Cancer Immunol Immunother (1998) 46: 113–119

ORIGINAL ARTICLE

Hisakazu Yamagishi · Yuji Ueda · Takahiro Oka

A case report of immunotherapy on a patient with advanced gastric cancer by adoptive transfer of OK-432-reactive HLA-matched allogeneic lymphocytes

Received: 19 May 1997 / Accepted 18 November 1997

Abstract Adoptive immunotherapy (AIT) for non-hematological malignancies, using HLA-matched donor lymphocytes, has been rarely reported. For a 35-year-old male patient with peritoneal disseminated advanced gastric cancer, we performed AIT using lymphocytes from his HLA-matched 37-year-old brother and a streptococcal preparation, OK-432, as an antigen. After the donor had been immunized by intradermal administration of OK-432, OK-432-reactive lymphocytes were induced in vitro and transferred to the patient intravenously with OK-432. Low-dose systemic immunochemotherapy, using interleukin-2, 5-fluorouracil and cyclophosphamide, was concurrently administered with AIT. As a result, the Schnitzler metastasis in the patient reduced in size without any significant graft-versus-host-related complications. One of the effector mechanisms of therapeutic benefit was suggested to be cytokine release from the transferred OK-432-reactive lymphocytes. Our findings suggest the safety and efficacy of AIT using lymphocytes from an HLA-matched sibling and OK-432 as an antigen. Further studies to investigate the use of tumor-associated antigen and an HLA-matched sibling's lymphocytes for AIT of advanced cancer are warranted.

Key words Adoptive immunotherapy · Allogeneic cell therapy · Gastric cancer · OK-432 · Interleukin-2 (IL-2)

Introduction

In adoptive immunotherapy (AIT) for immunosuppressed patients with advanced cancer, in vitro induction of potent effector cells from patient's lymphocytes is often difficult [5]. Thus, it may be reasonable to use effector cells induced from lymphocytes of a healthy sibling whose human leukocyte antigen (HLA) is matched to the type of the patient in antigen-specific AIT based on major histocompatibility complex (MHC) restriction. Some case reports have shown the effectiveness of AIT that uses HLA-matched donor lymphocytes, against recurrence after HLA-matched allogeneic bone marrow transplantation for hematological malignancies such as leukemia [18, 23]. However, AIT using allogeneic lymphocytes for non-hematological malignancies has rarely been reported [7]. In particular, no study has used lymphocytes that were collected from a donor immunized with an exogenous antigen and reacted in vitro with the same antigen for use in AIT.

We report here the first clinical application of AIT against advanced gastric cancer using lymphocytes from an HLA-matched sibling and OK-432 as an antigen, and discuss its clinical safety and antitumor effector mechanism. This is a pilot study for another MHC-restricted, tumor-specific AIT for immunosuppressed patients with advanced cancer, in which lymphocytes from an HLA-matched sibling and soluble tumor antigens [4] or synthetic peptides [2] are used.

H. Yamagishi (✉) · Y. Ueda · T. Oka
Second Department of Surgery, Kyoto Prefectural University of Medicine Kawaramachi Hirokoji, Kamigyo-ku, Kyoto 602, Japan
Fax: +81 75 223 6189

Patient and methods

Patient and lymphocyte donor

The patient was a 35-year-old man whose chief complaints were severe constipation and general fatigue. Upper GI tract examination (fluoroscopy and fiberscopy) and colorectal fluoroscopy revealed the presence of advanced gastric cancer with Schnitzler metastasis (Fig. 1a). He underwent laparotomy on 1 December 1994, which revealed that the cancer was located from the fornix to the lower body of the stomach and partially invaded the serosal surface. Severe peritoneal dissemination with Schnitzler metastasis and lymph node metastases were also observed [stage IV (T3N2M1)]. On the basis of these findings, total gastorectomy and double-barreled colostomy on the caecum were performed.

Histologically, the resected stomach revealed poorly differentiated schirrhous-type adenocarcinoma. After the operation, high-dose chemotherapy with peripheral blood stem cell transplantation was planned; however, the initial intraperitoneal chemotherapy using cisplatin (120 mg) and etoposide (100 mg) induced renal dysfunction, and the serum creatinine level increased to 2.5 mg/dl. Therefore, we

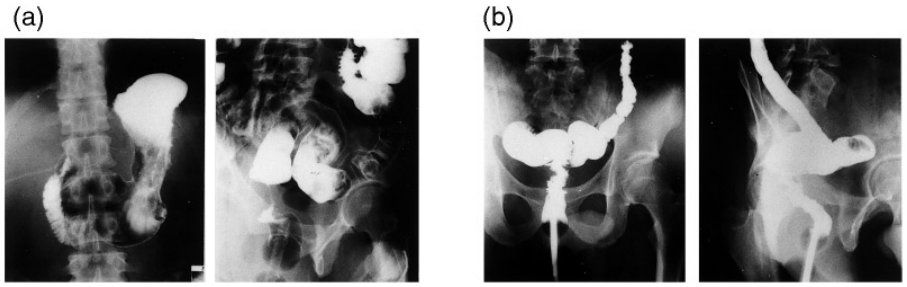


Fig. 1a Gastric and colorectal fluoroscopies before this study. Advanced gastric cancer with Schnitzler metastasis is depicted. **b** Colorectal fluoroscopies after four cycles of the therapy, showing a reduction of Schnitzler metastasis and widening of the intrarectal space

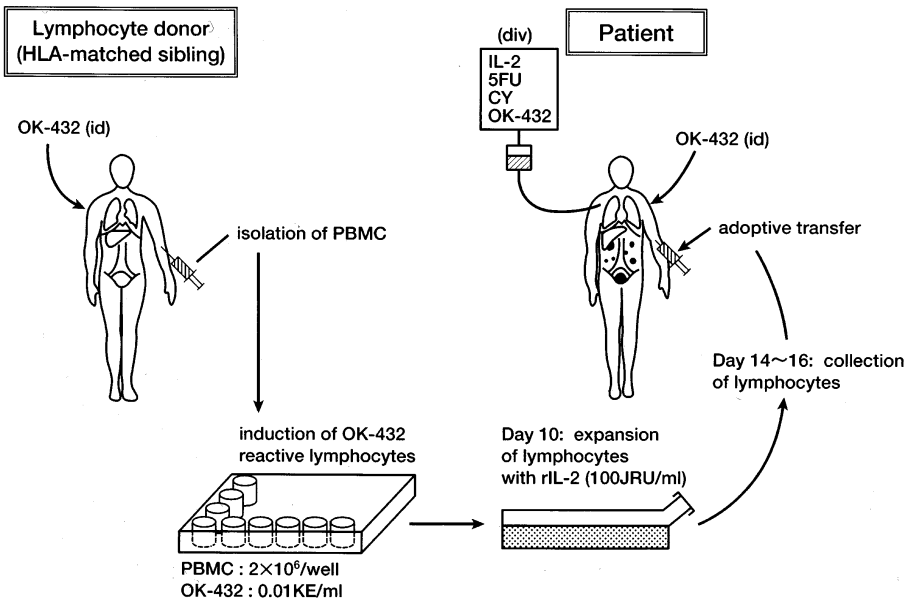


Fig. 2 Treatment procedures for adoptive immunotherapy using HLA-matched sibling's lymphocytes and OK-432 as an antigen. *CY* cyclophosphamide, *IL-2* interleukin-2, *PBMC* peripheral blood mononuclear cells, *id* intradermal administration, *div* intravenous drip administration

changed the treatment schedule to milder immunochemotherapy using 5-fluorouracil (5-FU), interleukin-2 (IL-2), OK-432 and adoptive transfer of in vitro activated lymphocytes.

The time between the intraperitoneal chemotherapy and the beginning of AIT was 40 days. Colorectal fluoroscopy immediately before the start of AIT did not reveal any changes in Schnitzler metastasis.

The lymphocyte donor for AIT was the patient's HLA-matched 37-year-old brother (HLA A-2,-; B-55, 61; Cw-1,3; DR-4,9; DQ-3,4), and mixed lymphocyte culture using the lymphocytes of the donor and of the patient demonstrated no significant proliferative response. After informed consent has been obtained from the patient and his brother, the brother's peripheral blood mononuclear cells (PBMC) were used for AIT.

In vitro induction of OK-432-reactive lymphocytes

The donor was injected with 5 KE OK-432 (Chugai Pharmaceutical Co., Tokyo, Japan; 1 KE = 0.1 mg protein) intradermally into the upper arm once a week for four weeks. One week after the last injection, fresh heparinized venous blood was collected and PBMC were separated on a Ficoll-Isopaque (Sigma) density gradient. PBMC (2×10^6 cells/well) were cultured in 24-well culture plates in 2 ml complete medium with 0.01 KE/ml OK-432 for 6 days in a moist 5% CO_2 incubator at 37 °C. The complete medium was RPMI-1640 (Gibco) supplemented with heat-inactivated 10% pooled human AB serum (Flow), L-glutamine, penicillin, and streptomycin (Sigma). On day 6, viable cells were collected and restimulated with 0.01 KE/ml OK-432 in the presence of the same number (1×10^6/well) of mitomycin-C-inactivated lipopolysaccharide (LPS) blasts as antigen-presenting cells (APC) and 100 JRU (Japanese reference units)/ml rIL-2 (Takeda, Osaka, Japan) in complete medium. A Japanese reference unit is approximately equal to a biological response modifier program unit (National Cancer Institute, National Institutes of Health, USA). LPS blasts were induced from donor PBMC in the presence of 20 μg/ml LPS (Sigma) for 2 days of culture, and were washed three times in RPMI-1640 medium before use as APC.

On day 10, cultured lymphocytes were transferred to 75-cm^2 flasks to which complete medium containing 100 JRU/ml IL-2 was added. On days 14–16, the OK-432-reactive lymphocytes were collected, washed twice in RPMI-1640 medium, and transferred intravenously to the patient (Fig. 2).

Immunological assays

Lymphocyte phenotyping

Lymphocyte phenotypes were analyzed by a direct immunofluorescence assay with the following fluoroscein-isothiocyanate- or phycoerythrin-conjugated monoclonal antibodies on a FACScan flowcytometer (Becton-Dickinson): Leu4 (CD3), Leu3a (CD4), Leu2a (CD8), Leu11a (CD16), Leu18 (CD45RA), and HLA-DR.

Proliferation assay

Proliferation of lymphocytes was assayed by standard tritiated thymidine ([^{3}H]dT) incorporation and scintillation counting. Samples of 10^4 lymphocytes were seeded in each well of flat-bottomed 96-well culture plates and various concentrations of IL-2 or OK-432 were added to the culture in a total volume of 0.2 ml. During the last 8 h of a 96-h culture

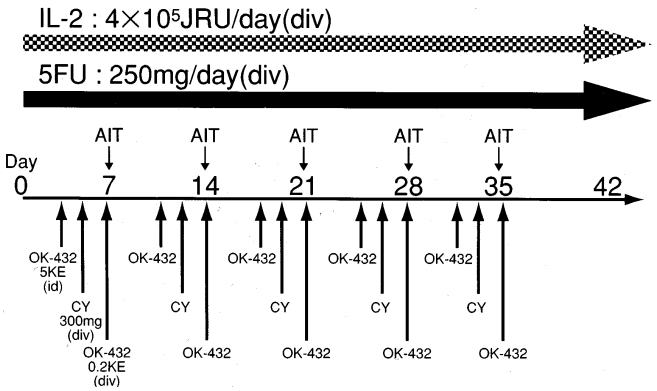


Fig. 3 Treatment regimen of each cycle of the therapy. *AIT* adoptive immunotherapy, *CY* cyclophsphamide, *id* intradermal administration, *div* intravenous drip administration

in a moist 5% CO_2 incubator at 37 °C, 37 kBq [^{3}H]dT was added to each well. Cells were then harvested and incorporation of [^{3}H]dT was counted in Beta Plate System (Pharmacia LKB).

Cytokine assay

To measure cytokines that were released in vitro from immunocompetent cells in response to OK-432 stimulation, a whole-blood cytokine assay, based on the method developed by Petrovsky et al. [15], was used. Fresh heparinized venous blood was collected from the patient and the donor. The blood (0.5-ml aliquots) was pipetted into 15-ml tubes and diluted tenfold in RPMI-1640 medium, which contained serial (0–0.1 KE/ml) concentrations of OK-432 without added serum. As a control, another biological response modifier, PSK (Kureha Chemical, Tokyo, Japan) was used as a stimulator. PSK is protein-bound polysaccharide and antigenically different from OK-432 [11, 14]. After mixing by repeated inversion of the tubes, they were incubated in a moist 5% CO_2 incubator for 24 h at 37 °C. The culture supernatant was then collected and stored at –80 °C until the assay for cytokines. Cytokines were measured by enzyme-linked immunosorbent assay (ELISA). The interferon γ (IFNγ), tumor necrosis factor α (TNFα) and IL-1β kits were supplied by the Otsuka Pharmaceutical Institute (Tokushima, Japan).

Treatment design and regimen

AIT for the patient using HLA-matched lymphocytes from his brother began on 2 February 1995. OK-432-reactive lymphocytes were induced from the donor's PBMC after in vivo sensitization by intradermal injection of 20 KE OK-432, and the lymphocytes were adoptively transferred to the patient intravenously with OK-432. Low-dose systemic immunochemotherapy using 5-FU, IL-2, and cyclophosphamide was administered to the patient concurrently with AIT (Fig. 2). 5-FU is an efficacious chemotherapy drug for gastric cancer, and better clinical effects can be obtained with IL-2 [12]. A relatively low dose of cyclophosphamide (300 mg) was administered intravenously 3 days prior to each AIT as a conditioning treatment in order to facilitate the acceptance of adopted lymphocytes (Fig. 3). Because our therapy transfers a large volume of activated allogeneic T cells, which is different from allogeneic bone marrow transplantation, the risk of severe graft-versus-host disease is quite high if complete immunosuppression occurs following a heavy pretreatment. One cycle of the immunochemotherapy used lasted 6 consecutive weeks. OK-432-reactive lymphocytes (3×10^9–6×10^9) were transferred to the patient once a week for 5 weeks (days 7, 14, 21, 28, 35 and 42) with intravenous (0.2 KE, days: 7, 14, 21, 28, 35 and 42) and intradermal (5 KE, days: 2, 9, 16, 23, 30 and 37) administration of OK-432; 4×10^5 JRU IL-2 and 250 mg 5-FU were continuously administered to the patient every day through a central-vein catheter (Fig. 3). OK-432 and IL-2 were administered to the patient in order to maintain the in vivo activity of adopted lymphocytes. The OK-432-reactive lymphocytes were washed twice with RPMI-1640 mdium immediately before the transfer to the patient.

After a 2-week interval, the next cycle of therapy was started. Until the middle of September 1995, the patient received five cycles of this immunochemotherapy using HLA-matched lymphocytes from his brother.

Results

Immune potentiality of the patient and the donor

Initially we planned to use the patient's autologous PBMC as a source of effector cells for AIT. However, he was in a severely immunosuppressed state because of cancer progression. Before this therapy, the patient's serum immunosuppressive acidic protein level was high, 615 μg/ml (normal range: <500 μg/ml, Fig. 4iii), and a complete blood count showed an increased percentage of monocytes

Fig. 4 Immune potentiality of the patient and the donor. *IAP* immunosuppressive acidic protein, *PLT* platelets, *Ht* hematocrit, *WBC* white blood cells, *lymph* lymphocytes, *neut* neutrophils, *mon* monocytes, *eos* eosinophils, *bas* basophils

(i) Complete blood count

	patient	donor
Ht (%)	31.2	46.4
PLT (×10³/μl)	481	184
WBC (×10³/μl)	4.4	4.6
lymph (%)	27.9	41.6
neut	39.5	46.7
mon	25.8	6.3
eos	6.2	5.2
bas	0.6	0.2

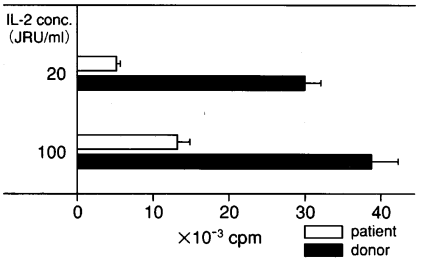
(ii) Phenotypes of peripheral blood lymphocytes

	patient	donor
CD3 (%)	76.8	43.7
CD4	50.4	32.2
CD8	23.5	19.7
CD16	11.9	47.6

(iii) serum IAP (μg/ml)

patient	donor
615	321

(iv) IL-2 responsiveness of PBMC



Each column and horizontal bar represents the mean and standard deviation of triplicate cultures

Table 1 Cytokine production in whole blood before and after OK-432 immunization of the donor and before and after OK-432 therapy of the patient. *IFN* interferon, *TNF* tumor necrosis factor, *IL* interleukin, *PSK* protein-bound polysaccharide

	Donor cytokines (pg/ml)						Patient cytokines (pg/ml)					
	Before			After			Before			After		
Stimulators	IFNγ	TNFα	IL-1β	IFNγ	TNFα	IL-1β	IFNγ	TNFα	IL-1β	IFNγ	TNFα	IL-1β
(–)	20>	20>	20>	20>	20>	20>	20	20>	20>	20>	20>	20>
OK-432 (KE/ml)												
0.001	135	30	116	3676	48	91	22	20>	124	5000<	186	343
0.01	222	102	217	4630	216	151	133	120	347	5000<	623	862
0.1	738	280	586	5000<	458	808	162	323	823	5000<	2171	4420
PSK (mg/ml)												
0.01	20>	50	66	20>	20>	65	20>	51	150	20>	20	32
0.05	33	222	63	20>	96	200	22	191	328	72	199	244
0.1	43	253	125	41	201	207	52	246	347	65	240	392

and a decreased percentage of lymphocytes in the white blood cells (Fig. 4i). In addition, the patient's PBMC showed very low proliferative response to IL-2 in vitro (Fig. 4iv), and no potent antitumor effector cells could be induced or expanded.

We therefore gave up the plan to use the patient's PBMC as a cell source for AIT, and decided to use his sole brother's HLA-matched PBMC. The donor's white blood cells in the peripheral blood contained many lymphocytes (Fig. 4i), and phenotypic analysis revealed that nearly half of the lymphocytes were $CD16^{(+)}$ natural killer cells (Fig. 4ii). The donor's PBMC responded well to IL-2 in vitro and expanded to a sufficient number for use in AIT (Fig. 4iv).

Characteristics of OK-432-reactive lymphocytes

After the donor had been immunized by intradermal injection of OK-432 (5 KE, four times), the whole-blood stimulation assay with OK-432 showed a strong cytokine release, in particular, that of IFNγ (Table 1). The donor's PBMC responded well to OK-432 and proliferated in vitro to optimal concentrations of 0.005–0.01 KE/ml (Fig. 5a).

After a 2-week culture, approximately 80% of the OK-432-reactive lymphocytes had a $CD4^{(+)}$ $CD45RA^{(-)}$ helper T cell phenotype (Fig. 5b) and secreted lymphokines such as IFNγ, TNFα, IL-2 in their culture supernatants, and they proliferated approximately 50-fold. When KatoIII cells (gastric cancer) were used as target cells, the 4-h ^{51}Cr-release assay revealed modest cytotoxicity of OK-432-reactive lymphocytes (data not shown). This suggested that OK-432-reactive lymphocytes had both helper and killer functions.

Clinical course

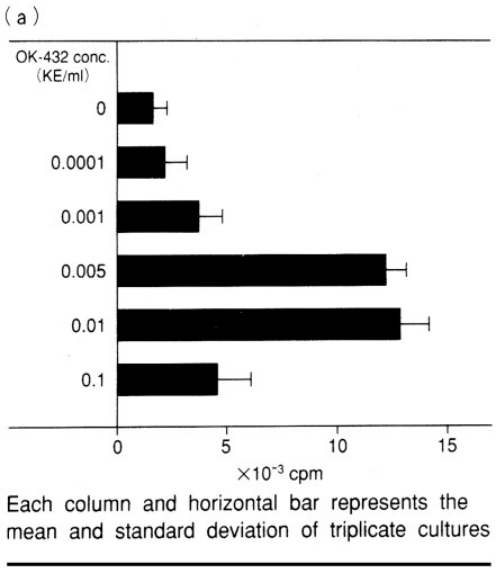
Between February and November 1995, five cycles of immunochemotherapy using OK-432-reactive lymphocytes were given to the patient. A total of 1.1×10^{11} OK-432-reactive lymphocytes were transferred intravenously. The interval between each treatment cycle was 2 weeks, during which time the patient was discharged from the hospital and took rest without having any treatment.

During the five cycles, the cancer progression was prevented and colorectal fluoroscopy after four cycles showed a reduction of the Schnitzler metastasis and widening of the intrarectal space (Fig. 1b). With the response to the therapy, the patient was able to continue taking food until the end of January 1996.

Side-effects of this treatment regimen were fever, transient renal dysfunction and anemia. Continuous but mild fever was observed after the initiation of therapy. High fever and chills were observed transiently after the transfer of lymphocytes, but these symptoms were easily controlled with indomethacin. Renal toxicity was observed but it was due to the basal renal hypofunction occurring after the initial chemotherapy with cisplatin. After the transfer of lymphocytes, the patient's serum creatinine level increased by 0.2–0.5 mg/dl transiently, but recovered within 2 or 3 days following the administration of flosemide. The anemia was treated with the transfusion of fresh whole blood offered from his brother (the lymphocyte donor).

Other hematological features were mild leukocytosis (8000–12000/mm^3) caused by OK-432, and prominent eosinophilia (30%–50% of the total white blood cells) caused by IL-2. No evidence of graft-versus-host-related complications were observed.

Even though we had planned to start the sixth cycle of the therapy from December 1995, severe pyelonephritis occurred at the end of November, the patient's serum creatinine level increased to 4.0 mg/dl, and it did not recover. Therefore, we gave up the immunochemotherapy with adoptive transfer of lymphocytes, and shifted the treatment regimen to a milder, weekly intravenous administration of 250 mg 5-FU, 10 mg epirubicin and intradermal administration of 5 KE OK-432. The peritonitis carcinomatosa progressed gradually from the beginning of 1996 and he died of disseminated intravascular coagulation syndrome on 22 April 1996.



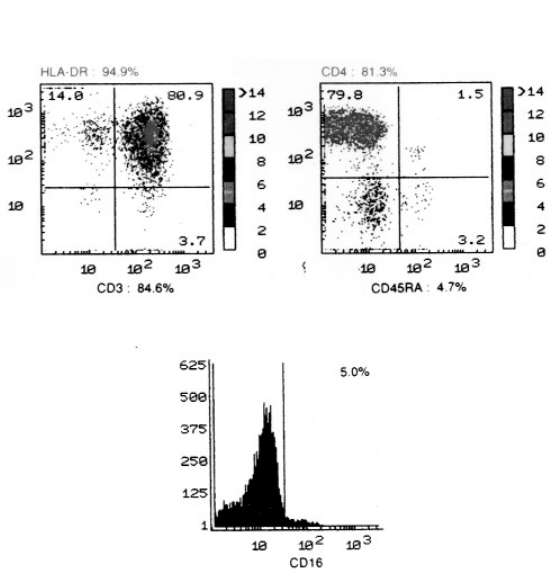


Fig. 5a OK-432 responsiveness of the donor's PBMC after OK-432 immunization. **b** Phenotypes of OK-432-reactive lymphocytes, which were induced after 14 days of culture: the $CD3^{(+)}$ $HLA\text{-}DR^{(+)}$ activated T cell phenotype (80.9%) and $CD4^{(+)}$ $CD45RA^{(-)}$ helper T cell phenotype (79.8%)

Cytokine assay before and after therapy

The whole-blood cytokine assays were performed before and after OK-432 immunization of the donor, and before and after OK-432 therapy of the patient (Table 1).

Before OK-432 immunization or OK-432 therapy, the immunopotent cells in the whole blood released low to moderate levels of cytokines in response to OK-432 and PSK stimulation. This cytokine release was considered to be the result of non-specific immunostimulatory activity of OK-432 and PSK.

One week after the last immunization with OK-432, the whole-blood cytokine responses of the donor to OK-432 were significantly augmented. Similarly, 1 week after the last day of the first cycle of the immunochemotherapy using OK-432 and OK-432-reactive lymphocytes, the patient's whole-blood assay revealed a marked increase in the cytokines released in response to OK-432, and the release of IFNγ was the most prominent. On the other hand, the IFNγ response to PSK was not affected by OK-432 immunization or OK-432 therapy.

Discussion

Since IL-2 became clinically applicable, AIT using patient's lymphocytes has actively been performed. However, there have been only a few case reports showing the usefulness of AIT for digestive-tract cancer [16]. Recent studies demonstrated *MAGE* gene expression in digestive-tract cancer and in vitro induction of MHC-restrictive cytotoxic T cells [10, 22]. This suggests that specific immunotherapy for digestive-tract cancer could be established. It is important to develop effective immunotherapy systems, including AIT for digestive-tract cancer, as there is a large population for treatment.

For effective AIT, the transfer of sufficient numbers of potent effector cells is important. However, candidates for AIT often have an advanced cancer that does not respond to conventional methods. Because of immunosuppression due to preceding chemotherapy or radiotherapy, it is often difficult to induce effector cells with high antitumor activity from a patient's lymphocytes. In addition, sufficient numbers of tumor-infiltrating lymphocytes cannot be collected from all patients [17]. Side-effects, such as anemia associated with leukapheresis, are great burdens for patients [9]. Therefore, we performed AIT using PBMC obtained from an HLA-matched sibling.

Recurrence after allogeneic bone marrow transplantation from an HLA-matched donor for hematological malignancies has been frequently treated with AIT using lymphocytes of the same donor and IL-2, and some effective cases have been reported [18, 23]. However, there have been few studies on AIT, not combined with allogeneic bone marrow transplantation, but using HLA-matched allogeneic lymphocytes for non-hematological malignancies. In 1985, Kohler et al. reported AIT using HLA haploidentical allogeneic lymphocytes for osteosarcoma and melanoma [7]. They used an immunosuppressive dose of cyclophosphamide (800 mg/m^2) and found no evidence of graft-versus-host-related complications in any of the patients. They mentioned that the absence of a graft-versus-host reaction might be attributable to incomplete immunosuppression of the patients. However, many of the donors in their study were first-degree relatives matched to the patient in one HLA haplotype. This involves a risk of lethal graft-versus-host disease [3]. On the other hand, Kimoto et al. performed AIT using lymphokine-activated killer cells induced from PBMC of some HLA-mismatched healthy donors in 12 patients with non-hematological malignancies, without any preconditioning treatment, and observed inhi-

bition of tumor growth in some patients without any graft-versus-host-related complications [5]. However, the safety and effectiveness of this method are unclear.

In our patient, AIT was performed under incomplete immunosuppression using relatively low-dose cyclophosphamide (1500 mg for one cycle of the therapy). In addition, concurrently administered OK-432 and IL-2 could activate the patient's antitumor immune response as well as the adopted lymphocytes, and these activations could then produce antitumor effects. This is only the third report following the cases described by Kohler et al. [7] and Kimoto et al. [5]. It is important for future studies to determine an optimal preconditioning treatment method and optimal cytokine use, in order to achieve safe and efficacious allogeneic lymphocyte therapy not combined with bone marrow transplantation.

We originally aimed to develop tumor-specific AIT based on MHC restriction using tumor antigens and lymphocytes of the patient's HLA-matched sibling. However, in our case, establishment of a tumor cell line in vitro was impossible. Therefore, as a pilot study, AIT using OK-432 as an exogenous antigen was performed. OK-432 is a streptococcal preparation and a biological response modifier widely used for gastric cancer patients in Japan [8], and recently the results of a phase IB trial for high-risk resected melanoma in the USA has been reported [6]. OK-432 is not only a non-specific immunopotentiator [6] but also an exogenous antigen that induces specific immune responses. Ozaki et al. established an OK-432-specific $CD4^{(+)}$ T helper cell clone in a murine system, and reported that the effects of its adoptive transfer together with OK-432 on tumor-bearing mice are attributable to OK-432-specific cytokine release and bystander tumor killing [13, 14]. It is also possible that OK-432-specific cytokine release in our patient after the transfer of OK-432-reactive $CD4^{(+)}$ T lymphocytes, together with non-specific immunoactivation by OK-432, produced antitumor effects. In addition, differences in minor histocompatibility antigens between the patient and donor could be involved in the tumor-killing mechanism [20].

We previously confirmed that the anti-OK-432 response (in terms of in vitro cytokine production on lymphocytes) in advanced gastric cancer patients is significantly lower than in healthy volunteers (unpublished data). On the other hand, cytokine production levels in our patient after receiving AIT using OK-432-reactive T cells were markedly higher than the levels in the donor (Table 1). Therefore, we consider that the strong anti-OK-432 response in our patient was induced by the synergetic effect of OK-432 and AIT, and not by OK-432 alone. In addition, cytophosphamide is reported to enhance delayed-type hypersensitivity against exogenous antigens under certain conditions [1]. In our patient, cytophosphamide would enhance the cellular immune response against OK-432, and would contribute to the appearance of the antitumor effect of AIT.

We also consider that the antitumor effect found in our patient was the result of the combination of AIT, OK-432, and IL-2, and not attributable to 5-FU: among 859 gastric cancer patients who were treated surgically in our institution between 1980 and the end of July 1997, 7 had very advanced gastric cancer with severe peritoneal dissemination, as seen in the patient reported here. These 7 patients received chemotherapy, mainly using 5-FU, but none of them survived for even 1 year. However, our patient survived for 508 days after the surgery. In addition, the effect of intraperitoneal chemotherapy (including its residual effect) was thought to be none or minor, because colorectal fluoroscopy immediately before the initiation of AIT revealed no changes in Schnitzler metastasis.

OK-432 and PSK are antigenically different biological response modifiers, and specific $CD4^{(+)}$ T cell lines can be established against each biological response modifier in murine systems [14]. Our findings suggest that OK-432 immunization or OK-432 therapy generates OK-432-reactive lymphocytes in vivo, that their restimulation in vitro produces cytokines in an antigen-specific manner, and that adoptive transfer of OK-432 reactive lymphocytes could augment this effect.

Studies using murine experimental tumor systems demonstrated that tumor-specific effector cells can be more efficiently induced in vitro by using lymphocytes of an immune mouse sensitized with the same tumor antigen than by using lymphocytes of a mouse bearing advanced cancer and in a severely immunosuppressive state [24]. Therefore, AIT using tumor antigens and lymphocytes of the patient's HLA-matched sibling, sensitized with the same tumor antigen, is thought to be a reasonable method. On the other hand, the procedure for such AIT is different from that of allogeneic bone marrow transplantation, which is performed under the complete suppression of the patient's immune system. This means that further studies on the involvement of migration and chimerism of adopted lymphocytes in the patient are necessary [7, 21].

At present, another clinical trial is underway in which PBMC are collected from each patient's HLA-matched sibling donor, who is sensitized with soluble tumor antigen extracted from the patient's tumor cell line [4]; cytotoxic T cells are induced in vitro by mixed lymphocyte/tumor cell culture, and the cytotoxic T cells together with IL-2 are transferred to the patient with advanced esophageal cancer. This AIT method could be an active specific immunotherapy for the prevention of cancer in a donor whose family shows a high incidence of cancer [19]. The clinical effects of this therapy will be clarified in the near future.

References

1. Berd D, Mastrangelo MJ, Engstrom PF, Paul A, Maguire H (1982) Augmentation of the human immune response by cyclophosphamide. Cancer Res 42:4862
2. Celis E, Tsai V, Crimi C, Demars R, Wentworth PA, Chesnut RW, Grey HM, Sette A, Serra HM (1994) Induction of anti-tumor cytotoxic T lymphocytes in normal humans using primary cultures and synthetic peptide epitopes. Proc Natl Acad Sci USA 91:2105
3. Ito K, Yoshida H, Yanagibashi K, Shimada Y, Imamura M, Tobe T, Akiyama Y, Saji H, Maruya E, Hosoi T (1988) Change of HLA phenotype in postoperative erythroderma. Lancet I:413

4. Kahan BD, Pellis NR, Legrue SJ, Tanaka T (1982) Immunotherapeutic effects of tumor-specific transplantation antigens released by 1-Butanol. Cancer 49:1168
5. Kimoto Y, Tanaka T, Tanji Y, Fujiwara A, Taguchi T (1994) Use of human leukocyte antigen-mismatched allogeneic lymphokine-activated killer cells and interleukin-2 in the adoptive immunotherapy of patients with malignancies. Biotherapy (Dordr) 8:41
6. Kirkwood JM, Wilson J, Whiteside TL, Bryant J, Doemelly S, Herberman RB (1997) Phase IB trial of picibanil (OK-432) as an immunomodulator in patients with resected high-risk melanoma. Cancer Immunol Immunother 44:137
7. Kohler PC, Hank JA, Exten R, Minkoff DZ, Wilson DG, Sondel PM (1985) Clinical response of a patient with diffuse histiocytic lymphoma to adoptive chemoimmunotherapy using cyclophosphamide and alloactivated haploidentical lymphocytes. A case report and phase I trial. Cancer 55:552
8. Maehara Y, Okuyama T, Kakeji Y, Baba H, Furusawa M, Sugimachi K (1994) Postoperative immunochemotherapy including streptococcal lysate OK-432 is effective for patients with gastric cancer and serosal invasion. Am J Surg 168:36
9. Margolin KA, Rayner AA, Hawkins MJ, Atkins MB, Dutcher JP, Fisher RI, Weiss GR, Doroshow JH, Jaffe HS, Roper M, Parkinson DR, Wiernik PH, Creekmore SP, Boldt DH (1989) Interleukin-2 and lymphokine-activated killer cell therapy of solid tumors: analysis of toxicity and management guidelines. J Clin Oncol 7:486
10. Nakao M, Yamana H, Imai Y, Toh Y, Tou U, Kimura A, Yanoma S, Kakegawa T, Itoh K (1995) HLA A2601-restricted CTLs recognize a peptide antigen expressed on squamous cell carcinoma. Cancer Res 55:4248
11. Nakazato H, Koike A, Saji S, Ogawa N, Sakamoto J (1994) Efficacy of immunochemotherapy as adjuvant treatment after curative resection of gastric cancer. Lancet 343:1122
12. Okuno K, Kobayashi S, Jinnai H, Lee YS, Shigeoka H, Yasutomi M (1996) IL-2 perfusion to the liver augments the hepatic extraction rate of accompanying anticancer drugs. Surg Today 26:662
13. Ozaki S, Suginoshita T, Watanabe T, Obayashi H (1990) Mechanism of tumoricidal activity of OK-432-specific L3T4^{+}Lyt2^{-} T cells. Cancer Res 50:4630
14. Ozaki S, Okazaki T, Nakao K (1995) Biological response modifiers (BRM) as antigens III. T cell lines specific for BRM kill tumor cells in a BRM-specific manner. Cancer Immunol Immunother 40:219
15. Petrovsky N, Harrison LC (1995) Cytokine-based human whole blood assay for the detection of antigen-reactive T cells. J Immunol Methods 186:37
16. Rosenberg SA (1992) The immunotherapy and gene therapy of cancer. J Clin Oncol 10:180
17. Rosenberg SA, Yannelli JR, Yang JC, Topalian SL, Schwarzentruber DJ, Weber JS, Parkinson DR, Seipp CA, Einhorn JH, White DE (1994) Treatment of patients with metastatic melanoma with autologous tumor-infiltrating lymphocytes and interleukin-2. J Natl Cancer Inst 86:1159
18. Slavin S, Naparstek E, Nagler A, Ackerstein A, Samuel S, Kapelushnik J, Brautbar C, Or R (1996) Allogeneic cell therapy with donor peripheral blood cells and recombinant human interleukin-2 to treat leukemia relapse after allogeneic bone marrow transplantation. Blood 87:2195
19. Sondel PM, Hank JA (1981) Alien-driven diversity and alien-selected escape: A rationale for allogeneic cancer immunotherapy. Transpl Proc 13:1915
20. Sondel PM, Hank JA, Wendel T, Flynn B, Bozdech MJ (1983) HLA identical leukemia cells and T cell growth factor activate cytotoxic T cell recognition of minor locus histocompatibility antigens in vitro. J Clin Invest 71:1779
21. Starzl TE, Demetris AJ, Murase N, Ildstad S, Ricordi C, Trucco M (1992) Cell migration, chimerism, and graft acceptance. Lancet 339:1579
22. Toh Y, Yamana H, Shichijo S, Fujita H, Tou U, Sakaguchi M, Kakegawa T, Itoh K (1995) Expression of MAGE-1 gene by esophageal carcinomas. Jpn J Cancer Res 86:714
23. Verdonck LF, Lokhorst HM, Dekker AW, Nieuwenhuis HK, Petersen EJ (1996) Graft-versus-myeloma effect in two cases. Lancet 347:800
24. Zou JP, Shimizu J, Ikegame K, Yamamoto N, Ono S, Fujiwara H, Hamaoka T (1992) Tumor-bearing mice exhibit a progressive increase in tumor antigen-presenting cell function and a reciprocal decrease in tumor antigen-responsive CD4^{+} T cell activity. J Immunol 148:648