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TWO COMPONENTS IN L1210 CELLS AND THEIR GROWTH CHARACTERIZATION

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ABSTRACT

L1210 cells were separated into two populations by the standardized method of mononuclear cell purification using Ficoll-Hypaque. One population (L-cells) was obtained in the usual mononuclear layer above Ficoll-Hypaque and the other (Bo-cells) was found at the bottom of the tubes below Ficoll-Hypaque. The biological characteristics of both populations were evaluated by the following parameters at every 24 hr and up to 168 hr after intraperitoneal inoculation of 10^5 L1210 cells into DBA/2J mice: growth kinetics *in vivo* and *in vitro*, viability, morphological features, cell volume, ^{3}H-thymidine (TdR) uptake, contents of protein, DNA and taurine, and modal chromosome number. Protein content was generally higher in Bo-cells than in L-cells throughout the observation period. The mean cell volume of Bo-cells increased and decreased more rapidly compared with L-cells, and its was significantly larger at earlier time points and smaller at later time points. DNA content and cell proliferation patterns *in vitro* and *in vivo* of Bo-cells showed similar changes. ^{3}H-TdR uptake was significantly higher in Bo-cells at any time point except for the last 168 hr, and taurine contents in both L- and Bo-cells showed almost mirror-image values of their respective ^{3}H-TdR uptakes. Viability and cytological findings did not show any specific difference between L- and Bo-cells. These studies demonstrated the heterogeneity of L1210 cells, which until now have been believed to be very homogeneous tumor cells. The correlation between cell growth pattern and effectiveness of antineoplastic agents was also discussed.

Key words: L1210 cells, Ficoll-Hypaque method, Heterogeneity of leukemia, Growth kinetics, Taurine

INTRODUCTION

L1210 lymphocytic leukemia is one of the most important screening tumors for the evaluation of candidates for antineoplastic agents. The National Cancer Institute (NCI) first reported the great predictive value of L1210 lymphocytic leukemia as a screening tumor in 1966[1] and endorsed the importance of its role by listing it on the new panel for screening method of antineoplastic agents in the Division of Cancer Treatment of NCI.[2]

L1210 cells have been recognized to consist of a homogeneous cell group with monotonous cytological features. These cells have high proliferative ability with short doubling time, and mice inoculated with L1210 cells show constant survival times.

We recently observed, unexpectedly, that the standardized method of mononuclear cell purification using Ficoll-Hypaque[5] separated L1210 cells into two populations. One population (L-cells) was found in the usual mononuclear cell layer above the Ficoll-Hypaque

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cushion and the other (Bo-cells) was at the bottom of the tubes below Ficoll-Hypaque where granulocytes or red blood cells should be present. It was confirmed that Bo-cells were neither dead cells nor granulocytes but living L1210 cells. Since it is known that tumor growth rate is related to responses to antitumor drugs,[6-8] we thought that it was of importance to confirm the heterogeneity of L1210 cells. Therefore, we tested the biological differences between these L- and Bo-cells in growth ability, including protein, DNA and taurine contents. Taurine, a highly acidic sulfur-containing amino acid, was reproted to be present at the highest concentration among the free amino acids in normal human leukocytes[9] and to have some relationship with leukemogenesis in L1210 cells and human leukemia cells.[10,11] In this paper we report the heterogeneity of L1210 leukemia cells in their cell proliferation pattern.

MATERIALS AND METHODS

Mice: DBA/2J female mice weighing 20—25g were obtained from Jakson Laboratory (Bar Harbour, Maine).

L1210 cells: L1210 leukemia cells were supplied by A.D. Little Company (Cambridge, Massachusetts) and were maintained in DBA/2J mice by weekly i.p. passage of 10^5 cells. Every 24 hr, up to 168 hr after inoculation of 10^5 L1210 cells, a group of 5 mice were sacrificed by cervical dislocation. The cells were collected by washing the peritoneal cavity with Hanks, balanced salt solution without Ca^{++} and Mg^{++} (HBSS). The cell suspension was centrifuged at 250 x g for 15 min. The pellet was resuspended in HBSS, and layered on Ficoll-Hypaque (Pharmacia Fine Chemicals, density = 1.077 g/ml), and centrifuged at 400 x g for 30 min. The cells layered in the interphase were designated as lymphocyte layer cells (L-cells) and were to be lighter than 1.077 g/ml. Additionally, the sedimented cells at the bottom of the tube were collected and referred to as bottom cells (Bo-cells). These cells in both groups were washed by HBSS at 160 x g for 15 min and resuspended in HBSS. The cell numbers of these samples were determined using both a hemocytometer and a Coulter counter Model ZBI. Aliquots of these cell suspensions were removed for the determination of viability, cell volume and morphology. The remaining cell suspensions were diluted to give a concentration of 5 to 20 x 10^6 cells/ml and sonicated for 30 sec at 250W using a Branson Cell Sonifier Model 350A. Samples were then stored at −80°C for further biochemical assays.

Normal lymphocytes: Lymphocytes were obtained from the lymph nodes of DBA/2J mice. The lymph nodes were minced by scissors in a Petri dish with HBSS, and particles were further dispersed by aspirating them through an 18 gauge needle. The cell suspension was then passed through a column which was lightly packed with glass wool, and the filtrate was layered on Ficoll-Hypaque. The cells were similarly separated into lymphocytelayer cells and bottom cells, and were designated as lymph-node L-cells and lymph-node Bo-cells, respectively.

Viability and Morphological findings of cells: The proportion of viable cells was determined by trypan blue (0.4% w/v in physiological saline) exclusion. The differentials of cells were examined on preparations stained by May-Gruenwald-Giemsa under a light microscope.

Cell volume analysis: The cell volume distribution and the mean cellular volume (MCV) were measured using a Coulter Channel Analyzer CHL2 and a Coulter counter Model ZBI. The aperture's diameter was 100 μm and calibration was performed by standard polystyrene

beads with a diameter of 9.69 μm. MCV was calculated by the following formula:

$$MCV = [\ (\text{mean channel No.} \times ww/100) \times BCT\ ] \times TF$$

ww: window width (100)
BCT: base channel threshold (5)
TF: threshold factor (10.34 was given by calibration)

The cell volume distribution curves were made when the peak channel had accumulated to 10^3 cells.

Protein assay: The cellular protein contents were measured by the protein-dye binding assay of Bradford.[12] The reagent was obtained from Bio-Rad Laboratories (Rockville Center, New York). The sample containing 1 to 10 x 10^5 cells was mixed with Bio-Rad reagent (final concentration: 0.01% Coomassie Brilliant Blue G-250, 4.7% ethanol and 8.5% phosphoric acid) and the absorbance at 595 nm was measured by a spectrophotometer. A standard curve was plotted for each experiment.

DNA assay: The DNA content of cells was determined using a fluorometric assay modified from Kissane and Robins.[13] The aliquot of a sample containing 1 to 10 x 10^5 cells was dried completely after treating with 10% trichloroacetic acid, ethanol and anhydrous ether; it was then reacted with diaminobenzoic acid-2HCl (Aldrich Chemical Co., Milwaukee, Wisconsin). The fluorescence was read by a Turner Spectrofluorometer at 410 nm of excitation and 520 nm of emission.

Taurine assay: The cellular taurine contents were determined by fluorometric assay using high performance liquid chromatography after deproteinization of samples by ethanol. The extracts from 2 to 10 x 10^3 cells were enough for this assay. The detailed method was described in our previous report.[10] Briefly, the sample was dissolved in 0.5% (v/v) acetic acid and passed through the column at a constant flow rate of 0.5 ml/min. The sample was reacted with the derivatizing agent containing o-phthaldialdehyde with 2-mercaptoethanol in a borate buffer of pH 10.4 and the fluorescence was measured. High performance liquid chromatography was performed using the following: a) Model UK 6 injector, b) Model 6000A solvent delivery system, c) Fatty acid analysis column, and d) Model 420-C and 420-E fluorometric detector (excitation at 325 nm, emission at 410 nm) from Waters' Associates (Milford, Massachusetts).

^{3}H-TdR uptake: L- and Bo-cells were prepared in McCoy's 5A medium instead of HBSS. The ^{3}H-TdR (specific activity 23 Ci/mmole) was added to the cell suspension to give a concentration of 100 μCi/ml, then it was incubated at 37°C for 2 hr. Unlabeled thymidine (84.3 μg/ml) was then added to wash the cells on the Millipore filter after incubation. The filter was placed in a scintillation vial and the radioactivity was counted using a Packard scintillation counter.

Chromosomal analysis: L- and Bo-cells at 96 hr were cultured in RPMI 1640 medium supplemented with 10% fetal calf serum (FCS) (GIBCO, Grand Island, New York) for 2 days at 37°C, and then 0.4 μg of colchicine was added. Two hours later, these cell suspensions were treated with a hypotonic solution of 0.075 M KCl for 20 min at room temperature and centrifuged at 400xg for 5 min, then fixed with a 3:1 mixture of methanol and acetic acid (v/v). The flame-dried preparations made from a drop of the above cell suspenson were stained by Giemsa. The chromosome number of 100 well-spread cells in metaphase was

counted.

In vitro cell growth: L- and Bo-cells at 96 hr were suspended in glass tubes at a cell density of 1 x 10^3 cells/ml in RPMI 1640 medium supplemented with 10% FCS, .12% of sodium bicarbonate, 100 μg/ml of kanamycin and 10 μM 2-hydroxyethyldisulfide (Aldrich Chemical Co.) The cell suspensions were incubated in a humid atmosphere of 5% CO_2 and 95% air at 37°C without medium change. The cell numbers were counted in triplicate using a Coulter counter Model ZBI during the successive 10 days after initiation of the culture.

RESULT

In the present experiments, a group of five mice were used at each time point and altogether 175 mice were used. The increase in cell numbers of L- and Bo-cells in vivo are shown in Fig. 1. The exponential increase in Bo-cell number occurred from 48 to 120 hr with a doubling time of 7.2 hr, while in L-cells it occurred from 72 to 144 hr with a doubling time of 10.7 hr. Bo-cells proliferated more rapidly and reached a plateau earlier than L-cells. It was noticeable that the decrease in cell number of Bo-cells at 48 and 144 hr was significant compared with that at 24 and 120 hr, respectively. The differentials of cells collected at 0 hr, i.e., the cells obtained from the peritoneal cavity before the inoculation of L1210 cells were composed of 90% lymphocytes and 5% monocytes in L-cells, and 70% lymphocytes and 20% mastocytes in Bo-cells. The cells collected at 24 and 48 hr were not L1210 cells but were mainly monocytes and lymphocytes. The percentage of L1210 cells in the ascites became 50% at 72 hr and reached 95% at 96 hr. At 96 hr both L- and Bo-cells were a mixture of L1210 cells with different sizes and different stages of maturation and were diffficult to distinguish morphologically from one another except that there was less variety in Bo-cells as shown in Fig. 2 a and b. However, when L- and Bo-cells were compared at a later period, much smaller cells were found in Bo-cells at 168 hr compared with L-cells.

The viability of L- and Bo-cells at each time point was examined to exclude the possible presence of dead cells among the Bo-cells. The viability was always more than 95% in both groups except that the viability of Bo-cells at 24 and 48 hr was around 80%.

Bo-cells were re-layered on the Ficoll-Hypaque cushion to exclude the possible effect of aggregation of cells by overloading. All cells sedimented to the bottom without leaving any cells above the Ficoll-Hypaque.

L- and Bo-cells could not be discriminated by a given cell size, since cells in both groups changed cell size independently, and one group of cells was larger at one time point and smaller at another time point as shown in Fig. 3. Change of MCV in Bo-cells occurred more rapidly and greatly than that in L-cells. In Bo-cells the most rapid increase of cell size was seen from 48 to 72hr, while in L-cells it was seen from 72 to 96 hr. These time points were coincident with the time points showing rapid increases in the numbers of L- and Bo-cells (Fig. 1). Fig. 4 clarifies that the change of MCV in L-cells was not due to a change in the modal number of relative cell volume but mainly due to a change in the percentage of large cells, while that in Bo-cells was due to a change in the modal number of relative cell volume; in other words, Bo-cells were more homogeneous with respect to cell size than L-cells at every time point except24 and 48 hr.

The chromosomal number was examined at 96 hr. The modal chromosomal number was 41 in both L- and Bo-cells as shown in Fig. 5. The distribution of chromosome numbers was

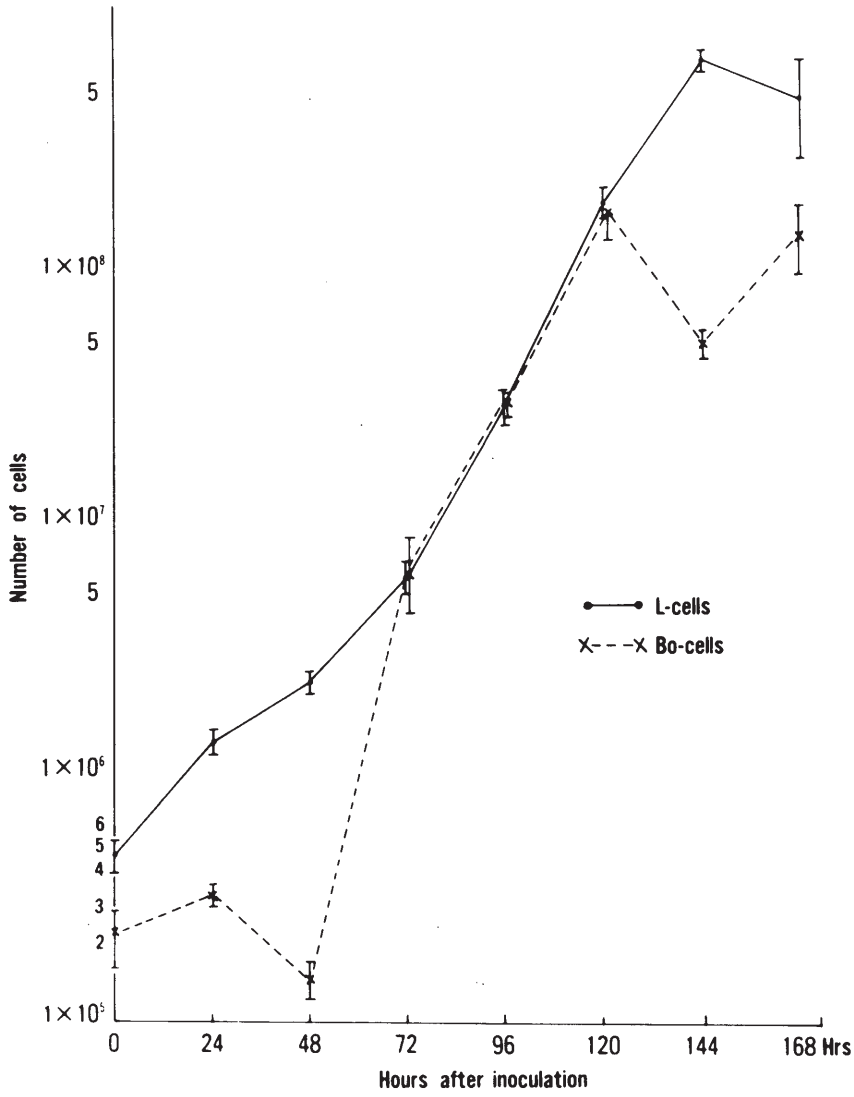


Fig. 1 L1210 cell growth in vivo. Mean cell numbers (±SE) of L- and Bo-cells collected from the peritoneal cavity of 25 mice are shown at 24 hr intervals after inoculation of 10^5 L1210 cells. The doubling time of L1210 cells in this experiment was 12 hr.

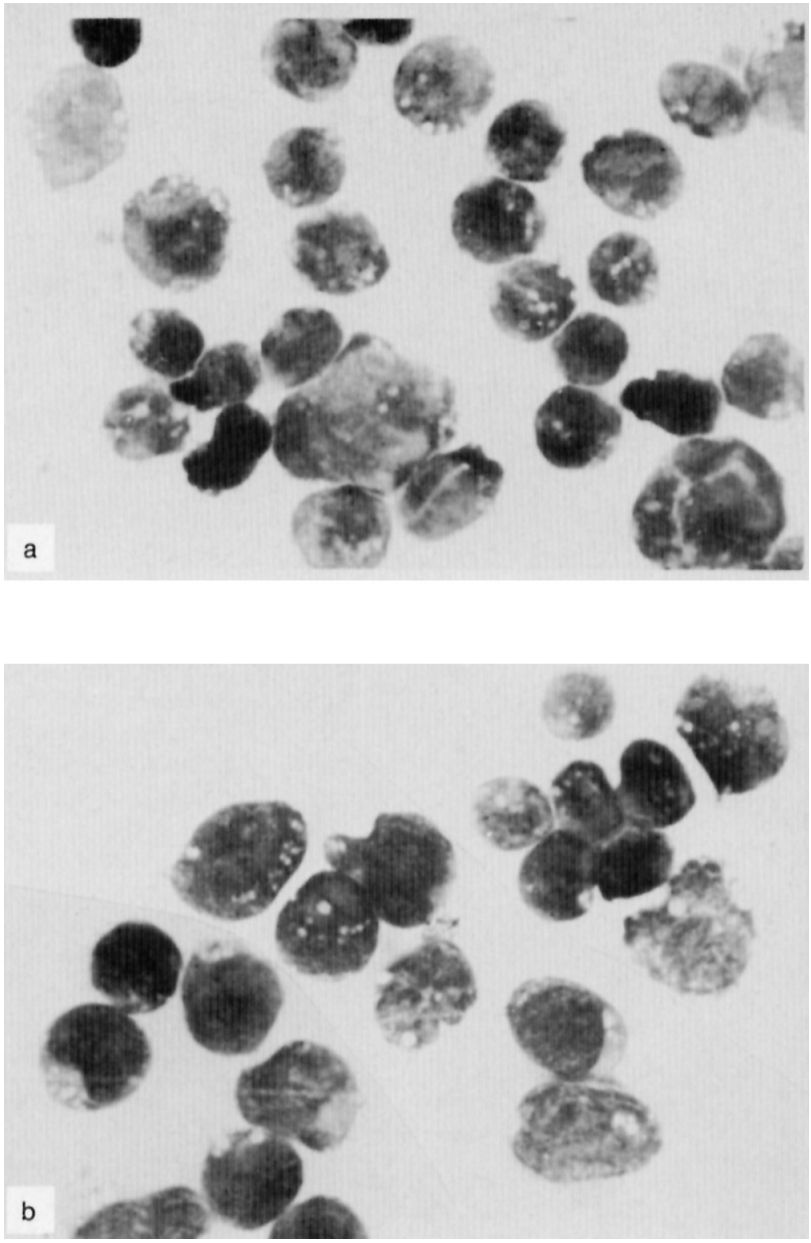


Fig. 2 Photographs a and b show L- and Bo-cells respectively in L1210 cells obtained from ascites at 96 hr after i.p. injection of L1210 cells. Both cells have the characteristic findings of large N/C ratio, clefting or lobulation of nucleus, hand-mirror shape and vacuolation in cytoplasma. Variation of cell size appears to be greater in L-cells than in Bo-cells. (May-Gruenwald-Giemsa stain, x1000)

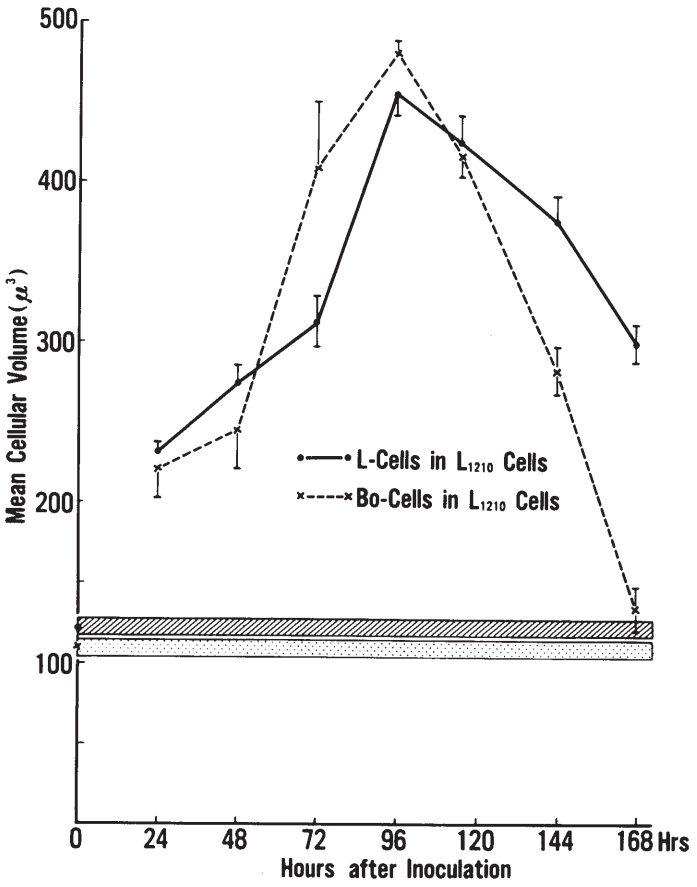


Fig. 3 Change of mean cellular volume after inoculation of L1210 cells. MCV of L- and Bo-cells (±SE) was obtained from 5—20 mice every 24 hr. MCV of Bo-cells shows a significantly higher level at 72 hr ($P<0.01$) and lower level at 144 and 168 hr ($P<0.001$) than that of L-cells. MCV of Bo-cells also demonstrates more rapid and grater change in time sequence. Lymph-node L-cells had larger MCV than lymph-node Bo-cells, but this was statistically not significant ($P>0.2$). Shaded area represents mean value of lymph-node L-cells ±SE. Dotted area represents that of lymph-node Bo-cells ±SE.

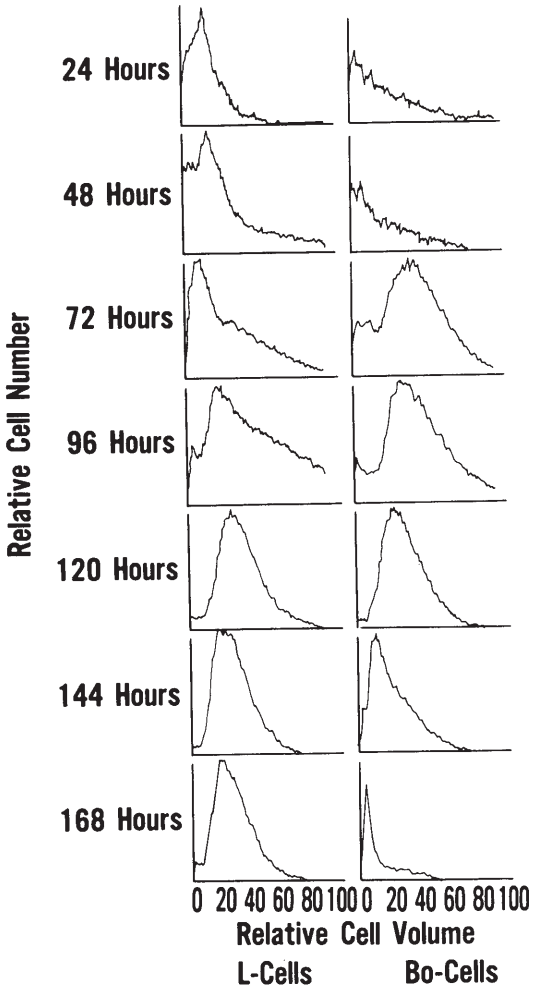
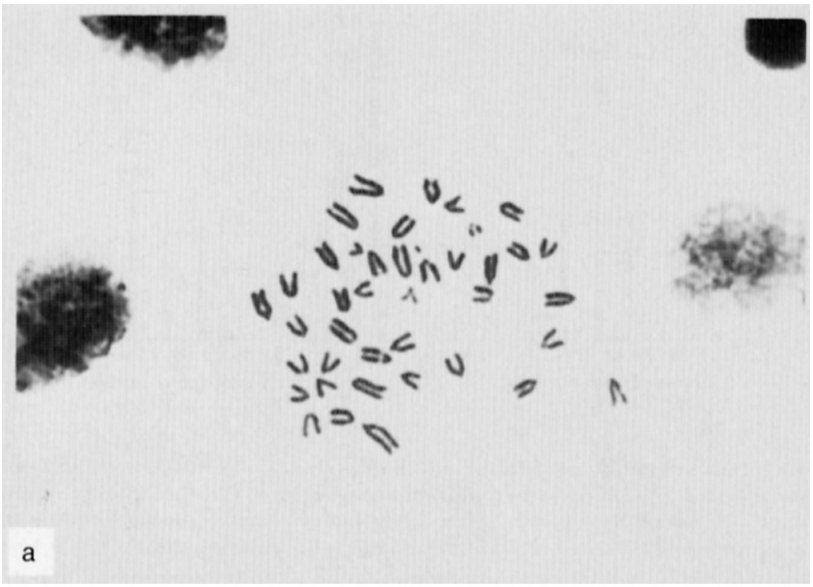
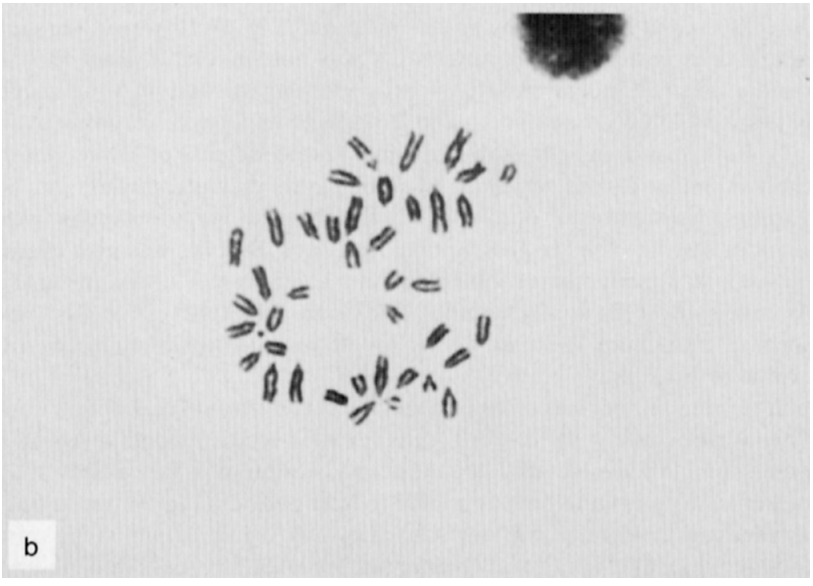


Fig. 4 Change in cell volume distribution of L1210 cells after inoculation. each figure demonstrates a typical cell distribution pattern of L-cells (left side) and Bo-cells (right side) at each time point. L-cells show little change of peak channel number (15 to 25) but a gradual increase (from 24 to 96 hr) and decrease (from 96 to 168 hr) in the population of larger cells are observed, while Bo-cells changed peak channel number from 40 (at 72 hr) to 5 (at 168 hr).


a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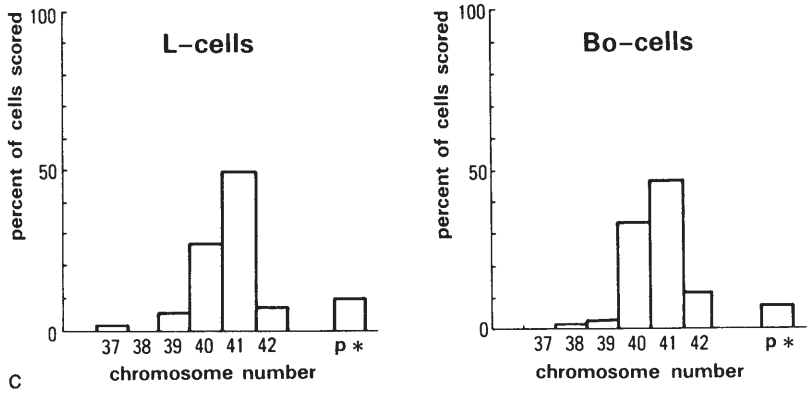


Fig. 5 Photographs of metaphase plate of L- (a) and Bo-cells (b) in L1210 cells at 96 hr (x1000). Both plates (a) and (b) show cells with diploid chromosome (2n = 41) consisting of 40 telocentrics and one minute marker chromosome. The modal chromosome number in L- and Bo-cells is 41 and the percentage of diploid cells is 90% in L-cells and 93% in Bo-cells as shown in (c). *P = polyploid cells

relatively wide and this observation was coincident with well-known findings in tumor cells. There was no remarkable difference in the distribution between L- and Bo-cells.

The comparison was further carried out biochemically. The protein content was generally higher in Bo-cells than in L-cells at any given time point (Fig. 6). However, the separation of Bo-cells from L-cells in a given protein content was not successful. Both Bo- and L-cells changed protein content independently, with a gradual increase in time sequence. The changing patterns of DNA content in L- and Bo-cells (FIg. 7) were very similar to those of MCV (Fig. 3). Both groups of cells showed the most rapid increase of DNA content from 48 to 72 hr, the content in L-cells remained at stable levels thereafter, while that in Bo-cells decreased rapidly. No significant difference in DNA content was observed between L- and Bo-cells except at 168 hr. The ^{3}H-TdR uptake in L- and Bo-cells was also examined. The uptake by Bo-cells was greater than L-cells at all time points except 72 and 168 hr (Fig. 8). The maximum uptake by Bo-cells was 24 hr and 30 folds higher than that of L-cells. The uptake by L-cells reached a maximum value at 72 hr, but it was still lower than one-fifth of the maximum value of Bo-cells.

The cellular taurine content was measured and normalized by 10^{6} cells (Fig. 9) and by DNA (Fig. 10). The taurine content per cell of L-cells revealed relatively high levels at earlier and later time points, and low levels at middle time points, while that of Bo-cells had a tendency to be low at earlier time points and high at middle to later periods (Fig. 9). These findings were emphasized when taurine content was normalized by DNA (Fig. 10) showing a mirror-image of ^{3}H-TdR uptake (Fig. 8). Significantly higher taurine levels in L-cells were observed only at 24 and 48 hr compared with those in Bo-cells.

The L- and Bo-cells which were separated from ascites at 96 hr were cultured and their *in vitro* growth rates were examined (Fig. 11). The relatively higher cell numbers were obtained from the culture of Bo-cells compared with those from the culture of L-cells at any time point

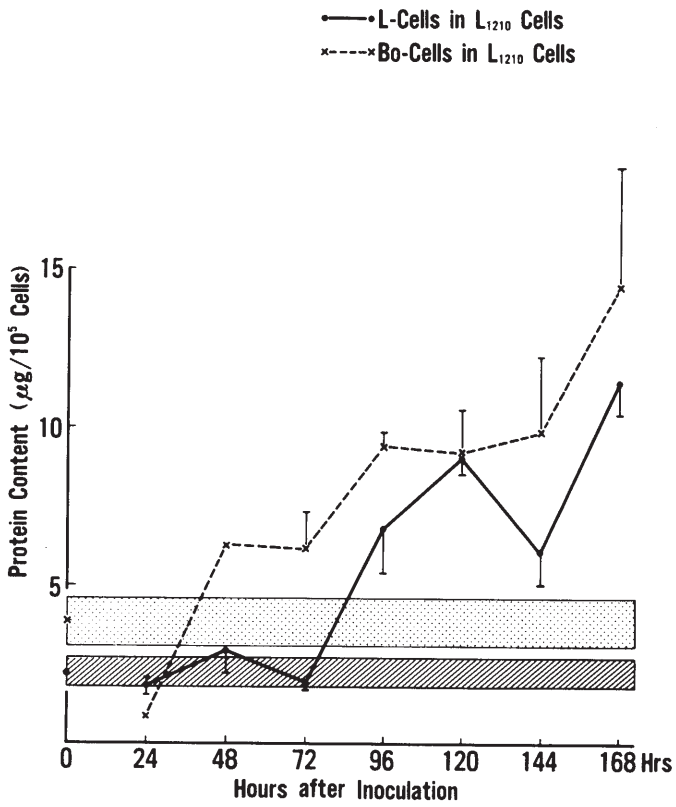


Fig. 6 Protein contents in L1210 cells. Mean cellular protein content (±SE) from 5—10 mice are shown. Bo-cells have higher protein content than L-cells at any time point and a significant difference was recognized at 72 hr ($P<0.01$). Lymph-node Bo-cells show higher protein level than lymph-node L-cells, but not a significantly higher level ($P>0.1$).

except the 10th day after the initiation of culture. As was observed in *in vivo* studies (Fig. 1, 3 and 7), Bo-cells also showed more rapid changes in *in vitro* than L-cells, i.e., the cell number of Bo-cells reached a plateau already by the 5th day and decreased on the 10th day. The *in vitro* doubling time of Bo-cells during the logarythmic growth, from day 0 to day 5, was 0.59 days, and that of L-cells, from day 0 to day 6, was 0.75 days.

Lymphocyte-layer cells and bottom cells were also obtained from cells of normal lymph nodes. The number of lymph-node Bo-cells was 2.9 times higher than lymph-node L-cells. The lymph-node Bo-cells had relatively higher protein and DNA contents than the lymph-node L-cells as shown in Fig. 6 and 7, and revealed lower MCV, ^{3}H-TdR uptake and taurine content as shown in Fig. 3, 8, 9 and 10. However, the differences were not significant. Lymph-node L- and Bo-cells generally had lower values in all parameters than L- and Bo-cells of L1210 cells except for taurine/DNA contents.

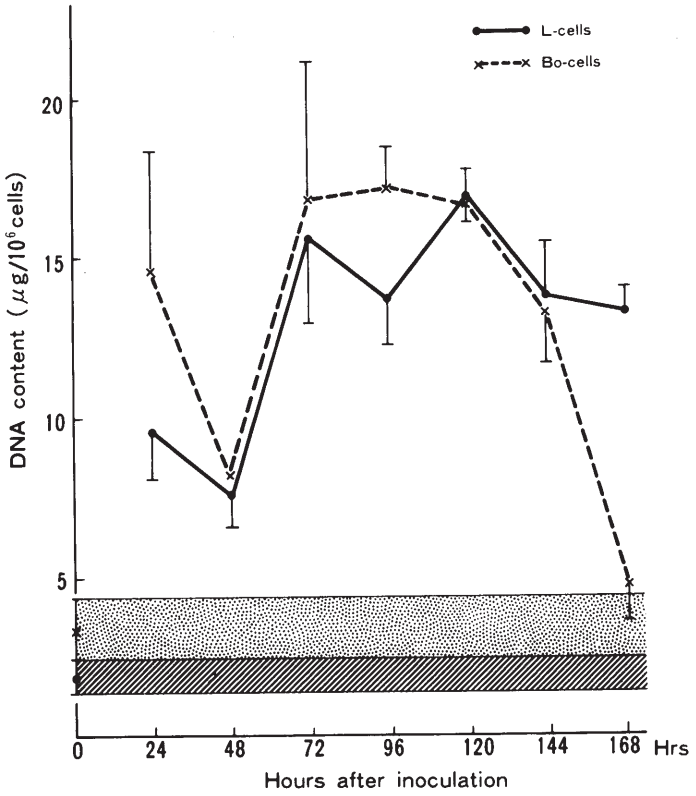


Fig. 7 DNA contents in L1210 cells. Mean cellular DNA content (±SE) from 5 mice are demonstrated. Alteration of the DNA levels in Bo-cells shows more rapid increase and decrease than that in L-cells. Significantly lower DNA content in Bo-cells is observed at 168 hr ($P<0.01$). Lymph-node Bo-cells had a higher level of DNA content than lymph-node L-cells, but not of significant value ($P>0.05$).

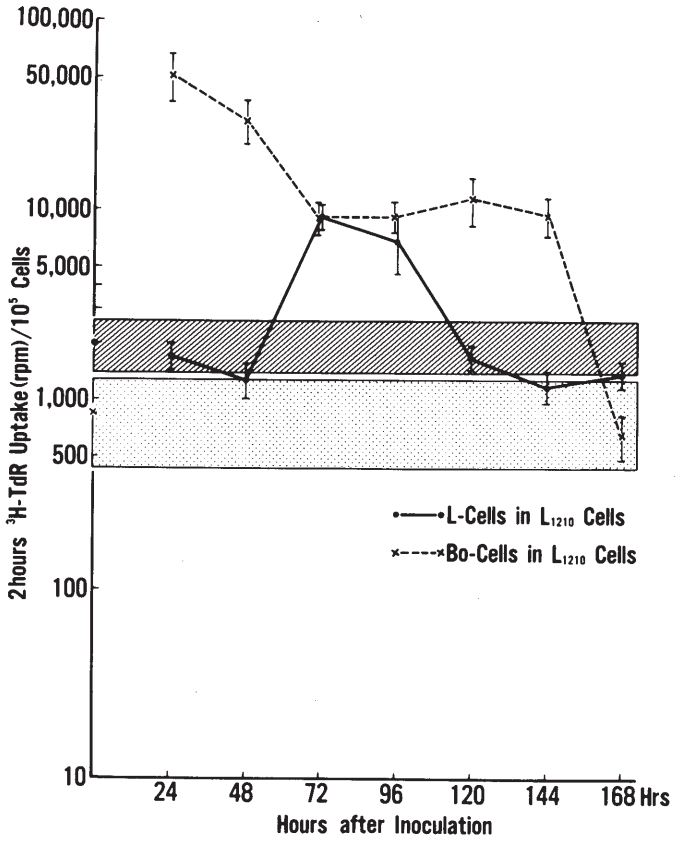
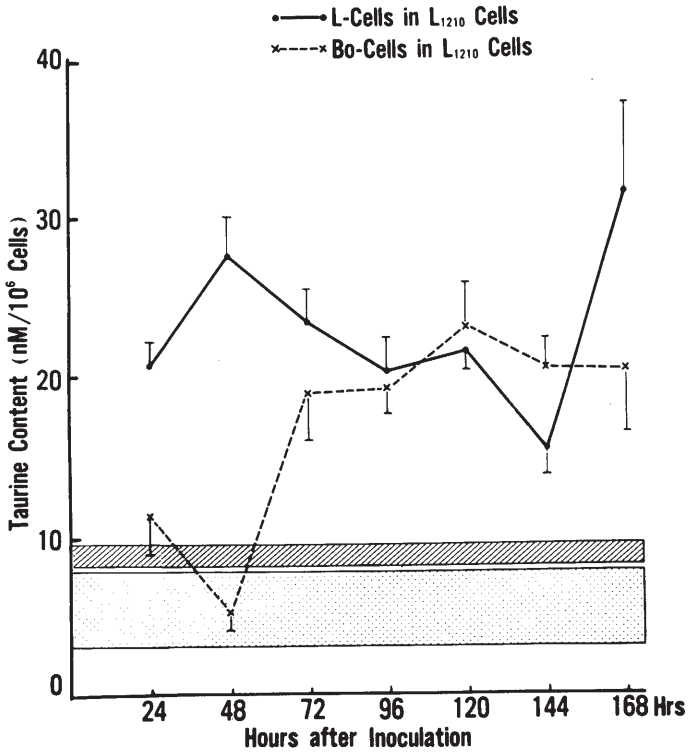
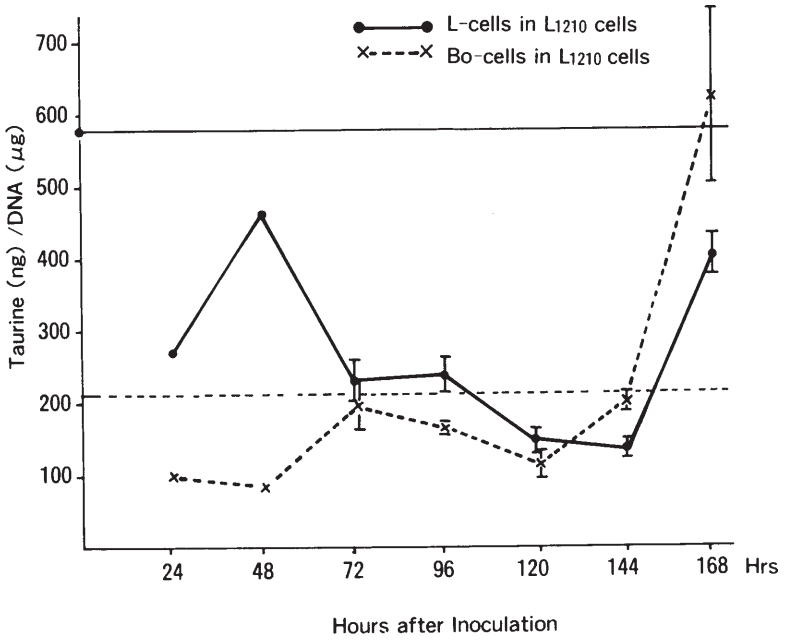


Fig. 8 ^{3}H-TdR uptake in L1210 cells. Mean cellular ^{3}H-TdR uptake (±SE) from 5 mice are shown. The data are obtrained from cells incubated for 2 hr after isolating them from the peritoneal cavity at each time point. Bo-cells have significantly higher uptake than L-cells at 24, 48, 120 and 144 hr ($P<0.001$—0.02), while lower uptake was shown at 168 hr ($P<0.02$). Lymph-node L-cells show higher uptake than lymph-node Bo-cells, but not of a significant level.


L-Cells in L1210 Cells
Bo-Cells in L1210 Cells
Taurine Content (nM/10⁶ Cells)
40
30
20
10
0
24
48
72
96
120
144
168 Hrs
Hours after Inoculation


L-cells in L1210 cells
Bo-cells in L1210 cells
Taurine (ng) /DNA (μg)
700
600
500
400
300
200
100
24
48
72
96
120
144
168 Hrs
Hours after Inoculation

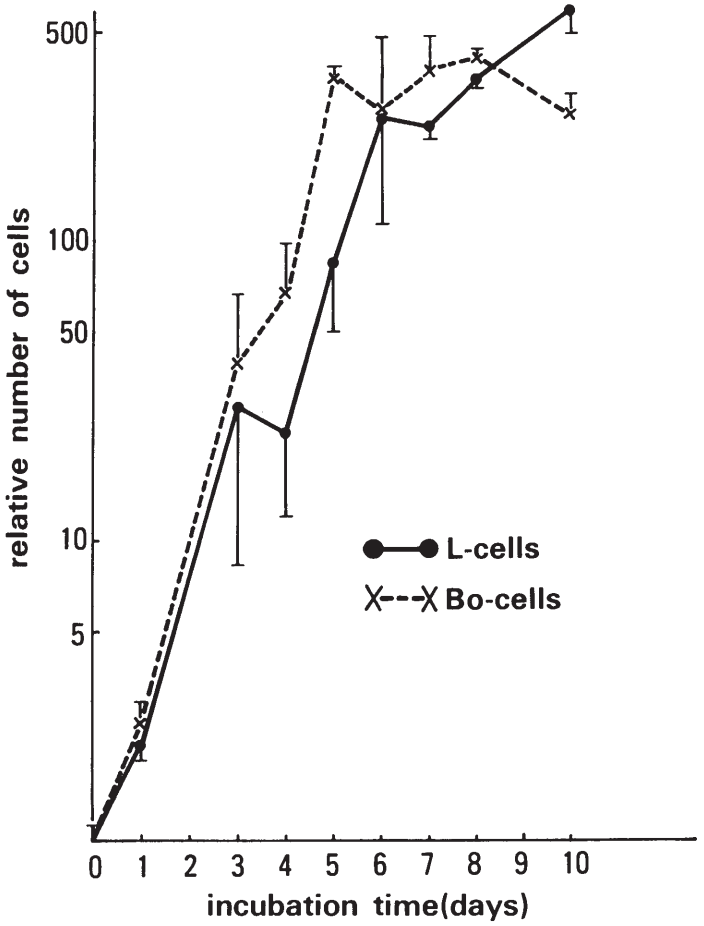


Fig. 9 Taurine content in L1210 cells. Mean taurine content/ 10^6 cells (±SE) from 10 mice are shown. L-cells have significantly higher levels than Bo-cells at 24 and 48 hr ($P<0.02$, $P<0.001$), then gradual decrease in value with the last rebounded high level at 168 hr. Bo-cells show a higher and constant taurine level from middle to late time points than at early time points. Lymph-node L-cells have higher taurine content than lymph-node Bo-cells, but not of a significant level ($P>0.3$).

Fig. 10 Taurine concentration of L1210 cells (normalized by DNA). Mean taurine content/ DNA content (±SE) from 5 mice are shown. L-cells have a significantly higher level than Bo-cells at 96 hr ($P<0.05$) and lowre at 144 hr ($P<0.01$). The lowest taurine level in L-cells is observed at middle phase of cell growth. The highest level in Bo-cells is at 168 hr. Lymph-node L-cells have a higher level than lymph-node Bo-cells. The solid line represents the value of lymph-node L-cells and the broken line represents that of Bo-cells.

Fig. 11 Growth curves of L1210 cells in vitro. Mean relative cell number (±SD), which was obtained by actual cell number versus cell number at 0hr from 3 samples, is shown at each incubation time after implantation of 10^3 L1210 cells isolated from the ascites at 96 hr. Significantly higher cell number in Bo-cells compared with L-cells is observed on the 5th ($P<0.01$) and 8th day ($P<0.02$), and it becomes significantly lower on the 10th day ($P<0.01$).

DISCUSSION

The Ficoll-Hypaque cell separation method is a standardized method for purification of mononuclear cells in human and animal blood cells. Since L1210 cells are lymphocytic leukemia cells, they should be collected in the usual interphase layer on the Ficoll-Hypaque cushion. In the density cell separation method, however, overloading of cells or contamination by large numbers of cell debris often causes aggregation of cells and consequently results in sedimentation of cells to the bottom of the tube as described by Shortman.[14] In the present study, Bo-cells as well as L-cells showed a high percentage of viability, and re-layering of Bo-cells on the Ficoll-Hypaque cushion resulted in resedimentation of all cells to the bottom without leaving in any L-cells. The possibility of overloading of cells was also refuted by the finding that an increase in cell number on the Ficoll-Hypaque cushion did not increase the percentage of Bo-cells. Thus, the possibility of the presence of dead cells or aggregated cells due to overloading could be excluded, and Bo-cells could be clearly demonstrated as not being the products of misengineering in the cell separation method employed in the present study.

Another possible explanation for the existance of Bo-cells is that they merely represent a different phase of the cell cycle in L-cells. However, the morphological features and cell sizes of both cells at different time points did not indicate that each group of cells consisted of a homogeneous population of cells representing a certain phase of the cell cycle. Moreover, cellular uptake of ^{3}H-TdR as well as protein, DNA and taurine contents did not indicate that each group belonged to a population in a certain phase of the cell cycle. Although protein content and ^{3}H-TdR uptake in Bo-cells tended to be higher than those in L-cells at each time point, sequential analysis of protein content and ^{3}H-TdR uptake showed independent changes in both cells and did not indicate that L-cells would be collected as Bo-cells when the protein contents and the ^{3}H-TdR uptakes of L-cells exceeded certain levels. Thus, Bo-cells did not seem to be a group of cells representing a different phase of the cell cycle in L-cells.

From the *in vivo* and *in vitro* proliferation patterns and the data of MCV and DNA content, Bo-cells could be regarded as a group of cells that showed more rapid and greater changes within a shorter period than L-cells. The higher protein content, the higher ^{3}H-TdR uptake, and the mirror-image levels of taurine and ^{3}H-TdR uptake in Bo-cells during the *in vivo* cell proliferations supported the above assumption that Bo-cells were the cells with increased proliferative capability. Our preliminary observations that the ratio of bottom cells to lymphocyte-layer cells of thymus cells is higher in younger mice compared with older mice may also support the above assumption.

Therefore, among L1210 cells there seem to be present two different cell fractions with their own growth behavior. The morphological features and the data of distribution of cell volume indicated that Bo-cells were a more homogeneous group of cells than L-cells.

Previously, we reported that taurine content per cell in human leukemia cells was lower than that in normal human lymphocytes.[10] However, taurine content per cell in L1210 cells was higher than that in normal murine lymphocytes. This was true both in L-cells and Bo-cells as shown in Fig. 9. The difference of taurine content per cell between human and murine leukemia cells could be explained by the difference of their respective cell sizes. L1210 celles are 3 to 5 times larger than normal murine lymphocytes, while human leukemia cells are not extremely large. Sizes of human acute leukemia cells are equal to or up to 1.5 times larger than those of normal human lymphocytes, and chronic lymphocytic leukemia cells are smaller

than lymphocytes. In fact, when the taurine content was normalized by DNA content, that of L1210 cells was found to be less than that of normal lymphocytes as shown in Fig. 10.

The observation that high ^{3}H-TdR uptake but low DNA content in Bo-cells at earlier time points and low ^{3}H-TdR uptake but high DNA content in L-cells at later time points could be explained by the difference in the ratio of active DNA with high ^{3}H-TdR uptake potential to inactive DNA at each time point as reported by Frenster et al.[18]

Another important point of the existence of Bo-cells is the fact that they are heavier than 1.077 g. ml. Since normal hematopoietic stem cells and leukemia colony forming cells in the spleen, bone marrow and peripheral blood are usually studied using cells lighter than 1.077,[19-21] both in mice and humans, the possible existence of cells heavier than 1.077, i.e. bottom cells, should also be tested in these studies.

Recently several reports have appeared on the heterogeneity of mouse and human leukemia cells,[22-25] but there has been no report on the heterogeneity of L1210 leukemia cells. The present study has proved that there is also heterogeneity in L1210 leukemia cells. It is already known that the difference of cell growth rate is related to responses to antineoplastic drugs.[6,8,26,27] Bruce *et al.* compared the sensitivity of normal spleen cells to various chemotherapeutic agents with that of lymphoma cells and demonstrated the higher sensitivity of the latter,[6] and Venditti described that tumor cells with shorter doubling time showed higher response to chemotherapy.[8] Since L1210 leukemia is one of the most important tumors in the screening of antineoplastic drugs for clinical use, it is of critical importance to understand that there are two components in L1210 cells with different proliferative activities. The understanding of these characteristics may possibly bring better chemotherapeutic regimens for the treatment of human neoplasias.

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