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Case Report

A Case of Renal Cell Carcinoma with Lung Metastases Treated with Adoptive Immunotherapy

Noboru YABUSAKI[1], Hideki KOMATSU[1], Akira UENO[1], and Kachio TASAKA[2]

Department of Urology[1], Department of Parasitology & Immunology[2], Yamanashi Medical College

Abstract: A patient with renal cancer and lung metastases who was treated with lymphokine-activated killer cells (LAK cells) is reported. A 52-year-old male was admitted because of asymptomatic hematuria in August 1986. Computed tomography showed a large renal tumor on the right side and the chest X-ray film revealed multiple lung metastases. Right nephrectomy was performed in September 1986. Histologically, the tumor was diagnosed as a renal cell carcinoma. Infusion of vinblastin via the bronchial arteries had no effect on the metastatic lesions. LAK therapy was started in April 1987. Peripheral blood lymphocytes were purified from the patient's leukocytes collected by leukapheresis and then cultured at a concentration of $1–2\times10^6$ cells/ml with 3–6 U/ml of human recombinant interleukin-2 in complete RPMI 1640 medium for 3–8 days. In the initial two treatments, LAK cells were infused intravenously, but from the third treatment they were infused via the bronchial arteries. The number of LAK cells infused was 4×10^8–3.2×10^9 per treatment and 16.1×10^9 in a total of 11 treatments. LAK therapy was stopped because of a toxic reaction which developed during the 11th infusion. The cause of this reaction was not obvious, but it was apparently an allergic mechanism. During LAK therapy the metastatic lesions slightly increased in size.

Key words: lymphokine-activated killer cell, interleukin-2, adoptive immunotherapy, lung metastases from renal cancer, plasma exchange

Since radiotherapy and chemotherapy have little effect on renal cell carcinoma, surgical excision has been thought of as the only effective method for treating this tumor. No method has been shown to be effective for disseminated renal cell carcinoma. In 1985, Rosenberg and his colleagues[4] reported the use of adoptive immunotherapy with lymphokine-activated killer (LAK) cells for patients with advanced cancer in whom other treatments had proven ineffective. Renal cell carcinoma was reported to be one of the best responders to this therapy[5]. For this reason we treated a patient with lung metastases from renal cell carcinoma by infusion of LAK cells via the bronchial arteries.

Tamaho, Nakakoma, Yamanashi 409-38, Japan
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CASE REPORT

A 52-year-old man was hospitalized in September 1986 for a right-sided renal tumor. Asymptomatic gross hematuria had occured 1 month previously, the tumor was detected by computed tomography (CT) at another hospital, and he was then referred to us. On physical examination a large mobile mass was palpable in the right upper quadrant. CT showed a large right renal mass with low density areas (Figure 1).

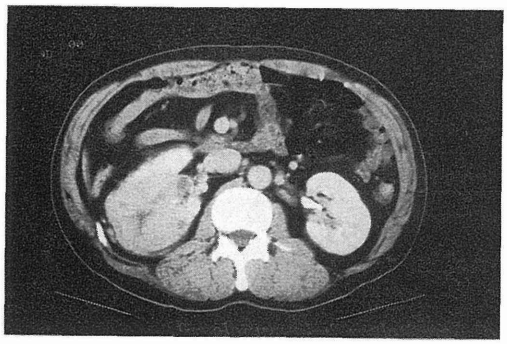

Fig. 1. Abdominal CT scan.

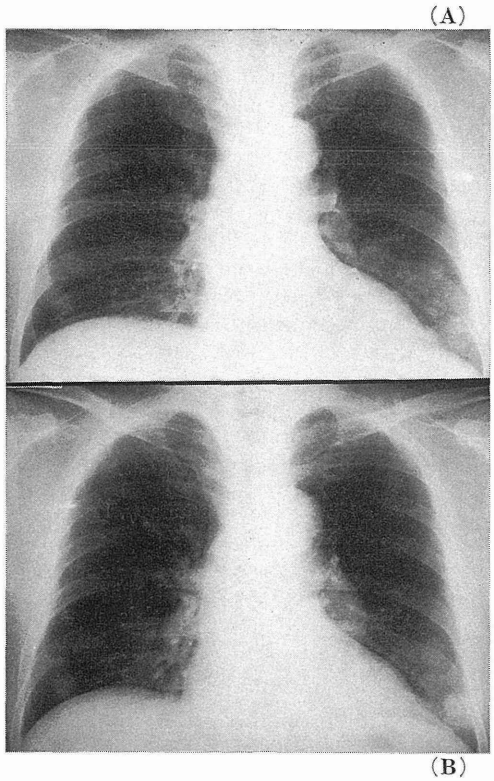

Fig. 2. Chest X-ray films.
before LAK treatment (A).
after LAK treatment (B).

Right renal arteriography demonstrated a hypervascular tumor occupying the lower pole of the right kidney. The chest X-ray film revealed multiple coin lesions in both lungs (Figure 2-A). On September 22nd, 1986, right nephrectomy was performed. Histologically, the tumor was diagnosed as a renal cell carcinoma, stage pT2b, pNO, M1 (the original tumor was in the renal capsule without lymph node metastasis, but with distant metastasis). On October 27th vinblastine was infused via the bronchial arteries for treatment of the metastatic pulmonary lesions. Since vinblastine had no effect on the pulmonary metastases, adoptive immunotherapy with LAK cells was started in April 1987.

Adoptive immunotherapy with LAK cells

Recombinant Interleukin-2 (rIL-2)

The rIL-2 used was TGP-3, which was kindly supplied by Takeda Pharmaceutical Co. Ltd., Osaka, Japan.

Induction of LAK cells

The lymphocyte-rich fraction was obtained from the peripheral blood of the patient using a blood cell separator (CS-3000, Travenol, Co. Ltd., Tokyo, Japan). From this fraction lymphocytes were further purified by Ficoll-Hypaque (Pharmacia Fine Chemicals, Uppsala, Sweden) gradient centrifugation, and were cultured at a concentration of $1–2\times10^6$/ml in roller bottles (Falcon, Becton Dickinson Co. Ltd., Oxnard, CA., USA) under 5% CO_2 at 37°C in a humidified atmosphere for 3 to 8 days. Complete RPMI 1640 with 5% autologous plasma or 5% human fresh frozen plasma containing 3–6 U/ml of human rIL-2 was used as the culture medium. After 3 to 8 days of cell culture, lymphocytes were collected and suspended in 20 ml of physiological saline with 1,000 U of rIL-2. This suspension was then administered to the patient.

Examination of the activity of LAK cells

The activity of the LAK cells was determined by a ^{51}Cr release assay, as previously reported[1]. The targets were Daudi cells (which are resistant to natural killer cells and sensitive to LAK cells), K562 cells (which are sensitive to natural killer cells),

the human renal cell carcinoma line CAKI-1, and autologous peripheral blood lymphocytes (PBL). After target cells were radiolabeled, effector and target cells were cultured together for 4 hours at the ratios of 6/1, 12/1, 25/1, and 50/1 in a CO_2 incubator (Tabai Espec, Co. Ltd., Osaka, Japan). Cytotoxic activity was calculated by the following equation:

$$\% \text{ Killng release} = \frac{\text{Experimental release} - \text{Spontaneous release}}{\text{Maximum release} - \text{Spontaneous release}} \times 100$$

Figure 3 shows the cytotoxic activity of LAK cells induced from the patient's PBL in July 1987. At an E/T ratio of 50/1, 80% of Daudi or K562 cells and 50% of CAKI-1 cells were killed, but LAK cells showed no cytotoxicity against autologous PBL.

Infusion of LAK cells

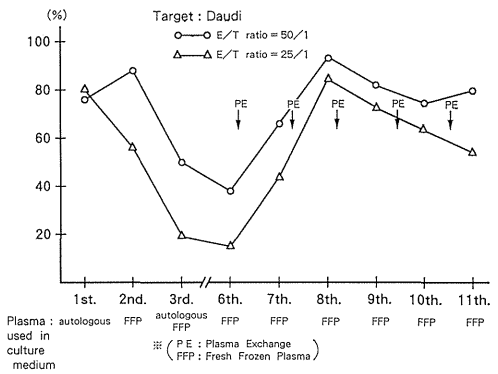

Fig. 3. Cytotoxicity of LAK cells.

LAK cells were administered to the patient 11 times in the period from April 1987 to June 1988. The first and second times the LAK cell suspension was infused intravenously, but from the third treatment we infused it via bronchial arteries to increase contact with the metastatic tumor. The method of bronchial artery infusion was as follows. A Seldinger catheter was inserted into the patient's femoral artery and the tip of the catheter was selectively advanced to a bronchial artery. Then 40 ml of the LAK cell suspension was infused over 30 minutes by syringe pump. Each time 2000 U/day of rIL-2 was given for 5 days. The LAK cell activity in our patient decreased gradually from treatment to treatment. We presumed that this was due to the presence of inhibiting factors against LAK activity which have been previously demonstrated[2]. Therefore, we stopped using the patient's plasma in the culture medium. Furthermore, we performed plasmapheresis the day before administration of LAK cells. Despite the use of fresh frozen plasma in the culture medium, LAK cell activity fell to a minimum at the 6th treatment, although from the 7th infusion it recovered slightly (Figure 4). This suggests that plasmapheresis removed the inhibiting factors to some extent. The number of transfered LAK cells at one time ranged from 4×10^8 to 3.2×10^9, and a total of 16.1×10^9 cells were administered in the 11 treatments.

Fever (38°C–39°C) developed after each infusion, and subsided three to four days after the infusion without treatment. At the 11th treatment symptoms such as chills, shivering, high blood pressure (230/140 mmHg), an acute rise of body temperature (38.7°C), and tachycardia (120/min) occurred 15 minutes after the beginning of LAK cell infusion via a bronchial artery. We stopped the infusion immediately. The symptoms disappeared soon after intravenous injection of methylprednisolone. The LAK cell suspension was examined for sterility by bacterial culture and for contamination with endotoxin by the Limulus test (Wako Chemicals, Osaka, Japan), but they were both negative. Then we investigated allergy to rIL-2. We injected rIL-2 intracutaneously into the forearm and observed local swelling and redness, the degree

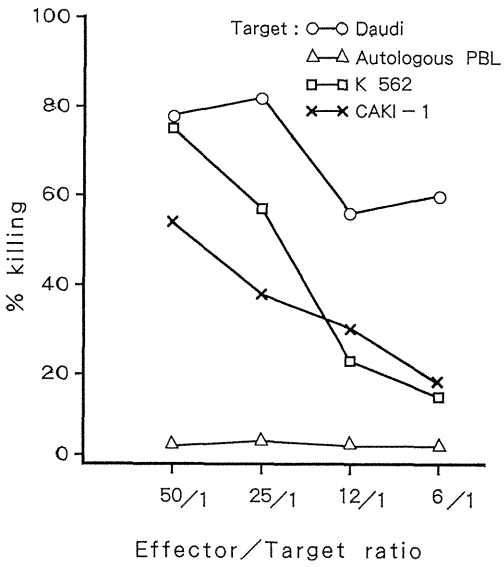

Fig. 4. Kinetics of LAK activity.

of which was dependent on the dose injected. Accordingly, we stopped the LAK treatment.

Figure 2-B shows the chest X-ray film after 11 LAK treatments. The multiple coin lesions were increased in size compared with before LAK treatment. The total of the diameters of five evaluable metastatic lesions increased from 61 mm to 78 mm, about a 28% increase in size. This change was evaluated as evidence of progressive disease (PD).

Discussion

In 1987, Rosenberg and his colleagues reported that they treated 108 patients with metastatic cancer by using LAK cells and human rIL-2, and achieved 8 complete remissions and 15 partial remissions[5]. Overall 22% of the patients responded to the treatment. Among them metastatic lesions from renal cancer responded particularly well to this treatment. Four complete remissions and 8 partial remissions were obtained among 36 metastatic renal cancer patients, a 33% response rate. Compared to their excellent result, the results of LAK treatment in Japan have been much poorer. According to a cumulative on the results of LAK treatment at many institutions in Japan[6], among 144 patients only 6 partial remissions were reported (a 4% response rate). The difference in response rates may be due to the frequency of LAK cell infusion, the number of LAK cells infused, or the dose of rIL-2. For example, Rosenberg scheduled the patients to receive five daily leukapheresis treatments, and infused all the induced LAK cells day by day for the following four days. The cumulative doses of LAK cells and rIL-2 amounted to $5–10\times10^{10}$ cells and $1–2\times10^{6}$ U/kg respectively. One unit of TGP-3 is reported to be equivalent to 300–400 units of the rIL-2 used by Rosenberg (personal communication). So $1–2\times10^{6}$ U/kg of rIL-2 equals about 3,000 to 6,000 U/kg of TGP-3. Their method required hundreds of liters of culture medium and an enormous amount of IL-2 which produced many side effects. Thus, their original method was modified for this patient.

A second reason why LAK therapy was not so effective in this case may relate to anatomical considerations. Generally, LAK therapy shows its best results in the brain, and we have reported one case of complete remission of a brain tumor[3]. The reason is that the brain is a confined space, so LAK cells can more easily reach the target and remain in its vicinity longer than in other organs. In this case, the frequency of infusion and the contact with the lesion may have been insufficient, even though LAK cells were injected selectively via the bronchial arteries.

As side effects of LAK treatment, Rosenberg and his coworkers reported chills, hyperbilirubinemia, and the so-called capillary permeability leak syndrome which induces elevation of creatinine level, hypo-

tension, weight gain, respiratory distress, and arrhythmias[5]. However, they did not report a toxic reaction to LAK therapy with IL-2 or to IL-2 alone. We presumed that some antibody against TGP-3 (manufactured rIL-2) was the cause of the symptoms in our patient, although we failed to demonstrate an anti-IL-2 antibody by ELISA.

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