Six-year Disease-free Survival of a Patient with Metastatic Eyelid Squamous Cell Carcinoma and Colon Adenocarcinoma after Repeated Postoperative Adoptive Immunotherapy

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INTRODUCTION

The rare phenomenon of spontaneous regression of malignant tumors has long been recognized. Everson (1) collected more than 1000 cases of spontaneous regression of cancer from the literature worldwide, of which only 130 cases met the definition in a strict sense. More than 50% of such cases belonged to certain types of cancer: neuroblastoma, renal cell carcinoma, choriocarcinoma and malignant melanoma, which are mostly the candidates for immunotherapy nowadays, and only four cases of colorectal cancer and none of eyelid squamous cell carcinoma were found in this study.

The underlying factors responsible for spontaneous regression may include some immune reactions among other known and unknown influences. The finding that lymphocytes mediate the anti-tumor immunity prompted many researchers to expand the T cell population. The T lymphocyte growth factor, IL-2, was discovered in 1976. The clinical use of lymphokine-activated killer cells (LAK) was begun in 1984 (2). Despite a partial favorable effect, the high cost and a low growth rate prevented the wider use of LAK therapy (2–8).

Therefore, it was essential to expand T cells in number effectively. In 1980, Van Wauwe et al. (9) succeeded in the selective expansion of CD3+ T cells which could attack tumor cells. Many studies along this line have been published since 1988 (9-16). Among them, Sekine et al.’s method is superior to the others in the proliferation rate (14,15).

In this paper, we discuss whether or not surgery could have completely removed all of the primary tumors and their metastases and whether the sole postoperative therapy with cultured autologous lymphocytes might have been really effective.

CASE REPORT

CARCINOMA OF THE EYELID

The patient was aware of a tiny nodule at the left lower eyelid in June–July 1991, for which a biopsy was carried out at the Department of Ophthalmology, Kanto-Teishin Hospital, Tokyo, in January 1992. Histopathology revealed granuloma telangiectaticum but scrutiny with 30 serial sections of a small suspected atypical epithelial portion could not establish a definitive diagnosis (Fig. 1). The residual tumor gradually enlarged, for which a biopsy was performed again at the Department of Ophthalmology, Tokyo Women’s Medical
College, on January 19, 1994, and this time the diagnosis of squamous cell carcinoma was made. For surgical treatment, he was admitted to the Ophthalmology Division, National Cancer Center Hospital, Tokyo, on February 16. The tumor had already grown deep into the orbit just behind the eyeball and was accompanied by swelling of regional lymph nodes. When the cancer diagnosis was substantiated, activated lymphocytes (CD3-AT cells) were prepared using his peripheral blood lymphocytes. He underwent exenteration of the left orbit and left modified radical neck dissection on February 22 (Fig. 2a). Histopathology revealed squamous cell carcinoma pT4N3M0 according to the TNM/pTNM Classification of Malignant Tumors (UICC) with lymph node metastases at the neck (5/19) (Fig. 2b). From the following day, adoptive immunotherapy with \((1–3) \times 10^{10}\) CD3-AT cells was started as the sole postoperative adjuvant treatment.

CULTIVATION AND HARVEST OF CD3-AT CELLS

The culture procedure and harvesting of lymphocytes have been described previously (14). In brief, mononuclear cells were separated from 20 ml of peripheral blood and cultivated with immobilized anti-CD3 monoclonal antibody (OKT3) and IL-2 in culture bottles for 6 days and then in gas-permeable bags with increasing volumes of culture medium for an additional 8 days. Freedom from microbial and endotoxin contamination was confirmed immediately before harvest. The growth rate of the lymphocytes was about 1000-fold in 2 weeks. Phenotyping of the CD3-AT cells showed that on average they consisted of 30% of CD4+ cells, 60% of CD8+ cells and 90% of CD3+/HLA-DR+ cells.

IMMUNOLOGICAL ACTIVITY OF CD3-AT CELLS

The autologous cytotoxic activity was compared by the \(^{51}\)Cr-release assay between the pre-activated peripheral blood lymphocytes and the CD3-AT cells against the short-term cultivated cancer cells from the patient’s eyelid tumor. The CD3-AT cells exhibited much higher activity than the peripheral lymphocytes against autologous cancer cells, but not against allogenic KB and TC 873 squamous cell carcinoma strain cells (Table 1). The pre-activated peripheral blood lymphocytes still possessed autologous cytotoxicity at a detectable level before the anti-CD3/IL-2 activation, probably because the patient had received repeated cell transfers for 6 years. In addition, the CD3-AT cells as well as the pre-activated peripheral blood lymphocytes showed a non-specific cytotoxicity (so-called LAK activity) against the Daudi lymphoma strain cells. Of particular interest in this experiment, the treatment of CD3-AT cells with the anti-HLA-ABC
and anti-CD8 antibodies almost completely inhibited the autologous cytotoxic activity (Table 2), and therefore it is very likely that the proliferated effector cells are the specific, MHC class I-restricted cytotoxic T lymphocytes (CTL), which were probably expanded by repeated cultivation without tumor cell stimulation. Unfortunately, we could not prepare the similar cytotoxic test on colon adenocarcinoma cells, because the autologous colon carcinoma cell strain was not available.

**CARCINOMA OF THE TRANSVERSE COLON**

On March 1, 1994, the following evening after the third adoptive immunotherapy, the patient suddenly suffered an abdominal pain, which was followed by a massive melena, hypotension and acute anemia to the level of Hb 8.9 g/dl. Colonoscopy revealed a stenosis due to a neoplastic growth with bleeding at the transverse colon 47 cm proximal to the anal verge, together with two other polyps. The biopsy revealed adenocarcinoma of the colon. A barium enema confirmed a circular narrowing 3.5 cm in length. No suspicious lesion of pulmonary or hepatic metastasis was detected on chest X-ray or ultrasonic images. He underwent resection of the transverse colon 30 cm in length with D2 lymph node dissection on March 10 (Fig. 3a). The macroscopic intraoperative staging was H3P1SENM(–) according to the General Rules for Clinical and Pathological Studies on Cancer of Colon, Rectum and Anus (Japanese Research Society for Cancer of Colon and Rectum) or T4N1M0 according to the TNM/pTNM Classification of Malignant Tumors (UICC). Histopathological diagnosis of the surgical specimen was moderately differentiated adenocarcinoma reaching to the depth of serosa and metastases to the regional nodes (2/14) and two peritoneal seedings on the transverse mesocolon (Fig. 3b). He received the adoptive immunotherapy twice before surgery and a further five times before discharge on March 31.

The therapy has been repeated 150 times in total, twice monthly at an interval of a week, at the time of writing. No accident has ever occurred in cell cultivation, harvesting and infusion. The average number of CD3-AT cells infused at each time was $\left(1.38 \pm 0.447 \text{ SD}\right) \times 10^{10} \text{ cells}$. Some clinical events occurred in the postoperative course, but none of them led to metastatic disease. In September 1997, he suffered from a slight fever, dizziness and a headache. One of the submandibular lymph nodes was enlarged and was suspected of a metastasis. However, a biopsy of the node revealed that it was an inflammatory reaction but not a metastasis. No abnormalities were found on chest X-ray and ultrasonograms done every 4 months. Laboratory data including liver function during this period were normal except for anemia (Hb 8.9–11.0 g/dl) and none of tumor markers such as AFP, CEA, CA19-9, CA125, SCC, ACP and PSA increased. Autologous skin grafting inside the orbit was undertaken three times (November 1994, March

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**Table 1.** Comparison of cytotoxic activity between peripheral blood lymphocytes and CD3-AT cells against autologous eyelid cancer cells and allogenic cancer cells*

<table>
<thead>
<tr>
<th>Effector cells</th>
<th>Lysis of target cells (%)†</th>
<th>Antibody treatment* of CD3-AT cells</th>
<th>Lysis of autologous SCCC (%)†</th>
<th>Inhibition of lysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>54.8 ± 8.1</td>
<td>None</td>
<td>54.8 ± 8.1</td>
<td>0</td>
</tr>
<tr>
<td>Anti-HLA-ABC</td>
<td>4.5 ± 0.5</td>
<td>Anti-HLA-ABC</td>
<td>4.5 ± 0.5</td>
<td>91.8</td>
</tr>
<tr>
<td>Anti-HLA-DR</td>
<td>50.3 ± 7.0</td>
<td>Anti-HLA-DR</td>
<td>50.3 ± 7.0</td>
<td>8.2</td>
</tr>
<tr>
<td>Anti-CD4</td>
<td>44.1 ± 7.2</td>
<td>Anti-CD4</td>
<td>44.1 ± 7.2</td>
<td>19.5</td>
</tr>
<tr>
<td>Anti-CD8</td>
<td>7.0 ± 2.6</td>
<td>Anti-CD8</td>
<td>7.0 ± 2.6</td>
<td>87.7</td>
</tr>
<tr>
<td>Anti-CD16</td>
<td>55.0 ± 7.4</td>
<td>Anti-CD16</td>
<td>55.0 ± 7.4</td>
<td>0</td>
</tr>
</tbody>
</table>

*Abbreviations: SCCC, squamous cell carcinoma cells; KB, epidermoid carcinoma cell strain from oral cavity; TC873, tongue cancer cell strain; Daudi, Burkitt lymphoma cell strain; PBL, peripheral blood lymphocytes.
†Cytotoxicity was detected by 6 h $\text{^{51}}\text{Cr}$-release assay at effector/target cell ratio of 20:1. Results are mean values ± SD.

**Table 2.** Inhibition of cytotoxicity of CD3-AT cells against autologous eyelid cancer cells by various antibodies

<table>
<thead>
<tr>
<th>Antibody treatment* of CD3-AT cells</th>
<th>Lysis of autologous SCCC (%)†</th>
<th>Inhibition of lysis (%)</th>
</tr>
</thead>
<tbody>
<tr>
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<td>54.8 ± 8.1</td>
<td>0</td>
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<tr>
<td>Anti-HLA-ABC</td>
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<tr>
<td>Anti-CD4</td>
<td>44.1 ± 7.2</td>
<td>19.5</td>
</tr>
<tr>
<td>Anti-CD8</td>
<td>7.0 ± 2.6</td>
<td>87.7</td>
</tr>
<tr>
<td>Anti-CD16</td>
<td>55.0 ± 7.4</td>
<td>0</td>
</tr>
</tbody>
</table>

*Antibodies were used at a concentration of 100 µg/ml protein.
†SCCC, squamous cell carcinoma cells. Results are mean values ± SD.
1996 and August 1996), but the graft is still partly unsuccessful. The patient has been free from recurrence and additional metastasis for 6 years after surgery. The only side effect of the therapy was a slight fever of about 37°C lasting 1–2 days after each infusion.

**DISCUSSION**

It is very difficult to evaluate the published reports on adoptive immunotherapy because of the many variables involved. For example, each treated patient was very different in background factors, such as gender, age, responsiveness, type of tumor, site of tumor and clinical stage. Most of the early studies utilized lymphocytes collected by leukapheresis but not proliferated due to poor culture conditions, inadequate criteria to evaluate therapeutic effects and a lack of an appropriate culture method for CD3-AT cells.

It is important to harvest as many naturally functioning T cells as possible, because recruiting T cells reactive to a specific antigen is fairly difficult. Sekine et al. (14,15) solved the above-mentioned problems by establishing their own culture method for CD3-AT cells.

The patient, as a basic researcher, always observed slides using only his left eye with a monocular fluorescence microscope for more than 30 years. The long-term exposure of his left eye to ultraviolet light (UV beams at and in the vicinity of 360 nm) might have had a causative effect in developing cancer in the lid. The initial diagnosis of inflammatory granuloma brought him a sense of relief. However, the nodule developed to a squamous cell carcinoma with some metastases in 2 years.

Squamous cell carcinoma of the eyelid is a relatively rare tumor and accounts for 9.2% of all eyelid malignancies on average in reported series of collected cases (19,20). As high as 21% of the tumor of this site has been predicted to metastasize to the preauricular or submandibular node (19,20). The reported mortality rate varied widely from 0 to 40% according to different authors (19,20). UV beams between 290 to 400 nm are known to induce pathological reactions including cutaneous carcinogenesis: sunlight exposure (20) and therapeutic UV lighting (21) are associated with an augmented risk of squamous cell carcinoma. However, to our knowledge, the development of eyelid squamous cell carcinoma due to the prolonged use of a fluorescence microscope has not been reported.

The frequency of concurrence of eyelid squamous cell carcinoma and colon adenocarcinoma seems very rare. The cumulative 4–5 year survival rate of colon cancer with P1 peritoneal dissemination has been reported as 24.3% in Japan (22).

There is little information on systemic chemotherapy of squamous cell carcinoma of the eyelid. Luxenberg and Guthrie (23) described the results of chemotherapy in a series of eight cases of eyelid cancers including two cases of squamous cell carcinoma, but the two sets of individual data only are not sufficiently encouraging.

Chemotherapy with a fluorinated pyrimidine and leucovorin or plus methotrexate or recently a platinum compound is commonly used for the treatment of colon cancer. The therapy provides some short-term improvement but no significant survival advantage (24,25). Moreover, chemotherapeutic agents have more or less immunosuppression and some adverse effects, sometimes fatal, on the patients. However, adoptive immunotherapy with activated autologous lymphocytes is free from such effects as described in this report.

Experimental data and clinical observations have recently revealed that adoptive immunotherapy may be effective and eliminate cancer cells.

The *in vitro* killing effect of CD3-AT cells on autologous carcinoma target cells has been described in this report and in another study (26).

Our group has treated four other advanced cancer patients having a massive primary tumor and measurable distant metastases [one hepatocellular carcinoma (15), one abdominal alveolar soft-part sarcoma (27) and two renal cell carcinomas (T. Sekine, unpublished observation)] by adoptive immunotherapy with CD3-AT cells, combined with radiotherapy or with surgery and chemotherapy or radiotherapy, and the patients achieved long-term survival and a remarkable clinical improvement. The immunotherapy has been repeated from once or twice bimonthly to twice or three times monthly, for 1 year and 9 months to 6 years and 6 months, depending on the state of each patient. All the patients currently survive in good condition after 3 years and 6 months to 6 years and 6 months, two without recurrence and the other two with residual metastasis (15,27). Such an unexpected gain in survival may imply the efficacy of this therapy.

Although this is a single case report without any control, considering the widespread lymph node metastases of double cancers in this case, it is unusual for the patient to enjoy such a long tumor-free survival. Cancer immunotherapy based on a modern scientific basis is expected for further development as an important aspect of cancer research and treatment.

**Acknowledgments**

The authors acknowledge Drs K. Yamaguchi and T. Shimoda for pathological diagnosis of the surgical specimens. This work...
was supported by Grants-in-Aid for Cancer Research from the Ministry of Health and Welfare.

References