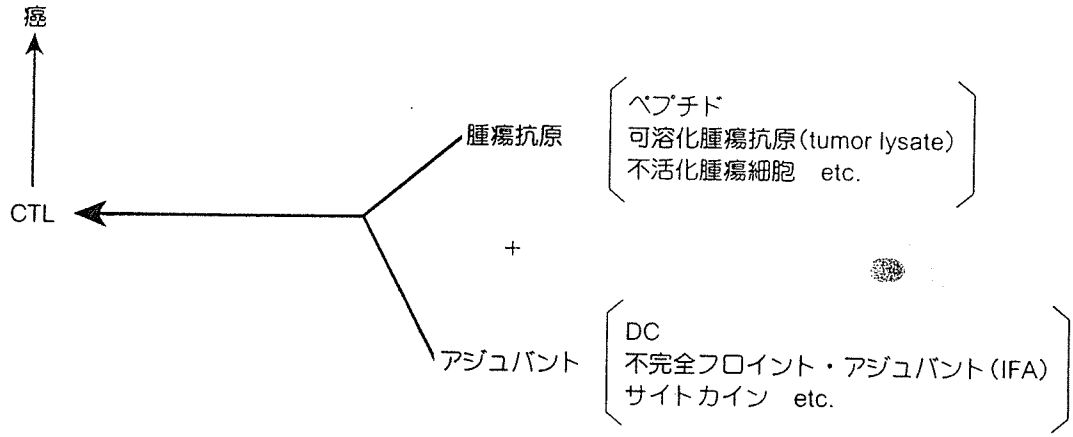

図2　癌ワクチン療法

患者の癌に由来する抗原を接種することにより，癌に対する特異的T細胞免疫応答を惹起し，癌の再発予防や退縮を期待する。

合体（major histocompatibility complex antigen：MHC）を抗原特異的に認識するが，T細胞が認識する抗原の本体はわずか9～15個ほどのアミノ酸残基からなるペプチドである。$CD8^+$T細胞はMHCクラス1分子と拘束性があり，その認識抗原ペプチド（$CD8^+$腫瘍特異的細胞傷害性T細胞〔cytotoxic T lymphocyte：CTL〕誘導性エピトープペプチド）が腫瘍拒絶抗原として，機能的DNA発現クローニング法を中心とした様々な手法を用いて数多く同定されてきた[2]。その代表的なものには，精巣と癌細胞にだけ発現する抗原（cancer/testis antigen）であるMAGEや，組織分化抗原（differentiation antigen）であるMART-1やgp100，癌に高発現する抗原（over expressed self antigen）であるCEAやHer2/neu等がある。これらの多くは，白人に多いHLA-A2拘束性あるいは日本人に多いHLA-A24拘束性ペプチドとして同定されている[3]。

しかし，いくら癌抗原の本質として分子レベルで同定された癌抗原ペプチドであるとはいえ，元来低免疫原性であるためその免疫効果は弱い。よって，実地臨床においては免疫効率を増強する

ための様々な補薬(アジュバント)が併用されることが多い。簡便であるため，その入手，調達が可能であれば，不完全フロイント・アジュバント（incomplete Freund adjuvant：IFA）やIFN，IL-2等のサイトカイン製剤が用いられることが多いが（欧米の臨床試験においては，IL-12やGM-CSFも用いられている），アジュバントとして間違いなく最も有能なものの1つは，ネーチャー・アジュバントと別称されるDCである。

DCはMHC分子や共刺激分子（CD80〔B7-1〕，CD86〔B7-2〕）を強発現し，抗原未感作のナイーブT細胞をも刺激することのできる，生体内で唯一の最も強力なAPCである。DCは流血中の単核細胞中に極微量の0.1%前後しか存在せず，その純化，増幅も困難であったため，その臨床応用が妨げられてきた。しかし，1994年にSallustoらにより，末梢血単球からGM-CSFとIL-4の存在下に比較的容易にDCを分化，誘導できることが明らかにされ[4]，腫瘍抗原ペプチドとDCの組み合わせによる癌ワクチン療法の臨床応用が開始された。9～15個のアミノ酸から構成されるペプチドをパルスされたDCは，MHCクラス1分子の溝にそれを提示し，強発現している共刺激分子とともに腫瘍特異的$CD8^+$CTLを効率的に刺激，誘導する。担癌宿主の細胞性免疫機構が正常に作動すれば，生体に投与されたDCは近傍のリンパ節に遊走し，そこでCTLの誘導を促し，リンパ流に乗ったCTLは体内を一巡して腫瘍を攻撃し，消退へと導く。これが，癌ワクチン療法の理論である。

3．癌ワクチン療法の現況

欧米(白人)ではメラノーマ患者が多く，なおかつメラノーマが抗原性が高く免疫療法が奏効しやすいという特性を有しているため，メラノーマに対するワクチン療法の臨床試験が積極的に展開されてきた。実際に有効症例が数多く報告されており，化学療法と腫瘍縮小効果，生存期間を比較検討するための第3相試験も開始されている[5),6)]。

一方，本邦においてはメラノーマ患者が少ないため，消化器癌に対する臨床試験が多く行われているのが特徴である。1997年以降，大学病院を中心とした多くの医療研究機関，その中でもとりわけ外科系の診療科において，ワクチン療法が試みられてきてい

表1　本邦における固形癌に対するワクチン療法の臨床試験

施　設	対象疾患	使用腫瘍抗原	併用アジュバント
九州大学生体防御医学研究所	消化器癌	ペプチド（MAGE）	DC
和歌山県立医科大学	消化器癌	ペプチド（CEA）	DC
京都府立医科大学	消化器癌，肺癌	ペプチド（CEA，MAGE）	DC，（IFN-α，OK-432）
	メラノーマ	ペプチド（MAGE，tyrosinase），tumor lysate	DC
国立がんセンター中央病院	前立腺癌	融合蛋白（GM-CSF/PAP）	DC
	メラノーマ	ペプチド（MAGE，tyrosinase，gp100）	DC
山梨医科大学	胃　癌	ペプチド（Her2/neu）	DC
東京女子医科大学	消化器癌	tumor lysate，ペプチド（CEA，Her2/neu，Muc1）	DC
慶応義塾大学	膀胱癌	ペプチド（MAGE）	DC
滋賀医科大学	乳癌，肺癌	ペプチド（Muc1）	DC
九州大学（腫瘍制御）	消化器癌	tumor lysate，ペプチド（CEA）	DC，OK-432
岩手医科大学	消化器癌，乳癌	tumor lysate，ペプチド（CEA，Muc1）	DC
東京大学医科学研究所	転移性皮下腫瘍	なし（腫瘍局所放射線照射）	DC，IL-2
産業医科大学	肺　癌	ペプチド（MAGE）	OK-432
	消化器癌，肺癌	ペプチド（SART，CypB，Lck，ART，etc.）	IFA*
久留米大学	泌尿器科癌	ペプチド（同上）	IFA*
	婦人科癌 etc.	ペプチド（同上）	IFA*
北海道大学	消化器癌	ペプチド（同上）	IFA*
山口大学	膵　癌	ペプチド（同上，Muc1）	IFA*，DC
東京慈恵会医科大学	脳腫瘍，消化器癌	融合細胞（腫瘍/DC）	
帝京大学	消化器癌	融合細胞（腫瘍/DC）	

＊ IFA：incomplete Freund adjuvant（不完全フロインドアジュバント）

るが，その代表的なものを表1に示した。使用されている腫瘍抗原としては，やはりHLA-A24拘束性のCTL誘導性ペプチドが最も多い。

九大生医研外科のグループは，消化器癌においてもMAGE遺伝

子の発現が比較的高頻度に認められることを明らかにした。さらにHLA-A24拘束性のMAGE-3ペプチドの同定にも成功し，本邦で最も早く1997年1月にMAGE-3ペプチドとDCを用いた消化器癌に対するワクチン療法の臨床試験を開始した。彼らはワクチンを静脈内投与しているが，実際に腫瘍が縮小した大腸癌症例1例，食道癌症例2例を報告している[7]。われわれの施設においても1998年10月よりHLA-A24拘束性のCEAペプチド（CEA652）とDCを用いた消化器癌，肺癌に対する臨床試験を開始し，多くの免疫学的効果（腫瘍特異的細胞性免疫能の増強），臨床効果（腫瘍マーカーの低下，長期間の腫瘍増殖抑制）を確認した[8), 9)]。また国立癌センター中央病院では，米国DENDREON社製のクローズドシステムでのDCワクチン療法の臨床試験を，1999年から進行前立腺癌に対して開始している。成分採血〔apheresis〕で採取したDCを多く含む単核細胞分画に，40時間の行程でGM-CSFと前立腺性酸性ホスファターゼ（prostatic acid phosphatase：PAP）の融合蛋白を取り込ませた後に投与する方法であり，本邦での同システムの権利を有するキリンビール(株)医薬事業部からの委託研究という形で臨床試験を推進している。産官が一体となった今後の本邦における細胞免疫療法の臨床研究のモデルケースとして注目される。他方後章において詳述されるが，DCを用いない非細胞療法としての癌ワクチン療法の臨床試験も，久留米大学のグループを中心に精力的に進められている。彼らは，扁平上皮癌抗原（SART）を中心として，数多くの腫瘍抗原ペプチドを同定してきているが，これらをIFAと組み合わせて患者に投与している。最終的な製剤化を目指し，新薬の開発に類似したスタンスで臨床試験を展開している。

4．癌ワクチン療法の今後の方向性（表2）

科学的理論に裏打ちされた先進医療としての癌ワクチン療法は，臨床応用への道が開かれたばかりである。よって，倫理的観点からも，まず合成ペプチドやDCを用いた治療手法の安全性と免疫学的効果を検証することを主目的とし，従来の確立された治療法に不応性の高度進行癌症例を対象として，臨床試験が遂行されてきた。しかし，ワクチン療法本来の目的は感染症，癌を問わずあくまでその発症予防，あるいは微少残存病巣からの再発予防

表2 癌ワクチン療法の今後の方向性

A）微少残存病巣からの再発予防療法として（非細胞療法として）

1）ペプチドワクチン単独療法
2）低容量化学療法との併用
3）分子標的治療薬との併用

B）難治性癌に対する先進医療として（細胞療法あるいは非細胞療法として）

1）分子標的治療薬との併用
2）進化し続ける細胞療法（樹状細胞療法，造血幹細胞移植療法 etc.）との連動
3）新たに開発が期待される免疫抑制因子制御療法との連動

であり，免疫能が極端に荒廃した進行癌症例においては，能動免疫療法であるワクチン療法が有効に作用し得るはずのないことは，火を見るより明らかである[9]。よって，根治を目指した外科療法，放射線療法，化学療法後の微少残存病巣からの再発予防を目的とし，できるだけ多くの患者に還元可能な治療戦略を確立するための臨床試験を推進していくことが，今後最も重要であろう。製剤化を目指したペプチドワクチン単独療法，低用量化学療法や新たに開発，承認されてきている分子標的治療薬との併用療法が，重要な選択肢である。

他方，難治性進行癌に対する新たなるワクチン療法の開発も，今後に課せられた大きな命題の1つである。この領域においては，改良された樹状細胞療法や造血幹細胞移植療法との連動が必要不可欠であろう[10]。また，簡便で効果的な免疫抑制因子制御療法の確立が，ワクチン療法の飛躍的な効果増強のブレークスルーになりうる可能性がある。われわれの施設では現在，ポリスチレン系極細繊維で構成された免疫抑制物質吸着カラム（血漿置換を伴わない体外循環用治療カラム）を研究開発中である[11]。

おわりに

樹状細胞療法や同種免疫療法としての造血幹細胞移植療法の臨床導入，分子標的治療薬の目覚ましい勢いでの創薬，承認と，近年の癌治療の進歩は大変急峻である[12]。しかし，この進歩に医療現場のインフラ整備が追いついていないのが現状である。ようやく昨年，薬事法改正に基づく医師主導型臨床試験が可能となった

が，新しい癌治療技術の開発研究には産官学の協力，協調が必要不可欠である。ワクチン療法のみならず，多くの新しく開発される癌治療戦略を，日常臨床にいち早く還元できるような臨床試験システムを構築していくことが，今後の大きな課題であろう。

（上田祐二，山岸久一）

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ANTICANCER RESEARCH *25*: 3575-3580 (2005)

MUC1 Peptide Vaccination in Patients with Advanced Pancreas or Biliary Tract Cancer

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Abstract. *Background: To evaluate the immunogenicity of MUC1 peptide vaccine in advanced pancreatic and bile duct cancers, a phase I clinical trial was conducted. Materials and Methods: A 100-mer MUC1 peptide consisting of the extracellular tandem repeat domain and incomplete Freund's adjuvant were subcutaneously administered to 6 pancreatic and 3 bile duct cancer patients at weeks 1, 3 and 5 and doses ranging from 300 to 3000 μg. Circulating intracytoplasmic cytokine-positive CD4$^+$ T cells and anti-MUC1 IgG antibodies were measured before and after vaccination. Results: There were no adverse events, except for mild reddening and swelling at the vaccination site. In 8 patients eligible for clinical evaluation, 7 had progressive disease and 1 stable disease with a tendency for increased circulating anti-MUC1 IgG antibody after vaccination. Conclusion: This phase I clinical trial revealed the safety of a vaccine containing 100-mer MUC1 peptides and incomplete Freund's adjuvant.*

MUC1 is a type I transmembrane glycoprotein with an extracellular domain composed of a polypeptide core containing multiple tandem repeats of a 20 amino acid sequence with numerous carbohydrate chains (1). The autoimmunogenicity of MUC1 was first shown by inducing HLA-unrestricted cytotoxic T lymphocytes (CTLs) against the tandem repeat region (2), which was confirmed by subsequent investigations (3-5). Thereafter, Domenech *et al.* demonstrated the presence of HLA-restricted CTLs against the tandem repeat sequence (6). The nanomer peptide STAPPAHGV, which corresponds to residues 9-17 of the 20 amino acid repeat sequence, was found to have significant binding affinity to several class I alleles, including HLA-A1, A2, A3 and A11, and to be able to elicit a MUC1-specific CTL response in an A11$^+$ cancer patient. On the other hand, a humoral immune response to MUC1 was also revealed (7, 8) and circulating antibodies against the tandem repeat peptides were detected in various cancers (9, 10). These findings suggested the potential application of MUC1 in cancer immunotherapy and led to clinical trials of a MUC1 peptide vaccination (11-14).

The first study, by Goydos *et al.*, demonstrated the safety of a vaccine composed of a synthetic MUC1 peptide with 5 repeats of the 20 amino acid sequence and BCG (11). Karanikas *et al.* then reported the results of a clinical trial with the MUC1 peptide of 5 repeats fused with mannan in 25 patients with advanced breast, gastric or colorectal cancer (12). They detected large amounts of IgG_1 anti-MUC1 antibodies in 13 of the 25 patients, and could induce HLA-A2-restricted CTLs, but a significant CTL response was only seen in 2 out of 10 patients tested. Gilewski *et al.* reported the results of a vaccination with the MUC1 peptide consisting of 1.5 repeats conjugated with keyhole limpet hemocyanin (KLH) together with the immune adjuvant QS-21 in 9 breast cancer patients (13). High IgM and IgG antibody titers against the MUC1 peptide were detected; however, there was no evidence of T cell activation. Another of their studies, using a 106-amino-acid-long MUC1 peptide conjugated with KLH plus QS-21 in 6 breast cancer patients, again showed that the T cell response against the MUC1 peptide was minimal and inconsistent (14). These clinical data suggested that the tandem repeat peptide of MUC1 could be useful for inducing anti-MUC1 antibodies rather than CTLs.

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Key Words: MUC1, peptide, vaccine, pancreas cancer.

Recently, von Mensdorff-Pouilly *et al.* have reported that a positive test result for both IgG and IgM antibodies in pretreatment serum was associated with significant disease-specific survival in stage I and II breast cancer patients (15). We also revealed that circulating anti-MUC1 IgG antibody was a favorable prognostic factor for cancer of the pancreas (16). These results suggest that the antibodies might protect the host against cancer progression. In this study, we attempted a phase I clinical trial of a 100-mer MUC1 tandem repeat peptide with incomplete Freund's adjuvant in patients with advanced pancreatic or bile duct cancer.

Materials and Methods

Trial eligibility. Five patients with inoperable pancreatic cancer, 2 with recurrent disease of bile duct cancer, 1 with recurrent disease of pancreatic cancer and 1 with inoperable bile duct cancer were enrolled in this study. They were required to have computed tomography (CT) or magnetic resonance imaging (MRI) for evaluating clinical stage or recurrent disease. The eligibility criteria were as follows: age of 85 years or less, serum creatinine of less than 1.4 mg/dl, bilirubin of less than 1.5 mg/dl, platelet count of 100,000/μl or more, hemoglobin of 8.0 g/dl or more, and total WBC of 3000/μl or more. Hepatitis B surface antigen and Hepatitis C antibody were negative in all patients. The patients were untreated for at least 4 weeks before entry into the study, and had to have an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 to 2 at the time of entry. Patients with evidence of other serious illness, immunosuppression, or autoimmune disease were excluded. Treatment of the enrolled patients was carried out at Yamaguchi University, Japan, from June 2000 through March 2004.

All patients were required to comprehend and sign an informed consent form approved by the Institutional Review Board of Yamaguchi University School of Medicine.

Vaccine preparation and administration. The MUC1 peptide, consisting of 100 amino acids (5 repeats) of the extracellular tandem repeat domain, was synthesized at the Peptide Synthesis Facility, University of Pittsburgh (Dr. O. J. Finn, Pittsburg, PA, USA), in accordance with the U.S. FDA Good Laboratory Practice Regulations and the Japanese GLP Standard. Montanide ISA-51 (incomplete Freund's adjuvant) was manufactured by Seppic, Inc. (Paris, France) and supplied in glass ampoules containing 3 ml of sterile adjuvant solution.

An appropriate amount of MUC1 peptide was diluted with sterile 0.9% NaCl solution and added in a 1:1 volume to Montanide ISA-51 and then mixed using a stopcock and two glass syringes for 5 min. The resulting emulsion was injected, using a glass syringe, subcutaneouly into the frontal thigh in a volume of 1 ml. Alternative thighs were used for a total of 3 injections, which were done 2 weeks apart. Skin tests were performed using 50 μg of the peptide in 0.9% NaCl solution injected intradermally in a volume of 100 μl using a 1-ml disposable syringe. The injection site was observed at 15 min and 48 h. For patients who requested the additional administration of MUC1 peptides, vaccination was repeated with monitoring for adverse events.

Evaluation of adverse events and clinical response. All adverse events were evaluated by the National Cancer Institute-Common Toxicity Criteria (NCI-CTC) version 2.0 (17) at every vaccination. All known sites of disease were evaluated by CT scan before and after 3 vaccinations. Patients were assigned to a response category according to the response evaluation criteria for solid tumors, given in a revised version of the WHO criteria published in June 1999 in the WHO Handbook for reporting results of cancer treatment.

Intracellular cytokine assays. Peripheral blood samples were collected and the proportions of $CD4^+$ T cells producing intracellular cytokines were determined using flow cytometry, as reported previously (18). In brief, peripheral blood samples were collected by venapuncture into syringes containing sodium heparin anticoagulant. Phycoerythin-cyanine 5 (PC5)-conjugated anti-CD3 monoclonal antibody (mAb) and energy-coupled dye (ECD)-conjugated anti-CD4 mAb were purchased from Coulter Immunology (Hialeath, FL, USA). Fluorescein isothiocyanate (FITC)-conjugated anti-IFN-γ mAb, phycoerythrin (PE)-conjugated anti-IL4 mAb and FITC/PE-conjugated control mAbs were purchased from Becton Dickinson (San Jose, CA, USA). PE-conjugated anti-interleukin (IL)-6 mAb and anti-IL-10 were purchased from R & D (Minneapolis, MN, USA) and PharMingen (San Diego, CA, USA), respectively. The proportions of CD3/CD4-positive lymphocytes producing IFN-γ, IL-4, IL-6 or IL-10 were measured using flow cytometry according to the instructions of the reagent manufacturer (Becton Dickinson). Briefly, 1 ml blood samples were treated immediately with 10 μg/ml of Brefeldin A (BFA) (Sigma Chemical, St. Louis, MO, USA) to block cytokine secretion, keeping the products within cells, and were kept at ambient temperature. Cell surfaces were stained with anti-CD3 and anti-CD4 mAbs. The red cells were lysed with 1 x FACS Lysing Solution (Becton Dickinson) for 10 min at room temperature. After washing with phosphate-buffered saline (PBS) containing 0.5% bovine serum albumin (BSA) and NaN_3, the cells were permeabilized with 0.5 ml of 1 x FACS Permeabilizing Solution (Becton Dickinson) for 10 min at room temperature. After two washes, the cells were incubated with optimal concentrations of anti-IFN-γ, anti-IL-4, anti-IL-6 or anti-IL-10 mAb. Samples were analyzed on an EPICS/XL flow cytometer (Coulter Electronics, Inc., Hialeath, FL, USA), and the data were analyzed using a System II software program (Coulter Electronics). The percentages of cytokine-producing $CD4^+$ T cells were calculated. Negative control reagents were used to verify the staining specificity of the experimental antibodies and to serve as a guide for setting markers to delineate positive and negative populations.

ELISA assays. An enzyme immunoassay for detecting antibodies was performed, as described previously (16). Briefly, the MUC1 peptide was coated onto 96-well microtiter plates (ASAHI TECHNO GLASS Corporation, Japan) at 100 μg/ml in PBS (pH 7.4) at 4°C for 12 h. The plates were washed with PBS, and non-specific binding sites were blocked with 3% HAS/PBS at 37°C for 1 h. The plates were then incubated with patient sera diluted 1:40 in 1% HSA/PBS at 37°C for 1 h. After washing with 0.05% Tween-20/PBS, they were incubated with the second antibody, a horseradish peroxidase-conjugated mouse anti-human IgG (DAKO Corporation, Carpinteria, CA, USA) diluted 1:5000 in 1% HSA/PBS, and washed with PBS. Substrate reaction using *O*-phenylenediamine dihydrochloride (DAKO) was determined at 492 nm in an autoreader (Labsystems, Helsinki, Finland). An anti-MUC1 mAb

Table I. *Patient characteristics and clinical response.*

Patient	Age/sex	Disease[a]	Prior therapy[b]	Dose of peptide (mg)	No. of vaccines received	Clinical response[c] (mos.)
1	77/M	PC	none	300	4	PD
2	66/M	BC	S	300	7	n.e.
3	58/F	BC	S, C, R	300	3	PD
4	65/M	PC	S	1000	3	PD
5	51/M	PC	R	1000	3	PD
6	57/M	PC	R	3000	3	PD
7	54/M	BC	none	3000	3	PD
8	49/M	PC	none	3000	3	SD (3)
9	56/M	PC	R	3000	3	PD

[a]PC, pancreas cancer; BC, biliary tract cancer.
[b]S, surgery; C, chemotherapy, R, radiotherapy.
[c]mos; months; PD, progressive disease; SD, stable disease; n.e., not evaluated.

E29 (DAKO) was used as a positive control. All of the serum samples were simultaneously measured in triplicate using one 96-well plate to compare each optical density (OD) value.

Results

Patient characteristics and clinical responses. Nine patients with advanced cancer of the pancreas or bile duct were enrolled in this phase 1 clinical study of a MUC1 peptide vaccination. The detailed characteristics of the patients are shown in Table I. The mean age of the patients was 59.2 years (range: 49-77 years). Six patients were in an inoperable state and 3 had recurrent diseases after surgery. The dose of MUC1 peptides ranged from 300 to 3000 μg; as no apparent toxicity was observed in patients 4 and 5 with a dose of 1000 μg, the highest dose (3000 μg) was started from patient 6.

It was difficult to draw any definitive results from this small-scale phase 1 study with regards to clinical responses and prognostic factor analysis. Nevertheless, the available results might be relevant from the point of view of developing a suitable peptide vaccine. In 9 patients who received MUC1 vaccinations, 8 were eligible for clinical evaluation. Of these, a stable disease (SD) in 1 patient (patient 8) and progressive diseases (PD) in 7 patients were diagnosed 2 weeks after the last vaccination (Table I). Patient 8 was diagnosed with SD by sequential CT scans and measurements of a tumor marker, CA19-9. The clinical response of patient 2 was unclear because recurrence was masked by bacterial cholangitis and subsequent liver abcesses during the observation period after vaccination. Patients 1 and 2 were vaccinated more than 3 times, to comply with their request.

Adverse events. All 9 patients were evaluated for adverse events according to the NCI-CTC (17). The vaccinations were generally well tolerated without hematological toxicity or symptoms of any autoimmune diseases. In all patients, mild reddening, swelling and itching at the vaccination site were observed, for which treatment was not required, and skin tests against MUC1 peptides were negative.

Immunological responses. Immunological responses could be evaluated in 7 out of 9 patients. Intracellular cytokine-positive $CD4^+$ T cell (%) and circulating anti-MUC1 antibody levels before and after vaccination are shown in Figure 1. IL-10 is a Th2 cytokine and IL-6 stimulates the proliferation of antibody-producing cells. In 5 out of 7 patients, both IL-10 and IL-6-producing $CD4^+$ T cell counts tended to decrease after vaccination (Figure 1a). Intracellular IFN-γ or IL-4-positive $CD4^+$ T cells were always under detectable levels (data not shown). The titer of circulating anti-MUC1 IgG antibodies also showed decrease or no change in 5 out of 7 patients. However, it tended to increase in the patient who showed SD for 3 months (Figure 1b).

Discussion

This phase I clinical trial revealed the safety of a vaccine containing 100-mer MUC1 peptides and incomplete Freund's adjuvant in advanced pancreas and bile duct cancer patients. The only adverse event observed was mild reddening and swelling at the vaccination site. A skin test against the MUC1 peptide before vaccination was negative in all patients. Although 1 pancreatic cancer patient showed SD with a modest increase of circulating anti-MUC1 IgG titer after vaccination, 7 other evaluable patients were PD, and the circulating cytokine-producing $CD4^+$ T cell and anti-MUC1 IgG levels tended to decrease in most patients. It seems that these results reflect a rapid progression of

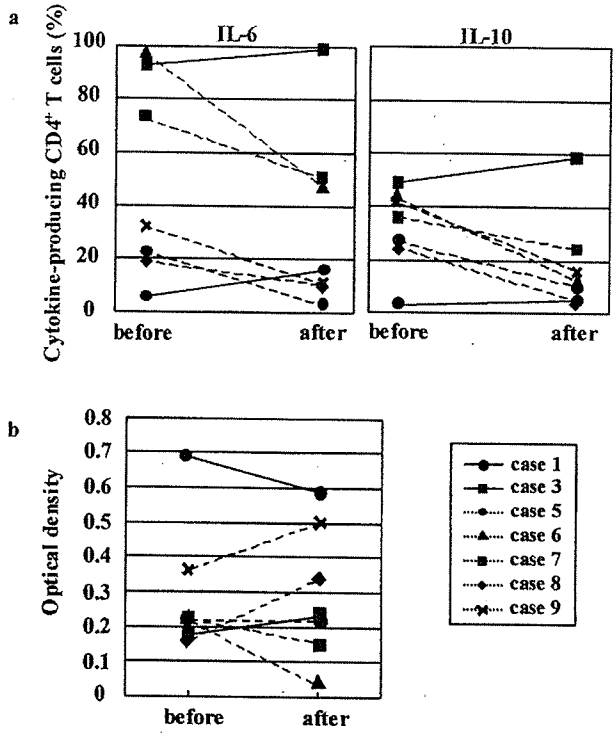

Figure 1. *Intracytoplasmic cytokine and anti-MUC1 IgG antibody assays before and after vaccination. (a) The effect of vaccination on the percentage of IL-6- or IL-10-producing CD4+ T cells in peripheral blood. (b) The effect of vaccination on circulating anti-MUC1 IgG antibody levels. All samples were measured simultaneously in each assay. Results are shown as the mean from triplicate wells.*

advanced pancreatic or bile duct cancer and the presence of a profound immunosuppressive status in those patients.

Pancreatic and biliary tract cancers are two of the worst cancers with regards to 5-year survival rates (19, 20). In pancreatic cancer, several mechanisms for escaping immune surveillance have been shown, including the secretion of immunosuppressive cytokines such as IL-10 and TGF-β, local hindrance of tumor infiltrating lymphocytes (TILs) and loss of the signal transducing CD3ζ chain of TILs (21). On the other hand, we have revealed that MUC1 is involved in the metastatic ability of pancreatic cancer cells (22) and is a poor prognostic factor for cancer of the pancreas (23). Recently, Monti *et al.* demonstrated that MUC1 mucins derived from pancreatic cancer cells suppress the maturation of dendritic cells, resulting in low immunostimulatory functions and the IL-10^{high}IL-12^{low} cytokine secretion phenotype of dendritic cells (24), suggesting that MUC1 *per se* could be a potent immunosuppressive factor. In this context, the findings of Hiltbold *et al.* should be noted. They showed that the efficiency of MUC1 processing by dendritic cells and the resulting strength of CTL activity were inversely correlated with the degree of MUC1 glycocylation (25), and that soluble MUC1 is not transported to late endosomes or MHC class II compartments for processing and binding to class II MHC (26). These suggest that the reduction of tumor burden, which leads to decreased immunosuppressive factors including MUC1, could be essential to cancer therapy with a peptide vaccine.

Ramanathan *et al.* recently reported the results of a phase I study of a MUC1 vaccine in patients with resected (n=15) or locally advanced (n=1) pancreatic cancer without prior chemotherapy or radiotherapy (27). Their MUC1 peptide was the same one as used in our study. Escalating doses of the peptide (100, 300, 1000 and 3000 μg) were admixed with SB-A2 and administered intramuscularly every 3 weeks for 3 doses. Two of 15 resected patients are alive and disease-free at follow-up of 32 and 61 months. Both patients were at stage T3N1M0 at surgical operation. Immunological parameters including delayed-type hypersensitivity, circulating $CD8^+$ T cell's number, the serum level of anti-MUC1 antibody and the cytokine (IFN-γ or IL-4) production of peripheral blood T cells were improved after vaccination in some patients. They observed an almost total suppression of the T cell's ability to make either IFN-γ or IL-4 in every patient before vaccination, which corresponds to our present results, but the production of cytokines increased significantly after vaccination in 5 patients. These findings suggested the importance of reduced tumor burden for peptide vaccine therapy in pancreatic cancer. A phase I study of MUC1 peptide vaccination for resected pancreatic cancer is now being prepared in our departments.

Acknowledgements

This work was supported by a Grant-in-Aid for Scientific Research on Priority Areas (C) from the Ministry of Education, Culture, Sports, Science and Technology, Japan (#12217097).

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Received May 9, 2005
Accepted June 21, 2005

ONCOLOGY REPORTS 13: 875-883, 2005

Immunological evaluation of personalized peptide vaccination for patients with pancreatic cancer

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Received September 30, 2004; Accepted December 2, 2004

Abstract. The prognosis of pancreatic cancer is extremely poor, and development of new treatment modalities is needed. One such treatment could be specific immunotherapy. To evaluate safety and immunological responses, we conducted a phase I study of personalized peptide vaccination for pancreatic cancer patients (n=11). Namely, pre-vaccination peripheral blood mononuclear cells were screened for their reactivity *in vitro* to each of 14 or 16 peptides in HLA-A24$^+$ or -A2$^+$ patients, and only the reactive peptides (maximum: 4) were vaccinated *in vivo*. This regimen was generally well tolerated, although inflammatory reactions at the injection site were observed in 7 patients. Delayed-type hypersensitivity to peptides used for vaccination was observed in 7 patients. Increased cellular and humoral immune responses to at least one of peptides used for vaccination were observed in the post-vaccination PBMCs and sera from 4 of 8 patients and 4 of 10 patients tested, respectively. The 6- and 12-month survival rates for patients who received >3 vaccinations (n=10) were 80% and 20%, respectively. Due to tolerability and capability of inducing specific immunity, further development of personalized peptide-based immunotherapy for pancreatic cancer patients is warranted.

Introduction

Patients with pancreatic cancer (PC) have a poor prognosis, and for this reason, PC is considered to be one of the deadliest types of malignancy. The median survival time (MST) after diagnosis is <12 months, with a 5-year survival rate of approximately 3-5% (1,2). There is no standard therapy for advanced PC, although many chemotherapeutic agents have been used in clinical trials in the past two decades (3-6). Among these chemotherapeutic agents, gemcitabine (GEM) is somewhat clinically effective, but the MST is still <6-9 months. Therefore, development of new treatment modalities is needed, one such treatment could be a peptide-based specific immunotherapeutic approach, as recent advances in tumor immunology have resulted in the identification of many tumor-associated antigens and epitopes recognized by HLA-class-I-restricted cytotoxic T lymphocytes (CTLs) from various cancers, including PC (7-10). However, clinical trials using those peptides have rarely demonstrated major clinical responses (11-13). This failure could be due to an insufficient induction of anti-tumor responses by these vaccine regimens, under which the peptide-specific memory T cells were not measured in pre-vaccination peripheral blood mononuclear cells (PBMCs). We have reported that personalized vaccinations based on pre-vaccination measurement of peptide-specific CTLs in the circulation induced potent anti-tumor immune responses in patients with cancers, such as lung, gastric, colorectal, prostate, and gynecologic cancers (14-19). Moreover, we previously reported that PC cells expressed tumor-associated antigens that encoded the peptides used for those clinical studies (20). Peptide-specific CTL precursors were also detectable in the majority of PC patients (20). In this report, we describe the safety and the immune responses to personalized peptide vaccination of PC patients.

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Key words: personalized peptide vaccination, pancreatic cancer, clinical trial, CTL precursors, anti-peptide antibody

Patients and methods

Patients and eligibility criteria. The Institutional Review Boards of Yamaguchi University and Kurume University approved this clinical protocol (#2031). Complete written informed consent was obtained from all of the patients at the time of enrollment. According to the protocol, the patients were required to be positive for HLA-A24 or -A2. All patients were clinically confirmed to have PC. Eligibility criteria included the following: age of ≤85 years, serum creatinine of <1.4 mg/dl,

bilirubin of <1.5 mg/dl, platelet count of ≥100,000/μl, hemoglobin of ≥8.0 g/dl, and total WBC of ≥3,000/μl. Hepatitis B surface antigen and Hepatitis C antibody were negative in all patients. The patients were untreated for at least 4 weeks before entry into the study, and had to have an Eastern Cooperative Oncology Group (ECOG) performance status (PS) of 0 to 2 at the time of entry. Patients with evidence of other serious illness, immunosuppression, or autoimmune disease were excluded. Treatment was carried out at Yamaguchi University and Kurume University Hospitals from March 2001 through November 2004.

Screening of peptide-specific CTL precursors. Peripheral blood (30 ml) was obtained before and after every 3 vaccinations. PBMCs were isolated by means of Ficoll-Conray density gradient centrifugation, and were then used for a CTL precursor assay, as reported previously (20). In brief, PBMCs ($1x10^5$ cells/well) were incubated with 10 μM of a peptide in wells of u-bottom-type 96-well microculture plates (Nunc, Roskilde, Denmark) in 200 μl of culture medium containing 100 U/ml of interleukin-2 (IL-2). Half of the medium was removed and replaced with new medium containing a corresponding peptide (20 μM) every 3 days. After incubation for 12 days, these cells were harvested and tested for their ability to produce interferon-γ (IFN-γ) in response to CIR-A2402 cells for HLA-A24$^+$ or to T2 cells for HLA-A2$^+$ patients, were pre-loaded with either a corresponding peptide or a HIV peptide (RYLRQQLLGI for HLA-A24$^+$ and SLYNTVATL for HLA-A2$^+$ patients) as a negative control. The level of IFN-γ was determined by enzyme-linked immunosorbent assay (ELISA) (limit of sensitivity: 10 pg/ml). Two-tailed Student's t-test was employed for the statistical analyses.

Peptides and vaccination. The peptides used in the present study were prepared according to good manufacturing practice conditions using Multiple Peptide System (San Diego, CA). The sequences of the peptides are shown in Table I. All of these peptides have the ability to induce HLA-A24- or HLA-A2-restricted and tumor-specific CTL activity in the PBMCs of cancer patients (7-20). Montanide ISA-51 adjuvant [known as incomplete Freund's adjuvant (IFA)] was purchased from Seppic, Inc. (Franklin Lakes, NJ). The peptides were supplied in vials containing 2 mg/ml sterile solution for injection. One ml of solution was added in a 1:1 volume to IFA, and then the solution was mixed in a vortex mixer (Fisher, Inc., Alameda, CA). The resulting emulsion was injected subcutaneously into the thigh using a glass syringe. The interval between vaccinations was 2 weeks, and a total of 3 injections were performed. For patients with a favorable clinical course, vaccination was repeated in order to further evaluate adverse events, immunological responses, and clinical responses.

Immunological assays. Skin tests were performed by intradermal injection of 50 μg of each peptide using a tuberculin syringe and a 27-gauge needle. Saline was used as a negative control. Immediate- and delayed-type hypersensitivity (DTH) reactions were determined at 20 min and 24 h after the skin test, respectively. At least 5 mm of induration or 10 mm of erythema was needed to score the skin test as positive. Cytotoxic activity was measured by a standard 6-h ^{51}Cr-release assay, as reported previously (20). In brief, cryopreserved pre- and post (3rd to 9th)-vaccination PBMCs were thawed at the same time, and then were cultured in the medium with 100 U/ml of IL-2 in the absence of peptides. On the 21st to 25th days of culture, the cells were harvested and used for the assay. MIA PaCa2 (HLA-A24$^+$A2$^-$ pancreatic carcinoma, which was obtained from Cell Resource Center for Biomedical Research Institute of Development, Aging and Cancer Tohoku University), PK-8 (HLA-A24$^+$A2$^-$ pancreatic carcinoma), YPK-1 (HLA-A24$^+$A2$^-$ pancreatic carcinoma) (19), Panc-1 (HLA-A24$^-$A2$^+$ pancreatic adenocarcinoma), and phytohemagglutinin (PHA)-blastoid T cells (HLA-A24$^+$ or HLA-A2$^+$) were used as target cells.

Table I. Vaccinated peptide and immune responses.

Peptide name	Sequence	No. of vaccinated patients[a]	Increased immune responses[b] CTL	IgG	DTH
HLA-A24-binding					
SART1-690	EYRGFTQDF	1	0/1	0/1	1
SART2-93	DYSARWNEI	1	1/1	1/1	1
SART2-161	AYDFLYNYL	2	0/1	0/1	1
SART2-899	SYTRLFLIL	1	0/1	0/1	0
SART3-109	VYDYNCHVDL	3	0/2	1/3	2
SART3-315	AYIDFEMKI	2	1/2	1/2	1
CypB-84	KFHRVIKDF	0	-	-	-
CypB-91	DFMIQGGDF	4	0/3	0/3	2
Lck-208	HYTNASDGL	6	0/6	1/6	4
Lck-486	TFDYLRSVL	5	1/5	0/5	3
Lck-488	DYLRSVLEDF	6	2/4	0/4	4
ART1-170	EYCLKFTKL	3	0/2	2/2	2
ART4-13	AFLRHAAL	1	0/1	0/1	0
ART4-75	DYPSLSATDI	0	-	-	-
HLA-A2-binding					
SART3-302	LLQAEAPRL	1	-	0/1	1
SART3-309	RLAEYQAYI	0	-	-	-
CypB-129	KLKHYGPGWV	0	-	-	-
CypB-172	VLEGMEVV	0	-	-	-
Lck-246	KLVERLGAA	1	-	0/1	1
Lck-422	DVWSFGILL	1	-	0/1	1
ppMAPkkk-294	GLLFLHTRT	0	-	-	-
ppMAPkkk-432	DLLSHAFEA	1	-	0/1	1
WHSC2-103	ASLDSDPWV	0	-	-	-
WHSC2-141	ILGELREKV	0	-	-	-
UBE2V-43	RLQEWCSVI	0	-	-	-
UBE2V-85	LIADFLSGL	0	-	-	-
UBE2V-208	ILPRKHHRI	0	-	-	-
HNRPL-140	ALVEFEDVL	0	-	-	-
HNRPL-501	NVLHFFNAPL	0	-	-	-
EIF4-51	RIIYDRKFL	0	-	-	-

[a]HLA-A24-binding and -A2-binding peptides were injected into 10 and 2 patients, respectively. [b]Immune responses to the peptide were compared at pre- and post-vaccination. Interferon-γ production of CTLs, peptide-specific IgG antibody density, and DTH responses were tested.

Table II. Patient characteristics.

No.	Age/ sex	HLA type	PS	Stage[a]	Histology[b]	Site of metastases[c]	Surgery[d]	Previous treatments Chemotherapy[e]	Radiotherapy
1	60/M	A24	0	T4N1M0 III	Adeno s/o	Abdominal LNs	-	-	-
2	63/F	A24	1	Recurrence	Tubular adeno	Neck LNs bone	PPPD	GEM	LNs bone
3	70/M	A24	0	Recurrence	Well diff. tubular adeno	Liver local	PD	5-FU MTX	-
4	63/M	A24	0	T3NxM1 (HEP) IV	Adeno s/o	Liver	-	5-FU CDDP	Local
5	59/M	A2	0	Recurrence	Moderately diff. tubular adeno	Peritoneum local	PD	-	-
6	69/M	A24	0	Recurrence	Well diff. tubular adeno	Abdominal LNs	DP	-	-
7	84/F	A24	0	T4N1M0 III	Adeno s/o	-	-	-	-
8	67/M	A24	0	T3N1M1 IV	Adeno s/o	-	-	-	-
9	68/F	A24	0	Recurrence	Well diff. tubular adeno	Abdominal LNs	PPPD	5-FU CDDP	-
10	60/M	A24	2	T3NxM1(HEP) IV	Acinar cell carcinoma s/o	Liver	-	-	-
11	71/F	A24	0	T3N0M0 IIA	Moderately diff. tubular adeno	-	PD	-	-

[a]UICC Classification of Pancreatic Cancer (6th edition, 2002). [b]s/o, suspected of; diff., differentiated; adeno, adenocarcinoma. [c]Ns, lymph nodes. [d]PPPD, pylorus-preserving pancreaticoduodenectomy; PD, pancreaticoduodenectomy; DP, distal pancreatectomy. [e]GEM, gemcitabine; 5-FU, fluorouracil; MTX, methotrexate; CDDP, cisplatin.

The serum levels of peptide-specific IgG were measured by ELISA, as previously reported (14-19). In brief, 100 µl/well of serum sample diluted with 0.05% Tween-20 Block Ace were added to the peptide (20 µg/well)-immobilized plate. After 2-h incubation at 37˚C, the plate was washed and further incubated for another 2 h with a 1:1,000-dilluted rabbit anti-human IgG (γ-chain-specific, Dako, Glostrup, Denmark). The plate was washed, then 100 µl of 1:100-diluted goat anti-rabbit Ig-conjugated horseradish peroxidase-dextran polymer (En Vision, Dako) was added to each well, and the plate was incubated for 40 min. After washing, 100 µl/well of tetramethyl-benzidine substrate solution (KPL, Guildford, UK) was added, and the reaction was stopped by the addition of 1 M phosphoric acid. To estimate the peptide-specific IgG levels, the optical density (OD) values of each sample were compared with those of serially diluted standard samples, and the values are shown as OD units/ml.

Evaluation of adverse events and clinical response. All adverse events were evaluated by the National Cancer Institute-Common Toxicity Criteria (NCI-CTC) version 2.0 at every vaccination. All known sites of disease were evaluated by vaccinations. Patients were assigned to a response category according to the response evaluation criteria for solid tumors, a revised version of the WHO criteria published in June 1999 in the WHO Handbook for reporting results of cancer treatment. Overall survival (OS) and progression-free survival (PFS) were evaluated from the first vaccination, and were analyzed in order to investigate correlations between clinical benefits and immune responses. Kaplan-Meier curves were described, and survival times were compared using the log-rank test.

Results

Patient characteristics. Eleven PC patients were enrolled in this phase I clinical study of personalized peptide vaccination. The detailed characteristics are shown in Table II. The mean age of the patients was 66.7 (range: 59-84). Six patients had undergone surgical resection of the primary lesion, and had histologically determined adenocarcinomas. For the remaining 5 inoperable patients, adenocarcinoma (n=4) and acinar cell carcinoma (n=1) were suspected by clinical evaluation and the laboratory findings, i.e. tumor markers, computer tomo-

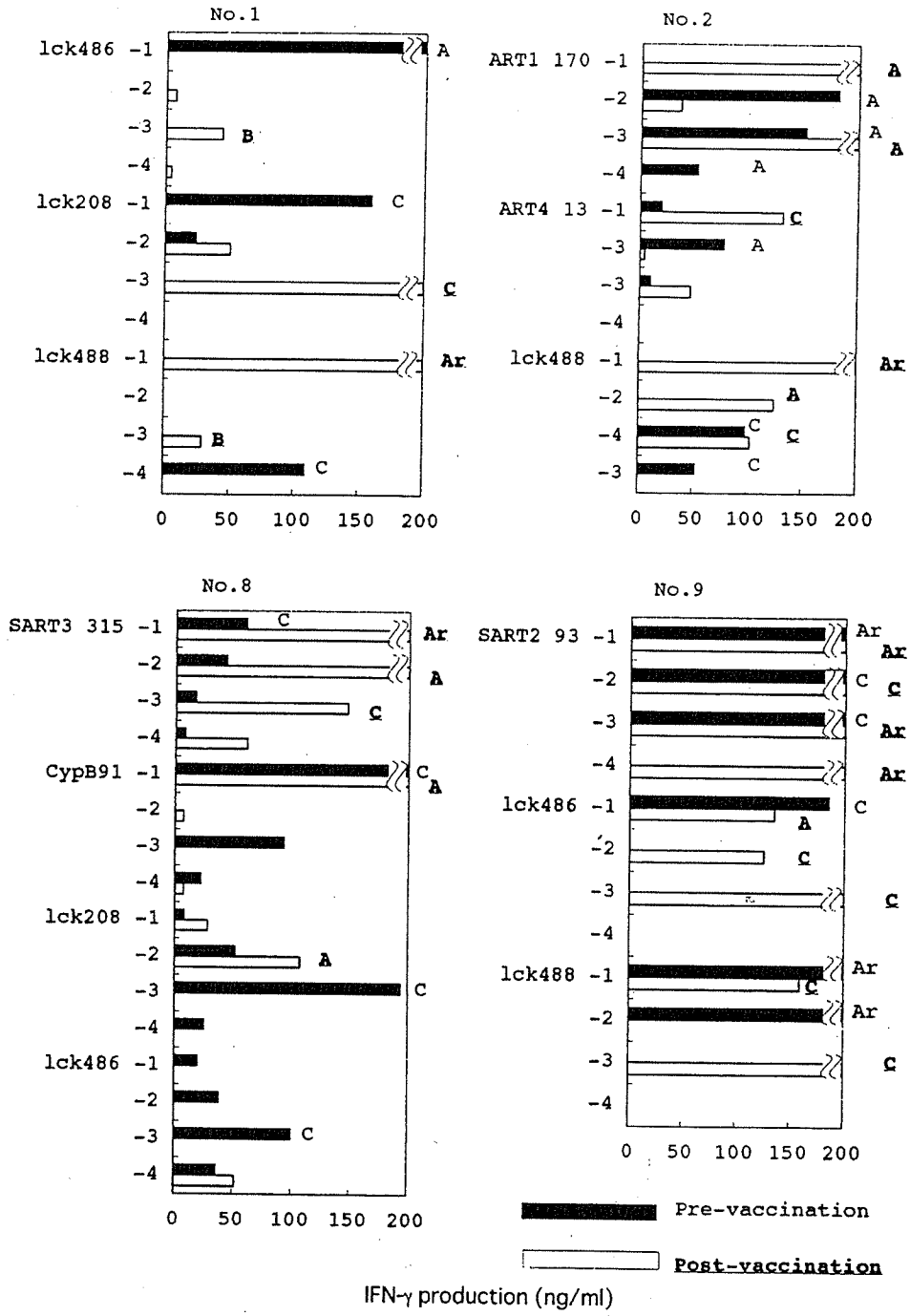

Figure 1. Representative results of peptide-specific CTL precursors in PBMCs of the pre- and post (6th)-vaccinations. Four sets of columns in each peptide indicate the results of quadruplicate cultures in the 4 wells. The value of each well was evaluated based on the following criteria. A level of armed response (Ar): p≤0.01 and ≥500 net value (the amount of IFN-γ in response to the corresponding peptide minus that in response to HIV peptide); A level of response (A): p≤0.05 and ≥50 net; B: p≤0.05 and ≥25 net <50; C: 0.05<p≤0.1 and ≥50 net; D: 0.05<p≤0.1 and ≥25 net <50.

chemotherapy, and 2 of them had received additional radiotherapy. The remaining 4 patients did not have any prior treatment. Ten advanced cases with confirmed recurrence entered into this trial, while the remaining one patient (case no. 11) received the vaccination 93 days after surgery as an adjuvant treatment without confirmed recurrence.

With regard to treatment after the vaccination, 9 patients did not receive any other combined form of therapy, but the remaining 2 patients received the vaccination combined with chemotherapy. Namely, case nos. 9 and 10 received GEM/5-fluorouracil/cisplatin after the 7th vaccination because of an elevation in tumor markers, and the 3rd vaccination because of progressive disease (PD), respectively.

Peptide screening, vaccination, and adverse events. Ten patients were HLA-A24 positive and were vaccinated with HLA-A24-binding peptides. Among the 14 peptides for HLA-A24$^+$ patients, Lck-derived peptides were most frequently used for the vaccination (Lck-208 and Lck-488 for 6 patients, and Lck-486 for 5 patients) (Table I). CypB-84 and ART4-75 were not used for the vaccination into any patient due to an immediate-type hypersensitivity reaction. Representative results

Table III. Immuno-responses and clinical outcome.

No.	Peptide	CTL response[a] Pre	CTL response[a] Post	Antibody to peptide[b] Pre	Antibody to peptide[b] Post	DTH[c]	Cyto-toxicity[d]	No. of vaccination	Clinical response at[e] 6th	Clinical response at[e] 12th	PFS[f] (days)	OS[g] (days)
	Lck-486	A	B	0.09	-	+(3)	Not					
1	Lck-208	C	C	0.05	0.03	+(3)	increased	10	PD	-	59	165
	Lck-488	C	ArB	-	-	+(3)						
	ART1-170	AAA	AA	-	-	-	Not					
2	ART4-13	A	C	-	-	-	increased	7	SD	PD	102	271
	Lck-488	CC	ArAC	-	-	-						
	SART1-690	ArA	B	0.31	0.37	+(5)						
3	SART2-161	A	A	0.12	0.12	+(5)	Not					
	CypB-91	A	-	0.14	0.23	+(5)	increased	8	PD	-	43	194
	Lck-208	A	A	0.27	0.22	+(2)						
	SART3-109	C		-	-	+(1)	Not					
4	Lck-488	D	NT	-	-	+(1)	increased	6	PD	-	57	232
	ART1-170	D		-	1.18	+(1)						
	SART3-302	C		-	-	+(2)						
5	Lck-246	Ar	NT	-	-	+(2)	NT	5	PD	-	43	73
	Lck-422	C		-	-	+(2)						
	ppMAPkkk-432	C		-	-	+(2)						
	ART1-170	ArBCC	-	-	2.06	+(3)						
6	Lck-488	AAA	-	-	-	+(3)	Not					
	Lck-208	AC	A	-	0.25	+(3)	increased	36	SD	SD	1058+	1058+
	SART3-315	C	-	-	0.03	+(3)						
	SART2-899	AA	-	-	-	-						
7	SART3-109	A	-	0.10	0.09	-	NT	8	PD	-	110	247
	Lck-208	A	A	-	-	-						
	Lck-486	A	B	-	-	-						
	SART3-315	C	ArAC	-	-	-						
8	CypB-91	C	A	-	-	-	NT	8	PD	-	96	206
	Lck-208	C	A	-	-	-						
	Lck-486	C	-	-	-	-						
	Lck-488	ArAr	CC	-	-	+(4)	Not			SD		
9	SART2-93	ArCC	ArArArC	-	0.04	+(4)	increased	7	SD	chemo+	96	623
	Lck-486	C	ACC	-	-	+(4)						
	SART2-161	A				-				PR		
10	Lck-488	AB	NT	NT	NT	-	NT	3	PD	chemo+	49	783+
	CypB-91	Ar				-						
	Lck-486	A	B	-	-	+(3)						
11	CypB-91	A	-	-	-	+(3)	Not					
	SART3-109	D	C	0.03	1.51	+(3)	increased	12	no rec	no rec	336	339
	Lck-208	D	-	-	-	+(3)						

[a]The peptide-specific CTL precursor cells were evaluated by quadricate assay in pre- and post (6th)-vaccination. NT, not tested. [b]Values indicate the fluorescent intensity of sera (x100 dilution). [c]Number of the vaccination when DTH to the peptide was detected for the first time. [d]Cytotoxicity to HLA-matched cancer cells of pre- and post-PBMCs was evaluated by ^{51}Cr-relase assay. [e]PD, progressive disease; SD, stable disease; PR, partial response; no rec, no recurrence. [f]PFS, progression-free survival; Pulse (+) mark, patients are alive (2004.9.15). [g]OS, overall survival.

for peptide selection in 4 patients whose post-vaccination PBMCs showed increased responses are shown in Fig. 1, and

All 11 patients were evaluated for adverse events according to the NCI-CTC. The vaccinations were generally well

Table IV. Adverse events.

	Grade 1	Grade 2	Grade 3
Fever	1	2	
Inflammatory reactions at the vaccination site	3	3	1
Anorexia	1		
Fatigue	1		

According to NCI-CTC criteria version 2.0.

any autoimmune diseases. The most frequently observed toxicity was an inflammatory reaction at the injection site in 7 of 11 patients. Three patients showed a grade 1, and 3 showed a grade 2 reaction. No treatment was required for these 6 patients. The remaining 1 patient showed a grade 3 inflammatory reaction with leg edema at the 31st vaccination, after which a non-steroidal antiphlogistic agent was administered. Fever (grades 2 and 1), anorexia (grade 1), and fatigue (grade 1) were observed in 3 (2 and 1), 1, and 1 patient, respectively (Table IV).

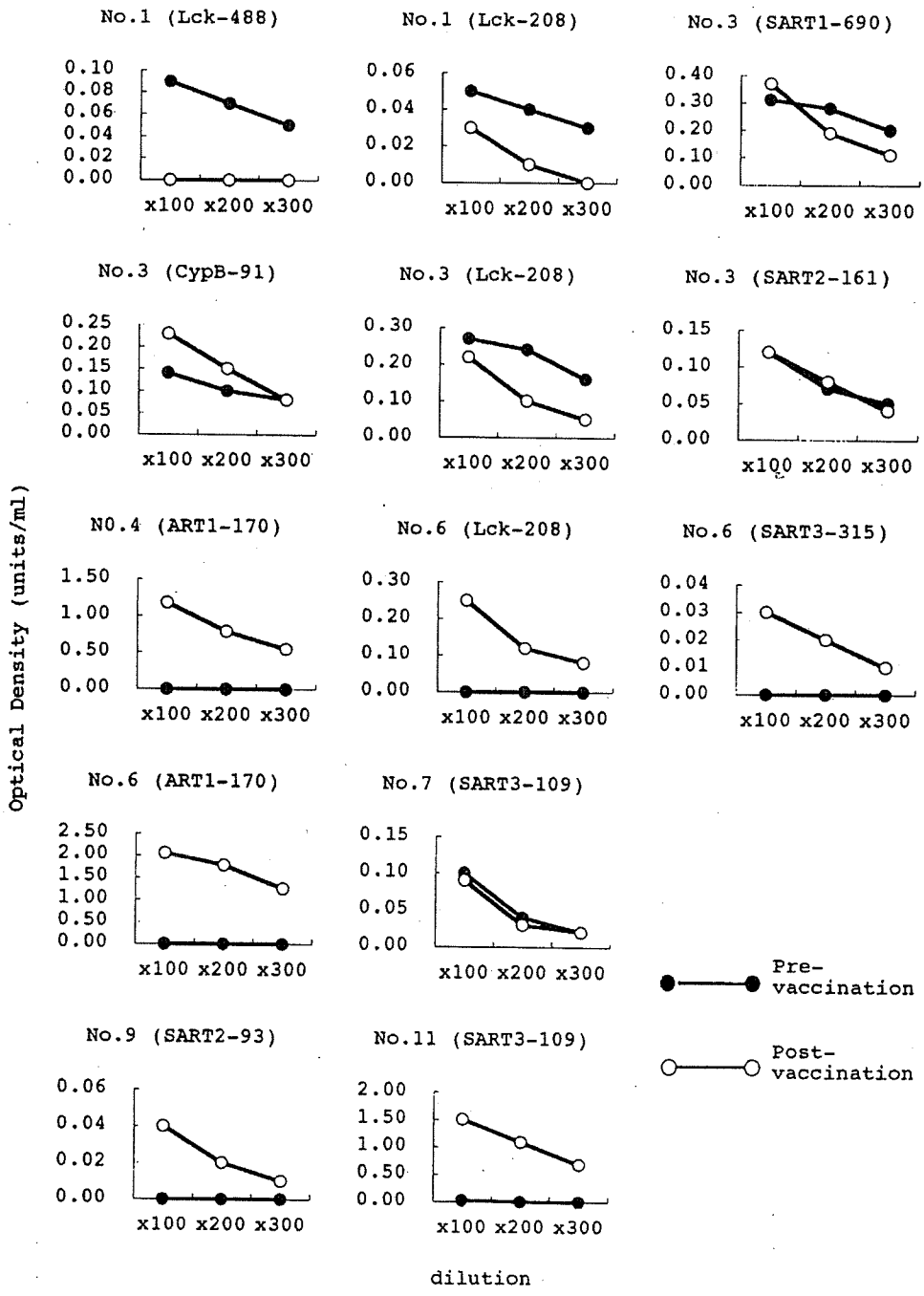

Figure 2. Kinetic study of IgG levels specific to the peptides administered to each patient. Pre- and post (6th)-vaccination sera were serially diluted and the levels of peptide-specific IgG were measured using ELISA, as described in Patients and methods. Results of 7 cases are shown in the figure. Horizontal lines indicate optical density (OD) and vertical lines the dilution of sera.

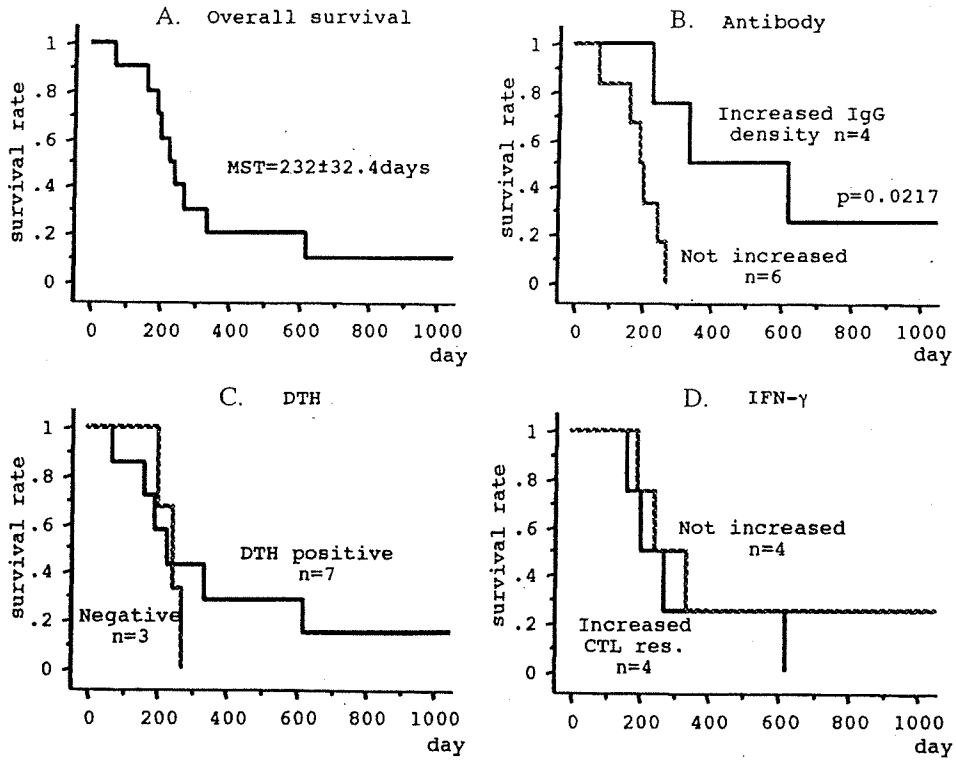

Figure 3. Antibody response and overall survival. Overall survival of 10 cases is given in (A). The group of patients with increased peptide-specific IgG levels (n=4) showed prolonged survival compared to the group of patients with no increase in peptide-specific IgG levels (n=6) (B). In contrast, neither the positive DTH response (C) nor the augmentation of CTL precursor reactive to peptide (D) influenced their survival.

Cellular immune responses. No DTH reaction against the peptides was observed prior to vaccination in any patient. DTH reactions were observed in 7 patients (nos. 1, 3-6, 9, and 11) until the 5th vaccination, the details of which are given in Table III. For example, in case no. 3, a DTH reaction to Lck-208 was observed after the 2nd vaccination and reactions to SART1-690, SART2-161, and CypB-91 were observed after the 5th vaccination.

Augmentation of CTL precursors reactive to at least one of the vaccinated peptides was observed in 4 of 8 patients tested (case nos. 1 and 2 for Lck-488, no. 8 for SART3-315, and no. 9 for SART2-93 and Lck-486; Fig. 1). Because of the limited availability of blood samples, we kinetically evaluated the anti-tumor cytolytic activity of pre- and post-vaccination PBMCs by a ^{51}Cr-release assay in 7 patients in response to each of the four different cells of PC cell lines, but none of the post-vaccination PBMCs showed increased cytotoxicity against PC cells in an HLA-class-I-restricted manner (data not shown). Significant and equal levels of cytotoxicity against PC cells were observed in an HLA-class-I-non-restricted manner in both the pre- and post-vaccination PBMCs from 4 patients (case nos. 2, 6, 9, and 11). In contrast, such cytotoxicity decreased in the post-vaccination PBMCs from the remaining 3 patients (case nos. 1, 3, and 4).

Humoral immune responses. We also examined whether or not peptide-specific IgG could be detected in the vaccinated patients. Peptide-specific IgG to the vaccinated peptides was detected in the pre-vaccination sera of 4 of 10 patients tested (Table III). Peptide vaccination increased the IgG levels in 1 Although no peptide-specific IgG was detected in the other 6 tested patients before the peptide vaccination, peptide vaccination resulted in the induction of peptide-specific IgG in 3 patients (case no. 4, anti-ART1-170; no. 6, anti-ART1-170, -Lck-208, and -SART3-315; no. 9, anti-SART2-93). The results are shown in Fig. 2. The peptide specificity of the IgG in the sera of these patients was confirmed by an absorption test, however, the data are not shown because the peptide specificity was reported previously (14-19).

Clinical outcome and prognostic factor analysis. It was difficult to draw any definitive results from this small-scale phase I study with regard to clinical responses and a prognostic factor analysis. Nevertheless, demonstration of the available results may be relevant from the point of view of developing a suitable peptide vaccine. In 10 patients who received >3 vaccinations and were eligible for clinical evaluation, stable disease (SD) of 3 patients (case nos. 2, 6, and 9) and PD of 6 patients were diagnosed at the time of 6th vaccination. The remaining one patient (case no. 11) with an adjuvant-setting vaccination had a recurrence in the bone marrow 336 days after the initial vaccination. The median time to progression (TTP) and the median survival time (MST) were 96±22.2 (± standard error) days and 232±32.4 days, respectively. Their 6-month and 1-year survival rates were 80% and 20%, respectively.

As regards the identification of a laboratory marker to predict long-term survival, the group of patients with increased peptide-specific IgG levels (n=4) showed prolonged survival compared to the group of patients with no increase in peptide-specific IgG levels (n=6)(MST 339 days vs. 194 days,

neither the augmentation of a CTL precursor reactive to a peptide nor a positive DTH response influenced survival.

Discussion

This study was conducted in order to evaluate the safety and biological responses of the personalized peptide vaccination. Severe toxicity was rarely associated with the peptide vaccination, and this regimen can be recommended for further evaluation. Cellular and humoral responses to the vaccinated peptides were observed in 50% and 40% of the post-vaccination PBMCs and sera, respectively. Therefore, this regimen is also recommended for further evaluation of the immunological responses. Although the clinical response was not the main objective, only 1 patient maintained a minor response for 3 years, this regimen is not recommended if used alone. No significant clinical response was obtained, although the MST was not as short as those of the other clinical trials investigating patients with advanced PC (3-5,21,22). Personalized peptide vaccination combined with chemotherapy might be recommended, since one such case had a desirable clinical course. Nevertheless, clinical studies in a phase II setting are needed to address this issue.

Increased rates of peptide-reactive cellular and humoral responses to the vaccinated peptides in the post-vaccination PBMCs and sera of advanced cancer patients other than PC patients were somewhat higher than those of the PC patients observed here (14-19). In addition, no increment in HLA-class-I-restricted CTL activity against PC cells was observed at all in the post-vaccination PBMCs from any of the 7 patients tested. In contrast, such an increment was observed in the post-vaccination PBMCs from patients with other types of cancer (14-19). These results suggest that immunity in advanced PC is more depressed than that in other epithelial cancers. Alternatively, a more suitable peptide repertoire might be provided for PC patients. Tumor-associated antigens and peptides derived from PC cell lines might be more suitable in use for personalized peptide vaccinations administered to PC patients. From this point of view, the 14 peptides provided for HLA-A24$^+$ patients were primarily derived from esophageal cancers (7-9). In contrast, the 16 peptides provided for HLA-A2$^+$ PC patients were primarily derived from a PC9 tumor cell line, as reported previously (10), but only one HLA-A2$^+$ patient was enrolled in this clinical study. Therefore, clinical study of additional HLA-A2$^+$ patients is needed to address this issue, which is now in progress.

We previously reported that the increase in IgG levels due to administration of a personalized peptide vaccination correlated well with long-term survival in patients with advanced stages of cancers other than PC (19). The statistically significant difference ($p=0.0217$) was also observed among PC patients, although the number of patients in this study was small. Further studies are therefore needed to confirm this issue.

The biological role of peptide IgG in anti-tumor immunity should be clarified by future basic and clinical studies. The mechanisms of peptide-specific IgG production, including the involvement of CD4 T helper cells and HLA-restriction, also need to be elucidated.

Goydos *et al* reported the data obtained from a phase I trial of a synthetic mucin MUC-1 peptide vaccine admixed BCG (21), and only 1 of 24 patients (4.2%) had SD. The MST data were not given in that report. Gjertsen *et al* presented the data from a clinical phase I/II trial involving PC patients who were vaccinated by intradermal injection of synthetic mutant ras peptides in combination with granulocyte-macrophage colony-stimulating factor (22). That report showed that, in the group with non-resectable cancer, 11 out 34 patients (32%) had SD and the 1-year survival rate was 6.3% (3 out of 48 cases). Our patients appeared to have better survival rates than those in previous clinical trials. However, this regimen by itself is not recommended for HLA-24$^+$ patients with advanced PC. Further basic and clinical studies should be conducted for developing therapeutically effective peptide vaccinations for advanced PC patients.

Acknowledgements

This study was supported in part by Grants-in-Aid from the Ministry of Education, Science, Sports, and Culture of Japan (no. 12213134 to K.I. and Research Center of Innovative Cancer Therapy of 21st Century COE Program for Medical Science to K.I. and S.S.), from the Japan Society for the Promotion of Science (no. 14570526 to S.S.), and from the Ministry of Health and Welfare, Japan (no. H14-trans-002 and 11-16).

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