Humoral Responses to Peptides Correlate with Overall Survival in Advanced Cancer Patients Vaccinated with Peptides Based on Pre-existing, Peptide-Specific Cellular Responses

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ABSTRACT

Purpose: The aim of this study is to find a laboratory marker for overall survival in advanced cancer patients who were vaccinated with peptides based on pre-existing, peptide-specific CTL precursors in the circulation.

Experimental Design: A group of 113 patients with advanced cancer (28 colorectal, 22 prostate, 15 lung, 14 gastric, and 34 other cancers) was enrolled in a Phase I clinical study of peptide vaccination in which peptide-specific CTL precursors of prevaccination peripheral blood mononuclear cells were measured, followed by vaccination with these peptides (maximum of four). For cellular responses, pre and postvaccination (sixth) peripheral blood mononuclear cells were provided for measurement of both peptide-specific CTL precursors by IFN-γ release assay and tumor reactivity by 51Cr release assay. Delayed type hypersensitivity was also measured. For humoral response, pre and postvaccination (sixth) sera were provided for measurement of peptide-reactive IgG by an ELISA.

Results: The median survival time and 1-year survival rate of the total cases were 346 ± 64.9 days and 44.6%, respectively, and those of patients vaccinated more than six times (n = 91) were 409 ± 15 days and 54.4%, respectively. In these 91 patients, the overall survival of patients whose sera showed increased levels of peptide-reactive IgG (n = 60) was significantly more prolonged (P = 0.0003) than that of patients whose sera did not (n = 31), whereas none of cellular responses correlated with overall survival.

Conclusions: Peptide-specific IgG in postvaccination sera could be a suitable laboratory maker for the prediction of prolonged survival in advanced cancer patients vaccinated with peptides based on pre-existing CTL precursors.

INTRODUCTION

Recent advances in tumor immunology have allowed the identification of a number of antigens and epitopic peptides capable of inducing tumor-reactive CTLs (1–14). Some of these peptides were used for clinical trials, but these initial trials obtained rare clinical responses, as well as dim levels of immune responses to peptides (15–20). One reason for this failure could be an insufficient induction of antitumor responses in these regimens, in which peptide-specific memory T cells were not measured in prevaccination peripheral blood mononuclear cells (PBMCs). Subsequently, we conducted Phase I clinical trials of peptide vaccination in which cancer patients received peptides (a maximum of four) based on information regarding pre-existing, peptide-specific CTL precursors in the circulation (21–25). The other reason for failure might in part be attributable to a lack of an appropriate laboratory marker either to measure immune responses or to predict clinical responses. Regardless of the extensive studies, there are few reproducible and appropriate laboratory markers for prediction of clinical benefits in recently developing peptide-based therapies (15–25) or in the other types of immunotherapies (26–28). In this study, we investigated the correlation of clinical benefits and immune responses to peptides in HLA-A24-positive or -A2-positive cancer patients who were vaccinated with these CTL-directed peptides and reported that humoral responses to peptides correlated with overall survival.

MATERIALS AND METHODS

Trial Eligibility. The ethical review boards of the Kurume University School of Medicine and the Hokkaido University School of Medicine approved the study protocol. Complete written informed consent was obtained from all patients at the time of enrollment. According to the protocol, patients were required to be HLA-A24 positive and/or HLA-A2 positive, have a histologically confirmed lesion of a malignant tumor, have been untreated for ≥4 weeks before the study, and have an Eastern Cooperative Oncology Group performance status of 0–2. Eligibility criteria included an age from 20 to 85 years, a...
creatinine level <1.4 mg/dl, a bilirubin level <1.5 mg/dl, a platelet count of >100,000/mm³, hemoglobin of >8 grams/dl, and total WBC count of >3000/μl. Hepatitis B and C antigens were required to be negative. No patient had received any concurrent treatments, steroids, or any other immunosuppressive drugs for 4 weeks before the initial vaccination. This clinical study was carried out from November 2000 through November 2002.

Peptides and Selection for Vaccination. The peptides used in the present study were prepared under conditions of Good Manufacturing Practice by the Multiple Peptide System (San Diego, CA). The peptide sequences are shown in Table 2. These peptides have the ability to induce HLA-A24- or -A2-restricted and tumor-specific CTL activity in the PBMCs of cancer patients and were frequently expressed on various tumor cell lines (5–14). These peptides were dissolved and stored at −80°C. Stock solutions were diluted with saline just before use. For the peptide screening, peptividation PBMCs were provided for assays of peptide-specific CTL precursors using methods reported previously (29). Peptide-specific IFN-γ production was calculated by subtraction of IFN-γ production of the peptide-stimulated PBMCs in response to a negative control (HIV peptide) from that in response to a corresponding peptide in quadric assays, and a two-tailed Student’s t test was used for the statistical analyses. As reported previously (21–25), positive wells were evaluated in the following order: (a) criteria Ar, the peptide-specific IFN-γ production was ≥500 pg/ml, and P was <0.1; (b) criteria A, the production was ≥50 pg/ml, and P was <0.05; (c) criteria B, ≥25 ≤ the production <50 pg/ml, and P was <0.05; (d) criteria C, the production was ≥50 pg/ml and 0.05 ≤ P < 0.1; and (e) criteria D, the production was >100 pg/ml and 0.1 ≤ P < 0.2. According to the results, up to four positive peptides were selected for each patient and were vaccinated as the CTL precursor-oriented peptide vaccine, if an immediate type hypersensitivity reaction against each peptide was not seen in a skin test performed before vaccination. The screening of peptide-specific CTL precursors was also performed after the sixth vaccination to evaluate the in vivo cellular responses to the peptides. Cellular responses to tumor cells in a HLA-A24- or -A2-restricted manner in pre and postvaccination (sixth) PBMCs were measured using a standard 51Cr-release assay whose methods were described elsewhere (19, 20).

Clinical Protocol. Skin tests were performed by intradermal injection of 10 μg of each peptide using a tuberculin syringe and a 26-gauge needle. Saline was used as a negative control for assessment of hypersensitivity. 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 read 24 h after injection were needed to score the skin test as positive. If immediate type hypersensitivity was negative, the peptide was vaccinated into the patients’ s.c. tissue in the site near each tumor’s regional lymph nodes, e.g., the upper arm in cases with lung cancer, lateral abdominal wall in cases with gastric cancer, or anterior thigh with the other cancers. Two milliliters of the peptide, which was supplied in vials containing 2 mg/ml sterile solution, were mixed with an equal volume of incomplete Freund’s adjuvant (Montanide ISA-51; Seppic, Paris, France) and emulsified in 5-ml sterilized syringes. Three milliliters of each prepared peptide emulsion (maximum of four peptides at one vaccination) were injected s.c. in individual site three times every 2 weeks. For patients showing a favorable clinical course, the vaccinations were continued every 2–4 weeks with informed consent from each patient.

Detection of Serum IgG Levels. An ELISA was used to detect the serum IgG levels specific to the administered peptides, as reported previously (20–25). Briefly, 100 μl/well serum samples diluted with 0.05% Tween 20-Block Ace were added to the peptide (20 μg/well)-immobilized plate, after which the plate was blocked with Block Ace (Yukijirushi, Tokyo, Japan) and washed. After a 2-h incubation, the plate was washed and further incubated for 2 h with a 1:1000-diluted rabbit antihuman IgG (DAKO, Glostrup, Denmark). The plate was washed, and 100 μl of a 1:100-diluted goat antirabbit immunoglobulin-conjugated horseradish peroxidase-dextran polymer (EnVision; DAKO) were then added to each well; the plate was incubated for 40 min. After washing, 100 μl/well tetramethyl-benzidine substrate solution (KPL, Guildford, United Kingdom) were added, and the reaction was stopped by the addition of 1 M phosphoric acid. To estimate peptide-specific IgG levels, the absorbance values of each sample were compared with those of serially diluted standard samples, and the values were shown as absorbance. The cutoff value of optimal density (OD) was determined as 0.02 at a serum dilution of 1:100, because the mean of the OD in response to an HIV peptide taken as a negative control was <0.02, as reported previously (21–25). Positive responses were judged by the two criteria. The first criterion is the case if the prevaccination serum showed no reactions, and the postvaccination serum showed at least significant levels of IgG (>0.02 of net OD value at a serum dilution of 1:100) specific to the vaccinated peptides. The other criterion is the case if an OD value of the postvaccination serum showed ≥2-fold increase at a serum dilution of 1:100 than that of the prevaccination serum.

Evaluation of Clinical Responses and Statistical Analysis. All known sites of disease were evaluated by computed tomography scan or X-ray examination. Patients were assigned a response category according to the Response Evaluation Criteria in Solid Tumors (RECIST criteria), the revised version of the WHO criteria published in the WHO Handbook for reporting results of cancer treatment (Geneva, 1979), June 1999 (Final). For prostate cancer patients without measurable lesions, serum prostate-specific antigen levels were used as a marker for evaluation, as reported previously (23, 25). Overall survival was evaluated from the entry date of these clinical trials, regardless of peptide vaccinations after Phase I trials, and analyzed to investigate correlation between clinical benefits and immune responses. Kaplan-Meier curves were described, and survivals were compared using the Log-rank test.

RESULTS

Patients’ Characteristics. A group of 113 patients with advanced malignant tumors was enrolled in this vaccination regimen. The types of cancer included the following: (a) colorectal cancer (HLA-A24: n = 22, HLA-A2: n = 6); (b) prostate cancer (n = 12, 10); (c) lung cancer (n = 10, 5); (d) gastric
cancer (n = 12, 2); (e) melanoma (n = 5, 2); (f) cervical cancer (n = 4, 2); (g) ovarian cancer (n = 1, 2); (h) breast cancer (n = 1, 2); (i) esophageal cancer (n = 1, 2); (j) uterine cancer (n = 2, 0); (k) pancreatic cancer (n = 2, 0); (l) leiomyosarcoma (n = 2, 0); (m) thyroid cancer (n = 1, 0); (n) chronic lymphocytic leukemia (n = 1, 0); (o) bladder cancer (n = 1, 0); (p) renal cell carcinoma (n = 1, 0); (q) peritoneal cancer (n = 0, 1); and (r) seminoma (n = 0, 1; Table 1). The average patient age was 61 years (range: 23–85). Patients’ performance status evaluated by Eastern Cooperative Oncology Group criteria was 0 (n = 68), 1 (n = 32), and 2 (n = 13). All of the patients showed failure to respond to chemotherapy, hormonal therapy, and/or radiotherapy with clinical stage IV or recurrence. Details regarding the characteristics of patients with each respective type of cancer were described in the other studies (21–24), currently in press (25) or under submission.

**Vaccinated Peptides and Immune Responses.** The number of patients receiving four, three, two, or one peptide without immediate type hypersensitivity were 50, 19, 8, or 1 in HLA-A24+ patients, respectively, and 21, 9, 3, or 2 in HLA-A2+ patients, respectively. CTL precursors reacting to peptides were detected in prevaccination PBMCs for vaccination; the frequency of vaccinated peptides is given in Table 2. The most frequently used peptide was the SART3109 (38 of 78 cases), followed by the lck208 (31 cases) in HLA-A24+ patients. In HLA-A2+ patients, the most frequently used peptide was the MAP294 (15 of 35 cases), followed by the MAP142 (14 cases). CTL activity was evaluated in postvaccination (sixth) PBMC to evaluate cellular immune responses to the vaccinated peptides. Increased cellular responses were most frequently observed when the SART3109 peptide was vaccinated (9 of 22 cases, 41%) followed by the SART3109 peptide (12 of 31 cases, 39%). Detailed results for each case have been reported elsewhere (21–25) and are summarized in Table 2. CTL activity to HLA-class I-restricted tumor cells was measured by the standard 51Cr-release assay in pre and postvaccination (third and sixth) PBMCs. Sixteen of 76 cases tested (21%) showed increased HLA-class I-restricted cytotoxicity. DTH response at the site of a skin test during the first to sixth vaccination was most frequently observed when the SART315 peptide was vaccinated (9 of 26 cases of HLA-A24+ patients). DTH response was most frequently observed in HLA-A2+ patients vaccinated with the lck122 peptide (6 of 12 cases), a summary of which is presented in Table 2. Humoral immune responses to the vaccinated peptides were simultaneously measured in both pre and postvaccination (third and sixth) sera. Increased levels of IgG antibodies reactive to peptides were most frequently observed when the SART3109 was vaccinated (19 of 37 cases), as summarized in Table 2. It is of note that the UBE43 peptide induced humoral immune responses in all five cases tested. Detailed results and the criteria of increased immune responses for each case have been reported elsewhere (21–25) and are under submission as separate studies.

**Clinical Responses and Prognostic Marker Analysis.** Of 113 cases, 5 cases showed partial response, 2 cases showed minor response, and the remaining 106 cases showed progressive disease. A median survival time of 113 cases was 346 ± 64.9 (±SE) days, and a 1-year survival rate was 44.6% (Fig. 1A). Twenty-two cases could not achieve one cycle of vaccination (six times) because of the rapid progression of tumors, whereas the remaining 91 cases received more than six vaccinations. The median survival time and 1-year survival rate of these 91 cases were 409 ± 15 days and 54.4%, respectively (Fig. 1B). In patients undergoing more than six vaccinations (n = 91), 60 cases had detectably increased levels of peptide-specific IgG antibody in their postvaccination sera against at least one peptide of at maximum four vaccination peptides, whereas the remaining 31 cases did not (Fig. 1C). Forty-two among 90 cases tested showed increased CTL activity response to at least one peptide of at maximum four vaccination peptides (Fig. 1D), and 16 of 73 cases tested showed increased CTL activity of HLA-class I-restricted cytotoxicity against tumor
cells (Fig. 1E) in their postvaccination PBMCs. Thirty-four of 91 cases showed DTH response to at least one peptide among at maximum four vaccination peptides until the sixth vaccination (Fig. 1F). None of the cellular responses (peptide-specific CTL precursors, tumor-reactive CTL activity, or DTH reaction) correlated with overall survival. In contrast, the overall survival of patients whose sera showed increased levels of peptide-reactive IgG antibodies (n/60) was more significantly prolonged (P/60.0003) than that of patients whose sera did not show such increased levels (n/31). In addition, multivariate analysis was carried out with factors of age, gender, performance status, HLA typing, increased peptide-reactive IgG antibodies levels, increased CTL responses, increased cytotoxicity, and observed DTH reaction. Among them, significantly contributed factors for overall survival of 91 cases were increased peptide-reactive IgG antibodies levels (P/60.0014) and performance status 0 or 1 (P/60.0046), although only 4 of 91 cases were performance status 2.

### Detailed Analysis of Antibody Responses and Survival Time.
To obtain a better understanding of the relationship between antibody response and survival time, representative results of serial measurements of IgG reactive to the peptides for ≤12 vaccinations in the patients whose sera showed the positive responses to the vaccinated peptides were shown in Fig. 2. The result on one peptide per patient was given if a serum reacted to several peptides to save space on the study. The data showed in the other studies that were cited in this one (21-25) or those under submission were not given to avoid double publication. Positive responses were judged by the two criteria. The first criterion is the case if the prevaccination serum showed no reactions, and the postvaccination serum showed at least significant levels of IgG (0.02 of net OD value at a serum dilution of 1:100) specific to the vaccinated peptides. This case was observed in 50 of 60 patients tested. Some of the cases are shown in Fig. 2. The other criterion is the case if the postvaccination serum showed 2-fold increase of the IgG level at a serum dilution of 1:100 than that of the prevaccination serum. This case was observed in the remaining 10 cases. There was, however, no apparent difference of the survival time between the two groups (Fig. 3A).

In regard to the kinetics, positive antibody responses were induced in sera after the third vaccinations in 19 cases and after the sixth vaccinations in the remaining 41 cases, respectively. There was, however, no apparent difference of the survival time between the two groups (Fig. 3B). Among 113 cases shown in

### Table 2 Vaccinated peptide and immune responses

<table>
<thead>
<tr>
<th>Peptide name</th>
<th>Sequence</th>
<th>No. of vaccinated patients</th>
<th>Increased immune reactions to peptide*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>CTL activity</td>
</tr>
<tr>
<td>HLA-A24-binding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SART2 93</td>
<td>EYRGFTQDF</td>
<td>19</td>
<td>3 /15 (20%)</td>
</tr>
<tr>
<td>SART2 161</td>
<td>AYDFLYNYL</td>
<td>21</td>
<td>2 /16 (15%)</td>
</tr>
<tr>
<td>SART2 899</td>
<td>SYTRELILIL</td>
<td>13</td>
<td>0 /7</td>
</tr>
<tr>
<td>SART3 109</td>
<td>VDYDYNCHVDL</td>
<td>38</td>
<td>12 /31 (39%)</td>
</tr>
<tr>
<td>SART3 315</td>
<td>AYIDFEMKI</td>
<td>26</td>
<td>9 /22 (41%)</td>
</tr>
<tr>
<td>CypB84</td>
<td>KFHRVIKDF</td>
<td>4</td>
<td>0 /3</td>
</tr>
<tr>
<td>CypB91</td>
<td>DFMIQGQDF</td>
<td>19</td>
<td>0 /17</td>
</tr>
<tr>
<td>Ick208</td>
<td>HYTNASDGL</td>
<td>31</td>
<td>7 /25 (28%)</td>
</tr>
<tr>
<td>Ick486</td>
<td>TDYDLYRSLVL</td>
<td>25</td>
<td>4 /21 (19%)</td>
</tr>
<tr>
<td>Ick488</td>
<td>DYLRSVLEDF</td>
<td>26</td>
<td>3 /21 (14%)</td>
</tr>
<tr>
<td>ART1 170</td>
<td>EYCLKFTKL</td>
<td>9</td>
<td>0 /7</td>
</tr>
<tr>
<td>ART4 13</td>
<td>AFRHLAAL</td>
<td>4</td>
<td>0 /1</td>
</tr>
<tr>
<td>ART4 75</td>
<td>DYPSSLSATDI</td>
<td>19</td>
<td>1 /16 (6%)</td>
</tr>
<tr>
<td>HLA-A02-binding</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>SART3 302</td>
<td>LLQAEAPRL</td>
<td>6</td>
<td>0 /5</td>
</tr>
<tr>
<td>SART3 309</td>
<td>RLAEQAYQY1</td>
<td>9</td>
<td>0 /7</td>
</tr>
<tr>
<td>CypB129</td>
<td>KLHKYGPGWV</td>
<td>6</td>
<td>0 /6</td>
</tr>
<tr>
<td>Ick246</td>
<td>KVERLGGAA</td>
<td>7</td>
<td>1 /4 (25%)</td>
</tr>
<tr>
<td>Ick422</td>
<td>DVWFSGILL</td>
<td>12</td>
<td>3 /11 (27%)</td>
</tr>
<tr>
<td>MAP294</td>
<td>GLFLHHTRT</td>
<td>15</td>
<td>1 /13 (8%)</td>
</tr>
<tr>
<td>MAP492</td>
<td>DSLHAFPA</td>
<td>14</td>
<td>3 /11 (27%)</td>
</tr>
<tr>
<td>WHSC103</td>
<td>ASLDSDPWV</td>
<td>8</td>
<td>0 /6</td>
</tr>
<tr>
<td>WHSC141</td>
<td>ILGELREK</td>
<td>8</td>
<td>1 /4 (25%)</td>
</tr>
<tr>
<td>UBE43</td>
<td>RLEWCEWSVI</td>
<td>6</td>
<td>1 /5 (20%)</td>
</tr>
<tr>
<td>UBE85</td>
<td>LIADLSGL</td>
<td>2</td>
<td>1 /2 (50%)</td>
</tr>
<tr>
<td>UBE208</td>
<td>ILPRKHHI</td>
<td>1</td>
<td>0 /1</td>
</tr>
<tr>
<td>HHNRL140</td>
<td>ALVVFEDVL</td>
<td>5</td>
<td>0 /5</td>
</tr>
<tr>
<td>HHNRL501</td>
<td>NVLHFFNAPLA</td>
<td>13</td>
<td>1 /11 (9%)</td>
</tr>
<tr>
<td>EIF51</td>
<td>RIYDRKFL</td>
<td>1</td>
<td>0 /1</td>
</tr>
</tbody>
</table>

*No. of tested cases and % positive in parentheses are shown.

**DTH**, delayed-type hypersensitivity.
In this study, a substantial number of cases have been receiving the peptide vaccination for >18 months with stable disease conditions in most cases, and the consistently higher levels are observed in sera of these cases (data not shown).

In regard to numbers of positive peptides, positive antibody responses to only one peptide and at least two peptides were seen in sera of the 35 and 25 cases, respectively. There was also no apparent difference of the survival time between the two groups (Fig. 3C). In detail, 35, 18, 6, and 1 showed increased humoral responses to one, two, three, and four peptides among all of the vaccinated peptides (at maximum four), respectively. However, because of too few cases for the analysis, the positive response to more than three peptides was not seen to contribute to overall survival.

In regard to the magnitude of humoral responses, the strong antibody responses by means of increase of IgG levels from the baseline to 0.5 OD values at a serum dilution of 1:100 were seen in of sera 2, 15, 6, and 1 cases after the 3rd, 6th, 9th, and 12th vaccinations, respectively. A part of the results are shown in Fig. 2. The peptides involved in the stronger responses were mainly UBE43, SART3109, and lck486.

The 91 cases who received the vaccination for more than six times were divided into the four groups based on their CTL and antibody responses. There was no apparent difference in the overall survival between the cases showing both CTL and antibody responses (n = 30) and those showing only antibody response (n = 30). Similarly, there was no apparent difference in the overall survival between the cases showing only CTL response (n = 12) and those showing neither CTL nor antibody response (n = 19; Fig. 3D).

We lastly studied the correlation between antibody response and overall survival in each of the four diseases. Antibody response did not significantly correlate with overall survival in colorectal cancer patients (Fig. 3E) or gastric cancer patients (P = 0.6059; data not shown), whereas it well correlated in hormone refractory prostate cancer patients (P = 0.0374; Fig. 3F) and lung cancer patients (P = 0.0486; Fig. 3G).

**DISCUSSION**

The detailed results of a Phase I study of CTL precursor-oriented peptide vaccines to each type of cancer have been reported elsewhere (21–25) or are currently under submission for colorectal cancer.9 In this study, immune responses and clinical benefits were mainly analyzed in all of the patients with various types of cancers under the same regimen to discover a laboratory marker for prediction of prognosis. The results of 91 patients who received more than six vaccinations (one cycle) were used for statistical analysis. Patients (n = 60) whose sera showed increased levels of humoral responses to at least one vaccinated peptide had significantly better prognosis than those whose sera did not show such increment.

We reported previously that some CTL-directed peptides have the ability to elicit both cellular and humoral immune responses in Phase I clinical studies (20–24). It is well known that humoral response is important for tumor regression. Indeed, we have reported that levels of antipeptide antibodies in postvaccination sera seemed

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9 Y. Sato et al., submitted for publication.
to correlate with the overall survival of advanced lung cancer patients who received peptide vaccination (21). The results shown in this study along with those from the initial results regarding lung cancer suggest that the elevation of humoral response to vaccinated peptides is a favorable factor for patients who received peptide vaccination beyond their different tumor origins. The correlation of humoral responses with better prognosis has also been reported in the other vaccination regimens in which whole tumor cells or epidermal growth factor is used for vaccination in melanoma patients or non-small cell lung cancer patients, respectively (30, 31).

We reported previously that IgG reactive against CTL epitope peptides is often detected in prevaccination sera of cancer patients and also in sera of healthy donors (21–25, 31). We also reported that IgG reactive to these CTL epitope peptides is either lacking or unbalanced in the sera of patients with atopic disease (32, 33). The results shown in this study along with those from noncancerous subjects suggest that these peptide-reactive IgGs play a role in host defense against various diseases, although the underlying mechanism in antitumor immune responses in cancer patients is unclear. These antipeptide IgGs did not react to the mother proteins and also failed to show either the direct inhibition of tumor cell growth in vitro or elicit antibody-dependent, cell-mediated cytotoxicity to tumor cells as far as tested (data not shown). Additional studies are needed to clarify their biological role, as well as their mechanism of action.

DTH response is a simple method with high reproducibility and has often been used as a laboratory marker to monitor immune responses in vivo for vaccination against infectious diseases and also malignant diseases. However, controversial results have been obtained regarding DTH response as a laboratory marker for either measuring immune responses to antigens or in the prediction of clinical benefits for vaccinated patients (15–28, 30, 31). In this study, DTH response did not correlate with either clinical course or overall survival. In addition, measurements of increased cellular immunity to either peptide or tumor cells did not correlate with overall survival. Collectively, none of the three assays for cellular immunity correlated closely with overall survival, regardless of the fact that the vaccinated peptides were screened by CTL precursor assay in prevaccination PBMCs. There may be several reasons for this unexpected result. One of them could have to do with reproducibility. CTL precursor frequency analysis, enzyme-linked immuno-spot assay, and cytotoxicity assays are generally used as

Fig. 2 Measurements of IgG reactive to the vaccinated peptides. Representative results of measurements of IgG reactive to the peptides for \( \leq 12 \) vaccinations in the patients whose sera showed the positive responses to the vaccinated peptides are shown. It was judged as positive when postvaccination serum showed at least significant levels of IgG (\( \geq 0.02 \) of net optimal density (OD) value at a serum dilution of 1:100) specific to the vaccinated peptides from a negative level in prevaccination serum. It was also judged as positive when postvaccination serum showed a \( \geq 2 \)-fold increase of the IgG level at a serum dilution of 1:100 than that of the prevaccination serum.
laboratory markers to measure cellular immune responses to vaccinated peptides (15–31, 34). Although these assays are well-established monitoring systems, none of them is highly reproducible, mainly because the CTL precursors in PBMCs are usually very low, and the precursor frequency is between 1/1000 and 1/100,000, whereas the limit of sensitivity of these assays for detection is 1/3000 to 1/10,000 cells (15–31, 34, 35). The lower reproducibility of these cellular assays might also be attributable to in vitro biases, including the cells’ condition of cryopreservation, the culture medium, culture conditions, and the numbers and viability of cells at the time of harvesting. In contrast, the measurement of humoral immune responses can be relatively reproducible, because antibody molecules reactive to peptides are generally stable and abundant in serum samples (21–25, 32, 33). One of the other reasons is that these CTL assays use PBMCs, not tumor-infiltrating lymphocytes, and thus do not necessarily reflect the CTL activity at tumor sites. It is well known that T cells in the circulation rarely infiltrate into tumor sites. In contrast, IgG molecules might easily reach either peri-tumor or intratumor sites. This assumption is in part supported by the recent observation that inflammatory responses were observed around prostate cancers at the time of surgery in patients who received peptide vaccinations based on information regarding antibodies reactive to peptides before radical prostatectomy.10

The feasibility of ELISA as a laboratory marker for monitoring immune responses to vaccinated peptides could be superior to any of the CTL assays from several different points of view. Serum samples are much easier to preserve than PBMCs. A small amount of sera (10 μl/peptide) is needed for the assay, whereas relatively large numbers of PBMCs (~10⁶ cells/peptide) are needed for CTL assays. The occupation time for ELISA is only 1 day, whereas CTL assays need 14–30 days. Running costs are another advantage of ELISA.

We have shown in this study new evidence that measurement of peptide-specific IgG in postvaccination sera is a suitable laboratory marker to predict prolonged survival for advanced cancer patients who received peptide vaccination based on pre-existing CTL precursors in the circulation. This evidence should be confirmed by other clinical trials regarding peptide-based immunotherapy for cancer patients.

REFERENCES


Fig. 3  Analysis of antibody responses and survival time. In A, 50 of 60 cases showed induction of peptide-specific IgG antibody response from negative to positive (>0.02) optimal density level, and the remaining 10 cases showed increased peptide-specific IgG antibody. In B, positive antibody responses were induced in sera after the third vaccinations in 19 of 60 cases and after the sixth vaccinations in the remaining 41 cases, respectively. In C, positive antibody responses to only one peptide and at least two peptides were seen in sera of the 35 and 25 cases, respectively. In D, 91 cases, who received the vaccination for more than six times, were divided into four groups based on their CTL and antibody responses. There was no apparent difference in the overall survival between the cases showing both CTL and antibody responses (n = 30) and those showing only antibody response (n = 30). E, antibody response and overall survival in colorectal cancer patients. F, antibody response and overall survival in prostate cancer patients (P = 0.0374). F, antibody response and overall survival in lung cancer patients (P = 0.0486).

10 M. Noguchi et al., unpublished data.
Peptide-Specific Antibody and Survival


Humoral Responses to Peptides Correlate with Overall Survival in Advanced Cancer Patients Vaccinated with Peptides Based on Pre-existing, Peptide-Specific Cellular Responses

Takashi Mine, Yuji Sato, Masanori Noguchi, et al.


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