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IMMUNOTHERAPY OF SOLID TOMOR BY INTRATUMORAL INFUSION OF LYMPHOKINE-ACTIVATED KILLER CELLS

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Fifty million lymphokine-activated killer (LAK) cells were infused into rat T9 gliosarcoma tumors for 1 hr at an infusion rate of 0.1 ml/hr. Cultured normal spleen cells were infused into similar tumors as a control. The LAK cell-treated tumors began to regress at approximately 3 weeks after infusion and disappeared by 6 weeks, while the cultured normal spleen cell-treated tumors grew progressively. Immunohistochemical analysis demonstrated prominent infiltration of cytotoxic/suppressor T cells in the LAK cell-treated tumors, while few lymphocytes were recognized in the control tumors. These data suggested that LAK cells infused intratumorally might be capable of mediating tumor regression by inducing host immunity against the tumor.

Key words: Lymphokine-activated killer cells — Interleukin-2 — Gliosarcoma

Efficacy of adoptive immunotherapy of cancer with lymphokine-activated killer (LAK) cells and interleukin-2 (IL-2) has been demonstrated in various human and animal models.[1-4] However, systemic administration of LAK cells to established metastasis in representative murine models has failed to provide complete cures of tumor-bearing hosts.[5, 6] The reason for this failure, particularly against solid tumors, may be low efficiency of access of lymphocytes to the tumor site.[7] High-dose IL-2 and LAK cell administration is very expensive, and causes substantial side effects.[8, 9] A possible alternative to this systemic LAK immunotherapy may be local administration of LAK cells in order to increase the availability of LAK cells to the tumor site and reduce side effects. In this communication, we describe significant antitumor effects of LAK cells given in combination with IL-2 by means of direct infusion of LAK cells into the tumor.

Inbred 6- to 10-week-old male Fischer rats (F 344) and syngeneic gliosarcoma cells (T9),[10] which were induced in F344 rats by weekly injection of N-nitrosourea for 8 months, were used in these experiments. Spleen cells obtained from normal F344 rats were cultured for 4 days in the presence of human recombinant IL-2 at a cell density of 2×10^6/ml to generate LAK cells. This human recombinant IL-2 (TGP-3) has a specific activity greater than 1.0 U/ml and was a gift from Takeda Chemical Industries, Ltd., Osaka. In order to determine the *in vitro* cytotoxicity of LAK cells, a short-term ^{51}Cr-release assay was employed. NK-sensitive (K562), NK-resistant (Daudi) or NK-resistant T9 tumor cells were labeled with 100 μCi of [^{51}Cr]sodium chromate and used as targets. Cytotoxicity was tested in V-shaped microwells (Costar 3898, Mass.) with 200 μl of assay medium containing 5×10^3 target cells and various numbers of effector cells. The control consisted of medium and target cells (spontaneous release). Target cells were incubated with 100 μl of 5% Nonidet P-40 detergent to determine maximal release. The isotope release was determined with a gamma counter (LKB Wallack Compugamma 1282). The percent specific lysis was calculated as follows:

$$\frac{\text{mean experimental cpm} - \text{mean spontaneous cpm}}{\text{mean maximal cpm} - \text{mean spontaneous cpm}} \times 100.$$

Ten million T9 tumor cells were inoculated subcutaneously in the dorsal area of F344

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rats. When the tumor reached approximately 10 mm in diameter, fifty million LAK cells suspended in 0.1 ml of phosphate-buffered saline were infused into the center of the T9 tumor at a speed of 0.1 ml/hr by means of an infusion/withdrawal pump (Harvard Apparatus, Mass.) thus allowing fifty million LAK cells to infiltrate into the tumor tissue in 1 hr. A 25 G spinal needle was used to inject LAK cells into the exposed tumor and was fixed by the use of stereotaxic instruments (1404 Heavy Duty Research, David Kopf Instruments, USA) during the infusion. For the following 10 days rats received 20 U of IL-2 intraperitoneally once a day. Control rats were infused with the same number of 4-day-cultured normal spleen cells with intraperitoneal injection of IL-2 for 10 days. The height (A), length (B), and width (C) of each T9 tumor were measured daily and the kinetics of tumor growth was evaluated in terms of the tumor volume, which was calculated as $1/2(A \times B \times C)$. Immunohistological analysis of the tumor tissue was carried out by means of the avidin-biotin-peroxidase technique using monoclonal antibodies specific for a subpopulation of rat T cells. Monoclonal antibodies, R1-3B3 and R1-10B5,[11] which detect rat lymphocyte antigens comparable with Lyt-1 and Lyt-2,3, respectively, were used in the experiments. Biotinylated goat anti-mouse Ig was purchased from Vector, Calif., and was absorbed with normal rat serum. Tumor tissues were excised at various times after infusion.

Experiments involving intratumoral infusion of LAK cells were repeated 6 times using a pair of rats to confirm the reproducibility of the effects. Figure 1 shows representative data. Tumors, whether they were infused with LAK cells or normal spleen cells, continued to grow and the growth curves were almost the same until 3 weeks after infusion. The tumors treated with LAK cells ceased to grow then, showed regression, and disappeared at approximately 6 weeks after infusion. On the other hand, the tumors treated with cultured normal spleen cells continued to grow until the tumor-bearing hosts died. At the time of infusion, cytotoxic activities of LAK cells against NK-sensitive (K562), NK-resistant (Daudi), or T9 cells in terms of 4-hr ^{51}Cr release assay were 52.7%, 22.0% and 28.1% (specific lysis), respectively, with effector-to-target ratios of 40 to 1. Cultured normal spleen cells exhibited less than 10% lysis of each tumor target.

We next analyzed local events occurring at the tumor site by histological and immunohistochemical techniques. The infused LAK cells or cultured normal spleen cells were observed in the center of the tumor tissue until day 5. In the LAK cell-treated tumors, lymphocyte infiltration around the tumor capsule was noticed from day 3, then it increased, and strong infiltration into the whole tumor was seen during the course of regression. Cultured normal spleen cell-treated tumors also showed infiltration by lymphocytes in the tumor capsule. However, the intensity of lymphocyte infiltration was weaker than that observed in LAK-treated rats. This lymphocyte infiltration began to disappear between the 2nd and 3rd weeks, in

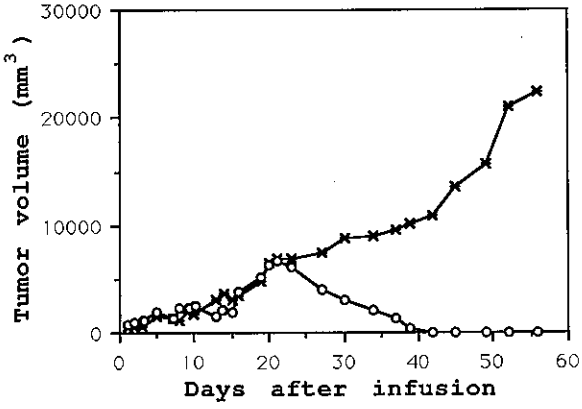

Fig. 1. Fffects of LAK cell (○) or cultured normal spleen cell (*) infusion plus IL-2 on the growth of T9 tumor. Fifty million LAK cells or cultured normal spleen cells were infused into the tumor at a rate of 0.1 ml/hr using a microinfusion pump. The rats were given 20 U of rIL-2 per dose intraperitoneally for the subsequent 10 days. Tumor growth is expressed as tumor volume (mm^3) calculated from the size of the tumor.

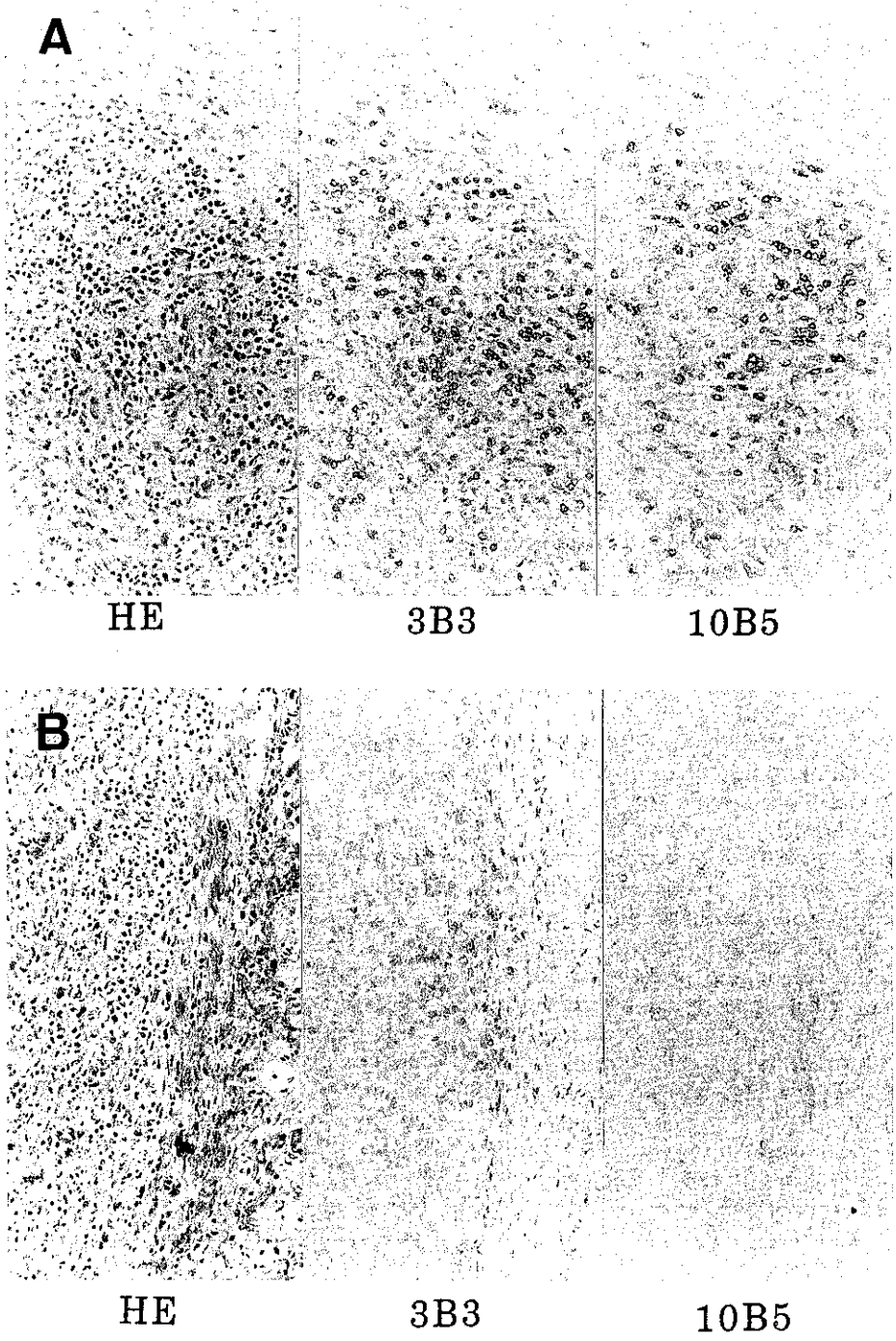

Fig. 2. Immunohistochemical analysis of T9 tumors by monoclonal antibodies specific for rat T cell subsets. A) Three weeks after LAK cell infusion. Prominent lymphocyte infiltration into T9 tumor is seen. HE. ×120. Most lymphocytes express pan T marker (3B3) and killer/suppressor T cell marker (10B5) on their cell surfaces. B) Three weeks after cultured normal spleen cell infusion. Few lymphocytes are found in the tumor tissue. HE. ×120.

clear contrast with the LAK cell-treated tumors (Fig. 2). Immunohistochemical analysis of tumor sections demonstrated that tumor-infiltrating lymphocytes were mostly R1-3B3- and R1-10B5-positive (killer/suppressor) T cells in the LAK cell-treated tumors.

Previous immunotherapy of tumors in animal models with adoptive transfer of tumor-sensitized lymphocytes[12, 13] or with sensitization of animals with tumor cells[14–16] demonstrated that anti-tumor immunity has great potential to cure a tumor-bearing host. However, neither therapy can be applied immediately to human cancer patients because of limitations in generating a sufficient number of specifically immunized cells from an immunoincompetent tumor-bearing host by stimulation with putative tumor antigens.[2] Lymphokine-activated killer cells have provided a new approach to treat human cancer patients in terms of practical immunotherapy of cancer. We employed LAK cells in combination with rIL-2 because the administration of LAK cells alone was reported to show little beneficial anti-tumor effect.[5, 6, 17, 18] Although systemic administration of LAK cells and IL-2 resulted in objective regression of metastatic cancer, it showed severe toxicity.[8, 9] Several attempts have been made to augment the LAK cell activity[19] or abrogate the toxicity of IL-2[20] to enhance the efficacy of the therapy. Our study presented in this communication achieved this end by transferring LAK cells locally into the tumor. Existence of effector cells at the tumor site is essential for the anti-tumor effect. In both animal and human cancers, the amount of tumor-infiltrating lymphocytes, particularly T cells, correlates with the prognosis of tumor-bearing hosts.[14–16] Infiltrating T cells of a regressing tumor were actually demonstrated to manifest tumor-specific cytotoxicity in rats.[16] The study of *in vivo* distribution of lymphoid cells grown in T cell growth factor (TCGF, currently known as IL-2) revealed that the radio-labeled lymphoid cells appeared in the lungs at 4 hr and subsequently they were found almost exclusively in the liver and spleen at 24 and 48 hr in both mice and humans.[7] Therefore, systemic administration of LAK cells involves a substantial reduction in therapeutic efficacy because of the poor local availability of LAK cells. In order to enhance the efficacy of LAK cell therapy with reduced side effects, local administration of LAK cells and IL-2 is desirable. The results of the present study showed an approximately 3-week latent period between LAK cell infusion and tumor regression. This indicates that LAK cells might not be the final effector cells to mediate eradication of the tumor. The peak in proliferation response of LAK cells administered *in vivo* to exogenous IL-2 is reported to appear by the fifth day, but to decline by day 7 despite continuation of IL-2.[21] In our model, the proliferating lymphoid cells found at the tumor tissue more than 1 week after the LAK cell infusion may not be intratumorally infused LAK cells. In addition, the results of an *in vitro* cytotoxicity test of LAK cells against T9 cells showed less than 30% specific lysis when the effector-to-target ratio was 40 to 1. The number of LAK cells infused into the tumor appears to be too small to achieve complete rejection of the tumor. Adoptive immunotransfer in a murine model also showed a one- to two-week latent period until tumor eradication was achieved.[22, 23] This latency might represent the period of induction of host tumor immunity or a proliferative stage of donor lymphocytes. Histologically, massive lymphoid cell infiltration into the tumor tissue occurred from the periphery of the tumor, which also suggests that those lymphoid cells were not the infused LAK cells. Immunohistochemical analysis of the tumor specimens demonstrated that the majority of tumor-infiltrating lymphoid cells were cytotoxic/suppressor T cells. From these findings it is conceivable that LAK cells might induce strong host immunity against the tumor. It is reported that amplification of immune reaction occurs in the microenvironment of the tumor when the tumor is rejected.[24–26] A cytokine cascade is responsible for the immune amplification, involving various kinds of soluble factors produced by a variety of immune-competent cells and immune-associated cells. Neutrophils, macrophages and helper T cells are known to produce factors such as IL-1, IL-2, IL-3, interferon, colony-stimulating factor, cytotoxic cell-generating factor and lymphocyte migration factor.[26, 27] The major asset of the intratumoral infusion of LAK cells may be high

efficacy of access of LAK cells to the tumor, presumably causing partial destruction of tumor tissues followed by migration and activation of immune-competent cells of host origin. Antigen presentation to the host immune systems should be better performed as a result of the availability of the processed tumor antigens obtained from the lysed tumor cells. Thus, immune reaction is assumed to be initiated by the lysis of the tumor cells by LAK cells followed by the cooperation of LAK cells and host immune cells to trigger the host reaction and further succeeded by the magnified reaction through the cytokine cascade, resulting in massive infiltration of cytotoxic T cells into the tumor tissue. It is possible that LAK cells can also produce soluble factors in the interaction with tumor cells to trigger host immune resistance against the tumor.

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