Locoregional Cellular Immunotherapy for Patients with Advanced Esophageal Cancer

Uhi Toh, Hideaki Yamana, Susumu Sueyoshi, Toshiaki Tanaka, Fumihiko Niiya, Katsuko Katagiri, Hiromasa Fujita, Kazuo Shirozou, and Kyogo Itoh

Departments of Surgery [U. T., H. Y., S. S., T. T., F. N., H. F., K. S.] and Immunology [U. T., K. K., K. I.], Kurume University School of Medicine, Kurume 830-0011, Fukuoka, Japan

ABSTRACT

The objectives of the present study were to determine the safety of locoregional administration of autologous lymphocytes stimulated with autologous tumor cells and interleukin (IL) 2 in vitro and to find laboratory markers to predict either clinical toxicity or clinical response. Eleven patients with advanced (n = 4) or recurrent (n = 7) esophageal cancers received the locoregional administration of these activated lymphocytes every 2 weeks for two to nine times (mean, 5.6 times), and mean numbers of the administered cells were 0.8 × 10^9 cells per treatment. The activated lymphocytes that were prestested for their surface markers and CTL activity were endoscopically injected into primary tumor sites (n = 4) or directly injected into metastatic lymph nodes (n = 2), pleural (n = 4) or ascitic (n = 1) regions. Grade 3 hypotension, grade 2 diarrhea, and grade 1 fever were observed in 1, 1, and 6 patients, respectively, and there was no adverse effect in the remaining three patients. The clinical outcome was as follows: one, complete response (CR); three, partial response (PR); two, stable response (SR); and five, progressive disease (PD). CTL activity in the administered cells was observed in 5 of the 11 patients (1 CR, 3 PR, and 1 PD) and was not observed in the remaining 6 patients (2 SR and 4 PD). Percentages of CD16^+ cells in the peripheral blood of the responder group (CR+PR) significantly increased when compared with those before treatment or with those of the nonresponder group before as well as after treatment. Because the clinical toxicity was moderate and tolerable, this new method of locoregional immunotherapy will be applicable for use in treatment of patients with advanced and recurrent esophageal cancers. Both CTL activity in the administered cells and the percentages of CD16^+ cells in the peripheral blood may be useful laboratory markers for predicting of clinical response.

INTRODUCTION

Cancer in the esophagus is one of most common malignant neoplasms in the world, particularly in the Pacific countries. Surgery remains the standard approach for patients with locoregional advanced disease that is resectable. Curative resection is feasible in only 50% of cases, and local or distant failure is common after resection (1–3). The 5-year survival is only ~30% for stage-III and -IV patients undergoing surgery. Some adjuvant multimodality therapies have been attempted to control both local and systemic disease (4–6). However, unresectable and relapsed esophageal cancers are still resistant to the presently available chemotherapy or radiation therapy regimens, and there is almost no clear advantage from these regimens for overall survival. Consequently, the development of a new effective therapeutic approach such as immunotherapy could be valuable to expand treatment modalities (7–9). Recently several reports presented the clinical efficacy of immunotherapy for advanced cancer in the digestive tract, but little clinical experience has been reported for advanced esophageal cancer (10–12). We have reported the presence of precursors of HLA class I-restricted and SCC3^+ -specific CTLs in both PBMCs and TILs of patients with esophageal cancer (13–15). In the present study, we investigated the clinical toxicity and clinical response of locoregional administration of PBMCs stimulated with autologous tumor cells in advanced and recurrent esophageal cancer patients. Laboratory markers for the prediction of toxicity or response were also determined.

MATERIALS AND METHODS

Patients.

Patients with unresectable primary or recurrent metastatic esophageal cancer were eligible to this pilot study. Patients were required to have disease assessable by physical or radiographic examination and life expectancies of at least 2 months. Patients’ characteristics are shown in a Table 1. No patients had been receiving corticosteroids or any prior immunotherapy; however, six patients had received prior chemotherapy and radiotherapy. The intervals between these prior treatments and immunotherapy were at least 1 month (range, 1–6

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2 To whom requests for reprints should be addressed, at Department of Surgery, Kurume University School of Medicine, 67 Asahi Machi, Kurume 830-0011, Japan. Phone: 81-942-31-7566; Fax: 81-942-34-0709; E-mail: hyamana@med.kurume-u.ac.jp.

3 The abbreviations used are: SCC, squamous cell carcinoma; PBMC, peripheral blood mononuclear cell; LN, lymph node; HLA, human leukocyte antigen; IL, interleukin; CT, computed tomography; CEA, carcinoembryonic antigen; TIL, tumor-infiltrating lymphocyte; CR, complete response; PR, partial response; SR, stable response; PD, progressive disease; LAK, lymphokine-activated killer; NK, natural killer.

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Immunotherapy for Esophageal Cancer

The tumor cells were irradiated with a dosage of 50 Gy over 10 min. Autologous tumor cells were prepared from biopsy samples by mincing and by enzymatic digestion with stirring in 50 ml of PBS containing 10 mg of type IV collagenase and 5 mg of DNase type I (Sigma) for 3–4 h at room temperature, followed by filtration through a layer of 100 nylon mesh and then by washing twice with PBS. The samples and effusion were applied on Ficoll-Hypaque solution and were centrifuged to isolate live tumor cells and normal cells used in these studies and their HLA class I alleles have been reported previously (13, 14).

**Table 2** Summary of the cellular immunotherapy

<table>
<thead>
<tr>
<th>Case no.</th>
<th>HLA class I A locus</th>
<th>Source of autologous tumor cell</th>
<th>Total no. of injected cells (total no. of injections)</th>
<th>Method of administration</th>
<th>CTL activity</th>
<th>Adverse effect</th>
<th>Clinical Response</th>
<th>Period of remission (mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>24/31</td>
<td>Primary tumor</td>
<td>2.1 × 10^6 (3)</td>
<td>Endoscopic</td>
<td>Yes Grade 3 (hypotension)</td>
<td>CR</td>
<td>20</td>
<td></td>
</tr>
<tr>
<td>2</td>
<td>2/</td>
<td>Primary tumor</td>
<td>5.3 × 10^5 (6)</td>
<td>Endoscopic</td>
<td>No Grade 1 (fever)</td>
<td>PD</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>3</td>
<td>24/26</td>
<td>Primary tumor</td>
<td>18 × 10^5 (9)</td>
<td>Endoscopic</td>
<td>Yes Grade 1 (fever)</td>
<td>PR</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>4</td>
<td>2/</td>
<td>Pleural effusion</td>
<td>1 × 10^5 (2)</td>
<td>Regional</td>
<td>None</td>
<td>PD</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>2/</td>
<td>Metastatic lymph node</td>
<td>5.8 × 10^5 (7)</td>
<td>Regional</td>
<td>Yes Grade 1 (fever)</td>
<td>PR</td>
<td>9</td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>26/33</td>
<td>Pleural effusion</td>
<td>3.9 × 10^5 (5)</td>
<td>Regional</td>
<td>Yes None</td>
<td>PD</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>NT*</td>
<td>Pleural effusion</td>
<td>1.2 × 10^5 (2)</td>
<td>Regional</td>
<td>No None</td>
<td>PD</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>8</td>
<td>24/31</td>
<td>Primary tumor</td>
<td>4.9 × 10^5 (6)</td>
<td>Endoscopic</td>
<td>No Grade 1 (fever)</td>
<td>SR</td>
<td>8</td>
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</tr>
<tr>
<td>9</td>
<td>24/11</td>
<td>Metastatic lymph node</td>
<td>7 × 10^4 (8)</td>
<td>Regional</td>
<td>Yes Grade 1 (fever)</td>
<td>PR</td>
<td>7</td>
<td></td>
</tr>
<tr>
<td>10</td>
<td>5/11</td>
<td>Pleural effusion</td>
<td>2 × 10^4 (3)</td>
<td>Regional</td>
<td>No Grade 1 (fever)</td>
<td>PD</td>
<td>11</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>2/</td>
<td>Ascites</td>
<td>3.6 × 10^4 (6)</td>
<td>Regional</td>
<td>No Grade 2 (nausea, diarrhea)</td>
<td>SR</td>
<td>13</td>
<td></td>
</tr>
</tbody>
</table>

* The term CTL activity was used in this study if the activated PBMCs produced the significantly higher levels of IFN-γ or percentage of cytotoxicity (P < 0.05 by a two-tailed Student’s t test) by recognition of the autologous tumor cells when compared with those in the allogeneic tumor cell lines.

+ Common toxicity criteria of the National Cancer Institute.

+ CR, (disappearance of all measurable mass); PR, (>50% reduction in the size of the mass); SR, (<50% reduction in the size of the mass); PD, (increase in the size of the mass).

+ Not tested.

months. The protocol was approved by the Institutional Review Committee of the Kurume University. The protocol was explained to each patient, and written informed consent to participate in the study was obtained from all of the patients who entered this study. Thirteen patients fulfilled the eligibility criteria, but two patients were eliminated before treatment because of inadequate growth of cultured PBMCs. Eleven patients with unresectable esophageal cancer (n = 4) or recurrent metastatic cancer (n = 7) received this treatment (Table 2). These tumors were histologically confirmed as SCCs by pathological examination. The mean age of the patients was 67.7 years. Six of the 11 patients (cases 4–8 and 10) received prior radiotherapy (48, 50, 52, 50, and 50 Gy, respectively; mean, 50 Gy) combined with two cycles of the following chemotherapy: 110 mg/m^2/day of cisplatin and 500 mg/m^2/5 days of 5-fluorouracil. The remaining five patients (cases 1–3, 9, and 11) did not receive any chemotherapy or radiotherapy before the immunotherapy.

**Cells.** Tumor samples used for the stimulation of PBMCs were obtained as follows: original esophageal tumors that were resected from patients for 2 weeks to prepare cells for the treatment. The yield of PBMCs that were obtained from the blood samples with the Ficoll-Hypaque gradient method (14) was 0.38–1.3 × 10^8 cells/ml (mean, 0.54) or 0.5–1.5 × 10^8 cells (mean, 1.0) in patients or healthy volunteers, respectively. The tumor cell lines and normal cell lines used in these studies and their HLA class I alleles have been reported previously (13, 14).

**Cell Culture.** One million PBMCs were incubated in 2 ml of the culture medium containing 10^5 irradiated autologous tumor cells in each well of a 24-well culture plate at 37°C in 5% CO_2. The culture medium consisted of RPMI 1640 with 10% fetal bovine serum, 100 U/ml recombinant IL-2, 100 units/ml penicillin, 100 μg/ml streptomycin, 50 μg/ml gentamicin, and 0.5 μg/ml fungizone. PBMCs stimulated with autologous tumor cells were restimulated on day 7 of culture, followed by washing and preparation for clinical use on day 14. PBMCs were also cultured with IL-2 alone and were used as a control for the in vitro analysis. Contamination in the cultured lymphocytes was checked by the Department of Laboratory Medicine according to the guideline for cellular therapy of our university. Endotoxin was not checked in this study, although the cells were repeatedly washed before injection.

**Assays.** The activated PBMCs were harvested after the second stimulation at day 14 of culture and were characterized for their phenotypes and cytotoxicity. Autologous tumor cells were separated from the single cell suspensions of biopsied tumor samples, pleural effusion, and ascites as mentioned above. These autologous tumor cells were cryopreserved in 90% human AB serum (Blood Center of Japanese Red Cross) plus 10% DMSO (Sigma) at −178°C in liquid nitrogen for restimulation and subsequent immunological assay. Heparinized peripheral blood samples (50–100 ml; mean, 78 ml) were collected from patients for 2 weeks to prepare cells for the treatment. The yield of PBMCs that were obtained from the blood samples with the Ficoll-Hypaque gradient method (14) was 0.38–1.3 × 10^8 cells/ml (mean, 0.54) or 0.5–1.5 × 10^8 cells (mean, 1.0) in patients or healthy volunteers, respectively. The tumor cell lines and normal cell lines used in these studies and their HLA class I alleles have been reported previously (13, 14).

plus 10% DMSO (Sigma) at −178°C in liquid nitrogen for restimulation and subsequent immunological assay. These cells were thawed, cultured for several days, and used as stimulator or target cells. A 6-h ^51 Cr-release assay was used to measure...
Fig. 1  Clinical findings of case 1. A barium esophagogram of the lower esophagus in case 1 before treatment (part 1a) showed a bulky irregular filling defect with destruction of mucosal folds in double contrast and persistent severe stenosis at the esophagogastric junction. Significant reduction in tumor size and significant improvement in the narrowing of the lumen in the same patient were observed after the third treatment (part 1b). The endoscopic appearance of the lower esophagus before treatment (part 2a) showed typical appearance of an exophytic polypoid carcinoma with a significant narrowing in the lumen of the esophagus. Significant reduction in the tumor size and improvement in the narrowing of the lumen were seen at 14 days after the first and second treatments (part 2, b and c, respectively). A feeding tube was observed on the right side of esophagus in part 2c. Complete tumor regression was observed in the lower esophagus after the third treatment (part 2d). Histology of the tissues in the biopsied specimen before treatment showed moderately differentiated SCC cells before treatment (part 3a), whereas the tumor cells were no longer observed and there were some inflammatory changes after the third treatment (part 3b). H&E stain; ×500.
The PBMCs were also measured for their IFN-γ production in response to various tumor cells by incubation of cells for 18 h with target cells at an E:T ratio of 3:1. The amounts of IFN-γ in cell-free supernatants were measured by an IFN-γ ELISA kit, and the limit of sensitivity of ELISA was 5 pg/ml as reported previously (14). The number of WBCs per mm³ was counted by the Department of Laboratory Medicine. Heparinized blood was applied for Ficoll-Hypaque solution and

Fig. 3  A CT scan of the liver in case 5. There were metastatic tumors in S2 (a-1, arrow) and S4 area (b-2, arrow) before treatment. There was marked tumor regression in the measurable mass of S2 (b-1, arrow) and S4 (b-2, arrow) after the fourth treatment into the metastatic LN located in the left supraclavicular LNs.

Fig. 4  A CT scan of the neck in case 9. There was marked tumor regression in the measurable mass of the left supraclavicular LN after the seventh treatment (a, before treatment; b, after treatment).
was centrifuged to obtain PBMCs, as reported previously (14). Viability of PBMCs was determined by a trypan blue dye-exclusion test. The surface phenotypes of PBMCs were tested by the two-color immunostaining technique with anti-CD3, -CD4, -CD8, and -CD16 monoclonal antibodies and FACScan flow cytometry at 4-week intervals, as reported previously (14). We used the term “CTL activity” in this study if the activated PBMCs produced significantly higher levels of IFN-γ production or percentage of cytotoxicity ($P < 0.05$ by a two-tailed Student’s $t$ test) by recognition of the autologous tumor cells compared with those in response to the allogenic tumor cell lines.

**Treatment Schedule.** The activated PBMCs were washed in PBS three times, resuspended in 5–10 ml of 0.9% saline, and administered by endoscopic intratumoral injection or direct regional injection (Table 2). Biopsy for preparation of the tumor cells was carried out before injection of the activated PBMCs in cases 1–3 and 8. These injections were repeated at least two times at 2-week intervals and for up to nine times until disease progression or severe toxicity was seen. The first clinical evaluation was performed 14 days after the second injection. The clinical outcome was evaluated by the following methods: esophagoscopy, esophagography and ultrasonography at 2-week intervals; CT scan at 4-week intervals; and serum CEA at 4-week intervals. A CR was evaluated as disappearance of all of the measurable tumor mass without the appearance of new lesions, and a PR was defined as a reduction in all of the measurable lesion of $50\%$ or more. A SR was defined as $<50\%$ reduction in all of the measurable lesions. Adverse effects were evaluated by history and physical examination and graded according to the National Cancer Institute common toxicity scale.

**Statistical Analysis.** Statistical analyses for lymphocyte phenotypic markers and IFN-γ production were performed using the two-tailed Student’s $t$ test or the Fisher’s exact test.

**RESULTS**

**Clinical Results.** Eleven patients with advanced ($n = 4$) or recurrent ($n = 7$) esophageal cancer received the locoregional cellular immunotherapy of the PBMC-activated autologous tumor cells and IL-2 every 2 weeks for 2–9 times (mean, 5.6 times). The mean number of administered cells per treatment was $0.8 \times 10^9$ cells (range, $0.5–2 \times 10^9$ cells). The patients’ profiles are summarized in Table 1, and the treatment schedule, CTL activity of injected cells, adverse effects, and clinical response are summarized in Table 2. Six of the 11 patients developed a transient febrile reaction ($\geq38°C$) within 72 h after the treatment. One patient (case 1) developed grade III hypotension that required a Neo-Synephrine pressure drip for recovery, and the other patient (case 11) had transient grade II nausea and diarrhea. No adverse effect was observed in the remaining three patients (Table 2). No toxicity was associated with the intrapleural administration (cases 6, 7, and 10) of activated cells. Transient grade II nausea and diarrhea were observed by the intra-abdominal administration of the cells in case 11. The clinical outcome was as follows: 1 CR, 3 PR, 2 SR, and 5 PD. Clinical findings of the four cases that showed major tumor regression (1 CR and 3 PR) are shown in the next paragraphs.
One patient (case 1) had a CR with no evidence of disease for >20 months after the last treatment. Esophagography of the lower esophagus before treatment (Fig. 1, part 1a) and after the third treatment (Fig. 1, part 1b) showed a marked tumor regression for tumor size. Endoscopic appearance before treatment (Fig. 1, , part 2a), after the first treatment (Fig. 1, part 2, b and c), and after the third treatment (Fig. 1, part 2d) also clearly indicated a significant reduction in tumor size followed by the complete disappearance of tumor. Moderately differentiated SCC cells observed in the biopsied sample before treatment

Fig. 7 CTL activity of the administered cells. In a, PBMCs, stimulated with autologous tumor cells, and IL-2 were measured on day 14 of culture for their activity to produce IFN-γ in response to the autologous tumor cells or to the cells of KE3 (A2/A24), TE10 (A24/A26) esophageal SCC cell line, and K562 erythroleukemia cell line. PBMCs cultured with IL-2 alone, taken as the control, were also measured for their activity. Values represent the mean of IFN-γ production of different effector cells (n = 2–9). Each sample was measured in triplicate determinants at an E:T ratio of 3:1. Background IFN-γ production (50–100 pg/ml) was subtracted from the values. Two-tailed Student’s t test was used for statistical analysis. In b, PBMCs, stimulated with autologous tumor cells, and IL-2 in cases 1 and 2 were measured for their cytotoxicity by a 6-h 51Cr-release assay against five different targets [autologous tumor cells, KE3, TE10, K562, and MKN28 (A31) gastric adenocarcinoma cell line]. PBMCs cultured with IL-2 alone, taken as the control, were also measured for cytotoxicity. Values represent the mean of the percentage of specific lysis of the different effector samples of the different experiments (n = 3 or 4). Each sample was measured in triplicate at an E:T ratio of 20:1. Bars, ±SD.
(Fig. 1, part 3a) were no longer observed in the biopsied sample after the third treatment (Fig. 1, part 3b). Instead, the marked infiltration of mononuclear cells was seen in the later sample. Similar histological changes were also observed in the samples after the treatment in the other responders (data not shown).

Three patients (cases 3, 5, and 9) had PRs. Esophagography before (Fig. 2, part 1a) and after treatment (Fig. 2, part 1b) in case 3 demonstrated a marked reduction in tumor size. Similarly, endoscopic appearance before (Fig. 2, part 2, a–1 and a–2) and after treatment (Fig. 2, part 2, b–1 and b–2) also clearly showed a marked reduction in tumor size. Marked infiltration of CD3+CD8+ T cells was observed in the biopsied sample of case 3 after treatment (Fig. 2, part 3b, a–1 and a–2). Injection of the activated cells into metastatic tumors at the left supraclavicular LN resulted in complete disappearance after the second treatment (data not shown). Furthermore, the tumor size of liver metastases in the S2 (Fig. 3a–1) and S4 (Fig. 3a–2) areas of case 5 was markedly reduced after the fourth treatment (Fig. 3, b–1 and b–2). This patient received regional injections of the activated cells into metastatic LNs in the left supraclavicular area but not into the liver. A significant decrease in size of the metastatic tumor on the neck in the supraclavicular LN was observed in case 9 after the seventh treatment (Fig. 4, a–1 and a–2). This patient received regional injections of the activated cells (cases 3, 5, 6, and 9; Fig. 7a) stomach cancer A24/A11, case 5 (HLA-A24/2; TE10, A24/A26; K562). PBMCs cultured with IL-2 alone in all of the 11 cases. A 6-h 51 Cr-release assay was used in cases 1 and 2, in which a relatively large number of cells were available for the study (Fig. 7b). The PBMCs of case 1 (HLA-A24/A26) showed significantly higher levels of cytotoxicity against HLA-A24+ esophageal cancer cells than those against MKN28 (HLA-A31) stomach cancer cells or K562 target cells. In contrast, there was no significant difference among the levels of cytotoxicity either by the stimulated PBMCs in case 2 or by the control PBMCs in cases 1 and 2.

Eleven patients were divided into the three groups as follows: responder group (n = 4, 1 CR + 3 PR; cases 1, 3, 5, and 9), SR group (n = 2; cases 8 and 11), and PD group (n = 5; cases 2, 4, 6, 7, and 10) to find laboratory markers useful for predicting the clinical response. Freshly isolated PBMCs (n = 57 from 11 cases) consisted of 71 ± 4% CD3+ T cells, 44 ± 3% CD4+ T cells, 18 ± 5% CD8+ T cells, and 5 ± 2% CD16+ NK cells (Table 3). The PBMCs stimulated with autologous tumor cells and IL-2 produced significantly higher levels of IFN-γ in response to the autologous tumor cells than those in response to any of the other three target cells (case 1) or to those in response to K562 target cells (cases 3, 5, 6, and 9; Fig. 7a). The stimulated PBMCs of case 3 (HLA-A24/A26), case 5 (HLA-A24/A26), and case 9 (HLA-A24/A11) also produced significantly higher levels of IFN-γ by recognition of autologous but HLA-A locus-matched tumor cells than those produced by recognition of K562 target cells. Four (cases 1, 3, 5, and 9) of these five patients responded to the treatment, whereas the remaining patient (case 6) had PD. There was no significant difference among the levels of IFN-γ production by the stimulated PBMCs in response to the four target cells tested in the remaining six patients who had either SR (n = 2) or PD (n = 4; data not shown). Similarly, there was no significant difference among the levels of IFN-γ production by the control PBMCs by recognition of the four target cells tested in any of the 11 patients. The results of PBMCs of the responders are shown in Fig. 7a.

These results suggested that the CTL activity was observed in the stimulated PBMCs of 5 cases (cases 1, 3, 5, 6, and 9), whereas LAK cell activity was observed both in the stimulated PBMCs of the remaining 6 cases and in the control PBMCs cultured with IL-2 alone in all of the 11 cases. A 6-h 51 Cr-release assay was used in cases 1 and 2, in which a relatively large number of cells were available for the study (Fig. 7b). The PBMCs of case 1 (HLA-A24/A26) showed significantly higher levels of cytotoxicity against HLA-A24+ esophageal cancer cells than those against MKN28 (HLA-A31) stomach cancer cells or K562 target cells. In contrast, there was no significant difference among the levels of cytotoxicity either by the stimulated PBMCs in case 2 or by the control PBMCs in cases 1 and 2.

<table>
<thead>
<tr>
<th>Group</th>
<th>% positive (mean ± SD)</th>
</tr>
</thead>
<tbody>
<tr>
<td>PBMCs cultured with</td>
<td>CD3</td>
</tr>
<tr>
<td>None (fresh)</td>
<td>71 ± 4*</td>
</tr>
<tr>
<td>(n = 57)</td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>Responders</td>
</tr>
<tr>
<td>SR</td>
<td>75 ± 20</td>
</tr>
<tr>
<td>PD</td>
<td>71 ± 5</td>
</tr>
<tr>
<td>Tumor and IL-2</td>
<td>(n = 57)</td>
</tr>
<tr>
<td>Group</td>
<td>Responders</td>
</tr>
<tr>
<td>SR</td>
<td>82 ± 23</td>
</tr>
<tr>
<td>PD</td>
<td>83 ± 7</td>
</tr>
<tr>
<td>IL-2 (n = 30)</td>
<td></td>
</tr>
<tr>
<td>Group</td>
<td>Responders</td>
</tr>
<tr>
<td>SR</td>
<td>76 ± 23</td>
</tr>
<tr>
<td>PD</td>
<td>80 ± 7</td>
</tr>
</tbody>
</table>

* Brackets indicate the two groups in which the values were statistically significant (P < 0.05 by a two-tailed Student’s t test).
NK cells in stimulated PBMCs of the responder group (17 ± 4%) and SR group (17 ± 5%) was significantly higher than that in the freshly isolated PBMCs of the responder group (7 ± 1%) and of the SR group (4 ± 2%), respectively, whereas that in the stimulated PBMCs of the PD group (15 ± 8%) was not significantly different from that of the freshly isolated PBMCs (6 ± 3%).

The mean number of administrated lymphocytes per case was 8.2 × 10^9 cells in the responder group, 4.2 × 10^9 cells in the SR group, and 2.7 × 10^9 cells in the PD group. The mean number of administrated lymphocytes per injection was 1.2 × 10^9 cells in the responder group, 0.7 × 10^9 cells in the SR group, and 0.7 × 10^9 cells in the PD group. A kinetic study showed no significant difference in the mean number of WBCs, PBMCs, or CD3^+, CD4^+, or CD8^+ cell counts that were measured before treatment and after the second and fourth treatments in these three groups (Fig. 8). In contrast, the mean number of CD16^+ lymphocytes in the peripheral blood after the second and fourth treatment in the responder group significantly increased compared with that before treatment in the responder group (Fig. 8). The number of CD16^+ lymphocytes was also significantly higher
than those after the second and fourth treatments in the PD group.

DISCUSSION

A number of clinical studies of adoptive immunotherapy using mostly LAK cells and TILs have shown a significant response rate in patients with melanoma, renal cell carcinoma, and some other cancers (16–21). However, there has been no report on the clinical studies demonstrating the obvious tumor regression of advanced esophageal cancers, to the extent that we searched in the literature. This study showed that locoregional cellular immunotherapy resulted in marked tumor regression in 4 of 11 patients with advanced or recurrent esophageal cancer. Our report seems to be the first report showing clear evidence for the potential of the application of immunotherapy for esophageal cancer patients.

The adverse events in all of the 11 cases were moderate and tolerable. There was no relationship between the number of administered cells and adverse effects. Grade 1 fever was often observed and might have been attributable to cytokine production from the injected activated lymphocytes. Other adverse effects were mainly grade 2 nausea and diarrhea in one other case. These adverse effects may also have been attributable to cytokine production from injected activated lymphocytes. These results suggest that this regimen of cellular immunotherapy was safe.

We provided the activated lymphocytes by stimulation of PBMCs with autologous tumor cells and IL-2 in vitro. These activated lymphocytes consisted of 42% CD8+ and 28% CD4+ cells. CTL activity was observed in these PBMCs from 5 (cases 1, 3, 5, 6, and 9) of the 11 cases, and 4 of them had significant tumor regression (1 CR and 3 PR), although the detailed studies of CTL activity, including the 51Cr-release assay against various target cells at different E:T ratios, were not carried out, mainly because of the limited number of cells for the in vitro analyses. LAK cell activity, instead of CTL activity, was observed in the PBMCs of the remaining six cases, and none of them showed major tumor regression (2 SR and 4 PD). LAK cell activity was observed in the control PBMCs cultured with IL-2 alone in all of the 11 patients tested. These results suggested that CTLs but not LAK cells were needed to achieve the tumor regression in this regimen of locoregional cellular immunotherapy. Consequently, the CTL activity of administered cells could be an appropriate laboratory marker to predict the clinical response. This phenomenon was supported by the fact that the mean percentage of CD16+ cells in the peripheral blood was highest among those of the different groups tested, and it was significantly higher than that of fresh PBMCs. The other laboratory marker for prediction of clinical response would be the percentage of CD16+ NK cells in the peripheral blood, which significantly increased in PBMCs of the responder group after treatment. These results suggest that both CTLs at the tumor sites and NK cells in peripheral blood were needed for tumor regression in this regimen. Therefore, both CTL activity in the injected cells and percentage of CD16+ cells in the peripheral blood might be useful laboratory markers for monitoring the clinical response to this locoregional cellular immunotherapy. These results, however, must be confirmed by a large-scale clinical study. The magnitude of CTL activity of the activated TILs was well correlated with clinical efficacy in metastatic melanoma patients in the regimens of adoptive cellular therapy with high-dose IL-2 (22). In contrast, no reliable laboratory markers for PBMCs, including the percentage of CD16+ cells, were found in the past decade, regardless of numerous trials in the field of immunotherapy using LAK cells, TILs, or cytokines.

It is of note that the injection of cells into the left supraclavicular LN in case 5 resulted in both the disappearance of tumors at the injection site and the decrease in tumor sizes at the un.injected site (liver). Some element of systemic immunity might have been achieved by local therapy in this case, but this type of tumor regression was not observed in any other cases tested. It is also of note that regional treatment of pleural effusions and ascites was ineffective in 5 of the 11 patients. Subsequently, the application of this locoregional immunotherapy to the control of cancer cells in pleural or abdominal regions is not recommended. In addition, prior chemoradiotherapy is not recommended for this immunotherapy. Three of five patients who received no prior chemoradiotherapy responded to the immunotherapy, whereas only one of six patients who received prior chemoradiotherapy responded to the immunotherapy. This could be in part attributable to the number of cells available for the treatment. The mean number of administered cells per injection was 1.2 × 10^9 cells in the responders, whereas it was 0.7 × 10^9 cells in the other groups. Prior chemoradiotherapy should suppress T-cell proliferation in response to IL-2 and autologous tumor cells.

In conclusion, we have shown that this treatment regimen was safe and resulted in tumor regression in 4 of 11 patients with advanced or recurrent esophageal cancer. A large-scale clinical trial is recommended to confirm the evidence from this Phase I clinical study.

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Locoregional Cellular Immunotherapy for Patients with Advanced Esophageal Cancer

Uhi Toh, Hideaki Yamana, Susumu Sueyoshi, et al.


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