Adoptive immunotherapy of human meningeal gliomatosis and carcinomatosis with LAK cells and recombinant interleukin-2

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Previous in vitro studies have demonstrated that peripheral blood lymphocytes activated with recombinant interleukin-2 (rIL-2) generated cells that were lytic for fresh autologous tumor cells but not for normal lymphocytes or lymphoblasts. Adoptive transfer of autologous lymphokine-activated killer (LAK) cells induced in vitro with rIL-2 was used in two patients: one with meningeal gliomatosis and the other with meningeal carcinomatosis. The adoptive transfer of LAK cells was very effective in reducing the clinical symptoms and signs, and in eliminating the malignant cells from the cerebrospinal fluid. Thus, this therapy is an attractive approach for the treatment of malignant tumors that have poor immunogenicity and are insensitive to several anti-cancer agents, and for patients with severe immunosuppressive conditions induced by repeated radiation therapy or chemotherapy.

KEY WORDS □9 adoptive immunotherapy □9 interleukin-2 □9 lymphokine-activated killer cells □9 meningeal gliomatosis □9 meningeal carcinomatosis

VARIOUS attempts have been made to carry out adoptive immunotherapy with cytotoxic T lymphocytes that have the capacity to specifically lyse autologous tumor cells. A serious problem has been to obtain tumor-specific cytotoxic T lymphocytes in large quantities. Culture of peripheral blood lymphocytes (PBL's) with recombinant interleukin-2 (rIL-2) in our laboratory resulted in the generation of lymphokine-activated killer (LAK) cells that were capable of lysing fresh autologous glioblastoma cells. The adoptive transfer of autologous LAK cells induced in vitro with rIL-2 has been used in treating two patients: one with meningeal gliomatosis and the other with metastatic meningeal carcinomatosis. Good results were obtained, as described below.

Materials and Methods

Human rIL-2* was used for the generation of LAK cells. For the production of LAK cells, patients' lymphocyte fractions were obtained through multiple leukophereses and PBL's were collected using lymphocyte separation medium.† The PBL's were activated to generate LAK cells by in vitro incubation for 3 to 5 days in complete medium consisting of RPMI 1640, containing 2% heat-inactivated human AB serum, L-glutamine (2 mM/dl), sodium pyruvate (0.01 mM/dl), 100x non-essential amino acids (1 ml/dl), 2-mercaptoethanol (5 × 10⁻⁵ M), and gentamicin (5 mg/dl). Human rIL-2 was added to the medium at a final concentration of 500 U/dl. After leukopheresis, 5 × 10⁸ PBL's were immediately cultured in complete medium with rIL-2 for an initial adoptive transfer, and the rest of the cells were cryopreserved. When subsequent adoptive transfer was scheduled, 5 × 10⁸ PBL's were thawed and cultured as described above. The patient received 1 × 10⁸ to 2 × 10⁸ LAK cells intracisternally through an Ommaya reservoir or a ventriculoperitoneal (VP) shunt valve twice a week. During the period of LAK cell administration, 500 U rIL-2, diluted in 50 ml normal saline, was infused intravenously over 15 minutes.

* Human rIL-2 was supplied by Takeda Chemical Industries, Ltd., Osaka, Japan.

† Phoresis system, Model V 50, manufactured by Haemnetics Corp., Natick, Massachusetts. Lymphocyte separation medium obtained from Litton Bionetics, Kensington, Maryland.
Case Reports

Case 1

This 29-year-old man presented in June, 1984, with a 4-month history of progressive neurological deterioration, which began with occasional nausea and vomiting. He developed double vision and clumsiness of the limbs on the right side.

Examination. He was dysphagic, with a left fourth, fifth, sixth, and seventh nerve palsy, marked impairment of swallowing, and truncal ataxia. A computerized tomography (CT) scan showed an area of slightly high density in the middle of the cerebellum adjacent to the roof of the fourth ventricle, which was shifted to the right. This area was about 2-cm in diameter and was markedly enhanced on CT scans with contrast medium.

Course. A tumor was removed subtotaly through a midline cerebellar approach. Tumor cells from this patient were histologically diagnosed as anaplastic astrocytoma. Subsequently, he developed increasing hydrocephalus, and a VP shunt was inserted. Radiotherapy with a total dose of 60 Gy in 30 fractions was delivered to the whole brain. The patient fully recovered after the operation. Five months later, he developed a severe headache and gait disturbance with bilateral ankle clonus. ACT scan revealed areas of slightly high density at the anterior horns of both lateral ventricles. These areas were remarkably enhanced on CT scans with contrast medium (Fig. 1). The cerebrospinal fluid (CSF) contained 78 cells and had a protein concentration of 196 mg/dl and a glucose level of 96 mg/dl. Numerous malignant glioma cells were found on cytological examination of the CSF, and a diagnosis of meningeal gliomatosis was made (Fig. 2). The patient was given $1.5 \times 10^9$ LAK cells intrathecally through the valve of the VP shunt over a period of 1 month. He improved steadily and led a normal life with only residual left ankle clonus. Malignant cells were no longer detected in his CSF. One week after this treatment, the CSF contained three cells and had a protein level of 131 mg/dl and a glucose concentration of 83 mg/dl. Seven months later, the patient was suddenly suffocated to death at home after a general convulsion.

Postmortem Examination. Systemic autopsy was not permitted, but brain examination was performed. The leptomeninges of the cerebrum were partially thickened but entirely clear. The anterior horn of the right lateral ventricle was occluded by the tumor mass. Within the parenchyma of the pons, invasive tumor was found macroscopically.

Case 2

This 56-year-old man presented in January, 1985, with a 2-week history of persistent headache, slight fever, and double vision.

Examination. He was alert, and had bilateral sixth nerve palsies and bilateral swollen cervical lymph nodes. Biopsy of the cervical lymph node led to a diagnosis of metastatic squamous-cell carcinoma from the tongue. A CT scan showed no abnormal findings. Analysis of the CSF revealed eight cells, a protein level of 2 mg/dl, a glucose concentration of 80 mg/dl, and many malignant cells. The patient was diagnosed as having meningeal carcinomatosis.

Course. An Ommaya reservoir was implanted subcutaneously in the right frontal region of his head, with

![Fig. 1. Case 1. Computerized tomography scans on second admission. Left: Plain scans showing an area of slightly high density at both anterior horns of the lateral ventricle (upper), and the tip of the indwelling ventriculoperitoneal shunt at the trigone of the right lateral ventricle (lower). Right: Scans obtained after administration of contrast material showing marked enhancement at the high-density area.](image)

![Fig. 2. Case 1. Photomicrograph of the cerebrospinal fluid showing many malignant glioma cells. Papanicolaou, x 135.](image)
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the tube inserted into the right anterior horn of the lateral ventricle. The patient received a total of $2 \times 10^9$ LAK cells intrathecally over 1 month. After this treatment, malignant cells were no longer detected in the CSF, which contained 30 cells and had a protein concentration of 12 mg/dl and a glucose level of 79 mg/dl. The patient improved steadily and has been leading a normal life without residual deficits during the 17 months since his therapy.

Discussion

The prognosis of patients with malignant gliomas has improved only slightly despite the combined use of surgery, radiation therapy, and chemotherapy, and any improvement is usually temporary. In the two cases reported here, an alternative therapeutic approach was used utilizing the adoptive transfer of tumoricidal effector cells generated from autologous PBL's. 13,14

Tumor-specific immunotherapy via adoptive transfer of monoclonal antibodies or cytotoxic T lymphocyte cells has been documented in the last few years. 2,4,7,10 However, clinical application of such tumor-specific immunotherapy may be limited owing to the time and difficulty involved in preparing individual agents that are specific for each patient. Recently, Rosenberg, et al., 11,12 reported adoptive immunotherapy using LAK cells in conjunction with interleukin-2 in the therapy of patients with advanced cancer. They observed a distinct clinical response (at least a 50% reduction in tumor volume) in 11 of 25 cases.

We have previously demonstrated that the incubation of murine spleen cells or human PBL's with rIL-2 generated lymphoid cells that were able to lyse cultured tumor cells resistant to natural killer cells but did not affect normal cells. 9,13,14 Several workers have reported that cytotoxic cells exhibiting potent cytotoxicity in vitro are not very effective in adoptive immunotherapy. 3,4,8 One possible reason that patients did not improve with immunotherapy using such cultured cells is that the effector cells did not reach the target cells of the patients in sufficient numbers after intravenous administration. Lotze, et al., 6 reported that activated killer cells that were injected into patients intravenously appeared in the lung first and were subsequently rapidly redistributed to the liver and spleen. In general, PBL's are not capable of penetrating the blood-brain barrier. 1

Therefore, in our patients we administered a total of $2 \times 10^9$ LAK cells intrathecally, with intravenous administration of 500 U of rIL-2 every day. After this treatment, the neurological findings of these two patients improved and malignant cells were no longer detected in the CSF.

The adoptive transfer of LAK cells to these two patients with intracisternally disseminated brain tumors was highly effective in reducing the clinical symptoms and signs, and in eliminating malignant cells from the CSF. This therapy is an attractive approach for the treatment of malignant tumors that have poor immunogenicity or are insensitive to anti-cancer agents, and for patients with severe immunosuppressive conditions induced by repeated radiation therapy or chemotherapy.

References


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