Dendritic Cells Pulsed with HER-2/neu-derived Peptides Can Induce Specific T-Cell Responses in Patients with Gastric Cancer

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ABSTRACT

Purpose: We have previously reported (K. Kono et al., Int. J. Cancer, 78: 202–208, 1998) that HER-2/neu-derived peptides are naturally processed as tumor-associated antigens recognized by tumor-specific, human leukocyte antigen (HLA)-A2-restricted CTLs in gastric cancer. In the present study, we described a Phase-1 vaccination trial in gastric cancer patients using dendritic cells (DCs) pulsed with the immunodominant HER-2/neu(p369) peptides.

Experimental Design: Nine enrolled patients, who had HER-2/neu-overexpressing tumors and who were HLA-A2 positive, received four vaccinations by DCs pulsed with HER-2(p369) peptide at 2-week intervals intradermally.

Results: There were no serious adverse effects noted in the immunized patients. Peripheral blood mononuclear cells, preimmunization and after the fourth immunization, were cultured with autologous, HER-2(p369)-pulsed antigen-presenting cells for 12 days. Thereafter, peptide specificity was evaluated by IFN-γ secretion assay from cultured T cells against T2 cells pulsed with HER-2(p369) peptide. HER-2/neu peptide-specific recognition could be demonstrated in six of nine patients after immunization, whereas there was no HER-2/neu peptide-specific recognition before immunization. The peptide-specific CTL lines isolated from two of the patients could also lyse a HER2/neu-transfected cell line. Furthermore, a peptide-specific delayed-type hypersensitivity response occurred in three of nine patients. One of the patients underwent a partial clinical response concurrent with a decrease of tumor marker. Another patient demonstrated a stabilization of disease status for a period of 3 months.

Conclusions: Taken together, tumor vaccination therapy with DCs pulsed with HER-2/neu-peptides may be a potential candidate for the novel treatment of gastric cancer patients.

INTRODUCTION

Gastric cancer is one of the most common cancers in Japan today. Despite the efforts to introduce new treatment modalities such as surgery combined with chemotherapy (1), hyperthermia (2), or chemoradiotherapy (3), control of gastric cancer at the advanced stage remains difficult. The utilization of antitumor T cells, as immunoadjuvant therapy for gastric cancer is, therefore, extremely appealing. We and others have reported that MHC class I-restricted CTLs from gastric-cancer patients can react specifically against autologous tumor cells (4–7). The occurrence of HLA-A1/A31- or HLA-A2-restricted, tumor-associated antigens, recognized by gastric-cancer-specific CTLs has been demonstrated (4, 6, 7). Therefore, immunotherapy using anti-gastric cancer CTLs or CTLs recognizing specific tumor-associated antigens, naturally presented by the tumor cells, could potentially be ideal candidates for immunotherapeutic approaches for gastric cancers.

In a previous study, we have provided evidence that HER-2/neu-derived peptides are naturally processed as tumor-associated antigens in gastric cancer and can be recognized by tumor-specific, HLA-A2-restricted CTLs (7). The HER-2/neu proto-oncogene encodes a M_185,000 transmembrane glycoprotein with tyrosine-specific kinase activity (8). HER-2/neu is amplified and overexpressed in ~30% of human ovarian and breast tumors (9) and in 20% of gastric cancers (10). HLA-A2-restricted CTL epitopes that are derived from HER2/neu and that are recognized by ovarian-cancer-specific (11, 12) and breast-cancer-specific (13) CTLs have previously been defined. Additional HLA-A2-restricted, CTL epitopes from HER-2/neu that can activate CTLs from healthy donors and patients with advanced ovarian carcinoma have also been reported (14, 15). On the basis of the above reports, it may be speculated that anti-HER-2/neu immune targeting may be used as a common approach to the immunotherapy of a variety of cancers.

DCs are the most potent APCs, capable of priming naive T cells to specific antigens (16). DCs have been demonstrated to be critical for the development of tumor-specific immune responses (17), and DC-based vaccination strategies have yielded encouraging results in experimental tumor models and clinical
In the clinical trial were as follows: (a) gastric cancer patients with recurrent or unresectable tumors; (b) their tumors were proved to overexpress HER-2/neu by immunohistochemistry; (c) HLA-A2-positive patients with adequate bone-marrow, cardiac, pulmonary, hepatic and renal functions; (d) no therapy 4 weeks before the initiation of the trial; (e) Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; and (f) age <80 years. The present study was approved by the ethical committee of Yamanashi Medical University, and written informed consent was obtained from all of the individuals. The trial was carried out in accordance with the Helsinki declaration on experimentation on human subjects.

Materials and Methods

Patients. The eligibility criteria of patients participating in the clinical trial were as follows: (a) gastric cancer patients with recurrent or unresectable tumors; (b) their tumors were proved to overexpress HER-2/neu by immunohistochemistry; (c) HLA-A2-positive patients with adequate bone-marrow, cardiac, pulmonary, hepatic and renal functions; (d) no therapy 4 weeks before the initiation of the trial; (e) Eastern Cooperative Oncology Group (ECOG) performance status of 0 or 1; and (f) age <80 years. The present study was approved by the ethical committee of Yamanashi Medical University, and written informed consent was obtained from all of the individuals. The trial was carried out in accordance with the Helsinki declaration on experimentation on human subjects.

Immunohistochemistry for HER-2/neu. Paraffin-embedded tissue sections were evaluated by immunohistochemical staining with the labeled streptavidin-biotin method (LSAB). A commercially available, mouse monoclonal antibody to the cytoplasmic domain of human HER-2/neu raised against a synthetic peptide corresponding to amino acids 1238–1255 (Serotec, San Diego, CA) was used as the primary antibody. The sections were incubated with the primary antibody (1:100 dilution) overnight at 4°C. The primary antibody was detected using the Universal Large Volume DAKO LSAB kit (DAKO, Carpinteria, CA) according to the manufacturer’s instructions. Diaminobenzidine was used as a substrate for 5 min to visualize peroxidase deposition at the antigenic sites. A positive staining of the tumor specimen, irrespective of the percentage of positive cells, was considered to be indicative of a HER-2/neu-overexpressing tumor (21).

Preparation of DCs Pulsed with HER-2/neu Peptides. PBMCs were separated from peripheral blood by centrifugation over Ficoll-Paque (Pharmacia, Uppsala, Sweden), and monocytes were enriched by adherence to a plastic tissue culture flask (Corning, Corning, NY) for 90 min at 37°C. Adherent cells were cultured for 7 days with 1000 units/ml GM-CSF (Peprotech EC Ltd., London, United Kingdom) and 1000 units/ml IL-4 (Peprotech EC Ltd) in RPMI (Life Technologies, Inc., Gaithersburg, MD) with 7% autologous serum. Thereafter, DCs were harvested with vigorous washing and examined morphologically by light microscopy. The phenotype of DC was determined by flow cytometry using CD14-PE (BD Pharmingen, San Diego, CA), CD80-FITC (BD Pharmingen), CD86-FITC (Dako, Glostrup, Denmark), MHC class I (Dako), and HLA-DR (Dako). Unlabelled monoclonal antibodies were visualized by using FITC-conjugated rabbit antimouse immunoglobulins (Dako). An average of 87.5% of the cultured cells expressed a DC phenotype (minimum, 76.1%; maximum, 90.5%), which was composed of high levels of MHC class I, HLA-DR, CD80, and CD86 and a low level of CD14.

DCs (1 × 10⁶/ml) were pulsed with 50 µg/ml HER-2(p369) peptide for 3 h at room temperature in normal saline with 1% human albumin. Thereafter, DCs were washed twice and resuspended in normal saline with 1% human albumin before administration.

Treatment Design. Four vaccinations with DCs pulsed with HER-2(p369) peptide were performed at 2-week intervals. Each vaccination consisted of 1 × 10⁷ peptide-pulsed DCs administered intradermally in a single site at a supraclavicular location. All of the patients were monitored clinically using imaging analysis with ultrasonography and computed tomography and were classified according to conventional criteria: CR, defined as the disappearance of all measurable tumor for at least 1 month; PR, defined as a ≥50% decrease of all measurable tumor for at least 1 month; minor response (MR), defined as a reduction of 25–49%; S.D., defined as a reduction of less than 25%; and PD, defined as an increase in the tumor burden. All adverse events were evaluated according to the WHO toxicity scale, and treatment was stopped if toxic effects of grade 3 or 4 developed.

Cell Lines. The C1R/A2 is a MHC class I-defective LCL cell line that expresses HLA-A2.1 (B35 low and Cw low), and C1R/A2HER-2 is a HER-2/neu transfectant cell line of C1R/A2 (7). HLA-A2 or HER-2/neu expression on these cell lines was confirmed by flow cytometric analysis as described previously (7). The T2 cell line is an HLA-A2+ Tap-line derived from a B-T-cell hybrid (22). All of the cell lines were kept in RPMI 1640 with 10% FCS, 50 units/ml penicillin and 2 mM L-glutamine.

Peptide. HER-2/neu peptide (nt. 369–377, KIFGSLAF; Refs. 7 and 11) was synthesized and purified (≥99% purity) by SAWADY Technology (Tokyo). The control HIV-derived (ILKEPVHGV, >98% purity; Ref. 23) and the Flu-derived peptide (GILGFVFTL, >99% purity; Ref. 24) were also synthesized in a similar manner. The endotoxin levels and bioburden of these peptides were tested and determined to be within acceptable levels prior to their use as vaccines.

Detection of HER-2/neu-specific Response in PBMCs. PBMCs were separated from the peripheral blood of patients by Ficoll Paque centrifugation and cryopreserved for later use. Autologous APCs were prepared from PBMCs by adherence to plastic for 60 min at 37°C. APCs were incubated with HER-2(p369) peptide (25 µg/ml) for 6 h at room temperature, then were irradiated (25 Gy), and washed twice before use as stimulators. PBMCs from preimmunization and after the fourth immunization were thawed simultaneously and cocultured with peptide-pulsed APCs in AIM-V medium (Life Technology, Grand Island, NY) with 50 IU/ml IL-2 (Shionogi, Osaka, Japan). APCs and PBMCs were cocultured at a ratio of 1:20.

After 12 days of culture, peptide specificity in the resultant T-cell population was evaluated by IFN-γ secretion assay. T2 cells, preincubated with HER-2(p369) peptide (25 µg/ml) or HIV-derived peptide (25 µg/ml) as an irrelevant negative control were used as stimulators in this assay. Cultured T cells (1 × 10⁶) and an equal number of peptide-pulsed T2 cells were cocultured for 12 h. IFN-γ content of the culture supernatant was determined by ELISA (Amersham Pharmacia Biotech, Uppsala, Sweden).
Table 1  Patient characteristics

<table>
<thead>
<tr>
<th>Age</th>
<th>Sex</th>
<th>Metastatic site</th>
<th>HLA A typing</th>
<th>HER-2/neu expressiona</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>36</td>
<td>M</td>
<td>Virchow</td>
<td>A2, A31</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>M</td>
<td>Para-aortic lymph node</td>
<td>A2, A2</td>
</tr>
<tr>
<td>3</td>
<td>72</td>
<td>M</td>
<td>Peritoneal dissemination</td>
<td>A2, A26</td>
</tr>
<tr>
<td>4</td>
<td>64</td>
<td>M</td>
<td>Para-aortic lymph node</td>
<td>A2, A33</td>
</tr>
<tr>
<td>5</td>
<td>42</td>
<td>M</td>
<td>Peritoneal dissemination</td>
<td>A2, A24</td>
</tr>
<tr>
<td>6</td>
<td>68</td>
<td>M</td>
<td>Retroperitoneal space</td>
<td>A2, A24</td>
</tr>
<tr>
<td>7</td>
<td>75</td>
<td>M</td>
<td>Peritoneal dissemination</td>
<td>A2, A24</td>
</tr>
<tr>
<td>8</td>
<td>75</td>
<td>M</td>
<td>Para-aortic lymph node</td>
<td>A2, A26</td>
</tr>
<tr>
<td>9</td>
<td>61</td>
<td>M</td>
<td>Para-aortic lymph node</td>
<td>A2, A24</td>
</tr>
</tbody>
</table>

a HER-2/neu expression was evaluated by immunohistochemistry as strong (+ +) or moderate expression (+).

Table 2  Recognition of HER-2/neu peptide after immunization as analyzed by IFN-γ release assay

The reactivity of T cells before or after immunization was evaluated by IFN-γ release assay against T2 cells pulsed with HER-2/neu peptide (+HER-2 pep) or T2 cells pulsed with HIV peptide (+HIV pep).

<table>
<thead>
<tr>
<th>Case</th>
<th>+HER-2 pep</th>
<th>+HIV pep</th>
<th>+HER-2 pep</th>
<th>+HIV pep</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>379 ± 21</td>
<td>295 ± 15</td>
<td>1652 ± 38b</td>
<td>301 ± 24</td>
</tr>
<tr>
<td>2</td>
<td>83 ± 19</td>
<td>79 ± 17</td>
<td>125 ± 12</td>
<td>69 ± 15</td>
</tr>
<tr>
<td>3</td>
<td>111 ± 12</td>
<td>99 ± 11</td>
<td>421 ± 45b</td>
<td>111 ± 21</td>
</tr>
<tr>
<td>4</td>
<td>56 ± 11</td>
<td>50 ± 13</td>
<td>396 ± 28b</td>
<td>79 ± 10</td>
</tr>
<tr>
<td>5</td>
<td>99 ± 21</td>
<td>110 ± 14</td>
<td>113 ± 15</td>
<td>149 ± 11</td>
</tr>
<tr>
<td>6</td>
<td>212 ± 19</td>
<td>198 ± 15</td>
<td>991 ± 21b</td>
<td>109 ± 14</td>
</tr>
<tr>
<td>7</td>
<td>151 ± 14</td>
<td>98 ± 19</td>
<td>250 ± 14</td>
<td>198 ± 14</td>
</tr>
<tr>
<td>8</td>
<td>175 ± 19</td>
<td>168 ± 18</td>
<td>541 ± 29b</td>
<td>268 ± 24</td>
</tr>
<tr>
<td>9</td>
<td>211 ± 20</td>
<td>209 ± 21</td>
<td>889 ± 31b</td>
<td>209 ± 23</td>
</tr>
</tbody>
</table>

a The content of IFN-γ in supernatant of T cells stimulated for 24 h is expressed as pg/ml.
b The values are considered as significant.

Buckinghamshire, England). IFN-γ levels at least twice that of the controls were considered as evidence of peptide-specific T-cell reactivity.

PBMCs of patients 6 and 9, collected after the fourth vaccination, were stimulated every 10 days with autologous, HER-2(p369)-pulsed APCs and 50 units/ml IL-2, for four cycles. Subsequently, the T cells were tested for their cytotoxic activity in a standard 51Cr release assay. 51Cr-labeled C1R/A2, HER-2(p369)-pulsed APCs and 50 units/ml IL-2, for four vaccination, were stimulated every 10 days with autologous, the controls were considered as evidence of peptide-specific HER-2 pep) or T2 cells pulsed with HIV peptide (HIV pep).

RESULTS

Patient Characteristics. The characteristics of the nine enrolled patients are shown in Table 1. HER-2/neu overexpression was confirmed in all of the primary or metastatic sites of the patients. Four cycles of immunization were carried out in all of the patients. The protocol described here was well tolerated, and no cardiac, hematological, hepatic, or renal toxicity was noted.

HER-2/neu Vaccination Induces Peptide-specific T-Cell Response. PBMCs, collected before immunization and after the fourth immunization, were stimulated with HER-2(p369)-pulsed autologous APCs and expanded as described in “Materials and Methods” in vitro at day 1. After 12 days of culture, peptide specificity was evaluated by IFN-γ secretion assay. As shown in Table 2, HER-2/neu peptide-specific T-cell reactivity could be demonstrated in six of nine patients after immunization, whereas there was no HER-2/neu peptide-specific recognition before immunization. These results indicate that the vaccination with peptide-pulsed DCs can induce a peptide-specific T-cell response in patients with HER-2/neu-overexpressing gastric cancer.

Because enough T-cell lines could be established from PBMCs of patients six and nine after their fourth vaccination by repeated in vitro stimulation with autologous, peptide-pulsed APCs, the cultured T cells were used as cytotoxic effectors in standard 51Cr release assays. As shown in Fig. 1, these T cells lysed the C1R/A2HER2 cell line but did not lyse C1R/A2 or K562, indicating that the T cells specifically recognized naturally processed epitopes of HER-2/neu (Fig. 1).
DTH Response. HER-2(p369) peptide-specific DTH responses were not observed in any patients before vaccination (data not shown). However, strong DTH responses against the HER-2(p369) peptide were recognized in three of nine patients after vaccination (Table 3). PBMCs of these three patients also demonstrated peptide-specific T-cell responses in vitro, which indicated that a HER-2(p369) peptide-specific DTH response correlated with the peptide-specific reactivity observed in in vitro assays.

Clinical Evaluation after Vaccination. One of the patients (patient 9) presented a PR in a metastatic para-aortic lymph-node at 3 months after the fourth vaccination (Fig. 2), concurrently with a decrease in CEA tumor markers (Table 4). Patient 1 showed S.D. in the metastasis of right supraclavicular lymph-nodes. In 3 of 9 patients, the tumor markers (CEA or CA19-9) were decreased after vaccination. Patients 1 and 9, who had clinical responses (PR and S.D., respectively) also generated DTH and peptide-specific T-cell responses after the vaccination. These results suggest that the induction of HER-2/neu-specific immunity may result in clinical responses.

Patient 9 had a recurrence in a para-Aortic lymph node 1 year after the gastrectomy for the advanced gastric cancer. At that time, he received chemotherapy for 3 months; however, the metastasis in the lymph nodes grew progressively and tumor markers (CEAs) increased (Fig. 3). After a 2-month interval without any treatment, he was enrolled in this clinical study. He showed a partial clinical response 3 months after the last immunization without any concurrent therapy; this manifested as a regression of a local lymph-node metastasis and a decrease of CEA tumor markers. The regression of lymph-node metastasis lasted for 6 months.

**DISCUSSION**

The focus of the present trial has been to investigate the toxicity of DCs pulsed with an immunodominant epitope of the HER2/neu and the efficacy of this approach in inducing an immune response in gastric cancer patients. Our results demonstrate that: (a) the majority of the patients developed a HER2/neu peptide-specific T-cell response; (b) the peptide-specific CTL lines isolated from two of the responder patients could also recognize a HER2/neu-transfected cell line; (c) a peptide-specific DTH response occurred only in the patients that responded in vitro against the HER2/neu peptide, but in none of the patients that did not respond in vitro to the same epitope; (d) there were no serious adverse effects detected in the immunized patients; and (e) one of the patients underwent a partial clinical response.

HER2/neu is a self-protein, detectable at low levels in human epithelial cells by immunohistochemistry in various normal tissue (25). The breaking of tolerance against this protein would, therefore, seem to require highly efficient methods of immunization. The use of a potent cellular adjuvant like DC, which has been previously shown to be effective for malignant
melanoma and B-cell lymphoma (26), is, therefore, eminently suitable for this purpose. We conclude that this mode of immunization indeed was effective in inducing T-cell immunity, because we were able to induce HER2/neu peptide-specific T-cell responses in six patients and peptide-specific DTH responses in three of the nine vaccinated patients. This relatively high response rate to HER-2/neu peptide, taken together with the modest therapeutic responses noted in two patients, is promising because all of the patients had advanced disease, with recurrent or unresectable tumors and a significant tumor burden.

In another Phase I trial (27), with 19 HLA-A2 patients with HER-2/neu-overexpressing cancers, a vaccine consisting of three putative HER-2/neu helper peptides (p369-384, p688-703, and p971-984), which also contained within these sequences HLA-A2-binding motifs (p369-377, p689-697, and p971-979), were administered intradermally (27) with recombinant human GM-CSF as an adjuvant. The results from this trial are encouraging, because the CD8 T-cell precursor frequency to the HLA-A2-restricted peptides, including the p369 epitope used in our trial, increased in the majority of the patients. These authors attribute their success (in generating high levels of peptide-specific CD8 precursors in the majority of the vaccinated patients) to their selection of patients, which, in contrast to many other Phase I studies including the present one, included only patients with low-level or nondetectable disease.

Most importantly, the peptide-specific T-cell lines or CD8 T-cell clones isolated in the study of Knutson et al. (27) were able to lyse HER-2/neu-expressing HLA A2 + transfectants or tumor lines. This is similar to our data, in which p369-specific CTL lines that were induced from two of the patients also were able to kill HER-2/neu transfectants but not their nontransfected control lines. This is in contrast to the findings of Zaks and Rosenberg (28), who treated four patients who had metastatic advanced breast, ovarian, or colorectal cancer that overexpressed HER-2/neu, with the same HLA A2 restricted peptide epitope 369 (7, 11). They reported that repeated immunization with 1 mg of this peptide epitope in incomplete Freunds adjuvant (IFA) s.c. every 3 weeks resulted in an increase in easily detectable peptide-specific CTL precursors. The main emphasis in their report is, however, that these peptide-specific CTLs failed to recognize HLA-A2+ HER-2/neu + tumors or HER2 COS.A2 transfectant, which have high surface expression of HLA-A2. On the basis of the lack of tumoricidal activity, the authors suggest that the p369 epitope is not presented on the surface of HER2/neu, with the same HLA A2 restricted peptide epitope 369 (7, 11). They reasoned that the discrepancy between their study and those of others may be related to differences in the mode of immunization.

The first study using an approach similar to the one used in the present report, with DCs pulsed with HER-2/neu-derived peptides including the p369 epitope, was recently carried out in patients with advanced and previously heavily treated breast and ovarian carcinomas (19). This study included 10 patients who were vaccinated with mature DCs pulsed with either two HER-2/neu-derived peptides (p369-377 and p654-662) or two MUC1-derived peptides, depending on the presence of these antigens in the tumor. Three vaccinations were required to observe any tumor regression in these patients, which then responded in 5 of 10 patients (2 with HER2 peptides) could be detected in the patients’ T cells by using

**Table 4 Clinical response after immunization**

<table>
<thead>
<tr>
<th>Case</th>
<th>Tumor marker</th>
<th>Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>↓ (CA19-9; 126 → 82)</td>
<td>S.D.</td>
</tr>
<tr>
<td>2</td>
<td>↑ (CA19-9; 444 → 598)</td>
<td>PD</td>
</tr>
<tr>
<td>3</td>
<td>↓ (CA19-9; 456 → 218)</td>
<td>PD</td>
</tr>
<tr>
<td>4</td>
<td>wnl</td>
<td>PD</td>
</tr>
<tr>
<td>5</td>
<td>↑ (CEA; 38 → 49)</td>
<td>PD</td>
</tr>
<tr>
<td>6</td>
<td>↑ (CA19-9; 198 → 498)</td>
<td>PD</td>
</tr>
<tr>
<td>7</td>
<td>wnl</td>
<td>PD</td>
</tr>
<tr>
<td>8</td>
<td>↑ (CEA; 29 → 88)</td>
<td>PD</td>
</tr>
<tr>
<td>9</td>
<td>↓ (CEA; 25 → 11)</td>
<td>PR</td>
</tr>
</tbody>
</table>

* Tumor makers were within normal limit (wnl) before or after immunization.
intracellular IFN-γ staining. In the HER-2/neu-peptide-immu-
nized patients, the major CTL response was induced with the
p369 peptide, which, in line with our study, confirms the
immunodominance of this epitope (7, 15). The response rate
among the patients in our study seems to be somewhat higher [6
of 9 as compared with 2 of 10 in the study by Brossart
et al. (19)]. This may have been because the heavily treated breast
and ovarian carcinoma patients in their study were more immuno-
compromised than the gastric cancer patients in our study.
Alternatively, this may also have resulted from their use of
mature monocyte-derived DCs as compared with the immature
monocyte-derived DCs in our study.

None of the patients in the present trial, or in any of the
other HER-2/neu trials mentioned here, demonstrated any ad-
verse effects. In particular, there was no clinical cardiac toxicity,
although the evaluation of cardiac function with such measures
as echocardiogram was not fully performed. The lack of immu-
nopathological reactions was noted despite the demonstrated
ability of the T cells from the vaccinated patients to recognize
naturally processed epitopes of HER-2/neu presented on normal
tissue. The apparent inability of specific T cells to recognize
HER-2/neu expressed in normal human or mouse epithelial
tissues may reflect a quantitative difference in HER-2/neu ex-
pression between normal and malignant cells (25, 30).

Tumor-associated peptides administered intradermally to
melanoma patients along with GM-CSF can elicit specific,
CD8+ T-cell responses that, in turn, may lead to tumor regres-
sion (31). We here report similar findings, demonstrating that
three of the nine patients immunized with DCs pulsed with the
HER-2/neu (p369) epitope also developed specific DTH. Of
interest, all three of the patients who demonstrated DTH also
recognized the p369 epitope in vitro in cytokine release assays.
This is concordant with the findings of Disis et al. (32), who
demonstrated that DTH is a predictor of peripheral blood T-cell
immunity after immunization with putative HER-2/neu helper
epitopes. Our results demonstrate that DTH may also be used
to predict a response after immunization with a MHC class
I-restricted CTL epitope. The finding that three of our patients
responded, as measured by in vitro T-cell assays but not by
DTH, indicates that specific cytokine release assays in vitro
provide a more sensitive method to detect specific T-cell re-
sponses. Alternatively, DTH responses may require activation
of T-cell subsets other than the subset(s) responding in IFN-γ
release assays.

One of the 9 patients showed a partial clinical response 3
months after the last immunization. The response was mani-
fested as regression of a local lymph node metastasis and a
decrease of circulating tumor marker. Concurrently, the patient
even demonstrated DTH and peptide-specific reactivity in vitro.
Another patient responded with stabilization of the disease
status for a period of 3 months. The relatively long period
between the last vaccination and the occurrence of clinical
responses was similar to that reported in a study in which
patients were immunized with the Mage 3.1 peptide (33).
This delay may reflect the time it takes for precursor T cells to
expand sufficiently to mediate tumor cell rejection.

The results of this trial suggest that a clinical response
could be evoked only transiently in a minority of the patients
with very advanced disease. It remains to be seen whether or not
additional MHC class I and T-helper cell epitopes, also from
other antigens expressed in gastric cancer, will enhance the
efficacy of this mode of vaccination. The selection of immuno-
compotent patients prescreened for DTH reactivity to nonspe-
cific recall antigens, may also enhance the frequency of clinical
and immunological responses.

In conclusion, our data clearly showed that HER-2(p369)
peptide is an immunodominant epitope for the induction of
HER-2/neu-specific immune responses in HER-2/neu overex-
pressing gastric cancer. In some patients, the induction of HER-
2/neu-specific immunity may result in clinical responses. Tumor
vaccination therapy with peptide-pulsed DCs may be a novel
approach to immunotherapy of gastric cancer.

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