

Lymphokine-activated Killer Cell Therapy Combined with High-dose Glucocorticoid Showed Clinical Efficacy towards Advanced Lung Carcinoma

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Abstract. We describe the case of a patient with terminal adenocarcinoma of the lung with no response to lymphokine-activated killer (LAK) cell therapy alone who distinctly responded clinically to a combination of high-dose glucocorticoids (GC) and LAK cell therapy, administered by chance. However, after decreasing the dose of GC because of gastrointestinal bleeding caused by the high-dose treatment, there was no response on restarting GC plus LAK cell therapy. The clinical course of this case strongly suggests that local inflammation in the vicinity of tumors should be adequately suppressed in patients with advanced cancer who receive immunotherapy.

We report a rare case of terminal adenocarcinoma of the lung in which micrometastasis detected at surgery did not respond to lymphokine-activated killer (LAK) cell therapy alone, but distinctly responded to a combination of LAK cell therapy and glucocorticoids (GC), given for palliative treatment. Although the mechanism of action is unclear, GC most likely suppressed immunosuppressive inflammatory activity in the vicinity of the tumor cells. The clinical course of this case may provide important clues to future ways of enhancing the therapeutic effectiveness of immunotherapy.

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Key Words: Combination therapy, inflammation, postsurgical, micrometastases, CEA.

Case Report

The patient was a 56-year-old man with adenocarcinoma of the lung who had undergone a left lobectomy in April 2005. Intrapulmonary metastases were detected intraoperatively, and the postoperative diagnosis was stage IV disease. Combined chemotherapy with cisplatin and paclitaxel was started in June 2005. The patient received four cycles of chemotherapy, with no decrease in carcinoembryonic antigen (CEA) tumor marker levels and no shrinkage of pulmonary metastases (5 mm in diameter) on imaging studies. Because the patient complained of severe adverse effects of anticancer treatment, chemotherapy was subsequently discontinued. Between August 2005 and February 2006, LAK cells produced by culturing peripheral blood leukocytes of the patient with anti-CD3 antibody and interleukin-2 were administered systemically 9 times. The serum CEA level was 19.9 ng/ml before LAK cell therapy and rose to 36.6 ng/ml after 9 doses of LAK cell therapy. Subsequently, the patient's condition deteriorated, but markedly improved after starting treatment with gefitinib in March 2007. However, after 8 months of gefitinib treatment, the serum CEA level began to increase again, and terminal disease with pulmonary lymphangitis was diagnosed in October 2008 (Figure 1A).

In the same month, the patient was transferred to his home, where he was treated by a hospice physician. Oxygen inhalation therapy and GC treatment (starting dose: 12 mg/day of betamethasone, equivalent to 120 mg/day of prednisolone) were begun. On day 17 of treatment with GC, the serum CEA level had increased to 1,859 ng/ml, as compared with 818 ng/ml before treatment. In November

2008, the same regimen of LAK cell therapy administered previously was restarted at the insistence of the patient. After two doses of LAK cell therapy, the serum CEA level fell to less than half of the pretreatment value. The serum CEA level decreased further after three doses of LAK cell therapy. The maximum decrease was equivalent to less than 70% of the pretreatment value. A decrease of 50% or higher was maintained for 4 months. The patient's general condition also improved, and in January 2009 he was able to play golf. However, gastrointestinal bleeding occurred in the same month, necessitating a reduction in the GC dose and the withdrawal of LAK cell therapy. In March 2009, the patient requested LAK cell therapy again, and three doses were administered. Concomitant GC treatment (prednisolone 40 mg/day) was given for a total of only 7 days before and after LAK cell therapy. The serum CEA level did not decrease in response to the last three doses of LAK cell therapy (Figure 1B). The patient died of cancer in June 2009.

Discussion

Only one randomized clinical trial has reported that LAK cell therapy is effective for adjuvant treatment in patients with liver cancer (1). Other studies have failed to demonstrate effectiveness for other types of advanced cancer (2). However, in Japan many patients with advanced cancer in whom standard therapy is ineffective or cannot be used because of adverse events request and receive LAK cell therapy as an alternative treatment.

LAK cell therapy was developed about 30 years ago, but its mechanism of antitumor action remains uncertain. Studies in mice at the time of development reported that the *in vitro* and *in vivo* antitumor activities of LAK cells produced by tumor-bearing and non-tumor bearing mice were similar (3), thus providing no evidence for specific cytotoxic activity. Although the mechanism is unclear, a nonspecific direct effect of LAK cells on cancer cells cannot be ruled out. However, the present case showed no response of micrometastases to LAK cell therapy after resection of the primary tumor, suggesting that nonspecific direct cytotoxic activity was probably not involved.

Cytokines produced by LAK cells, such as interferon- γ and tumor necrosis factor (TNF)- α (4), might indirectly activate other cytotoxic mechanisms in the vicinity of tumor cells, thereby suppressing tumor growth. One direct mechanism of cytotoxic activity may involve natural immunity associated with macrophages and other cells. Interferon- γ and TNF- α have been shown to disturb cancer cells by increasing the expression of TNF related apoptosis inducing ligand (TRAIL) on macrophages (5) and by inducing the production of nitric oxide (4). However, tumor-associated macrophages (TAMs) are widely known to locally

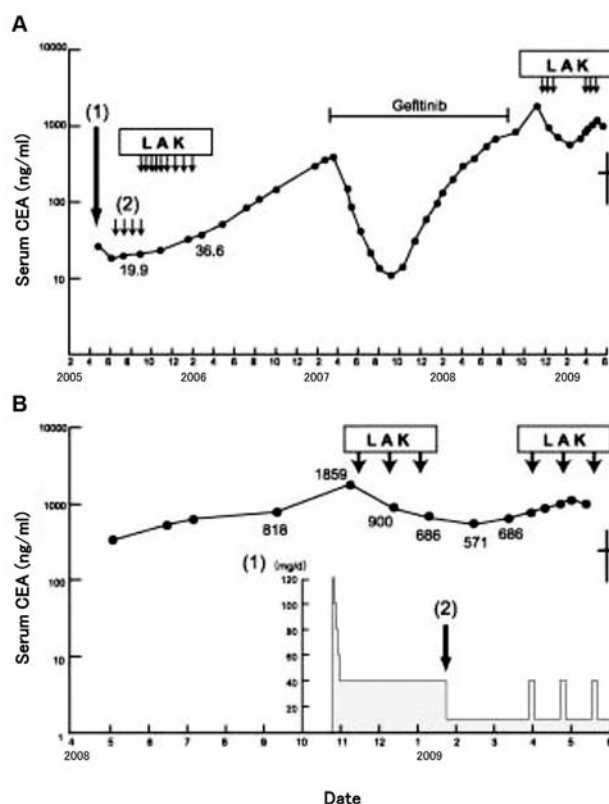


Figure 1. Time courses of treatment and serum CEA levels in a patient with adenocarcinoma of the lung. A: Clinical course from the start of therapy to death from cancer. (1) Lobectomy, (2) cancer chemotherapy. B: Course during combined treatment with GC and LAK cell therapy, given as palliative treatment during the terminal phase of cancer. (1) Bar graph showing the daily dose of GC as equivalent dose of prednisolone, (2) gastrointestinal bleeding.

participate in tumor growth and progression of metastasis in advanced cancer. Even at the basic research level, methods to alter the characteristics of TAMs from activities promoting tumor growth to those reducing tumor mass, namely the methods to control the ambivalent behavior of macrophages to tumor at will, have yet to be established (6).

In our patient with terminal cancer, the distinct antitumor effectiveness of GC combined with LAK cell therapy may have been related to three factors. Firstly, the serum CEA level continued to increase even after GC treatment, but then decreased by up to 70% while the patient received GC plus three doses of LAK cell therapy. A decrease in the serum CEA level by >50% continued for 4 months. Secondly, the patient survived for 8 months after the start of terminal hospice care at his home. The usual duration of hospice care for terminal cancer is 4 weeks on average. Patients surviving for 3 months have rarely been reported (7). Survival for 8 months clearly indicates that treatment was effective. Thirdly, the patient's general condition

transiently improved from a performance status of 3 before treatment to a performance status of 1 after three doses of LAK cell therapy.

Among the six administered doses of LAK cell therapy, the first three doses were significantly effective clinically, whereas the latter three, given after reducing the dose of GC, were ineffective. These findings may provide important clues to the mechanism of action of GC combined with LAK cell therapy and suggest future directions for the development of adjuvant immunotherapy. Although experimental studies have reported that GC does not attenuate the effectiveness of tumor-specific lymphocytes activated *ex vivo* (8), it is unlikely that concomitant LAK cell therapy augmented the antitumor activity of lymphocytes *in vivo*, given that the dose of GC was comparable to that used previously for suppression of acute rejection in recipients of renal transplants. A previous study has shown that suppression of TAMs by GC persists during long-term, high-dose treatment, but is only transient during single-dose treatment (9). Therefore, during the first three doses of LAK cell therapy, continuous treatment with high-dose GC may have reduced the activity of immunosuppressive M2 macrophages, thereby allowing M1 macrophages with cytotoxic activity to be activated by LAK cells in the vicinity of the tumor cells. In contrast, during the last three doses of LAK cell therapy, administered after reducing the dose of GC, inhibition of M2 macrophages was probably insufficient and M1 macrophages were unable to exert their cytotoxic activity. These factors may have been involved in the mechanism of action of combination therapy in our patient.

Many studies have reported that M2 macrophages play a major role in the functions of TAMs. Recently, detailed genetic analyses have demonstrated mixed expression of M1 and M2 genes in a murine fibrosarcoma (10). The same study reported that it is unclear whether both types of genes are expressed on the same cells or if two types of cell systems coexist. However, the results of *in vivo* immunohistochemical analyses suggested that cells possessing M2 molecules are numerous and diffusely distributed, whereas cells possessing M1 molecules exist in small clusters. Because the status and distribution of M1 and M2 TAMs may depend on the type and stage of cancer, and individual differences among patients, clinical elucidation is difficult at present. Clinically, however, a relation between outcomes and different local distributions of TAMs has been reported for multiple types of cancer (11, 12). The development of treatments that can locally control immunosuppressive macrophages as well as locally activate macrophages with cytotoxic activity is considered an important factor in the development of future anticancer therapies.

Previous studies have reported that bulky tumors do not respond well to immunotherapy (13). Although various types of combination therapy have been tried to overcome this

problem, only one animal study has examined GC combined with immunotherapy. That study found that dendritic cell therapy was effective only after pretreatment with a single dose of GC (14). However, antitumor effectiveness was limited to the suppression of proliferation, and the best regimen for GC remains unclear. In our patient, LAK cell therapy was effective only during continuous treatment with high doses of GC. To reduce the side-effects of GC and enhance the clinical effectiveness of adjuvant immunotherapy, drug delivery systems that can locally deliver GC to tumors should be developed (9).

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Received June 8, 2010

Revised July 6, 2010

Accepted July 8, 2010