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Detection of Cytotoxicity of Freshly Obtained Lymphocytes and of Lymphocytes Activated with Recombinant Interleukin II (rIL-2) against Lung Cancer Cell Lines by Human Tumor Clonogenic Assay (HTCA)

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Abstract—*The cytotoxicity of freshly obtained human peripheral blood lymphocytes and lymphocytes activated with human recombinant interleukin* II (*rIL-2*) *was evaluated against lung cancer cell lines by the human tumor clonogenic assay. Colony formation of all human lung cancer cell lines, PC-*1, 3, 6, 7, 9, 13 *and* 14 *were suppressed by lymphocytes activated with* 100 units/ml *of human rIL-2 for* 72 hr. *However, the degree of the suppression of colony formation by lymphocytes activated with rIL-2 was different for each cell line. The per cent inhibition of colony formation obtained by HTCA correlated well with the per cent cytolysis obtained by* 51*Cr-release assay for all cell lines. HTCA provides a very useful tool for the detection of cytotoxicity of lymphocytes against clonogenic tumor cells.*

INTRODUCTION

NATURAL killer (NK) cells, a subpopulation of peripheral blood lymphocytes (PBL), are involved in 'immune surveillance' against tumor cells, defence against viral infection, and regulation of hemopoiesis [1]. Although NK cells express cytotoxicity against K-562 cells, most epithelial tumors are resistant to NK cells, and the potential role of spontaneous cytotoxicity by NK cells in human cancer is unclear.

Due to recent progress in genetic engineering, purified human recombinant interleukin II (rIL-2) is readily available. Lymphokine activated killer (LAK) cells, generated by *in vitro* exposure of normal lymphocytes to IL-2, have been reported to recognize and lyse fresh autologous NK-resistant tumor cells [2]. LAK cells can also be induced by *in vivo* administration of rIL-2 [3]. There are several trials examining the possible use of rIL-2 and/or LAK cells in the treatment of human tumors.

Previously we have reported that NK activity against K-562 cells and antibody dependent cellular cytotoxicities against PC-9 cells can be detected by a modification of human tumor clonogenic cell assay (HTCA) and that the lymphocytes-induced colony inhibition detected by HTCA correlated well with the cytotoxicity of lymphocytes detected by ^{51}Cr-release assay ($P < 0.001$) [4]. In this report, the cytotoxicity of lymphocytes activated with rIL-2 against various lung cancer cell lines were evaluated by HTCA, and the colony inhibition detected by HTCA was compared to the cytotoxicity detected by the ^{51}Cr-release assay.

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MATERIALS AND METHODS

Preparation of freshly obtained lymphocytes

PBL was obtained by venopuncture from normal healthy volunteers with a needle attached to a plastic syringe containing heparin. The blood was diluted with Eagle's minimum essential medium

(MEM), and the mononuclear cells were separated by centrifugation on a Ficoll–Conray cushion (1080g) according to Boyum's method [5]. The interface was collected and the cells were washed twice with MEM and once with RPMI-1640 medium containing 10% heat-inactivated fetal calf serum (RPMI-FCS). The mononuclear cells in RPMI-FCS were incubated in a Falcon 3003 plastic dish (Falcon Plastics Co., U.S.A.) in a humidified atmosphere of 5% CO_2, 95% air, at 37°C for 1 hr. Later non-adherent cells were collected by repeated extensive washing with MEM. More than 95% of these non-adherent cells were lymphocytes. The number of cells was adjusted (E/T ratio was 12.5 : 1, 25 : 1 or 50 : 1) before the cytotoxicity assay.

Preparation of lymphocytes activated with rIL-2

For the preparation of lymphocytes activated with rIL-2, non-adherent lymphocytes suspended in RPMI-FCS (2×10^6/ml) were incubated with rIL-2 (from Shionogi Pharmaceutical K.K., Osaka, Japan) in flat-bottomed multiwell plates (Nunc 24 wells 16mm diameter, Copenhagen, Denmark).

To determine the effect of the concentration of rIL-2 and incubation time of lymphocytes with rIL-2 on the cytotoxicity, lymphocytes were incubated with 1–1 $\times 10^4$ I.U. of rIL-2 for 72 hr, or with 1×10^2 rIL-2 for 0–144 hr. The effect of the concentration of human rIL-2 and the time of incubation on the cytotoxicity of lymphocytes against K-562 and PC-9 cells detected by ^{51}Cr-release assay are presented in Table 1 and Table 2. The cytotoxicity of lymphocytes against K-562 and PC-9 cells reached maximum levels by incubating PBL with 10 and 1×10^2 units of human rIL-2 for 72 hr, respectively (Table 1). Table 2 shows that the cytotoxicity of lymphocytes against K-562 and PC-9 cells reached maximum levels by incubating PBL with 1×10^2 units of rIL-2 for 72 hr. In further experiments of HTCA and ^{51}Cr-release assay the non-adherent lymphocytes were incubated with 1×10^2 units of rIL-2 for 72 hr. Three different E/T ratios (12.5 : 1, 25 : 1 and 50 : 1) were used in subsequent experiments.

Table 1. *Effect of the concentration of human rIL-2 on per cent specific ^{51}Cr-release*

Units of rIL-2 (/ml)	Per cent specific ^{51}Cr-release	
	K-562 cells (mean ± S.E.)	PC-9 cells (mean ± S.E.)
Control	38.2 ± 14.0	11.2 ± 4.5
1	66.7 ± 5.8	15.2 ± 4.2
10	78.4 ± 0.8	39.8 ± 6.4
1×10^2	78.0 ± 1.1	49.1 ± 2.8
1×10^3	78.3 ± 1.3	48.2 ± 1.3
1×10^4	76.6 ± 0.9	48.7 ± 5.8

K-562 cells and PC-9 cells were incubated for 5 hr with lymphocytes activated for 72 hr with human rIL-2.
Effector/Target = 50 : 1 Data are from four independent experiments.
Control lymphocytes were incubated without rIL-2 for 72 hr.

Table 2. *Effect of the time of incubation of lymphocytes with rIL-2 on per cent specific ^{51}Cr-release*

Incubation time with rIL-2 (hr)	Per cent specific ^{51}Cr-release	
	K-562 cells (mean ± S.E.)	PC-9 cells (mean ± S.E.)
Control	28.3 ± 5.1	16.3 ± 2.5
24	65.8 ± 3.1	39.0 ± 4.0
48	75.0 ± 2.0	60.0 ± 4.0
72	80.1 ± 0.9	69.2 ± 4.8
96	79.5 ± 0.6	74.0 ± 3.2
120	79.8 ± 0.8	77.1 ± 0.8
144	76.8 ± 1.7	76.2 ± 5.9

K-562 cells and PC-9 cells were incubated for 5 hr with lymphocytes activated with 1×10^2 units of human rIL-2.
Effector/target ratio = 50 : 1.
Data are from three independent experiments.
Control lymphocytes were incubated without rIL-2 for 144 hr.

Tumor cells

The human cell line, K-562 cell derived from the pleural effusion of a patient with chronic myelogenous leukemia in blastic crisis, and PC-1, 3, 6, 7, 9, 10, 13, and PC-14 cells derived from carcinoma of the lung (kindly donated by Professor Y. Hayata, Tokyo Medical College), were used as target cells in the cytotoxicity assay. The detailed characteristics including clonogenic activities of these lung cancer cell lines were shown in Table 3. PC-1, 7, 9, 13 and 14 were derived from adenocarcinoma of the lung. PC-3 and 10 were derived from squamous cell carcinoma of the lung and PC-6 was from small cell carcinoma of the lung.

Table 3. *Characteristics of human lung cancer cell lines*

Cell line	Histology	Plating efficiency (%)	Plating cell number	Days of culture
PC-1	Adeno	0.5	1×10^5	14
PC-3	Squamous	0.4	1×10^5	14
PC-6	Small	0.6	1×10^5	21
PC-7	Adeno	11.3	1×10^4	14
PC-9	Adeno	36.0	1×10^4	7
PC-10	Squamous	not suitable to HTCA		
PC-13	Adeno	19.6	1×10^4	7
PC-14	Adeno	17.5	1×10^4	7

All cell lines were derived from human lung cancer. The plating numbers of tumor cells were determined according to the growth characteristics of each cell lines. The plating efficiency (%) was calculated by the following formula:

$$\frac{\text{number of colonies}}{\text{number of cells plated}} \times 100.$$

The days of culture were determined in each cell line based on the day that colony numbers reach maximum.

Labelling of tumor cells

Target cells (2.5×10^6/0.25ml) were incubated with 0.25 ml of 125 μCi of $Na_2{}^{51}CrO_4$ (Japan Radioisotope Association, Tokyo) for 60 min, and washed twice with 15 ml of MEM and once with 15 ml of RPMI-FCS to remove unbound ^{51}Cr. Finally, the cells were suspended at a concentration of 10^5/ml in RPMI-FCS.

^{51}Cr-release assay

For determination of the cytotoxicity of freshly obtained lymphocytes and lymphocytes activated with rIL-2 by ^{51}Cr-release assay, 0.1 ml quantities of the target cell suspension (10^5/ml) were mixed with 0.1 ml of the lymphocytes suspension (1.25×10^6/ml, 2.5×10^6/ml and 5×10^6/ml) which produced final *E/T* ratio of 12.5 : 1, 25 : 1 and 50 : 1, respectively.

The reaction mixtures were carried out in the cells of 96-well V-bottomed microtitre plate (Limbro Scientific Co., Hamden, CT, U.S.A.). These plates were incubated in a humidified atmosphere of 5% CO_2, 95% air at 37°C for 5 hr. After incubation all the plates were centrifuged at 400 ***g*** for 10 min, and 0.1 ml of the supernatant from each well was removed and its radioactivity was counted by an auto-γ-counter. Spontaneous target cell release was determined from the supernatant of the target cells cultured without effector cells. The maximum release of ^{51}Cr was obtained by treatment with five cycles of freezing and thawing in a dry-ice/alcohol mixture and hot water. Triplicate cultures were used throughout.

The percentage of cytotoxicity was calculated as:

$$100 \times \frac{\text{experimental release} - \text{spontaneous release}}{\text{maximum release} - \text{spontaneous release}}$$

and all data were expressed as mean ± S.E. (standard error).

Human tumor clonogenic assay

The HTCA used in this study was a modification of the double agar method developed by Hamburger and Salmon [6]. The bottom layer was 0.5% agar (Bacto; Difco, Detroit, MI) in RPMI-1640 medium which contained 10% heat inactivated FCS and 10% heat-inactivated horse serum (HS). The top layer was 0.3% agar in RPMI-1640 medium which contained 10% FCS, 10% HS, glutamine (0.05 mmol/ml), Na-pyruvate (0.5 mmol/ml), serine (0.01 mmol/ml), and insulin (Novo Industry, Copenhagen, Denmark) (1.6 units/ml).

For the determination of the cytotoxicity of lymphocytes by HTCA, 3 or 30×10^4 (final plating numbers per well of each cell lines is listed in Table 3) of tumor cells were mixed with lymphocytes at *E/T* ratio 12.5 : 1, 25 : 1 and 50 : 1 respectively and incubated for 5 hr at 37°C in 2.7 ml top medium without agar.

After incubation, 0.3 ml of 3% agar was added to each tube. One ml of the resultant mixture was plated in 35-mm Falcon 1008 plastic dishes. The colony formation of tumor cells incubated without lymphocytes was considered to be the control number of colonies. The dishes were incubated in a humidified atmosphere of 5% CO_2, at 37°C for 7–14 days depending on the growth characteristics of tumor cells. Colonies at least 60 μm in a diameter were counted with a colony analyzer (CP2000, Siraimatsu Instrument Ltd., Osaka, Tokyo, Japan). Each experiment was performed in triplicate. Percent inhibition of colony formation was determined by the following formula:

$$100 - \frac{\text{Number of colonies per test tube}}{\text{Number of colonies per control plate}} \times 100$$

Statistical analysis

All data were analyzed for significance by the two-tailed Student *t*-test. *P* values were calculated by comparison of experimental groups.

RESULTS

As the growth rates of these lung cancer cell lines were different, we determined the optimal cell number for plating. Cells from several lung cancer cell lines were plated at different cell numbers ranging from 5×10^3 to 5×10^5 /dish based on several incubation times respectively. Figures 1a and 1b show the example of the results of experiments determining the appropriate plating number of tumor cells. Figure 1a shows the growth characteristics of PC-3. Figure 1b shows the growth characteristics of PC-14. When the numbers of tumor cells plated were too small, the inhibition of colony formation by lymphocytes was not reproducible (standard deviations of more than 10%). If the numbers of cells plated were too great, the number of lymphocytes plated was also too great, and the reasonable data were not obtained because of depletion of nutrients [7]. Based on this evidence, the plating numbers of tumor cells were determined according to the growth characteristics of each cell line. As 2000 was the upper limit of colonies in each dish, we choose the conditions that would yield about 1500 colonies per dish for each cell line. For instance, for the PC-3 cell line, which has a low plating efficiency, it was necessary to plate 1×10^5 cells per dish and for the PC-9 cell line, which has high plating efficiency, 1×10^4 cells per dish were plated. The incubation periods for the colony counts to reach a plateau phase were 14 and 7 days for PC-3 and PC-9 cells, respectively. The

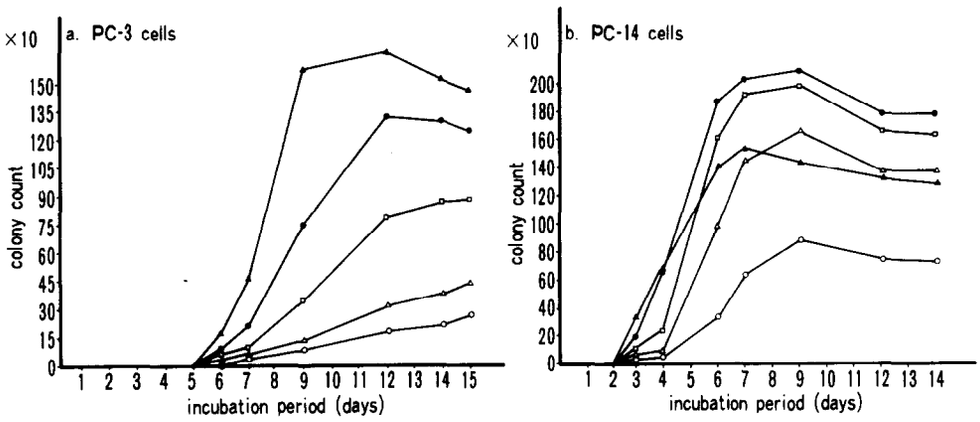

Fig. 1. Growth curves of PC-3 cells (a) and PC-14 cells (b) by different cell number plating in each dish ○: 5 × 10^3/*dish*, △: 1 × 10^4/*dish*, □: 2.5 × 10^4/*dish*, ●: 5 × 10^4/*dish*, ▲: 1 × 10^5/*dish*. *Each point represents the mean of triplicate.*

final plating number per plate and plating efficiencies of each cell line in such experimental condition are listed in Table 3. The optimal days of culture period was determined for each cell line based on the days that the colony numbers reached a plateau (Table 3).

Table 4 shows the cytotoxicity of freshly obtained lymphocytes and of lymphocytes activated with rIL-2 against eight lung cancer cell lines. It was difficult to label PC-6 cells with ^{51}Cr, and PC-10 formed no colonies by HTCA. These PC cell lines were generally resistant to cytotoxicity of freshly obtained lymphocytes except for PC-13 which shows the cytotoxicity of 46.9 ± 6.9% at the *E/T* ratio of 50 : 1 by ^{51}Cr-release assay. The cytotoxicity of lymphocytes against PC-9 and PC-10 cells was less than 10% even at the *E/T* ratio of 50 : 1. In freshly obtained lymphocytes, the cytoto-

Table 4. Cytotoxicity of lymphocytes activated with rIL-2 and freshly obtained lymphocytes against several lung cancer cell lines

Cell lines	E/T	Cytotoxicity of lymphocytes activated with rIL-2 HTCA	^{51}Cr	Cytotoxicity of freshly obtained lymphocytes HTCA	^{51}Cr
PC-1	12.5/1	92.2 ± 1.4**	40.2 ± 2.2	0 ± 0	6.5 ± 0.7
(6)*	25/1	94.4 ± 0.6	49.7 ± 1.3	1.0 ± 0.7	11.3 ± 0.7
	50/1	93.0 ± 0.7	54.4 ± 1.5	4.2 ± 0.7	16.8 ± 1.2
PC-3	12.5/1	90.0 ± 0.9	56.6 ± 1.7	0 ± 0	9.8 ± 0.9
(5)	25/1	92.4 ± 1.5	61.4 ± 2.1	3.8 ± 1.2	14.7 ± 0.2
	50/1	87.4 ± 0.6	64.2 ± 2.1	3.6 ± 0.7	20.6 ± 0.4
PC-6	12.5/1	–	–	6.6	–
(1)	25/1	–	–	9.7	–
	50/1	89.6 ± 0.6	–	12.0	–
PC-7	12.5/1	54.4 ± 1.9	43.7 ± 2.6	0 ± 0	7.3 ± 1.6
(3)	25/1	57.1 ± 0.9	51.1 ± 0.6	0 ± 0	6.9 ± 0.1
	50/1	63.0 ± 3.1	53.2 ± 2.4	0 ± 0	12.4 ± 0.6
PC-9	12.5/1	20.1 ± 5.2	27.4 ± 5.5	1.9 ± 1.3	0.9 ± 0.5
(6)	25/1	20.9 ± 6.0	36.8 ± 6.9	0 ± 0	0.5 ± 0.3
	50/1	27.6 ± 5.7	49.2 ± 5.9	0 ± 0	0 ± 0
PC-10	12.5/1	–	44.7 ± 4.6	–	7.7
(2)	25/1	–	50.4 ± 2.8	–	4.5
	50/1	–	60.0 ± 10.7	–	5.9
PC-13	12.5/1	94.4 ± 0.9	73.9 ± 1.5	5.8 ± 2.7	28.6 ± 2.5
(3)	25/1	97.1 ± 0.8	77.3 ± 1.9	11.3 ± 4.3	38.0 ± 5.7
	50/1	96.4 ± 1.6	80.3 ± 1.0	15.2 ± 0.7	46.9 ± 6.9
PC-14	12.5/1	44.8 ± 7.1	45.8 ± 5.2	3.8 ± 0.8	11.1 ± 0.7
(6)	25/1	66.8 ± 8.8	53.3 ± 5.7	6.5 ± 1.1	14.1 ± 0.5
	50/1	71.8 ± 10.2	58.6 ± 5.9	15.0 ± 2.8	18.0 ± 2.1

( )* No. of donors.
** mean ± S.E.
Cytotoxicity of lymphocytes activated with rIL-2 and freshly obtained lymphocytes against each cell lines were determined by HTCA and ^{51}Cr-release assay.

xicity as measured by ^{51}Cr-release assay was higher than the colony inhibition in HTCA for all cell lines. In contrast, all cell lines were killed by lymphocytes activated with rIL-2. Colony inhibition measured by HTCA was higher than the cytotoxicity in ^{51}Cr-release assay in lymphocytes activated with rIL-2 for all cell lines except PC-9. However the values of HTCA and ^{51}Cr-release assay from several donors were significantly correlated in each cell line (data not shown). In PC-1, 3, 7, 9, 13 and 14 cell lines, ^{51}Cr-release assay and HTCA were performed simultaneously using the same lymphocytes and correlation between the two assays was also evaluated. Number of donors for each cell line was 5, 5, 3, 6, 3 and 6 in PC-1, 3, 7, 9, 13 and 14 cell lines, respectively. Figures 2a, b and c show the positive correlation of per cent inhibition of colony formation obtained by HTCA and per cent cytolysis obtained by ^{51}Cr-release assay in every *E/T* ratio of 12.5 : 1 ($r = 0.90$, $P < 0.001$), 25 : 1 ($r = 0.91$, $P < 0.001$) and 50 : 1 ($r = 0.89$, $P < 0.001$). The per cent inhibition of colony formation by lymphocytes activated with rIL-2 obtained by HTCA ranged from 20.1 to 94.4, from 20.9 to 97.1 and from 27.6 to 96.4 at *E/T* ratio 12.5 : 1, 25 : 1 and 50 : 1 respectively, and per cent cytolysis obtained by ^{51}Cr-release assay by lymphocytes activated with rIL-2 ranged from 27.4 to 73.9, from 36.8 to 77.3 and from 49.2 to 80.3 at *E/T* ratio 12.5 : 1, 25 : 1 and 50 : 1, respectively.

DISCUSSION

Nomori *et al.* reported previously that the NK activity and antibody dependent cellular cytotoxicity of lymphocytes on K-562 and PC-9 cells could be detected with HTCA [4].

In the present study, the cytotoxicity of lymphocytes against PC-1, 3, 6, 7, 9, 10, 13 and 14 cell lines were evaluated by HTCA and ^{51}Cr-release assay. PC-10 formed no colony by HTCA, and it was impossible to label PC-6 cells with ^{51}Cr. All of PC cell lines except PC-13 were resistant to freshly obtained lymphocytes. On the other hand, these cell lines were sensitive to lymphocytes activated with rIL-2. The degree of colony inhibition evaluated by HTCA as well as the cytotoxicity detected by ^{51}Cr-release assay were different for each of the six cell lines. These differences may depend on the differences in the numbers of the determinant against lymphocytes activated with rIL-2 on each tumor cell.

There was a positive correlation between per cent inhibition of colony formation obtained by HTCA and per cent cytolysis obtained by ^{51}Cr-release assay in lymphocytes activated with rIL-2. However, there exist several important differences between the results of these two assays. First, in most of the cell lines except PC-9 cells, HTCA was more sensitive in detecting the cytotoxicity of lymphocytes activated with rIL-2. Second, inhibition of colony formation was not observed in HTCA if the cytotoxicity of lymphocytes expressed as per cent cytolysis was less than 10–15% by ^{51}Cr-release assay in most cell lines. The exact reasons of these phenomena are unclear, but these phenomena seem to be derived from the difference of methodology of these two assays. With HTCA, it is possible to expose tumor cells to lymphocytes for a long-term period. Long-term incubation in ^{51}Cr-release assay is difficult because of the increased spontaneous release. In addition, the target cells detected by the two assays differ. Third, non-lethally damaged cells may release ^{51}Cr, and still regrow in soft agar. In addition, HTCA allows growth of human tumor cells while preventing the growth of normal host cells. HTCA may be able to solve problems arising in the determination of lymphocytes cytotoxicity for autologous freshly isolated tumor cells by the ^{51}Cr-release assay.

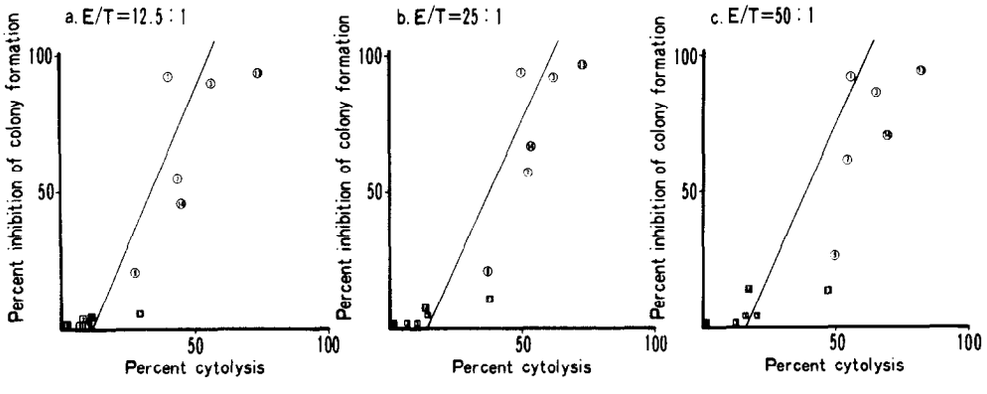

Fig. 2 Correlation of cytotoxicity of freshly obtained lymphocytes (□) and lymphocytes activated with rIL-2 (○) between per cent inhibition of colony formation obtained by HTCA and per cent cytolysis obtained by ^{51}Cr-release assay in PC-1, 3, 7, 9, 13 and 14 (Table 4). Figures in (□) and (○) represent number of PC cell lines. The correlation was highly significant in E/T = 12.5 : 1 (a) ($r = 0.90$, $P < 0.001$), E/T = 25:1 (b)($r = 0.91$, $P < 0.001$) *and* E/T = 50 : 1 (c) ($r = 0.89$, $P < 0.001$). *The solid lines represent the least squares regression lines.*

The clonogenic tumor cells have been considered to be related to tumor stem cell that metastasize and regrow after surgery, radiation therapy and chemotherapy. Therefore, HTCA may provide an important tool for the detection of cytotoxicity of lymphocytes against clonogenic tumor cells.

REFERENCES

1. Trinchieri G, Perussia B. Human natural killer cells: biologic and pathologic aspects. *Lab Invest* 1984, **50**, 489–513.
2. Rosenstain M, Yron I, Kaufmann Y, *et al.* Lymphokine-activated killer cells: lysis of fresh synergistic natural killer-resistant murine tumor cells by lymphocytes cultured in interleukin II. *Cancer Res* 1984, **44**, 1946–1953.
3. Saijo N, Ozaki A, Sakurai M, *et al. In vivo* augmentation of the cytotoxicity of spleen lymphocytes against synergistic B-16 melanoma cells and the suppression of the artificial metastases in C57BL/6 mice by subcutaneous multiple injection of high dose human recombinant interleukin-2. *Jpn J Cancer Res* (*Gann*) 1986 (submitted).
4. Nomori H, Saijo N, Fujita J, *et al.* Detection of NK activity and antibody-dependent cellular cytotoxicity of lymphocytes by human tumor clonogenic assay – its correlation with the 51Cr-release assay. *Int J Cancer* 1985, **35**, 449–455.
5. Boyum A. Separation of leukocytes from blood and bone marrow. *Scand J Clin Lab Invest* 1968, **21**, (suppl.97), 77.
6. Hamburger AW, Salmon SE. Primary bioassay of human myeloma stem cells. *Science* 1977, **197**, 461–463.
7. Lee YC, Saijo N, Sasaki Y, *et al.* Clonogenic pattern of human pulmonary adenocarcinoma cell lines (PC-9, PC-13 and PC-14) and how they influence on the result of chemosensitivity in human tumor clonogenic assay. *Jpn J Clin Oncol* 1986 (in press).