Jpn. J. Cancer Res. (Gann), 79, 390–399 ; March, 1988

Generation and Characterization of Lymphokine-activated Killer Cells against Fresh Human Leukemia Cells

Tohru TAHARA,[*1, *4] Rieko ISEKI,[*2] Yasuo MORISHIMA,[*1] Shozo YOKOMAKU,[*3] Ryuzo OHNO[*1] and Hidehiko SAITO[*1]

*[1]First Department of Internal Medicine and *[2]Department of Dermatology, Nagoya University School of Medicine, 65 Tsurumai, Nagoya 466 and *[3]Department of Internal Medicine, Aichiken-Shokuin Hospital, 3-2-1 Marunouchi, Nagoya 460*

Lymphokine-activated killer (LAK) cells generated from 15 acute leukemia patients in remission showed significant levels of cytotoxicity against Daudi 1A4, a natural killer-resistant cell line. This indicates that lymphocytes of leukemia patients in remission could respond to interleukin-2 to generate conventional LAK cells. However, LAK cells caused lysis of autologous leukemia cells at considerably lower levels in seven out of the 15 patients, with the exception of one case (48.6% cytolysis). None of the remaining eight patients exhibited LAK activity against autologous leukemia cells. On the other hand, patients' LAK could lyse allogeneic leukemia cells including those resistant to autologous LAK. Thus, patients' LAK seem not to be defective in lysis of leukemia cells. In the cold target competition analysis, the binding of patients' LAK to leukemia cells could be inhibited by autologous and allogeneic leukemia cell competitors, implying that almost all leukemia cells could be recognized by patients' LAK. Most LAK cells from normal donors showed significant lysis of allogeneic leukemia cells, but some leukemia cells were found to be resistant to lysis. LAK cells against both leukemia cells and Daudi 1A4 were phenotypically heterogenous, and were predominantly observed in the $T3^-$ fraction in the precursor phase. In the effector phase, whereas LAK activity against leukemia cells was also predominantly shown in the T cell-depleted fraction, similar levels of LAK activity against Daudi 1A4 were found in both the T cell-depleted and -enriched fractions.

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

Interleukin-2 (IL-2)-activated killer cells, known as LAK (lymphokine-activated killer) cells, represent a unique cytotoxic system distinct from cytotoxic T lymphocytes and natural killer (NK) cells based on characteristics including kinetics of activation, target cell specificity, the stimulus responsible for activation, and phenotypes of the precursor and effector cells.[1–5] When cultured in the presence of IL-2 without any other stimulation, lymphocytes acquire the ability to lyse a wide range of tumor targets that include not only cultured tumor cell lines but also autologous and allogeneic fresh tumor cells. These data suggest that the LAK system may have possible clinical applications, such as the adoptive immunotherapy of tumors.[6–11]

The target spectrum of LAK cells is extremely broad and none of the cultured cell lines is resistant to LAK. Conversely, it has been observed that LAK activity sometimes faild to be generated against autologous fresh tumor cells.[1, 5, 9, 12] Lymphocytes from tumor patients may be defective in response to IL-2 to induce LAK, and/or some of the fresh tumor targets may not be recognized by LAK cells. While LAK cells generally appear not to lyse nonmalignant cells,[1, 7, 9–13] they have sometimes been reported to lyse normal fresh lymphocytes.[5, 7] Thus, it seems to be likely that malignant cells are not always susceptible to LAK and that nonmalignant cells are not always resistant to LAK cells. Most recently, LAK from leukemia patients in complete remission have been reported by two different groups of investigators. Dawson *et al.*[14] described one successful case out of two in which they tried to generate LAK against autologous leukemia cells. Oshimi *et al.*[15] reported that in 11 out of 13 patients, leuke-

*4 To whom communications should be addressed.

mia cells were susceptible to autologous LAK cells.

In the present study, we examined autologous and allogeneic LAK systems against fresh noncultured leukemia cells as the target cells. All of the LAK cells from leukemia patients in complete remission and normal donors could lyse Daudi 1A4, an NK-resistant cell line, at high levels. However, leukemia cells examined were not efficiently lysed by autologous LAK cells. Most allogeneic LAK cells from both patients and normal donors could lyse leukemia cells at relatively lower levels compared to the levels with Daudi 1A4 as targets. However, some leukemia cells were resistant to allogeneic LAK. These results indicate that all malignant cells are not always lysed by LAK cells. We also demonstrated that such LAK cells against leukemia cells do not represent a single cell population of lymphocytes.

Materials and Methods

Lymphocytes Anticoagulated whole blood samples were obtained from 15 acute leukemia patients (13 acute nonlymphoblastic leukemia, ANLL; 2 acute lymphoblastic leukemia, ALL) during complete remission and 28 healthy volunteer donors. Because it is not known whether chemotherapy affects LAK precursors, blood was taken at least 4 weeks after the cessation of therapy. Peripheral blood lymphocytes (PBL) were separated by Ficoll-Hypaque (Pharmacia, Uppsala, Sweden) density gradient centrifugation (400*g*, 20 min), washed three times with phosphate-buffered saline (PBS), and then resuspended in RPMI-1640 medium (Gibco Laboratories, Grand Island, NY) supplemented with 10% heat-inactivated human AB serum, penicillin (100 U/ml), streptomycin (100 μg/ml) and L-glutamine (2m*M*). Cells at $1\text{–}2\times10^6$/ml were incubated in the presence or absence of recombinant IL-2 (TGP-3 Lot 046A; Takeda Chemical Industries, Ltd., Osaka) in 2-cm^2 Corning 25820 24-well plates (Corning Glass Works, Corning, NY) for 5 days in 5% CO_2 humidified incubator at 37°, and then resuspended in RPMI-1640 containing 10% fetal bovine serum (Gibco) (RPMI-FBS) as effector cells for cytotoxicity assay.

Concentration of IL-2 Non-adherent (NAd) cells were obtained from PBL of healthy donors by two passages through nylon wool columns. Cells at 2×10^6/ml were cultured in RPMI-1640 medium supplemented with 10% human AB serum. Recombinant IL-2 was added at the concentrations ranging from 0.001 μg/ml to 1.0 μg/ml. Five days later, the cultured cells were harvested, washed, and examined for their cytolytic effect on ^{51}Cr-labeled Daudi 1A4 and cryopreserved fresh leukemia cells (blast A from Pt.A) as the target cells in a standard 4-hr cytotoxicity assay.

Target Cells Target cells were obtained from blood leucocytes containing 75–95% leukemia cells of 22 patients (ANLL 18, ALL 4) before the initiation of therapy and were cryopreserved in liquid nitrogen. Frozen cells were thawed rapidly at 37° and gradually supplied with RPMI-FBS on ice, then washed three times with RPMI-FBS. To remove cell debris, these cells were centrifuged on Ficoll-Hypaque. As a negative control in the cytotoxicity assay, autologous PBL (7 cases) were prepared for target cells. Daudi 1A4 (subclone of Daudi, an NK-resistant cell line) was also prepared as positive control target cells. Daudi 1A4 at 5×10^5, leukemia cells and PBL at 1×10^6 in 50 μl of RPMI-1640 containing 20% FBS were incubated with 100 μCi of $Na_2{}^{51}CrO_4$ (New England Nuclear, Boston, MA) for 2 hr at 37°, then washed twice with RPMI-FBS and resuspended in 1 ml of RPMI-FBS.

Cytotoxicity Assay Aliquots of 10 μl of target cell suspensions (leukemia cells and PBL at 10^4, Daudi 1A4 at 5×10^3 in 10 μl) were dispensed into 96-well round-bottomed microtiter plates (Nunc, Roskilde, Denmark) with 200 μl of effector cells at effector-to-target (E/T) ratios of 10:1, 20:1, and 40:1. Following incubation for 4 hr in 5% CO_2 at 37°, the microtiter plates were centrifuged at 400*g* for 5 min. Aliquots of 100 μl of supernatant were collected from each well into Shionogi tubes (Shionogi Pharmaceutical Co. Ltd., Osaka), and counted in a gamma counter (Aloka ARC-500, Aloka Co. Ltd., Tokyo). Spontaneous release and maximum release of ^{51}Cr were determined by incubating target cells with RPMI-FBS alone, or 2% Triton X, respectively. Leukemia cells as the targets were found to give between 9.3 and 30% spontaneous release during the 4-hr assay. Each variable was tested in triplicate and the data are expressed as percentage cytotoxicity, which was calculated from the following formula:

$$\%\ \text{cytotoxicity} = [(\text{experimental cpm} - \text{spontaneous cpm})/(\text{maximum cpm} - \text{spontaneous cpm})]\times100.$$

LAK activity was considered successful when lysis was positively detected in ^{51}Cr release assay.

Cold Target Competition Analysis The cold target competition analysis was performed according to the method previously described.[13] Briefly, a quantity of LAK effectors was added to the well in 100 μl volumes at an effector:target ratio of 25:1, followed by addition of 100 μl containing a dilution of cold competitor cells. This mixture of inhibitors and effectors was incubated at 37° for 30 min. Next

the ^{51}Cr-labeled targets were added in a 10 μl volume containing 5×10^3 cells. Cold target inhibition conditions were always tested in triplicate.

Characterization of LAK Cells In order to examine the phenotypes of LAK cells, fractionation of PBL was done at the precursor phase and effector phase as follows.

Precursor phase: NAd cells from healthy donors were obtained by passage through scrubbed nylon wool columns twice. These cells (2×10^7) were mixed with 3×10^9 sheep red blood cells (SRBC) (Nippon Bio-Test Laboratories Inc., Japan) in 1 ml of FBS, spun at 200*g* for 5 min, and incubated at 29° for 1 hr to form high-affinity rosettes.[16] After incubation, the cell mixtures were gently resuspended in PBS, and centrifuged on Ficoll-Hypaque. The T cell-depleted fraction which did not form high-affinity rosettes (Eh^-) was harvested from the interface. The T cell-enriched fraction which formed Eh rosettes (Eh^+) was recovered from the pellets of Eh rosette-formed cells after lysis of SRBC with Tris-HN_4Cl. Eh^- cells were treated with OKT3 (Ortho Diagnostic Systems Inc., Raritan, NJ) for 30 min on ice, and then incubated with 1/2-diluted rabbit complement (Hoechst-Behring, Behringwerke AG, Marburg, W. Germany) for an additional 90 min at 37°. The resultant cells were referred to as $T3^-$. On the other hand, Eh^+ cells were incubated with anti-Leu11b (Becton Dickinson, Mountain View, CA) plus rabbit complement to deplete contaminant NK cells. The recovered cells were referred to as $T3^+$. The purity of both $T3^-$ and $T3^+$ subpopulations was determined by indirect immunofluorescence analysis on a fluorescence-activated cell sorter (FACS analyzer, Becton Dickinson). These cells were cultured in the presence or absence of IL-2 as described above. After 5 days' culture, these cells were prepared as effector cells for cytotoxicity assay.

Effector phase: NAd cells which passed through nylon wool columns twice were cultured in the presence or absence of IL-2. After 5 days' culture, cells were treated with OKT3 plus complement or anti-Leu11b plus complement as described above. The number of contaminant cells was assessed by the FACS analyzer. Thereafter, the resultant cells were prepared as effector cells for cytotoxicity assay.

RESULTS

Optimal Concentration of IL-2 Required for LAK Induction The addition of IL-2 to the culture of NAd cells resulted in a dose-dependent increase in cytotoxicity against both Daudi 1A4 and leukemia cells (Fig. 1). At doses of IL-2 greater than 0.05 μg/ml, there was no further significant increase in cytotoxic activity against either cells. A concentration of IL-2 of 0.1 μg/ml was, thus, used in subsequent experiments for the induction of LAK activity.

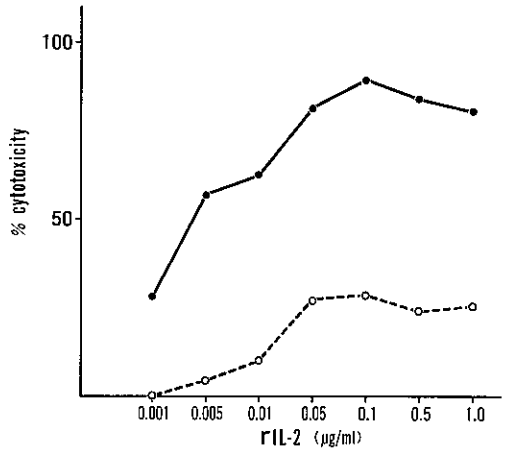

Fig. 1. NAd cells were cultured at 2×10^6/ml in the presence of various concentrations of rIL-2 for 5 days, and then these cells were assessed for cytotoxicity against Daudi 1A4 (●) and cryopreserved leukemia cells from patient A (○). Data were expressed as percent cytotoxicity at an E/T ratio of 30/1.

Generation of LAK Cells from Leukemia Patients and Normal Donors against Daudi 1A4 LAK activities generated from 14 leukemia patients in remission stage and from 12 normal donors were assessed against Daudi 1A4 which is a subclone of Daudi, an NK-resistant cell line shown to be resistant to fresh NK cells in Fig. 4. It is shown in Fig. 2 that LAK cells from leukemia patients caused lysis of Daudi 1A4 targets (% cytotoxicity, mean$\pm$SD; 68.1$\pm$26.4% at E/T=40/1) to about the same extent as the cells isolated from normal donors (69.7$\pm$13.6%). Thus, it appears that PBL from leukemia patients in remission exhibited a normal response to IL-2 to induce LAK activity.

LAK from Leukemia Patients against Autologous Leukemia Cells Figure 2(a) shows the generation of LAK cells from leukemia patients against autologous leukemia cells. No LAK activity was observed against autologous leukemia cells in eight out of the 15 patients examined, although significant lysis

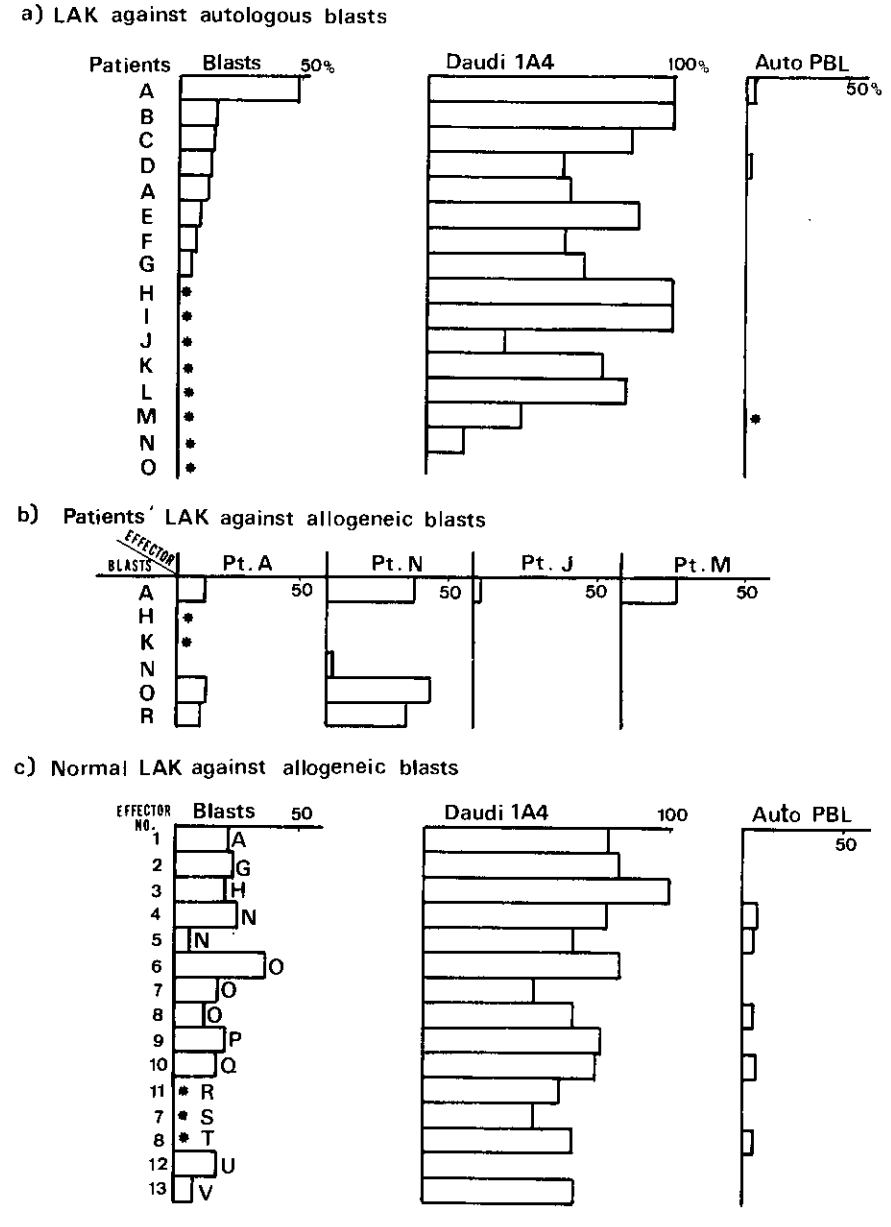

Fig. 2. LAK activity induced from PBL of leukemia patients and normal donors. PBL were cultured in the presence of 0.1 μg/ml of IL-2 for 5 days, and then were tested as effector cells for cytotoxicity assay. LAK activity was expressed as percent cytotoxicity at an E/T ratio of 40/1. (a) Patients' LAK against autologous blasts, Daudi 1A4, and autologous PBL. (b) Patients' LAK against allogeneic blasts. (c) Normal LAK against allogeneic blasts, Daudi 1A4, and autologous PBL. Blasts I, K, Q, and S were obtained from ALL patients. Blasts N, Q, U, and V were freshly isolated, and immediately used as the target cells. Thus, the freezing and thawing procedure seemed not to alter the ability of cells to be lysed. * $\leqq$0% cytotoxicity.

of Daudi 1A4 was induced in all cases. LAK cells caused lysis of autologous leukemia cells in the remaining seven patients. However, levels of cytolytic activity were considerably lower than those observed against Daudi 1A4 and were less than 15% of the cytotoxicity at a 40/1 E/T ratio, with the exception of one case. Repetition of the experiments with blood collected from the same patients indicated that these results were reproducible (data not shown).

LAK from Leukemia Patients against Allogeneic Leukemia Cells In order to determine whether LAK from leukemia patients are unable to lyse only their own autologous leukemia cells or alternatively whether such LAK are unable to lyse any leukemia cells, the efficacy of LAK cells from leukemia patients was tested against allogeneic leukemia cells. As shown in Fig. 2(b), patients' LAK which were unable to lyse autologous leukemia cells (blasts N, J, and M) could lyse allogeneic leukemia cells (blasts A, O, and R). Thus, LAK effectors from leukemia patients seemed not to be defective in lysing leukemia cells. On the other hand, two (blasts H and K) out of four blasts could not be killed by allogeneic LAK which well lysed autologous blast A. Blast H cells were resistant to both autologous and allogeneic patients' LAK but not to allogeneic normal LAK (Fig. 2). These results suggested the possibility that LAK recognize leukemia cells "selectively."

Recognition of Leukemia Cells by Patients' LAK Low susceptibility of leukemia cells to autologous LAK may be attributed to the failure to recognize some leukemia cells by patients' LAK. The approach used to test this hypothesis was a cold target competition analysis. The results are shown in Fig. 3. In the right-hand panel, LAK from patient A were incubated with ^{51}Cr-labeled autologous blast A target. Cold blasts A and R inhibited killing in a dose-dependent manner, but cold blast O caused only partial inhibition. When LAK from patient N which were unable to lyse autologous blast N were incubated with hot blast A target (the left-hand panel), cytotoxicity was inhibited by cold blasts A, R. O and N. Blast A competed better than the other three blasts. Interestingly, however, leukemia cells (blast N) resistant to autologous LAK (from Pt. N) had binding ability to autologous LAK. Thus, these results suggest that almost all leukemia cells including both those sensitive and resistant to autologous LAK could be recognized by autologous and allogeneic LAK.

LAK from Normal Donors against Allogeneic Leukemia Cells LAK activities from normal donors are shown in Fig. 2(c). Most LAK cells from normal donors showed significant lysis of allogeneic leukemia cells. The levels of cytolytic activity observed in the allogeneic systems (mean $\pm$ SD; 18.0 $\pm$ 8.5% at E/T = 40/1) were slightly higher than those in the autologous systems (14.6 $\pm$ 14.2), but were considerably lower than those against Daudi 1A4 (69.7 $\pm$ 13.6%). Leukemia cells obtained from 3 of the 12 patients examined were not

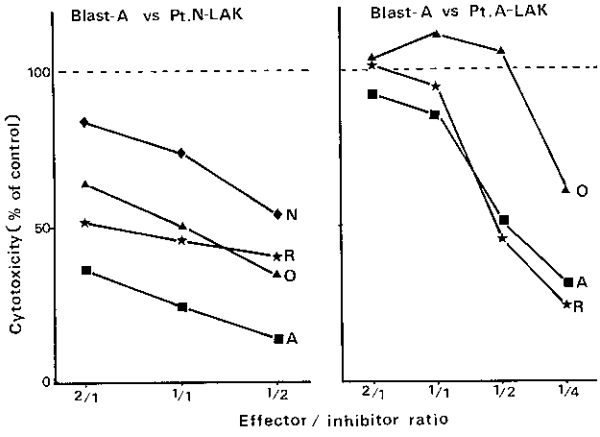

Fig. 3. Cold target competition analysis of LAK lysis autologous (right) and allogeneic (left) blasts. LAK prepared from PBL of patients A and N were tested for lysis of blast A at an E/T ratio of 25/1. Unlabeled blasts A, N, O, and R were titrated into the assay as competitors. The degree of inhibition was expressed as a percentage of lysis observed in control cultures with no competitors. Lysis in control cultures was in the range of 20 to 35%.

lysed. Blast O cells were lysed by LAK cells from three different normal donors, but the levels of cytotoxicity observed were variable (36.3%, 17.1% and 11.0% at E/T=40/1). Effector No. 7 lysed blast O but not blast S target cells, and effector No. 8 lysed blast O but not blast T target cells. These results indicate that some leukemia cells are not well lysed by conventional LAK cells.

Characterization of LAK Cells against Leukemia Cells In order to characterize the phenotypes of LAK cells against allogeneic and autologous leukemia cells, further experiments were undertaken. Three kinds of leukemia cells, blast A, O, and P cells, were utilized as target cells. Blast A cells from patient A were significantly lysed by both autologous and allogeneic LAK (Fig. 2), whereas blast O and P cells were susceptible only to allogeneic LAK. LAK cells were generated from several normal donors and one leukemia patient (Pt. A). The fractionation of cells was done as described in "Materials and Methods." The population referred to as $T3^+$ cells (NAd→Eh^+→Leu 11+C) consisted of ≧97.4% of $CD3^+$ cells, and the $T3^-$ cell fraction (NAd →Eh^-→OKT3+C) contained $CD16^+$ cells (≧48.1%), and $CD3^+$ cells (≦12.5%). Freshly isolated $T3^+$ and $T3^-$ cells were assessed for NK activity against K562 target cells. As shown in Fig. 4, NK activity observed in the $T3^+$ fraction was negligible, implying that the $T3^+$ cell fraction contained very few NK cells. NAd, $T3^+$, and $T3^-$ cells were cultured in the presence or absence of IL-2 for 5 days, and then assessed for cytotoxicity against Daudi 1A4 and leukemia cells. As is evident in Table I, LAK activity both against Daudi 1A4 and leukemia cells

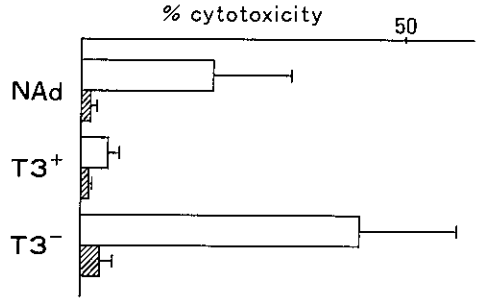

Fig. 4. Freshly isolated NAd, $T3^+$ and $T3^-$ (see "Materials and Methods") were assayed for cytotoxicity against K562 (□) and Daudi 1A4 (▨) at an E/T ratio of 20/1.

Table I. Phenotypic Analysis of LAK Cells against Fresh Leukemia Cells and Daudi 1A4 in the Precursor Phase

Target cells		% cytotoxicity on target cells (E/T=30/1)		
		NAd	$T3^+$	$T3^-$
Blast A	N=5	24.4±10.0[a] (100)	11.4±7.4 (44.3±23.1)*	27.2±4.9 (125.4±50.5)*
Daudi 1A4	N=5	54.9±10.9 (100)	44.6±20.1 (77.6±29.9)	63.4±11.3 (119.1±31.2)
Blast O		22.8	6.2	54.1
Daudi 1A4		57.5	39.6	79.4
Blast P		39.7	25.7	59.0
Daudi 1A4		83.0	64.5	87.2
Blast A	(autologous)	28.5	6.3	16.3
Daudi 1A4		67.2	40.5	53.3

NAd, $T3^+$ (NAd→Eh^+→Leu11+C) and $T3^-$ (NAd→Eh^-→OKT3+C) cells were obtained as described in "Materials and Methods," cultured with IL-2 for 5 days, and then prepared as the effector cells. The cytolytic assay both against leukemia cells and Daudi 1A4 was performed simultaneously using the same effector cells. Numbers in parentheses indicate the proportion to the lysis by NAd cells.

a) Mean±SE.

* Significantly different at $P<0.01$ (Student's *t*-test).

Table II. Phenotypic Analysis of LAK Cells against Fresh Leukemia Cells and Daudi 1A4 in the Effector Phase

Target cells		% cytotoxicity on target cells (E/T=40/1)		
		C	Leu11+C	OKT3+C
Blast A	N=5	22.9±9.2[a] (100)	17.4±8.6 (74.2±7.0)*	35.9±13.5 (163.0±50.8)*
Daudi 1A4	N=5	56.1±10.8 (100)	51.6±13.4 (91.3±9.8)	40.2±14.8 (71.8±21.0)
Blast O		5.8	8.1	25.3
Daudi 1A4		35.7	25.9	24.0
Blast P		14.5	12.8	46.2
Daudi 1A4		49.1	55.1	40.2
Blast A	(autologous)	18.6	13.9	29.1
Daudi 1A4		64.3	57.9	54.6

NAd cells were cultured with IL-2 for 5 days, then treated with monoclonal antibody plus complement to enrich or deplete $T3^+$ cells, and prepared as the effector cells. The cytolytic assay both against leukemia cells and Daudi 1A4 was performed simultaneously using the same effector cells. Numbers in parentheses indicate the proportion to the lysis by NAd (C control) cells.

a) Mean±SE.

* Significantly different at $P<0.01$ (Student's *t*-test).

could be induced from either $T3^+$ or $T3^-$ cells. However, distinctive levels of LAK activity were observed between the two cell fractions. Higher levels of cytolysis were observed from the $T3^-$ fraction than $T3^+$ fraction against Daudi 1A4, allogeneic leukemia cells, and autologous blast A target cells. When blast A cells were used as allogeneic target cells, significantly different levels ($P<0.01$) of cytolysis were found between LAK from $T3^+$ and LAK from $T3^-$ cells.

In the effector phase, LAK activity against blast A target cells was also significantly different ($P<0.01$) between the T cell-enriched fraction (Leu11+C; ≧94% $T3^+$ cells) and the T cell-depleted fraction (OKT3 +C; ≦5% $T3^+$ cells) (Table II). In contrast to the precursor phase, LAK activity against Daudi 1A4 from the T cell-enriched fraction was slightly higher than that from the T cell-depleted fraction in the effector phase. These results indicate that LAK cells both against Daudi 1A4 and leukemia cells are heterogenous in either the precursor or effector phase, and that LAK cells against fresh leukemia cells are slightly different from those against Daudi 1A4.

Discussion

In this study, we have demonstrated that the induction of LAK activity against fresh noncultured leukemia cells may prove to be difficult in autologous effector-target systems. These results are clearly distinct from recent reports that LAK cells can lyse most fresh solid tumors.[1-4, 6] Failure of LAK induction against autologous leukemia cells was also observed by other investigators. Oshimi *et al.*[15] have reported that in 11 out of 13 patients, leukemia cells were sensitive to autologous LAK, but in the remaining two, LAK failed to lyse autologous leukemia cells. Dawson *et al.*[14] described a low frequency of induction of LAK against leukemia cells. The low response of the LAK system against leukemia cells in the current study may be attributable to defects in the effector and/or target cells. In leukemia patients who have formerly received chemotherapy, lymphocytes may be defective in response to IL-2 to induce LAK. This is unlikely. In our study, LAK cells generated from both leukemia patients in complete remission and normal donors

showed significant levels of cytotoxicity against Daudi 1A4, an NK-resistant cell line. This indicates that lymphocytes of leukemia patients in remission respond to IL-2 to generate conventional LAK cells. Moreover, patients' LAK which were unable to lyse autologous leukemia cells could lyse allogeneic leukemia cells resistant to autologous LAK. Thus, patients' LAK effectors seem not to have defects in lysis of leukemia cells. These results rather suggested that LAK may recognize leukemia cells with selectivity. However, the cold target competition analysis demonstrated that leukemia cells including those resistant to autologous LAK were recognized by both autologous and allogeneic LAK. Therefore, although leukemia cells could be recognized by LAK, some of them were unable to be lysed. This could be explained as follows: LAK may bind leukemia cells with much lower avidity in some combinations of the effector versus target, or multiple binding molecules may be required when LAK lyse leukemia cells. Whether all leukemia cells can be recognized by any LAK or not remains unclear. It appears to be probable that the multiple structures recognized by LAK may play a role as the restriction molecules when LAK lyse leukemia cells.

We have demonstrated here that both $T3^+$ and $T3^-$ cells can generate LAK cells against leukemia cells and Daudi 1A4. Whereas LAK against leukemia cells was predominantly observed in $T3^-$ cells both in the precursor and effector phase, $T3^+$ cells contributed to LAK activity against Daudi 1A4 similarly to $T3^-$ cells. These results conflict with earlier reports[1-4] that the precursor cells of LAK do not express the T3 molecules on their surfaces. Most recently, three groups of investigators have reported that IL-2-activated killer cells are derived from phenotypically heterogenous precursors.[17-19] NK activity observed in the $T3^+$ fraction was negligible, whereas that in the $T3^-$ fraction was substantial, as shown in Fig. 4. Thus, it is unlikely that the contaminant NK cells in the $T3^+$ fraction might contribute to the observed LAK activity. Our data support the evidence that the precursor and effector cells of LAK are heterogenous. In many investigations, LAK activity was studied against cultured tumor cell lines,[12, 20-22] whereas in this study, the surface phenotypes were examined on the LAK cells against fresh noncultured leukemia cells. When leukemia cells were employed as the targets, LAK activity was predominantly induced from $T3^-$ cells and was observed at significantly higher levels in the $T3^+$-depleted fraction of the effector phase. It is of particular interest that $T3^-$ cells predominantly lysed fresh leukemia cells on which the class I MHC molecules were expressed (data not shown). Daudi 1A4 which do not express the class I and class II molecules on their surfaces were lysed by $T3^+$ and $T3^-$ LAK cells to the same extent. Recently, it was reported that NK cell-mediated cytotoxicity was negatively affected by the presence of the class I molecules on the target cells.[23] This result was supported by our observation that fresh leukemia cells with the class I molecules could not be lysed by fresh NK cells at all (data not shown). However, the $T3^-$ fraction which was highly enriched with NK cells (Fig. 4) exhibited significantly higher LAK activity against class I-positive fresh leukemia cells. These results suggest that the cytolytic mechanisms of IL-2-activated NK cells are distinct from those of fresh NK cells and that memory cytotoxic T lymphocytes are not a major component of LAK cells.

At the same effector-to-target ratio, fresh leukemia cells were less sensitive to lysis by allogeneic LAK than the cultured cell line, Daudi 1A4. These data suggest that the number of effective cells which actually participate in the lysis of fresh leukemia cells may be considerably less than that in the case of Daudi 1A4.

Taking these results together, it is clear that LAK activity could be induced from some heterogenous populations, and it appears that the populations of actually effective cells for the target cell lysis may be different between fresh leukemia cells and cultured Daudi 1A4 as the targets. Moreover, it is also probable that some populations contribute to the LAK lysis of leukemia cells with multiple lytic mechanisms. Thus, the different susceptibility of leukemia cells to autologous and allogeneic LAK may be attributable to the LAK heterogeneity.

ACKNOWLEDGMENTS

We are grateful to Drs. Koichi Miyamura and Yoshihisa Kodera of The Japanese Red Cross Nagoya First Hospital for providing leukemia cells and peripheral blood of leukemia patients in remission. This work was supported in part by a grant from the Ministry of Health and Welfare of Japan.

(Received Sept. 10, 1987/Accepted Dec. 25, 1987)

REFERENCES

1) Grimm, E. A., Mazumder, A., Zhang, H. Z. and Rosenberg, S. A. Lymphokine-activated killer cell phenomenon. Lysis of natural killer-resistant fresh solid tumor cells by interleukin 2-activated autologous human peripheral blood lymphocytes. *J. Exp. Med.*, **155**, 1823–1841 (1982).

2) Grimm, E. A., Remsey, K. M., Mazumder, A., Willson, D. J., Djeu, J. Y. and Rosenberg, S. A. Lymphokine-activated killer cell phenomenon II. Precursor phenotype is serologically distinct from peripheral T lymphocytes, memory cytotoxic thymus-derived lymphocytes, and natural killer cells. *J. Exp. Med.*, **157**, 884–897 (1983).

3) Grimm, E. A., Robb, R. J., Roth, J. A., Neckers, L. M., Lachman, L. B., Wilson, D. J. and Rosenberg, S. A. Lymphokine-activated killer cell phenomenon III. Evidence that IL-2 is sufficient for direct activation of peripheral blood lymphocytes into lymphokine-activated killder cells. *J. Exp. Med.*, **158**, 1356–1361 (1983).

4) Itoh, K., Tilden, A. B., Kumagai, K. and Balch, C. M. Leu-11$^+$ lymphocytes with natural killer (NK) activity are precursors of recombinant interleukin 2 (rIL-2)-induced activated killer (AK) cells. *J. Immunol.*, **134**, 802–807 (1985).

5) Lotze, M. T., Grimm, E. A., Mazumder, A., Strausser, J. L. and Rosenberg, S. A. Lysis of fresh and cultured autologous tumor by human lymphocytes cultured in T-cell growth factor. *Cancer Res.*, **41**, 4420–4425 (1981).

6) Hersey, P., Bindon, C., Edwards, A., Murray, E., Phillips, G. and McCarthy, W. H. Induction of cytotoxic activity in human lymphocytes against autologous and allogeneic melanoma cells *in vitro* by culture with interleukin 2. *Int. J. Cancer*, **28**, 695–703 (1981).

7) Jacobs, S. K., Wilson, D. J., Melin, G., Parham, C. W., Holcomb, B., Kornblith, P. L. and Grimm, E. A. Interleukin-2 and lymphokine activated killer (LAK) cells in the treatment of malignant glioma: clinical and experimental studies. *Neurol. Res.*, **8**, 81–87 (1986).

8) Mazumder, A., Eberlein, T. J., Grimm, E. A., Wilson, D. J., Keenan, A. M., Aamodt, R. and Rosenberg, S. A. Phase I study of the adoptive immunotherapy of human cancer with lectin activated autologous mononuclear cells. *Cancer*, **53**, 896–905 (1984).

9) Rayner, A. A., Grimm, E. A., Lotze, M. T., Chu, E. W. and Rosenberg, S. A. Lymphokine-activated killer (LAK) cells. Analysis of factors relevant to the immunotherapy of human cancer. *Cancer*, **55**, 1327–1333 (1985).

10) Rosenberg, S. A. Adoptive immunotherapy of cancer: accomplishments and prospects. *Cancer Treat. Rep.*, **68**, 233–255 (1984).

11) Rosenberg, S. A., Lotze, M. T., Muul, L. M., Leitman, S., Chang, A. E., Ettinghausen, S. E., Matory, Y. L., Skibber, J. M., Shiloni, E., Vetto, J. T., Seipp, C. A., Simpson, C. and Reichert, C. M. Observation on the systemic administration of autologous lymphokine-activated killer cells and recombinant interleukin-2 to patients with metastatic cancer. *N. Engl. J. Med.*, **313**, 1485–1492 (1985).

12) Shiiba, K., Suzuki, R., Kawakami, K., Ohuchi, A. and Kumagai, K. Interleukin 2-activated killer cells: generation in collaboration with interferon γ and its suppression in cancer patients. *Cancer Immunol. Immunother.*, **21**, 119–128 (1986).

13) Grimm, E. A. and Wilson, D. J. The human lymphokine-activated killer cell system V. Purified recombinant interleukin 2 activates cytotoxic lymphocytes which lyse both natural killer-resistant autologous and allogeneic tumor and trinitrophenyl-modified autologous peripheral blood lymphocytes. *Cell. Immunol.*, **94**, 568–578 (1985).

14) Dawson, M. M., Johnston, D., Taylor, G. M. and Moore, M. Lymphokine activated killing of fresh human leukaemias. *Leuk. Res.*, **10**, 683–688 (1986).

15) Oshimi, K., Oshimi, Y., Akutsu, M. Takei, Y., Saito, H., Okada, M. and Mizoguchi, H. Cytotoxicity of interleukin 2-activated lymphocytes for leukemia and lymphoma cells. *Blood*, **68**, 938–948 (1986).

16) Timonen, T., Ortaldo, J. R. and Herberman, R. B. Characteristics of human large granular lymphocytes and relationship to natural killer and K cells. *J. Exp. Med.*, **153**, 569–582 (1981).

17) Damle, N. K., Doyle, L. V. and Bradley, E. C. Interleukin 2-activated human killer cells are derived from phenotypically heterogenous precursors. *J. Immunol.*, **137**, 2814–2822 (1986).
18) Ortaldo, J. R., Mason, A. and Overton, R. Lymphokine-activated killer cells. Analysis of progenitors and effectors. *J. Exp. Med.*, **164**, 1193–1205 (1986).
19) Phillips, J. H. and Lanier, L. L. Dissection of the lymphokine-activated killer phenomenon. Relative contribution of peripheral blood natural killer cells and T lymphocytes to cytolysis. *J. Exp. Med.*, **164**, 814–825 (1986).
20) Itoh, K., Shiiba, K., Shimizu, Y., Suzuki, R. and Kumagai, K. Generation of activated killer (AK) cells by recombinant interleukin 2 (rIL 2) in collaboration with interferon-γ (IFN-γ). *J. Immunol.*, **134**, 3124–3129 (1985).
21) Hoyer, M., Meineke, T., Lewis, W., Zwilling, B. and Rinehart, J. Characterization and modulation of human lymphokine (interleukin 2) activated killer cell induction. *Cancer Res.*, **46**, 2834–2838 (1986).
22) Trinchieri, G., Matsumoto-Kobayashi, M., Clark, S. C., Seehra, J., London, L. and Perussia, B. Response of resting human peripheral blood natural killer cells to interleukin 2. *J. Exp. Med.*, **160**, 1147–1169 (1984).
23) Piontek, G. E., Taniguchi, K., Ljunggren, H., Gronberg, A., Kiessling, R., Klein, G. and Karre, K. YAC-1 MHC class I variants reveal an association between decreased NK sensitivity and increased H-2 expression after interferon treatment or *in vivo* passage. *J. Immunol.*, **135**, 4281–4288 (1985).