INTERLEUKIN 5 ENHANCES INTERLEUKIN 2-MEDIATED LYMPHOKINE-ACTIVATED KILLER ACTIVITY

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After 3–6 d of in vitro incubation with IL-2, human PBL and mouse splenic lymphocytes become cytotoxic to a variety of tumor cells, including not only NK-sensitive, but also NK-resistant tumor cells (1–3). These IL-2-dependent lymphocytes consist of a heterogeneous population of effectors (4–6). They also show non-MHC-restricted cytotoxicity, which has been referred to as the lymphokine-activated killer (LAK) phenomenon (2).

On the other hand, murine IL-5 (mIL-5) was previously described as a T cell–replacing factor (TRF) (7, 8) and as a B cell growth factor II (9) based on its induction of proliferation and Ig secretion by activated B cells. This lymphokine has been shown to exert several other effects, including the induction of growth and differentiation of eosinophils from bone marrow stem cells (10), the induction of differentiation of peanut agglutinin–positive (PNA$^+$) thymocytes into CTL (11), and the induction of mIL-2-R expression on both splenic B cells and PNA$^+$ thymocytes (12, 13).

In this study, we examined the effect of mIL-5 on the in vitro induction of LAK activity as a basic study for modified adoptive immunotherapy.

Materials and Methods

Mice.   Male C57BL/6 mice, 6–8 wk old, were supplied by the Shizuoka Animal Center (Shizuoka, Japan).

Tumors.   203 glioma is a malignant glioma cell line originally induced by 20-methylcholanthrene from the C57BL/6 strain. The tumor cells were maintained in a monolayer form in tissue culture with Dulbecco's modified MEM (Nissui, Japan) supplemented with 10% heat-inactivated FCS (Gibco Laboratories, Grand Island, NY) and antibiotics (penicillin G, 200 U/ml; streptomycin sulfate, 50 µg/ml). Methylcholantherene-induced mastocytoma (P815), originating from DBA/2 mice, and Moloney leukemia virus–induced T cell lymphoma (YAC-1), originating from A/Sn mice, were maintained in RPMI 1640 (Nissui, Japan) with 10% heat-inactivated FCS and antibiotics.

Lymphokines.   Human rIL-2 (TGP-3; 4.2 × 10^4 U/mg protein) was kindly supplied by Takeda Chemical Industry, Ltd., Osaka, Japan.

rmIL-5 was the culture supernatant of X63·Ag8·653 myeloma cells transfected with mIL-5 complementary DNA (14, 15). Culture supernatant (SN) (1,200 U/ml) was filtered and aliquots were stored at −70°C. Activity was determined by polyclonal IgM plaque-forming cell (PFC) assay using TRF-responding BCL$_1$ cells (16). This culture SN contained no IL-1, IL-2, IL-3, IL-4, IL-6, IL-7, or CSF (data not shown). Control culture SN was collected from the original myeloma cell (X63·Ag8·653).

Generation of LAK Cells. Spleens were removed aseptically and crushed with the hub of a syringe in RPMI 1640. Spleen cells were incubated with mIL-5 at a concentration of 2 × 10⁶ cells/ml for 3 d in the presence of IL-2 in RPMI 1640 with 10% heat-inactivated FCS and antibiotics. The culture was performed in 5-ml wells of a 6-well plate (Corning Glass Works, Corning, NY). The cells were harvested, washed twice, and used as effectors.

Cytotoxicity Assay. A 4-h ^{51}Cr release assay in round-bottomed microtiter plates (Corning Glass Works) was used to measure cytotoxicity. Target cells were labeled with 0.1 mCi ^{51}Cr (Amersham Corp., Arlington Heights, IL) for 60 min at 37°C. Effectors were coincubated with the targets at various E/T ratios for 4 h at 37°C in humidified air with 5% CO_2. The radioactive SNs were harvested and counted with a gamma counter. The cytotoxic activity was calculated from the average of triplicate cultures as: percent of cytolysis = 100× (experimental release – spontaneous release)/(maximum release – spontaneous release). Spontaneous release was determined by adding culture medium only to the target cells and maximum release was counted in detergent. All determinations in each experiment were performed in triplicate.

Results

The Effect of rIL-5 on the Induction of LAK Cells. Freshly isolated spleen cells were incubated with IL-2, rIL-5, or a combination of IL-2 and rIL-5. As shown in Fig. 1, rIL-5 enhanced IL-2-induced LAK activity, although rIL-5 alone induced no cytotoxic activity. Control SN obtained from X63·Ag8·653 myeloma cells did not show such enhancement (data not shown). Freshly isolated spleen cells showed slight cytotoxicity against YAC-1, but little cytotoxicity against P815 and 203-glioma (data
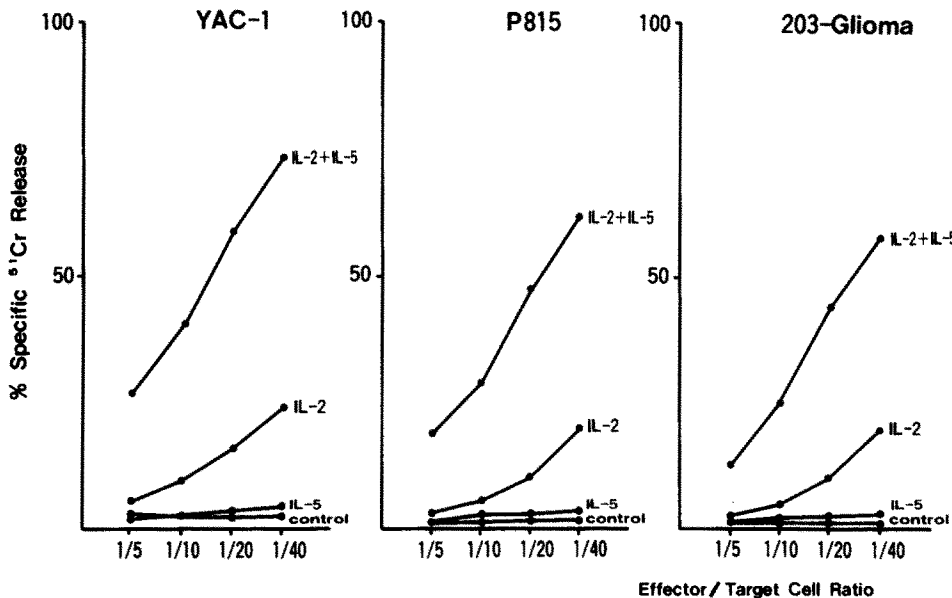

FIGURE 1. rIL-5 enhances rIL-2-mediated LAK activity. C57BL/6 mouse splenocytes were cultured at 2 × 10⁶ cells/ml in 5 ml of complete medium containing rIL-2 (1 U/ml) and/or rIL-5 (50 U/ml). After 3 d, effectors were tested for cytotoxicity against ^{51}Cr-labeled tumor cells in a 4-h assay. Control values represent the killer activity of effectors cultured in complete medium without any lymphokines. Results are presented as the means of at least three experiments.

not shown). Spleen cells cultured without lymphokines for 3 d were ineffective in lysing YAC-1, P815, and 203-glioma.

The Effect of rIL-5 on the Induction of LAK Activity Depends on the Concentration of IL-2. IL-2 mediated LAK cell activity is also dependent on IL-5 concentration. To determine the optimal concentration of IL-2 or rIL-5 to obtain the maximum cytotoxic activity, double titration experiments were carried out. In Fig. 2, these killer activities show dose dependency at IL-2 concentrations from 0.01 to 10 U/ml and at rIL-5 concentrations from 0.01 to 100 U/ml.

The Effects of Various Time Schedules of Administration of IL-2 and rIL-5 on the Induction of LAK Activity. As shown in Fig. 3, rIL-5 alone did not show killer activity when IL-2 was not present from the onset of culture (Fig. 3, *j* and *k*). If IL-2 was present from the onset of culture, however, rIL-5 enhanced LAK activity, even when added 24-48 h after culture initiation (Fig. 3, *h* and *i*). Finally, the presence of IL-2 for the first 24 or 48 h and rIL-5 during the last 48 or 24 h, respectively, did not enhance LAK activity (Fig. 3, *d* and *e*). These results suggest that rIL-5 acts on the late stage of the LAK induction in the presence of IL-2.

Discussion

The present study demonstrates that mIL-5 enhances the LAK activity in the presence of IL-2, though mIL-5 alone can not induce killer activity (Fig. 1). We also found that in vitro the cell numbers are greater from cultures containing IL-2 plus mIL-5 than those containing IL-2 alone (data not shown). As mIL-5 induces the expression of IL-2-R on both splenic B cells and PNA$^+$ thymocytes (12, 13), it may also induce the expression of IL-2-R on LAK cells. Although mIL-5 alone can
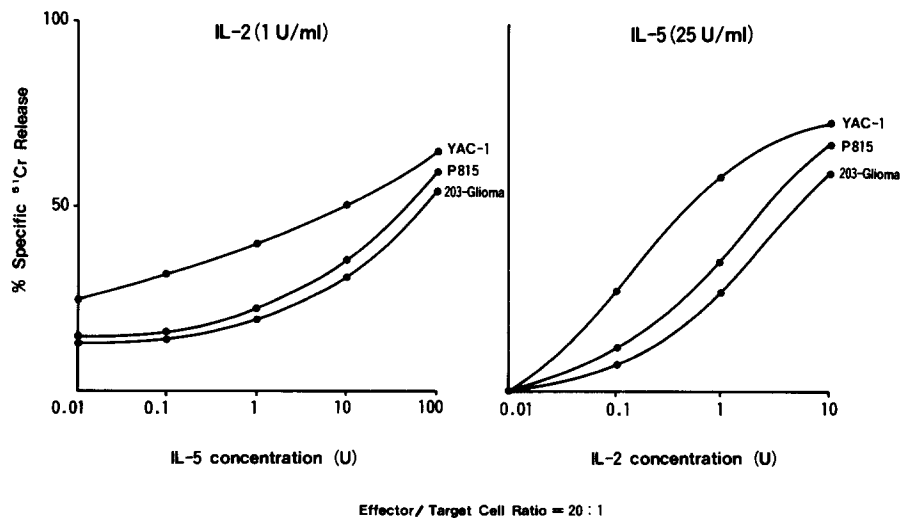

FIGURE 2.   Generation of LAK activity. Dose-response cytotoxic activity by rIL-5 and rIL-2. C57BL/6 mouse splenocytes were cultured at various concentrations of rIL-5 and rIL-2 for 3 d. Cytotoxic activities against YAC-1, P815, and 203-Glioma were measured at an E/T ratio of 20:1. All determinations in each experiment were performed in triplicate.
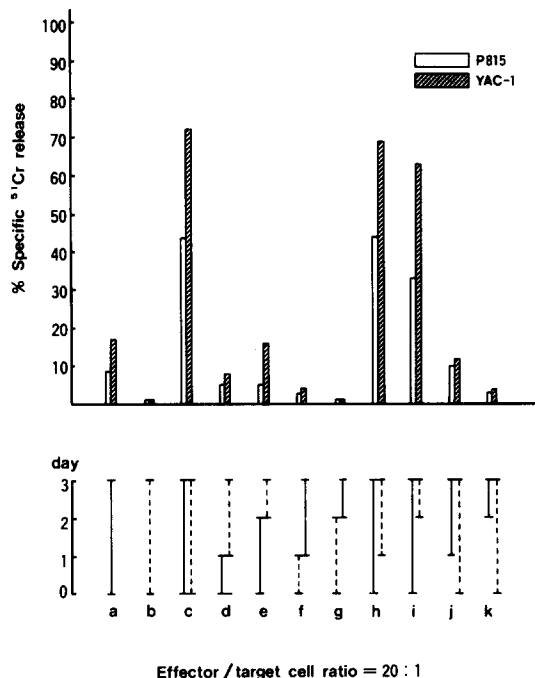
FIGURE 3. Effect of various time schedules of administration of rIL-5 and rIL-2 on LAK induction. C57BL/6 mouse splenocytes were cultured. rIL-5 (25 U/ml) or rIL-2 (1 U/ml) was present in the culture for limited periods as indicated. After 3 d, cytotoxic activities against YAC-1 and P815 were measured at an E/T ratio of 20:1. All determinations in each experiment were performed in triplicate. (———) IL-2; (——————) IL-5.

not induce killer activity, some mechanisms involving IL-5-R probably may augment IL-2 signal transduction pathways.

mIL-5 may act on the late stage of LAK induction in the presence of IL-2. As shown in Fig. 3, *c*, *h*, and *i*, if IL-2 is present from the onset of culture, mIL-5 enhances LAK activity, even when mIL-5 is added at the late stage of culture. If mIL-5 is present from the onset of culture, however, killer activity is not so enhanced, even when IL-2 is added at the late stage (Fig. 3, *j* and *k*). Hence, it might be possible that IL-2 activates precursor cells in the first stage, and mIL-5 augments the activated precursor cells. Moreover, this effect cannot take place in the absence of IL-2 (Fig. 3, *f* and *g*).

Mule et al. (17) found that mIL-4 alone induces killer activity as well as IL-2-mediated killer activity. mIL-5, on the other hand, enhances IL-2-mediated killer activity, but mIL-5 alone does not induce the killer activity. These results suggest that the mechanism by which LAK activity is augmented by mIL-5 is different from that of mIL-4.

Unlike IL-2, mIL-4 has little capacity to generate LAK activity from mouse PBL, whereas both lymphokines induce this activity from splenocytes (17). Whether or not mIL-5 and IL-2 similarly generate LAK activity from mouse PBL remains to be determined.

Hergen et al. (18) found that human IL-4 blocks IL-2-generated LAK activity. The reason for the discrepancy between human and mouse IL-4 is unclear. Apart from the possibility that the ability of IL-4 to block the induction of LAK activity is species restricted, it might be possible that mouse spleen cells contain already acti-

vated precursor cells. Hergen et al. (18) found IL-4 inhibits IL-2-induced LAK activity in spleen cells.

It would be interesting, for the purpose of adoptive immunotherapy, to see whether human IL-5 also enhances IL-2-induced LAK cell activity in PBL or not.

Summary

IL-5 expresses various biologic effects on several types of lymphocytes, including B cells, eosinophils, and T cells. We demonstrated that the incubation of resting splenocytes from C57BL/6 mice in murine rIL-5 enhances IL-2-mediated lymphokine-activated killer (LAK) activity against various tumor cells. IL-5 alone, however, does not induce killer activity. IL-2-mediated LAK activity increases in proportion to the dose of IL-5. During the late phase of the culture period, IL-5 seems to have some effect on the induction of IL-2-mediated LAK activity. We expect that IL-5 will prove useful for adoptive immunotherapy.

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