Augmented Accumulation of Transferred Lymphokine-Activated Killer (LAK) Cells at Murine Tumor Sites through Production of LAK-Attractant Facilitated by Chemotherapy

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HOSOKAWA, M., WAKIZAKA, Y., KURAMITSU, Y., MICALLEF, M., TOGASHI, Y. and KOBAYASHI, H. *Augmented Accumulation of Transferred Lymphokine-Activated Killer (LAK) Cells at Murine Tumor Sites through Production of LAK-Attractant Facilitated by Chemotherapy.* Tohoku J. Exp. Med., 1992, 168 (2), 413-416 — We observed that effects of adoptive immunotherapy with lymphokine-activated killer (LAK) cells on BMT-11, a fibrosarcoma in C57BL/6 mice were improved by combination with cyclophosphamide (CY)-chemotherapy corresponding to enhanced accumulation at tumor sites of LAK cells. On the other hand, cytotoxic T lymphocytes (CTLs) which were able to accumulate at tumor sites more densely than LAK cells produced significant therapeutic effects by themselves. We have also found observed that LAK-attractant activity was detected in conditioned medium (CM) of CY-treated tumor tissue but not in the CM of untreated tumor tissue. These findings reveal that CY-chemotherapy facilitates LAK-attractant-production and enhances the accumulation in tumor tissue of LAK cells and that therapeutic effects of adoptive transfer of LAK cells are augmented by cancer chemotherapy through the enhanced accumulation of LAK cells. — lymphokine-activated killer; fibrosarcoma; cytotoxic T lymphocyte; adoptive transfer; cyclophosphamide

We have previously reported that lymphokine-activated killer (LAK) cells do not accumulate within tumor tissues when LAK cells are adoptively transferred into untreated tumor-bearing mice and that transferred LAK cell-accumulation is enhanced in the tumor tissue of mice treated by cancer chemotherapy (Hosokawa et al. 1988; Kawata et al. 1990). The enhanced accumulation of transferred effector cells at tumor sites might improve the effects of adoptive immunotherapy. We attempted, therefore, to investigate therapeutic effects of LAK cell-transfer when combined with cyclophosphamide (CY)-chemotherapy corresponding to their accumulation at tumor sites and mechanisms responsible for the enhanced

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accumulation at the tumor tissue of LAK cells transferred after chemotherapy.

MATERIALS AND METHODS

C57BL/6 mice inoculated s.c. with BMT-11 (a syngeneic fibrosarcoma) cells were treated with adoptive immunochemotherapy. Cyclophosphamide (CY; 150 mg/kg) was administered i.v. as chemotherapy on Day 10 when the tumor had grown to a mean diameter of 5 mm. Four days after the chemotherapy, effector cells were transferred i.v. followed by 5 days i.p. administration of recombinant human interleukin 2 (rIL-2; 10^4 JRU/day) as adoptive immunotherapy. Three mice from each group were inoculated with ^{111}In indium oxine-labeled effector cells and examined the effector cell-accumulation at tumor sites by counting radio activity in each tumor tissue 24 hr after the transfer. Effector cells used were lymphokine-activated killer (LAK) cells which had been prepared by a 4-days culture of normal syngeneic spleen cells in RPMI-1640 medium with rIL-2 at a concentration of 1,000 JRU/ml and cytotoxic T lymphocytes (CTLs) specific to BMT-11 cells which had been prepared by a mixed lymphocyte-tumor culture (MLTC) of spleen cells obtained from mice hyperimmunized with BMT-11 cells. In order to detect LAK-attractant produced by CY-treated or untreated tumor tissues, we collected conditioned medium (CM) after 24 hr incubation of tumor tissue in serum free medium (AIM-V, GIBCO). The LAK-attractant activity in the various CMs was assayed under the agarose migration method.

RESULTS

Therapeutic effects. As summarized in Table 1, no significant therapeutic effect was brought about by CY alone or LAK·IL-2 alone. When combined with CY-chemotherapy, LAK·IL-2 immunotherapy gave the strongest therapeutic effects. Complete cures of tumor were obtained in 3 out of 8 mice (38%) and prolongation of mean survival time (MST) of dead mice was observed by CY plus LAK·IL-2. On the other hand, CTL·IL-2 alone brought about significant prolongation of MST of mice. The effects of CTL·IL-2 was further augmented by combination with CY.

Accumulation of effector cells at tumor sites. The accumulation (%Dose/g)

TABLE 1. *Therapeutic effects of adoptive immunotherapy in combination with cyclophosphamide (CY) on BMT-11 tumors in C57BL/6 mice corresponding to the accumulation of effector cells at tumor sites*

Treated with	Cured/treated (%)	MST $\pm$ S.D. of dead mice	Accumulation of effector at tumor site (%Dose/g)
None	0/8 (0)	45.3 $\pm$ 6.7	
CY alone	0/8 (0)	51.8 $\pm$ 5.3	
LAK · IL-2 alone	0/8 (0)	45.9 $\pm$ 5.6	2.73 $\pm$ 0.50
CY plus LAK · IL-2	3/8 (38)	64.4 $\pm$ 3.6*	19.70 $\pm$ 4.11 (7.22 folds)
CTL · IL-2 alone	0/8 (0)	54.1 $\pm$ 6.7*	9.14 $\pm$ 0.45
CY plus CTL · IL-2	2/8 (25)	63.3 $\pm$ 3.9*	13.90 $\pm$ 0.59 (1.52 folds)

*Statistically significant difference from non-treated group.

TABLE 2. *LAK-attractant activities in CM of CY-treated tumor tissue*

CM of		No. of mice	Directed migration of LAK cells (Mean $\pm$ s.d. in mm)
Tumor	Treated with		
BMT-11	None	7	1.00 $\pm$ 1.29
	CY	7	4.57 $\pm$ 1.72
3LL	None	4	0.25 $\pm$ 1.50
	CY	8	3.18 $\pm$ 1.13

The LAK-attractant activities is expressed as directed migration under agarose assay for 24 hr in which random migration to a control medium was 3.14 mm in BMT-11 and 3.20 in 3LL, respectively.

of LAK cells at tumor sites was only 2.7 when they were transferred into untreated mice and was definitely enhanced by combination with CY (19.7) and was 7 folds as compared with that of the LAK $\cdot$ IL-2 alone group. On the other hand, that of CTLs was sufficiently dense even in the CTL $\cdot$ IL-2 alone group (9.1) and slightly enhanced by combination with CY (13.9). These results in the accumulation of effector cells in tumor sites corresponded to therapeutic effects as seen in Table 1.

LAK-attractant in various CMs. The LAK-attractant activity was detected in the CM of CY-treated BMT-11 and 3LL tumor tissues but not in the CM of untreated tumor tissues (Table 2). The LAK-attractant activity was detected initially 3 days after CY, reached a peak 5 days and disappeared 10 days after CY-treatment. Then we separated host reactive cells from CY-treated tumor tissues and observed that LAK-attractant was produced in CM of host reactive cell enriched fraction but not in that of tumor cell rich fraction. We now are investigating the nature of LAK-attractant and preliminary results indicate that the LAK-attractant activities are located in about 10,000 dalton fraction of the CM and are sensitive to pH 2 or 100°C treatment, but resistant to 56°C treatment for 30 min.

CONCLUSIONS AND DISCUSSION

The findings in this work reveal that the effects of adoptive immunotherapy depend upon the accumulation of transferred effector cells at tumor sites and that the production of LAK-attractant by tumor tissue facilitated by chemotherapy is one of the mechanisms responsible for enhanced LAK-accumulation at tumor sites. The production of LAK-attractant by CY-treated tumor tissues was observed temporarily after chemotherapy. We suggest, therefore, the transfer of LAK cells should be carried out a short time after the chemotherapy. This study was initiated because of our previous experiments in which we had tried to treat 3LL lung cancer in C57BL/6 mice with a low dose rIL-2 (Hosokawa et al. 1988) in order to avoid severe side effects of observed in patients treated with high dose

rIL-2 (Rosenberg et al. 1985). Although the immunotherapy with rIL-2 was able to induce LAK cell-activity in spleen of tumor bearing mice, it did not bring about any therapeutic effect by itself. The effects of the rIL-2-immunotherapy were definitely improved when combined with CY-chemotherapy. We have also reported that LAK cell-accumulation at 3LL tumor tissue was enhanced by pretreatment of tumor-bearing mice with antitumor drugs such as CY, adriamycin and nimustine (Kawata et al. 1990). The enhanced accumulation of LAK cells by CY has been also observed in other tumor than 3LL tumor and in this study, we have substantiated that therapeutic effects are improved by the enhancement of LAK-accumulation within tumor tissues.

Regarding the improvement in the effects of adoptive immunotherapy, another trials using bispecific antibody which reacts to both tumor cells and effector cells have been reported (Nitta et al. 1988). Trials with bispecific antibodies will be promising as far as we can prepare an antibody specific to tumor cells. Our trials on the combination of immunotherapy with chemotherapy are also promising, since chemotherapy is effective to eliminate suppressor cell activity which may interfere with transferred effector cell activities (North 1982).

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