手順書 2.0 版	実施計画書 2.1 版 同意説明文書 監査手順書 2.0 版
2011/2/21	院内の重篤有害事象	—	—
2011/3/22	継続審査，安全性情報	継続審査，安全性情報	継続審査，安全性情報
2011/4/18	実施計画書 2.2 版 治験薬概要書 2.0 版 同意説明文書	実施計画書 2.2 版 治験薬概要書 2.0 版	実施計画書 2.2 版 治験薬概要書 2.0 版
2011/5/16	同意説明文書	同意説明文書	同意説明文書
2011/9/20	実施計画書 2.3 版 同意説明文書 緊急逸脱報告書	実施計画書 2.3 版 同意説明文書	実施計画書 2.3 版 同意説明文書

図3　3大学から千葉大学中央IRBへ提出され審議された内容

れている国々があるが，その設置の基準や役割はさまざまである。海外の状況（⑥）を検討するためにわれわれは実際に規制当局や医療機関を訪問し，調査を行った（**図1**）。このなかで，施設固有の問題点を審議するローカルIRBと計画書の審議をする中央IRBがそれぞれ業務を分担し審議を行っていることを知った。さらに，規制当局との連携も重要な点であった。また，米国ではコマーシャルIRBが数多く設置されているが，それぞれのIRBではその質の高さと独立性を示すための取り組み（認定の取得，業務内容の公開，FDAの調査を受けた時期の公開）を行っていることが明らかになったが，一方で施設固有の問題点を審議することについては難しい状況であることが判明した。

医師主導治験の中央IRBについて

2010年7月千葉大学医学部附属病院治験審査委員

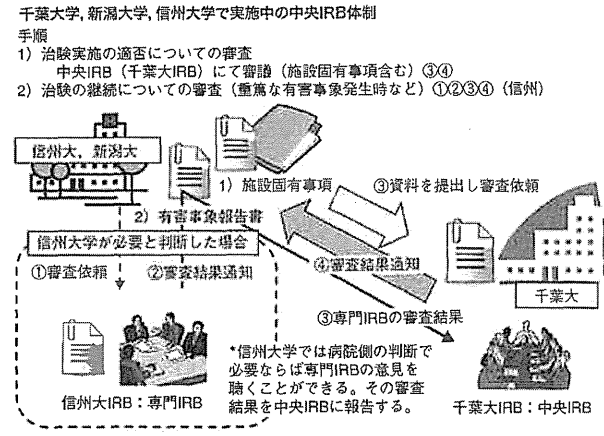

図2　WGで検討された中央IRBのフロー

会では，信州大学医学部附属病院長および新潟大学医歯薬総合病院長から千葉大学医学部附属病院長への医師主導治験の審議依頼を受け審議を開始した。これは国立大学病院としては初めての取り組みである。この審議を実施するにあたり3大学ではWGで検討された中央IRBの審議のフロー（**図2**）をもとにそれぞれ手順書の改訂（⑦）契約書の作成とこれに伴う審査費用の算定（⑧）を行った。ここでは申請元の大学のIRBを「施設で発生した重篤有害事象を審議する専門IRB」と位置づけている。(⑨)3大学から中央IRB事務局へ提出される書類は内容がすべて同一でないことより中央IRBで承認された内容や時期が異なっている（**図3**）。このことは治験事務局と中央IRB事務局との独立性を意味し，推進する立場とこれを監督する立場のそれぞれの事務局の独立性が確保された状況となっている。(⑩) 医師会の作業班の提案する各大学の治験事務局を廃止し中央に委託することについては，作業の効率性に加え，技術的に千葉大学の事務局が各大学の業務を引き受けることは困難との結論に至った。

ま　と　め

およそ10の課題について検討を行いながら，具体的に中央IRBを運用するに至った。規制当局との連携については今までまったく議論をされていな内容である。つまり，その本質において単なる効率化ではなく真の意味での中央IRBの役割を考えることが重要であり，欧米での取り組みを十分考慮のうえ，進めて行く必要がある。大学病院臨床試験アライアンスではわが国を代表するグループとして欧米からも信頼される中央IRB体制の構築を実施する。

〔千葉医学　88：1～9，2012〕

〔原著〕

脊髄障害性疼痛に対する顆粒球コロニー刺激因子（G-CSF）の治療効果

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要　旨

脊髄障害性疼痛を有する13例を対象として，顆粒球コロニー刺激因子（G-CSF）を用いた神経保護療法の臨床試験を施行した。対象症例は，脊髄症の急性増悪と関連する疼痛を認めた6例（脊髄症急性増悪群）および脊髄症の後遺症と考えられる疼痛を認めた7例（脊髄症後遺症群）の二群に分けられた。G-CSFを5 μg/kg/日×5日間（1例），10μg/kg/日×5日間（11例），10μg/kg/日×3日間（1例）の投与量・期間で点滴静注投与し，投与前後での疼痛の変化をVisual analogue scale（VAS）にて評価した。11例で投与後に疼痛が軽減し，2例では完全に消失した。疼痛軽減効果は全例，投与開始から1週間以内で認められた。脊髄症後遺症群では無効例が2例存在した。VASは，脊髄症急性増悪群で投与前平均63.3㎜から投与後1週間で平均25.0㎜（$P<0.05$）に，脊髄症後遺症群で投与前平均67.1㎜から投与後1週間で平均44.3㎜（$P<0.05$）に減少した。投与後6ヵ月間の観察期間中に7例で疼痛軽減効果の減弱が見られた。効果の減弱は投与後3ヵ月時に4例，6ヵ月時に3例で認められた。今回の検討から，G-CSFが脊髄障害性疼痛に対して疼痛軽減効果を有することが示唆された。

Key words: 脊髄障害性疼痛，顆粒球コロニー刺激因子，神経保護療法

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Ⅰ．緒　　言

脊髄障害性疼痛とは，2009年以降に本邦で使用されるようになった新しい概念である。厳密な定義は定められてはいないが，ヘルニア・脊柱靭帯骨化症などによる圧迫性脊髄症や，急性脊髄損傷などの脊髄障害に起因すると考えられる多彩な痛みを総称している[1,2]。侵害受容性疼痛に比べ神経障害性疼痛の占める割合が大きいのが特徴であり，通常では痛みを起こさない程度の刺激が強い痛みを引き起こすアロディニア（異痛症）や，障害脊髄高位より尾側の締め付けられるような自発痛などに代表される。非ステロイド性抗炎症薬（NSAIDs）の効果は低く，最近では末梢神経障害性疼痛で得られたエビデンスをもとに，プレガバリンなどの抗てんかん薬や一部の抗うつ薬などの有効性が解明されてきたが[3-5]，多くの症例で必ずしも十分な除痛効果が得られるわけではない。病態が十分に解明されておらず，症例によって薬剤に対する反応性が異なるため，治療に難渋することがしばしばである。本邦では平成22年度に厚生労働省により脊髄障害性疼痛症候群が研究奨励分野に指定され，研究が進められている。

我々は，2008年から急性期脊髄損傷患者および圧迫性脊髄症急性増悪患者に対する顆粒球コロニー刺激因子（Granulocyte-colony stimulating factor: G-CSF）を用いた神経保護療法の臨床試験を進めている[6-9]。G-CSFは血球系に作用する増殖因子であり，顆粒球系細胞の分化・増殖・生存促進などの作用を有する[10]。本邦ではがん化学療法による好中球減少症や，末梢血幹細胞移植時の造血幹細胞の末梢血への動員などの目的で臨床使用されている。中枢神経系においては，骨髄細胞を脳・脊髄中へ動員する作用や[11]，脳卒中モデルに対する神経保護作用などが報告されており[12]，海外では脳梗塞に対する臨床試験が報告されている[13]。以上より，脊髄損傷に対してもG-CSFが治療効果を発揮しうる可能性が想定されたため，われわれはマウスおよびラットの脊髄損傷モデルを用いてG-CSFの有効性およびその作用機序につき報告してきた[14-18]。その結果，G-CSF投与により後肢運動機能が有意に改善した。その機序に関しては，①G-CSFにより動員された骨髄由来幹細胞が脊髄損傷部に生着する，②直接的に神経細胞死を抑制する，③Oligodendrocyteの細胞死を抑制し，髄鞘を保護する，④炎症性サイトカイン（TNF-α，IL-1β）発現を抑制する，⑤血管新生を促進する，などが想定された[14-18]。これらの根拠から，慢性圧迫性脊髄症の急性増悪に対してもG-CSFが神経保護作用を有する可能性が示唆されたため，圧迫性脊髄症急性増悪患者に対する治療薬としてのG-CSFの安全性・有効性を確認するため臨床試験を開始した[6,7]。結果として，G-CSFの安全性が確認され，全例で神経症状の改善が認められた。さらに，予期せぬ効果として，G-CSF投与後に脊髄障害に起因する疼痛が軽減した例を少なからず経験した[19]。このことからG-CSFが脊髄障害性疼痛に対して何らかの疼痛軽減効果を有することが示唆された。今回は，臨床試験例におけるG-CSF投与後の脊髄障害性疼痛の変化を検討した。

Ⅱ．方　　法

2009年１月から2010年２月の期間に，脊髄障害に関連する疼痛を認めた16例に対し，G-CSFを用いた神経保護療法の臨床試験を施行した。今回は，その中で投与から６ヵ月以上経過観察しえた13例を対象として検討した（表１）。症例１～６では，後縦靭帯骨化症（OPLL）および黄色靱帯骨化症（OYL）による圧迫性脊髄症の急性増悪（日本整形外科学会頚髄症治療判定基準［JOAスコア］が直近の１ヵ月で２点以上悪化）に対してG-CSFの投与が行われた。この６例での脊髄障害性疼痛は，脊髄症の急性増悪との関連が強く示唆されたため，脊髄症急性増悪群として分類し検討を進めた。G-CSFの投与量・期間は，症例１で５μg/kg/日×５日間，症例２～６で10μg/kg/日×５日間の点滴静注であった。６例全例で，G-CSF投与後1.5～８週で脊髄症に対する後方除圧固定術または椎弓切除術が行われた。

症例７では，頚椎症性脊髄症に伴うと考えられる疼痛が持続しており，症例８～13では，脊髄症に対する手術の術後に脊髄関連の疼痛が遺残していた。したがって，症例７～13を脊髄症後遺症群