

Adoptive Immunotherapy for Pancreatic Cancer Using MUC1 Peptide-pulsed Dendritic Cells and Activated T Lymphocytes

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Abstract. *Background:* Pancreatic cancer has a poor prognosis. The clinical efficacy of immunotherapy using both dendritic cells pulsed with MUC1 peptide (MUC1-DC) and, cytotoxic T lymphocyte (CTL) sensitized with a pancreatic cancer, YPK-1, expressing MUC1 (MUC1-CTL) was evaluated. *Patients and Methods:* Twenty patients with unresectable or recurrent pancreatic cancer were enrolled. Peripheral blood mononuclear cells (PBMCs) were separated into adherent cells for induction of MUC1-DCs and floating cells for MUC1-CTLs. MUC1-DCs were generated by culture with granulocyte monocyte colony stimulating factor (GM-CSF) and interleukin-4 (IL-4) and then exposed to MUC1 peptide and TNF- α . MUC1-CTLs were induced by co-culture with YPK-1 and then with interleukin-2 (IL-2). MUC1-DCs were injected intradermally and MUC1-CTLs were given intravenously. *Results:* Patients were treated from 2 to 15 times. One patient with multiple lung metastases experienced a complete response. Five patients had stable disease. The mean survival time was 9.8 months. No grade II-IV toxicity was observed. *Conclusion:* Adoptive immunotherapy with MUC1-DC and MUC1-CTL may be feasible and effective for pancreatic cancer.

Pancreatic cancer has the poorest prognosis among gastrointestinal cancer because of its low resectability, malignant behavior and low sensitivity to anticancer agents (1). Treatments for pancreatic cancer, including surgery, chemotherapy and radiotherapy, have failed to improve the

prognosis (2, 3). Eighty percent of patients are not eligible for surgical resection because of local spread or metastatic disease at the time of diagnosis. Even with curative surgery, the mean survival time (MST) does not exceed 2 years, with a 5-year survival of 15%-25% (4-6). New treatment strategies are necessary to improve the outcome of patients with pancreatic cancer.

Immunotherapy has an advantage over radiation and chemotherapies because it can act specifically against the tumor without damaging normal tissue. Immunotherapeutic approaches to pancreatic cancer have included the use of monoclonal antibodies (7, 8), cytokines (9), vaccines (10, 11) and lymphokine-activated killer (LAK) cells (12). We have also reported the efficacy of adoptive immunotherapy (AIT) with cytotoxic T lymphocytes (CTLs) stimulated by autologous pancreatic tumors (13) and vaccination with a variety of peptides (14, 15).

Mucins are large glycoproteins that are expressed by a variety of normal and malignant epithelial cells (16). MUC1, a mucin polypeptide, is unique in its transmembrane expression at the cell surface (17). Cancer-associated MUC1 is incompletely glycosylated and has truncated carbohydrate chains composed largely of 1-6 sugar units (18). It has been proposed that normally hidden CTL epitopes in the MUC1 core protein are unmasked by underglycosylation in tumor cells and that highly multivalent epitopes of tandemly repeated peptides on a single MUC1 molecule crosslink the T-cell receptors of anti-MUC1 CTLs (19). CTLs against MUC1 have been induced in patients with pancreatic, breast and ovarian cancer (20-22). Interestingly, unlike conventional CTLs, anti-MUC1 CTLs recognize MUC1 molecules in an HLA-unrestricted manner (20). Such immune responses against MUC1 may be useful for immunotherapy. It has been reported that MUC1 apomucin is the major type of mucin in invasive ductal carcinoma of the pancreas (23-26). Therefore, MUC1 may serve as a target antigen for the treatment of pancreatic cancer. We have reported the use of AIT with CTLs stimulated by a MUC1-expressing human pancreatic

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cancer cell line, YPK-1 (27), for unresectable pancreatic cancer (28). In that study, we reported that induced CTLs were cytotoxic against five MUC1-expressing pancreatic cancer cell lines and one breast cancer cell line, regardless of their HLA phenotype. Clinically, AIT with MUC1-CTLs for unresectable pancreatic cancer did not improve survival, although no patient without liver metastasis developed liver metastasis during the study. Likewise, the adjuvant setting of this AIT for patients with curatively resected pancreatic cancer can prevent hepatic recurrence but may not be able to prevent local recurrence and peritoneal dissemination. CTLs may distribute mainly to the liver. AIT with MUC1-CTLs alone for pancreatic cancer was not sufficiently effective to prevent local progression of disease. To overcome these limitations, we developed an AIT using a combination of MUC1-CTLs and dendritic cells (DCs) pulsed with MUC1 peptide.

DCs are the most potent antigen-presenting cells in the human body and are involved in the regulation of both innate and adoptive immune response (29-31). Vaccination with DCs loaded with tumor-specific antigens or tumor-associated antigens (TAAs) has been shown to induce protective immune responses in several animal models (32, 33). The results of several clinical trials that have investigated the use of tumor antigen-pulsed DC vaccines for the treatment of various advanced malignancies have been reported (34). One important issue in applying DC-based cancer immunology is the choice of the appropriate TAA.

To create a more effective therapy, we conducted combined AIT using DCs pulsed with MUC1 peptide (MUC1-DC) added to our previous AIT with CTLs stimulated by MUC1-expressing YPK-1 cells (MUC1-CTL). The objective of this study was to evaluate the clinical efficacy of combination AIT with MUC1-CTLs and MUC1-DCs to treat unresectable or recurrent pancreatic cancer.

Patients and Methods

Patients. During the period 2001-2006, 20 patients with unresectable or recurrent pancreatic cancer histologically confirmed as invasive ductal carcinoma were treated. The clinicopathological features of these patients are described in detail in the Results section. Patients were treated at the Department of Digestive Surgery and Surgical Oncology (Department of Surgery II) of the Yamaguchi University Graduate School of Medicine (Japan). The study protocol was approved by the Institutional Review Board for Human Use of Yamaguchi University School of Medicine. Written informed consent was obtained from all patients.

Pretreatment evaluation and follow-up. Pretreatment evaluation included a complete medical history, physical examination, imaging of measurable tumors, complete blood cell count, biochemical screening profile. During treatment, patient monitoring included assessment of clinical toxicities, complete blood cell count, serum chemistry and physical examination. Adverse effects were evaluated

according to the Common Terminology Criteria for Adverse Events v3.0 (CTCAE) (35). Evaluation of treatment response by computed tomography (CT) scan was repeated every 4 weeks according to the Response Evaluation Criteria in Solid Tumors (RECIST) Committee (36).

Cell lines. Human cancer cell lines used in the experimental study were YPK-1 and -3 (pancreatic cancer) and YES-1 and -2 (esophageal cancer) established in our department. All cell lines were maintained in Dulbecco's modified Eagle's medium (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan) containing 10% heat-inactivated fetal bovine serum (FBS) (Invitrogen, Carlsbad, CA, USA), 100 units/mL penicillin and 0.1 mg/mL streptomycin, and cultured in a 5% CO₂ atmosphere at 37°C. YPK-1 and -3 were MUC1⁺, whereas YES-1 and -2 were MUC1⁻. The HLA-A typing status was as follows: YPK-1, A24/0201; YPK-3, A0201/-; YES-1, A0201/-; and YES-2, A24/-.

Separation of adherent and non-adherent cells. Peripheral blood mononuclear cells were harvested with the COBE Spectra Apheresis System (COBE BCT, Inc., Lakewood, CO, USA) every 2 to 4 weeks. PBMCs from 2,500 ml of blood were enriched by density gradient centrifugation with Ficoll-Paque (Amersham Pharmacia Biotech, Uppsala, Sweden). The PBMCs were incubated for 45 min in a 5% CO₂ atmosphere at 37°C in serum-free AIM-V medium (Gibco, Paisley, Scotland). Plastic-adherent cells were used for generation of DCs, while non-adherent cells were used for generation of CTLs. On day 10, patients were injected with MUC1-CTLs and MUC1-DCs. Patients were repeated by received this AIT until progressive disease was recognized.

Generation of MUC1-CTLs. The PBMCs harvested from each patient were incubated for 45 min in a plastic plate. Non-adherent cells were cultured in AIM-V with the MUC1-expressing pancreatic cancer cell line YPK-1 inactivated with 0.2 mg/mL mitomycin C (Kyowa Hakko Kogyo Co., Ltd., Tokyo, Japan). The effector-to-stimulator cell ratio was 1,000:1. After 3 days of culture, the cells were cultured with 10 Japan Reference Units (JRU)/mL recombinant human interleukin (rhIL)-2 (Shionogi Pharmaceutical Co., Tokyo, Japan) in a 5% CO₂ atmosphere at 37°C for 7 days. These cells were termed MUC1-CTLs. Cultures were examined for endotoxins, mycoplasma and bacterial contamination prior to administration. On day 10, MUC1-CTLs were washed 3 times with saline, suspended in 100 mL saline and administered intravenously as maximum available cell products.

Generation of MUC1-DCs. The PBMCs harvested from each patient were incubated for 45 min in a plastic plate. Adherent cells were cultured in AIM-V medium containing 800 units/mL granulocyte macrophage colony-stimulating factor (Osteogenetics GmbH, Wurzburg, Germany), and 500 units/mL interleukin-4 (Osteogenetics GmbH). On day 6, immature DCs were pulsed with MUC1 peptide. The MUC1 peptide, which was a 100-mer peptide consisting of the extracellular tandem repeat domain, was provided by Olivera J. Finn (Department of Immunology, University of Pittsburgh, Pittsburgh, PA, USA). On day 7, immature DCs were cultured in AIM-V medium containing 300 units/mL tumor necrosis factor- α (R&D Systems, Minneapolis, MN, USA). Cultures were checked for endotoxins, mycoplasma and bacterial contamination prior to administration. On day 10, floating and

Table I. Characteristics and treatment of the patients with unresectable pancreatic cancer.

No.	Age (years)	Gender	HLA-A	TNM stage	Location of metastasis	Total number of CTLs (x10 ⁹)	Total number of DCs (x10 ⁷)	Clinical response (TTP*, months)	Survival (months)
1	52	F	24/26	IV	Liver	5.7	31.0	SD (6)	12
2*	63	M	02/31	IV	Lung	3.2	15.0	CR	75
3	63	M	24/-	IV	Liver	1.9	7.1	PD	2
4	64	M	02/26	IV	Liver	0.8	3.4	PD	2
5	57	M	24/02	IV	Peritoneum	0.9	4.3	PD	3
6	58	M	11/31	IV	Liver	0.7	4.0	PD	4
7	73	M	02/-	IV	Liver, peritoneum	0.6	4.4	PD	3
8	84	M	24/26	IV	Liver	1.1	6.4	SD (3)	11
9	83	M	11/31	IV	Bone, liver	1.0	6.0	PD	6
10	58	M	02/31	III		4.0	1.1	PD	5
11	51	M	02/-	IV	Liver	6.8	5.2	PD	3
12*	57	F	24/33	III	Lymph node	4.7	17.0	SD (17)	24
13	58	F	02/31	III		4.0	11.0	PD	5
14	51	F	02/-	IV	Liver	0.7	5.2	SD (9)	12
15	73	F	33/-	IV	Liver	0.5	4.5	PD	2
16	58	M	02/33	III		1.3	11.0	PD	9
17	67	F	24/-	IV	Liver, peritoneum, bone, brain	1.8	12.1	PD	5
18	66	M	11/24	IV	Peritoneum	1.2	4.9	PD	4
19	68	M	11/24	III		1.5	6.0	SD (2)	6
20*	63	M	24/26	IV	Peritoneum	1.8	5.3	PD	2

Patient No. 2 and No. 12 received pancreato-duodenectomy as curative operation; Patient No. 20 received gastro-jejunostomy; Patient No. 2 had a complete response after AIT and is still alive. TTP: Time to progression. SD: stable disease; PD, progressive disease; CR, complete response.

loosely adherent cells were collected as mature MUC1-DCs. DCs were washed three times with saline, suspended in 5 mL saline and injected intradermally in the inguinal region as maximum available cell products.

Analysis of CTL subsets. Induced CTL subsets were analyzed with monoclonal antibodies against surface antigens of human lymphocytes. All monoclonal antibodies were purchased from Coulter (Hiialeah, FL, USA). Fluorescein isothiocyanate (FITC)-conjugated anti-CD3 (T3), -CD4 (T4), -CD20 (B1), -CD25 (IL-2R1), -CD56 (NKH-1) -HLA-DR (I2), and -CD11b (MO1) were used. Phycoerythrin (PE)-conjugated anti-CD8 (T8) and -CD62L were also used according to the manufacturer's instructions. Two-color analysis was performed with a combination of CD62L+/CD4+ (helper T cell) and CD8+/CD11b- (cytotoxic T cell). Samples were analyzed with an EPICS Flow Cytometer (Coulter) at a fluorescence excitation wavelength of 488 nm at 200-500 mW. For each sample, 5,000 lymphocytes were analyzed.

Analysis of DC subsets. Induced DC subsets were analyzed with monoclonal antibodies against surface antigens. All monoclonal antibodies were purchased from Coulter. FITC-conjugated anti-CD80 (B7-1), -CD83 (HB-15), -CD14 (B1), -HLA-ABC and -HLA-DR (I2) were used. PE-conjugated anti-CD86 (B7-2) and -CD40 were also used according to the manufacturer's instructions. Samples were analyzed with an EPICS Flow Cytometer (Coulter) at a fluorescence excitation wavelength of 488 nm at 200-500 mW. For each sample, 5,000 DCs were analyzed.

Induction of MUC1 peptide-pulsed DC-activated killer (MUC1-DAK) cells. MUC1-DAK cells were induced from PBMCs of a healthy

volunteer with HLA-A24/26 stimulated by co-culture with MUC1-DC at a ratio of 100:1 (PBMCs:MUC1-DC) for 7 days in AIM-V medium. Activated lymphocytes are referred to as MUC1-DAK (37).

Cytotoxicity assay. Pancreatic cancer cell lines YPK -1 and -3, and esophageal cancer cell lines YES-1 and -2 were used as target cells. Target cells (1x10⁶/mL) were labeled for 60 minutes at 37°C with 100 µCi/mL radioactive sodium chromate (⁵¹Cr) (Amersham Japan, Tokyo, Japan). The cells were then washed 4 times in RPMI 1640 medium (Nikken biomedical laboratory, Kyoto, Japan). Labeled cells were resuspended in culture medium at a density of 1x10⁵ cells/mL. Effector cells consisting of induced CTLs were suspended at 0.5, 1.0, or 2.0x10⁶/mL, and 0.1 mL effector cell suspension was added to a micro plate (Falcon Plastics, Cockeysville, MD, USA) with 0.1 mL target cells to yield an effector-to-target cell ratio of 5:1, 10:1, or 20:1. All experiments were performed in triplicate. Plates were incubated for 4 hours at 37°C in a CO₂ incubator. The amount of ⁵¹Cr released into each well was determined with a gamma counter (Auto Well Gamma System ARC-202, Aloka, Tokyo, Japan). The percentage of cytotoxicity was calculated as follows:

$$\% \text{ Cytotoxicity} = \frac{[\text{experimental release} - \text{spontaneous release}]}{[\text{maximum release} - \text{spontaneous release}]}$$

To measure spontaneous ⁵¹Cr release by target cells in the absence of effector cells, target cells were mixed with 0.1 mL culture medium. To measure maximal ⁵¹Cr release, target cells were treated with 0.1 mL 0.1 N hydrochloric acid.

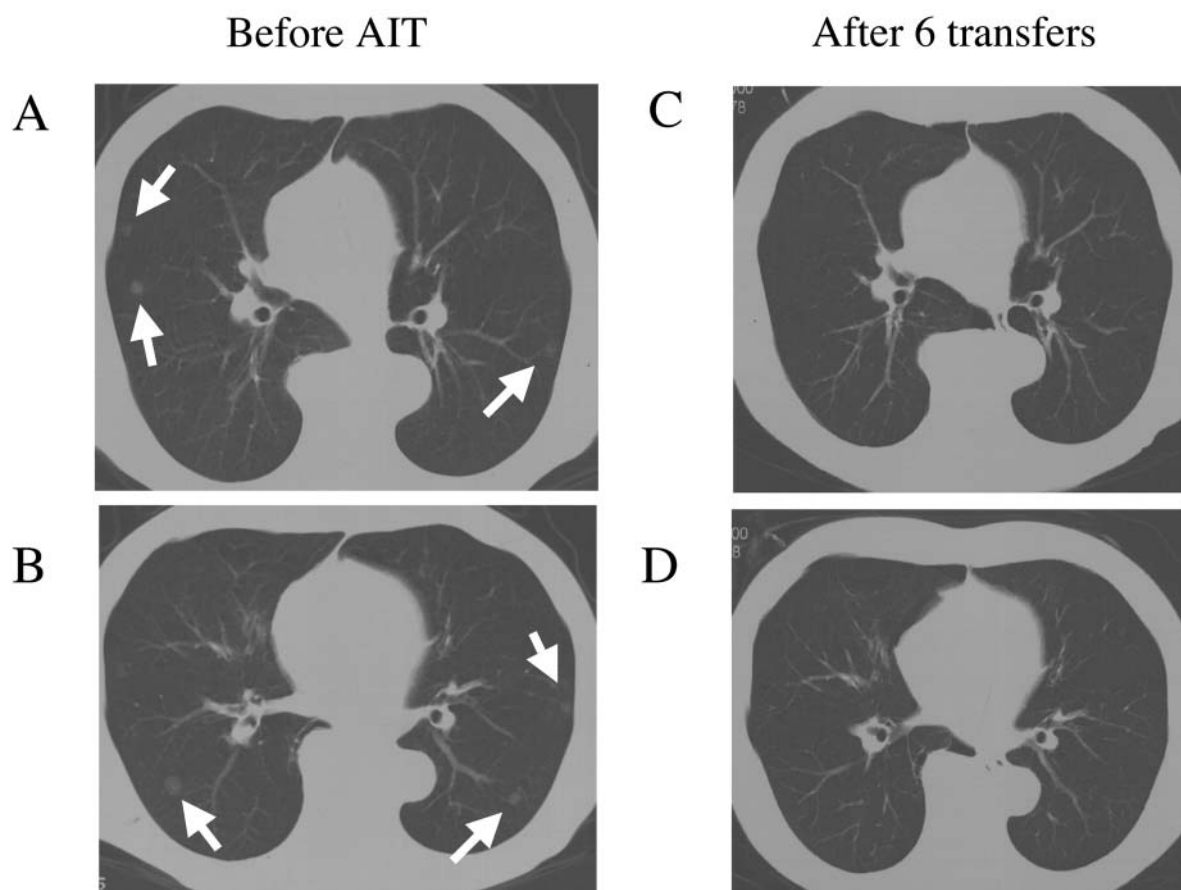


Figure 1. Chest CT scans of patient No. 2 before AIT (A and B) and after 6 transfers (C and D). After AIT, multiple lung nodules disappeared completely.

Statistical analysis. Changes in surface markers were assessed with Student's *t*-test for paired or unpaired means. A *p*-value of less than 0.05 was considered significant. Values are presented as mean \pm standard error (SE). Kaplan-Meier analysis was used to estimate cumulative survival.

Results

Patient characteristics. The patients' clinicopathological features are listed in Table I. Eleven of the 20 patients showed liver metastasis and 5 showed peritoneal dissemination. One patient showed lung metastasis, but no liver metastasis or peritoneal dissemination. Fifteen of the 20 patients were stage IV and 5 were stage III according to the TNM staging of the UICC (6th edition) (38), and all showed histologically confirmed invasive ductal carcinoma of the pancreas. Sixteen of the twenty patients did not receive chemotherapy. Four patients had received gemcitabine prior to the present AIT.

Patients were treated from 2 to 15 times with both cell types (Table I). Total numbers of MUC1-DCs and MUC1-

CTLs administered were 1.1×10^7 to 3.1×10^8 and 5.0×10^8 to 6.8×10^9 , respectively (Table I).

Efficacy. One of the 20 patients (patient 2), who had multiple lung metastases after curative surgery, had a complete response. Figure 1 shows the chest CT scans of patient 2 before AIT and after 6 transfers. After AIT, multiple lung nodules disappeared completely. Five of twenty patients had stable disease. The duration of stable disease is given in Table I as time to progression (TTP). Patient 1, who had unresectable pancreatic cancer with multiple liver metastases, had stable disease for at least 6 months. Figure 2 shows the abdominal CT scans of patient 1 before AIT and after 8 treatments. After AIT, there was no progression of the primary pancreatic cancer. One hepatic metastasis decreased in size, although another increased. The clinical outcome of mixed efficacy was considered as stable disease according to RECIST criteria. No new lesion was observed in patient 1 for 6 months. The survival times of the 20 pancreatic cancer patients who

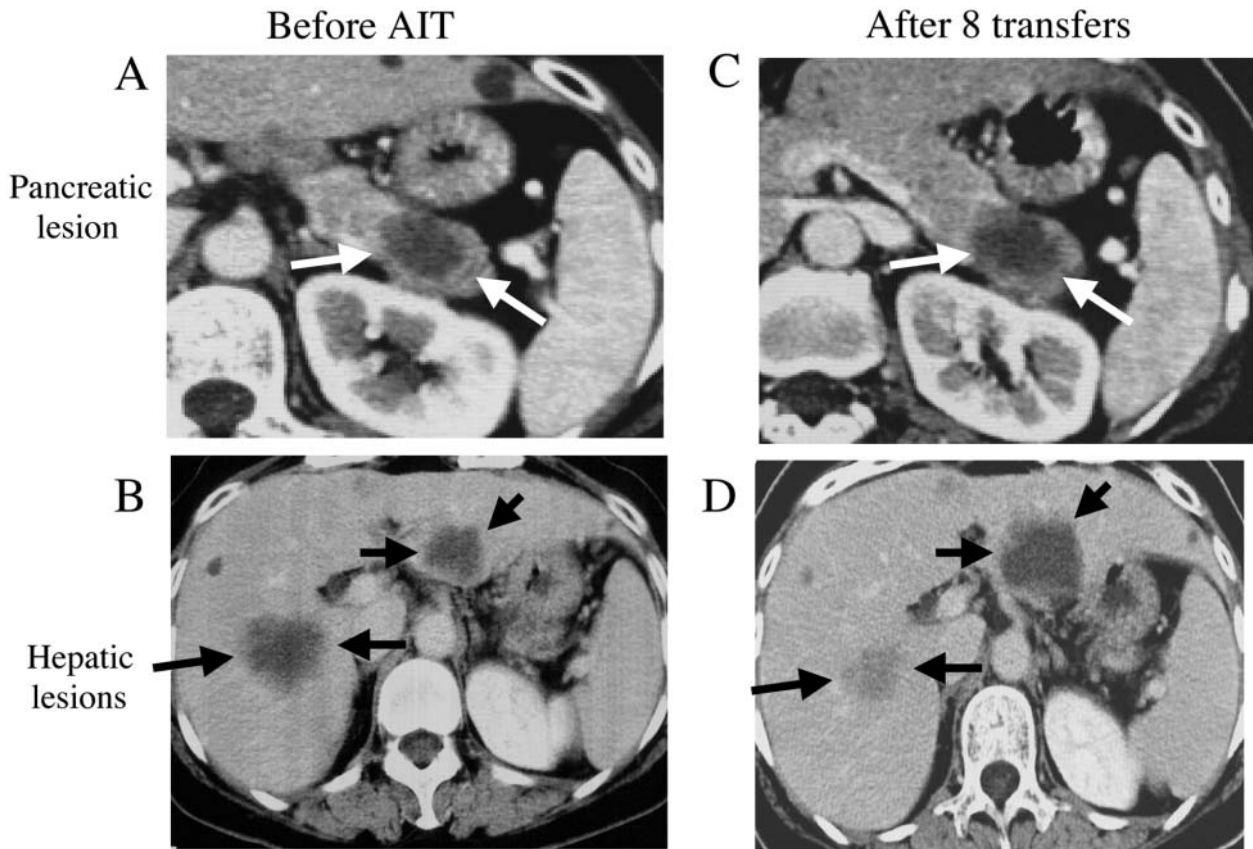


Figure 2. Abdominal CT scans of patient No. 1 before AIT (A and B) and after 8 treatments (C and D). After AIT, there was no progression of primary pancreatic cancer. One hepatic metastasis decreased in size, although another increased. Clinical outcome of mixed efficacy was considered stable disease according to RECIST criteria. No new lesion was observed for 6 months.

underwent AIT with MUC1-CTLs and MUC1-DCs ranged from 2 to 75 months, with a mean survival time of 9.8 months. One-, two-, and three-year survival rates after AIT were 20.0%, 10.0%, and 5.0%, respectively.

Toxicity. No grade II-IV toxicity according to CTCAE was observed in any patient after injection of MUC1-DC and MUC1-CTL. Only one patient developed transient systemic itching.

Characteristics of MUC1-CTLs. We assessed changes in lymphocyte subsets before and after culture of PBMCs. The proportions of CD3⁺ cells (63.3%±1.6% before culture to 80.8%±2.4% after culture), CD4⁺ cells (37.8%±1.7% to 54.5%±2.8%), HLA-DR⁺ cells (16.0%±1.1% to 22.5%±2.9%), and CD8⁺/CD11⁻ cells (cytotoxic T-cells) (23.9%±1.3% to 32.3%±1.8%) increased significantly after culture ($p<0.05$), whereas the proportion of CD56⁺ cells (NK cells) (24.5%±1.7% to 17.4%±1.7%) decreased significantly ($p<0.05$).

Differentiation of DC surface marker expression. Differentiation of DC surface marker expression on the basis of a short survival time (shorter than 6 months' survival after AIT: n=12) and a long survival time (longer than 6 months' survival after AIT: n=8) is shown in Table II. DCs displayed the mature DC phenotype, CD80⁺, CD86⁺, HLA-ABC⁺, HLA-DR⁺, CD40⁺, CD14⁻ and CD83⁺. The long survival group showed significantly higher expression of CD83 than did the short survival group ($p=0.038$).

Cytotoxic activity of MUC1-DAKs. MUC1-DAKs were induced from PBMCs of a healthy volunteer with HLA-A24/26 as described the Patients and Methods. The profile of cytotoxicity of MUC1-DAKs against pancreatic cancer and esophageal cancer cell lines is shown in Figure 3. MUC1-DAKs showed strong cytotoxicity against the pancreatic cancer cell lines which expressed MUC1 antigen on the cell surface in an HLA-independent manner. However, cytotoxicity against the esophageal cancer cell lines, which did not express MUC1, was low.

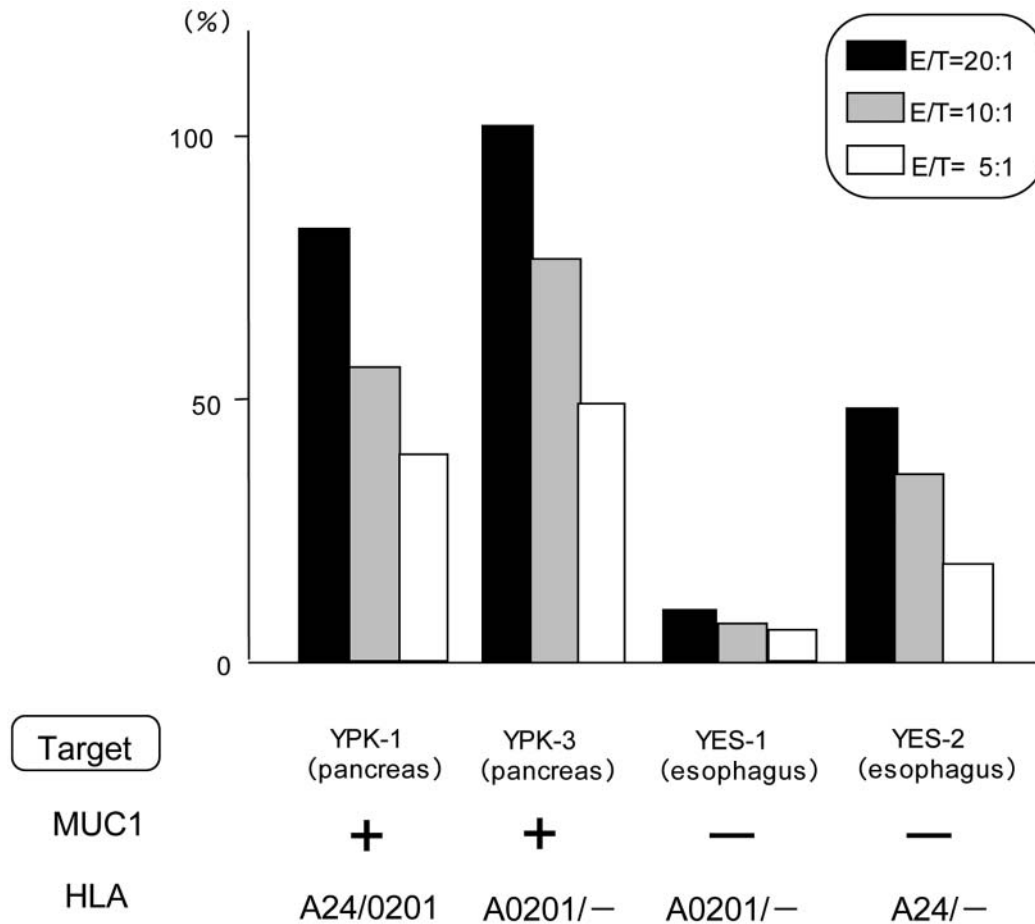


Figure 3. Cytotoxicity of MUC1-DAK. MUC1-DCs and MUC1-DAKs were induced from a healthy volunteer with HLA A24/26. MUC1-DAKs have strong cytotoxicity against cell lines that express MUC1 antigen on the cell surface (YPK-1 and -3) independent of HLA-A24. MUC1-DAKs also show stronger cytotoxicity against cell lines that express HLA A24 (YES-2) than against cell lines that do not express HLA A24 (YES-1).

Discussion

In the present clinical study, AIT with MUC1-DCs and MUC1-CTLs resulted in greater than 20% 1-year survival for patients with unresectable or recurrent pancreatic invasive ductal carcinoma. One of the 20 patients, who had multiple lung metastases after curative surgery, experienced a complete response that has lasted more than 5 years (Figure 1) and 5 patients had stable disease. One of these five patients, who had unresectable pancreatic cancer with multiple liver metastases, had stable disease for 6 months (Figure 2). The long survival group (longer than 6 months' survival after AIT) showed significantly higher expression of CD83 than did the short survival group. MUC1-DCs induced CTLs having strong cytotoxicity against pancreatic cell lines, which expressed MUC1 antigen on the cell surface, in an HLA-independent manner.

MUC1 is a target antigen for tumor-reactive CTLs from pancreatic, breast and ovarian cancers and multiple myeloma (20, 39). These CTLs recognize MUC1 directly in an MHC-unrestricted manner (21, 22, 40). Clinical trials have been performed with MUC1 peptide (41) and MUC1 peptide-sensitized DCs (42-44) for patients with adenocarcinoma, however, there have been few studies for pancreatic cancer (10). The present study is the first to confirm the efficacy of MUC1 peptide-pulsed DCs for treatment of pancreatic cancer. We previously reported that MUC1-CTLs are induced from PBMCs by co-culture with a pancreatic cancer cell line that expresses a high level of MUC1 (28). Blocking assays showed that cytotoxicity of MUC1-CTLs is MHC unrestricted, which means that these cells can be used for all patients with tumors expressing MUC1 antigen. We also reported that invading or metastatic pancreatic cancer cells express MUC1 all along

Table II. Differentiation of dendritic cell surface marker expression between the short survival group and long survival group (mean±SE).

Surface marker	Expression of short survival group (n=12) (%)	Expression of long survival group (n=8) (%)	P-value
CD80	74.4±6.4	77.6±10.1	NS
CD86	94.6±2.0	96.3±1.0	NS
CD83	36.7±5.7	57.7±4.0	0.038
CD40	98.0±0.5	99.1±0.2	NS
HLA-DR	84.9±3.6	91.9±1.7	NS
HLA-ABC	98.0±0.5	98.5±0.3	NS
CD14	3.7±2.4	1.0±0.2	NS

Short survival group: survival shorter than six months after AIT; long survival group: survival longer than six months after AIT.

the cell membrane (26). Therefore, we hypothesized that MUC1-CTLs may be therapeutically useful for pancreatic cancer. In a preliminary study (28), AIT with MUC1-CTLs for unresectable pancreatic cancer did not improve survival, although no patient without liver metastasis developed liver metastasis. Likewise, the adjuvant setting of this AIT for patients with curatively resected pancreatic cancer can prevent hepatic recurrence but may not be able to prevent local recurrence and peritoneal dissemination. It has been reported that intravenously injected peripheral blood lymphocytes and LAK cells are taken up mostly by the liver and spleen (45). Therefore, CTLs may distribute mainly to the liver. AIT with MUC1-CTLs only for pancreatic cancer was not sufficiently effective to prevent local progression of disease. To overcome these limitations, we developed an AIT using a combination of MUC1-CTLs and DCs pulsed with MUC1 peptide.

DCs are potent antigen-presenting cells that are integral to the initiation of T-cell immunity (29-31, 42-44, 46). In the present study, we found that DCs pulsed with a 100-mer MUC1 peptide induced MUC1-DAK cells with strong cytotoxicity against pancreatic cancer cell lines that express MUC1 but not against MUC1-negative esophageal cancer cell lines (Figure 3). In our previous AIT with MUC1-CTLs, MST was only 5 months, and none of the patients survived more than 1 year (28). In contrast, MST was 9.8 months for the present combination therapy with MUC1-CTLs and MUC1-DCs. Moreover, the 1-, 2- and 3-year survival rates after combination AIT were 20%, 10% and 5%, respectively. This combination AIT can influence not only liver metastases but also local lesions and lung metastases. The efficacy of this combination therapy appears to be due to the additional effects of MUC1-DCs.

The analysis of the DC subsets showed that the population of mature DCs that expressed CD83 was higher in patients surviving more than 6 months than in patients

surviving less than 6 months (Table II). CD83 serves as a useful and specific marker for mature DCs in human blood (47). CD83⁺ DCs display all the phenotypic and morphological characteristics of mature DCs and are the most potent antigen-presenting cells (48). Therefore, expression of CD83 by DCs is a predictive marker for the efficacy of DC-based therapy. We reported that the percentage of pancreatic cancer patients (1 of 15, 6.7%) who had CTL precursors reactive to EBV peptide was significantly lower than that of healthy volunteers (8 out of 10) (15). This result suggests that the cellular immunity of these patients might be depressed and therefore other supportive immunotherapies may be needed for these patients to increase their general level of immunity prior to specific immunotherapy. A strategy to improve the immune status of patients with suppressed immune function is necessary. Several studies have suggested that gemcitabine improves the immunity of patients with advanced pancreatic cancer (49, 50). A study of colon cancer patients suggested that a multi-drug regimen that includes gemcitabine is a powerful antitumor and immunomodulating regimen that can make the tumor cells a suitable means of inducing an antigen-specific CTL response (51). AIT may not only be successfully used in combination with gemcitabine for the treatment of unresectable pancreatic cancer but may also be effective in preventing local recurrence or metastasis in post-operative patients.

Finally, AIT with both MUC1-DCs and MUC1-CTLs may be a practical, safe and effective treatment for pancreatic cancer patients. Further randomized control studies of large numbers of patients are needed to confirm the efficacy of this combination AIT for pancreatic cancer.

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