Jpn. J. Cancer Res. (Gann), 79, 59–68; January, 1988

Heterogeneous Origin of Established Non-T,Non-B Cell Lines from Two Adolescent Patients with Acute Lymphoblastic Leukemia

Miharu NAGASAKA-YABE,[*1, *4] Sakan MAEDA,[*1] Osamu MABUCHI,[*2] Hideo MISU,[*2] Shinobu NAKAMURA,[*2] Yukio KOBAYASHI,[*3] Hisamaru HIRAI[*3] and Taketoshi SUGIYAMA[*1]

[*1]*Department of Pathology, Kobe University School of Medicine, 7-chome, Kusunoki-cho, Chuo-ku, Kobe 650,* [*2]*Department of Hematology, Hyogo Prefectural Children's Hospital, 2-chome, Takakuradai, Suma-ku, Kobe 654 and* [*3]*The 3rd Department of Internal Medicine, Faculty of Medicine, University of Tokyo, Hongo, Tokyo 113*

Three leukemic cell lines were established from the bone marrow cells of two adolescents with non-T,non-B acute lymphoblastic leukemia (ALL) at relapse. Two cell lines from a 14-year-old girl and one from an 11-year-old boy were designated as KH-3A, KH-3B and KH-4, respectively. Leukemic cells started to grow attached to the bone marrow stromal (BMS) cells. KH-3A was positive for OKIa1 and positive at low percentages for B1, Leu-1 and J5 antigens; KH-3B reacted with OKIa1 and J5. Except for OKIa1, these two cell lines showed no surface marker change after 12-O-tetradecanoylphorbol-13-acetate (TPA) treatment. On TPA treatment, clones (KH-3A-2 and KH-3A-3) isolated from KH-3A in agarose showed the induction of differentiation into T and B cell lineage. KH-4 was positive for OKIa1 and positive at low percenteges for B1 and J5, and showed a strong reaction with OKIa1, B1 and J5 after TPA treatment. T cell receptor (TCR) β-chain gene and immunoglobulin gene (J_H and C_μ) rearrangements were found in KH-3A, KH-3B, and sublines isolated from KH-3 (KH-3A-2 and -3) simultaneously. These findings indicate that BMS cells are useful for the establishment of leukemic cell lines and that some common ALL (cALL) cell populations may be heterogeneous.

Key words: Non-T,non-B cell line — Common acute lymphoblastic leukemia — Differentiation — Clonal cell growth — Leukemia cell culture

Although the hematopoietic microenvironment has been the subjects of extensive investigation aimed at long-term culture of mouse and human normal bone marrow cells,[1, 2] several attempts at developing long-term suspension cultures of human leukemic cells have met with limited success. Several leukemic cell lines, NALM-1,[3] NALM-6 and 16,[4] KM-3 and SH2,[5] KOPN 1-8,[6] Reh,[7] NALL-1,[8] Laz-221,[9] HPB-Null,[10] P30/Ohkubo,[11] and HOON and HYON,[12] have been reported as non-T,non-B ALL[*5] lines. They all have an Ia-like antigen and CALLA as common characteristics.[13] However, the NALM-6, KOPN 1-8, HPB-Null, and Laz-221 lines are considered to be pre-B-ALL subtypes, containing cIg.[14] On the other hand, some lines could be induced to differentiate, showing a T-cell or B-cell surface antigen.[15, 16]

We report here the establishment and characterization of three leukemic cell lines (KH-3A, KH-3B and KH-4) by using BMS cells derived from two adolescent patients with non-T,non-B ALL.

Materials and Methods

Patients Case 1. A 14-year-old girl with mediastinal ganglioneuroma had been operated on in 1979. In 1982, she was admitted because of high fever, petechia and hepatomegaly. A bone marrow puncture revealed 85.1% blast cells, and ALL was diagnosed. Combination chemotherapy, 2,400 rads of cranial irradiation, and intrathecal injection of

[*4] Present address: Department of Pediatrics, Tokai University School of Medicine, Bohseidai, Isehara 259-11; to whom correspondence should be addressed.

[*5] Abbreviations used: ALL, acute lymphoblastic leukemia; TPA, 12-O-tetradecanoylphorbol-13-acetate; CALLA, common ALL antigen; BMS, bone marrow stromal; TCR, T cell receptor; PAS, periodic acid Schiff; sIg and cIg, surface and cytoplasmic immunoglobulin; TdT, terminal deoxynucleotidyl transferase; EBNA, Epstein-Barr virus-associated nuclear antigen; IMDM, Iscove's modified Dulbecco's medium.

methotrexate induced complete remission. Although in March, 1983, she had a relapse with 74% blasts in the bone marrow, strong combination chemotherapy again induced complete remission. Two weeks later, however, 85.1% cells in the bone marrow were blasts, and she died of sepsis 11 months after the onset of the disease.

Case 2. An 11-year-old boy complained of dizziness in August, 1982. In November, he was admitted because of slight fever, foot pain, and facial pallor. A bone marrow puncture revealed 44.2% blasts, and ALL was diagnosed. Although he was treated with combination chemotherapy, it did not induce remission and he died of leukemic cell invasion to the brain, testis, and eye fundus 9 months after the onset of the disease.

Cell Culture Cell culture was initiated from the bone marrow samples from Case 1 at 1st (74% blasts) and 2nd (85.1% blasts) relapses. In Case 2, cell culture was initiated from the bone marrow cells (73% blasts) when the leukemic cells were bursting to grow. Bone marrow cells from both cases were separated by Hypaque-Ficoll gradient centrifugation. Cells were washed three times in RPMI-1640 medium (Nissui Seiyaku Co., Ltd., Tokyo) and then suspended in the same medium with 20% heat-inactivated fetal bovine serum (GIBCO, NY) and 1% non-essential amino acids (M.A. Bioproducts, MD) in Petri dishes (Falcon No. 3002). The cells were cultured at 37° in a humidified incubator containing 5% CO_2, and a partial medium change was made twice a week. While cell growth was dependent upon the presence of BMS cells, the autologous BMS cells, which could be obtained in suspension by strong pipetting, were transferred to new dishes together with leukemic cells from the primary or the previously passaged cultures.

Cytochemical Staining Fresh leukemic cells from both cases and the established cell lines were examined by PAS, Sudan Black B, α-naphthyl butyrate esterase and acid phosphatase staining.

Electron Microscopy An aliquot of 2×10^8 cells centrifuged at 900g was immediately fixed in 2.5% glutaraldehyde, then in 2% buffered osmium. After dehydration in alcohol, the cells were embedded in

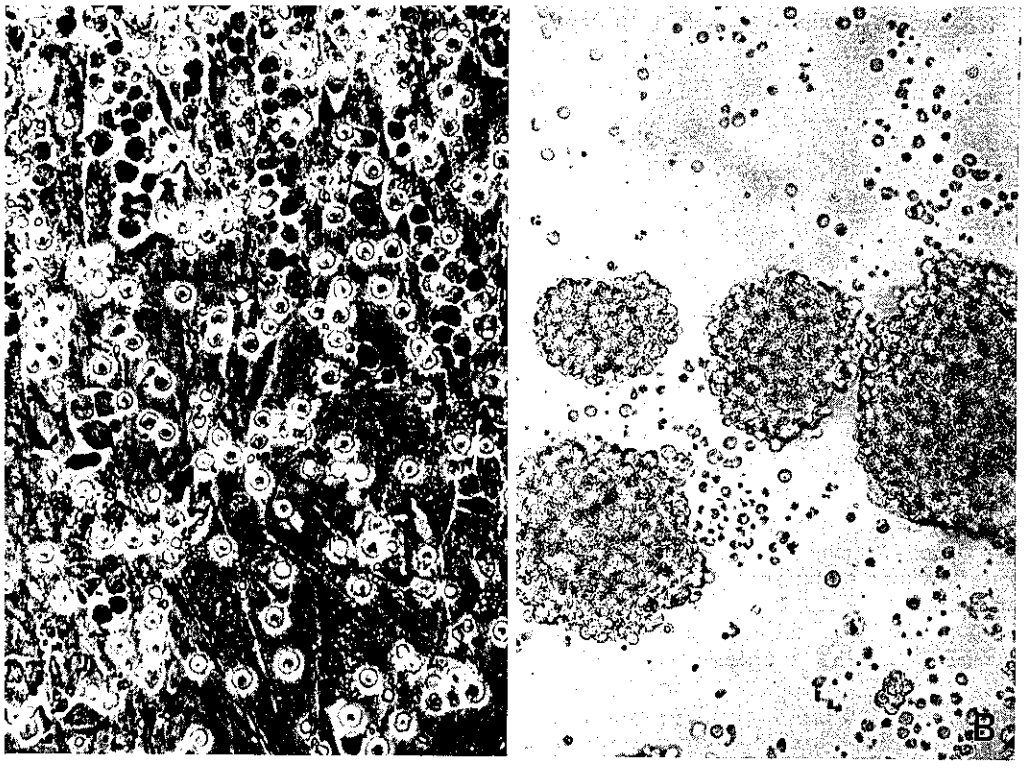

Fig. 1. Phase-contrast microphotograph of cultured cells. A, KH-3B cells growing with fibroblast-like adherent cells at the bottom of dishes (×100). B, KH-4 cells growing in clusters of hundreds of cells (×50).

epoxy resin. Ultrathin sections were examined under a JEOL 1200EX electron microscope after uranyl and lead staining.

Cytogenetic Analysis Bone marrow cells cultured for 12–24 hr were exposed to 0.01 μg/ml of colcemid for 2 hr, treated with 75m*M* KCl at 37° for 25 min and fixed with acetic acid:methanol (1:3). From the conventionally air-dried chromosome slides, about 30 metaphases were analyzed for modal chromosome number, and several selected metaphases were further analyzed by the Giemsa banding technique.[17]

Colony Formation in Soft Agar, Methylcellulose, and Agarose Colony formation was assayed in a soft gel system using agar,[18] methylcellulose,[19] or agarose[20] as previously described.

Immunofluorescence Studies Spontaneous rosette formation with sheep erythrocytes (ERFC) was tested by the usual method, and the receptor for complement was assayed in terms of the formation of zymosan beads. Cell s-Ig and c-Ig were detected by direct immunofluorescence with fluorescein-conjugated rabbit anti-human Ig. Cells were examined for CALLA by indirect immunofluorescence using the J5(CD10) mouse monoclonal antibody (Coulter Electronics Inc., Hialeah, FL) and fluorescein-conjugated goat anti-mouse Ig (Coulter). The Ia-like antigen, using OKIa1 (Ortho Pharmaceuticals, Raritan, NJ), the T-cell antigen, using Leu-1(CD5)(Becton-Dickinson, Sunnyvale, CA), 10.2(CD5) (New England Nuclear, Seattle, WA) and T11 (CD2) (Coulter), and the B-cell antigen, using B1(CD20) (Coulter), were similarly detected by means of indirect immunofluorescence. OKM1(CD11) (Ortho) was used to detect a myeloid-macrophage antigen.

TdT was examined by indirect immunofluorescence, using a TdT assay kit (Bethesda Research

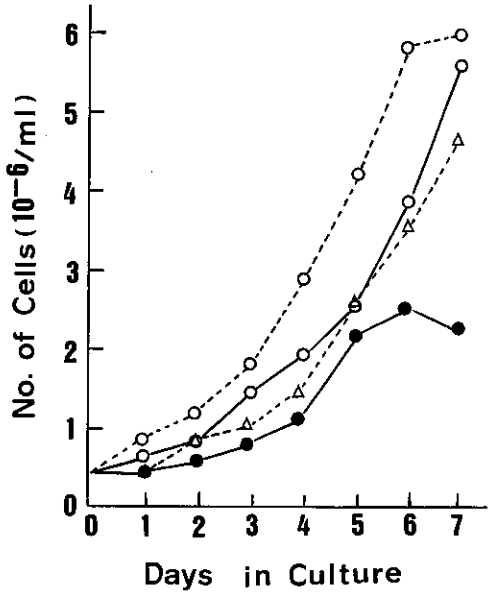

Fig. 2. Growth characteristics of KH-3A, KH-3A-2, KH-3A-3 and KH-4. Cells (5×10^5) were seeded in 5 ml of medium in 60 mm Petri dishes and counted daily with a hemocytometer. ○---○, KH-3A; ●—●, KH-4; ○—○, KH-3A-2; △---△, KH-3A-3.

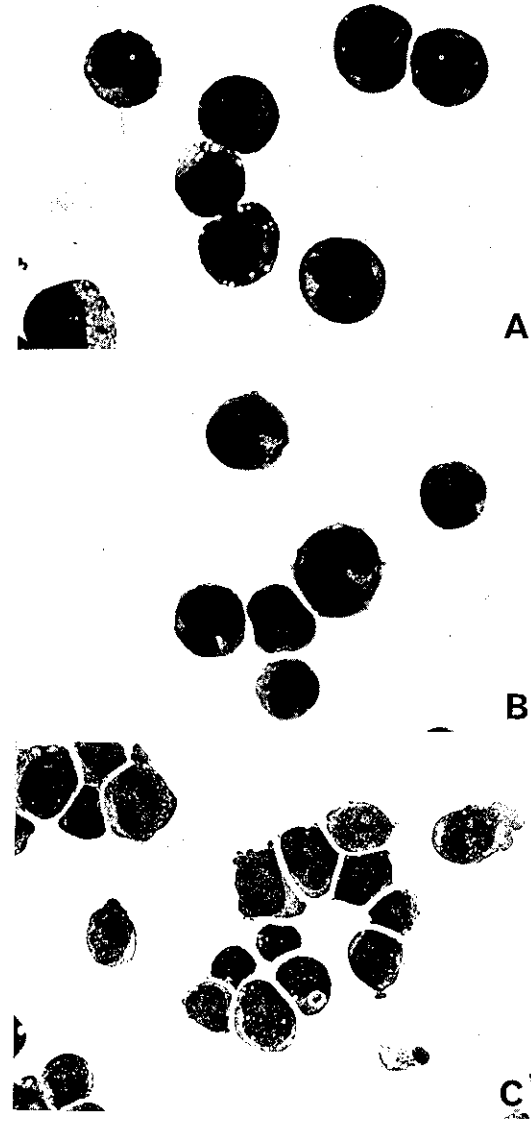

Fig. 3. May-Grunwald-Giemsa staining of KH-3A (A), KH-3B (B) and KH-4 (C) (×1,000).

Laboratories, MD). EBNA was assayed by an anti-complement immunofluorescence method according to Reedman and Klein.[21]

Induction of Differentiation KH-3A and KH-3B cells (5×10^6) were cultured in 5 ml of RPMI-1640 medium supplemented with 20% FBS and $10^{-7}M$ TPA in 60-mm Petri dishes. For KH-4 cells, Iscove's modified Dulbecco's medium (IMDM) (Flow Laboratories, Irvine, Scotland) was used instead of RPMI-1640 medium. Three days after TPA treatment, the viability of cells in each treated dish was more than 90%. At that time, 1×10^7 cells were washed three times with a phosphate-buffered saline solution, and surface markers were examined using various monoclonal antibodies.

TCR and Ig Gene Rearrangements The DNAs of cultured cells from KH-3A, KH-3A-2, KH-3A-3, and KH-3B were subjected to Southern blot analysis for the detection of TCR β-chain and Ig (J_H and C_μ) gene rearrangements as previously described.[22, 23]

Results

Establishment of 3 Cultured Lines and Cell Cloning At the beginning of culture, the leukemic cells showed slow but definite multiplication, being attached to the BMS cells on the bottom of the dishes (Fig. 1A). They could be cultured as single cell suspensions after two weeks or a month at the first and second relapses of Case 1, and they were designated as KH-3A and KH-3B, respectively. In Case 2, they were very difficult to grow

Table I. Cytochemical Staining before and after Cell Culture

Staining method	KH-3A[a]		KH-3B		KH-4	
	before	after	before	after	before	after
Peroxidase	−	−	−	−	−	−
Sudan Black B	−	−	−	−	−	−
Non-specific E[b]	−	−	−	−	−	−
PAS	±	−	−	−	±	±
Acid phosphatase	±	+	+	+	+	+

a) −, Negative; ±, weakly positive; +, positive.
b) Esterase.

Table II. Surface Marker Analysis of Uncloned and Cloned Leukemia Cells before and after TPA Treatment

Patient (time of sample collection)	E	C3b	s-Ig	B1	OKIa1	OKM1
Case 1						
1st relapse	0.5%	5.0	0	2.0	40.9	1.4
KH-3A	0	0	0	16.8	90.0>	0
KH-3A after TPA[a]	6.3	0	0	2.0	90.0>	2.0
KH-3A-2	0	0	0	0	90.0>	0
KH-3A-2 after TPA	0	0	0	0	90.0>	0
KH-3A-3	0	0	0	2.0	90.0>	0
KH-3A-3 after TPA	0	0	0	33.8	90.0>	0
2nd relapse	0	0	0	0	83.5	3.0
KH-3B	0	0	0	0	90.0>	0
KH-3B after TPA	0	0	0	0	90.0>	0
Case 2						
Diagnosis	0.5	0	0	0	29.5	4.8
KH-4	0	0	0	8.4	90.0>	0
KH-4 after TPA	0	0	0	90.0>	90.0>	0

a) Cells were treated with $10^{-7}M$ TPA for 3 days.
b) Not tested.

without BMS cells on the bottom of the dishes, but they grew as suspension cells after 4 months. They grew better after a medium change from RPMI-1640 to IMDM, and were designated as KH-4. Even after KH-3B and KH-4 cells started to grow in suspension culture, the proliferating cells were transferred to new dishes containing the appropriate BMS cells where they grew for about 2 weeks and 2 months, respectively. Therefore, cells grew without BMS cells.

The three cell lines did not form any colonies in soft agar even when 10^6 cells per dish were seeded. When 10^5 cells of KH-3A were seeded in agarose, 20 colonies were obtained and isolated for cloning 20 days after seeding. Five colonies grew in suspension culture and were designated as KH-3A-1-5. When 10^5 cells were seeded in 0.8% metylcellulose, three colonies in the case of KH-3A and 44 colonies in the case of KH-3B were observed. In KH-4, 5×10^5 cells in methylcellulose formed 107 colonies. Five and 10 clones from KH-3B and KH-4, respectively, were isolated.

Growth Characteristics KH-3A and KH-3B cells grew as single-cell suspensions; on the other hand, KH-4 cells grew in clusters of hundreds of cells (Fig. 1B). The growth curves are shown in Fig. 2. KH-4 cells were seeded in IMDM and their growth was slow in contrast with that of KH-3A. KH-3A and KH-3B showed almost the same growth curves, with KH-3B growing a little more slowly (data not shown). The doubling times of KH-3A and KH-3B were about 48 hr and that of KH-4 was about 72 hr.

Morphological and Cytochemical Characteristics KH-3A and KH-3B were round, medium-sized lymphoid cells with large, slightly convoluted nuclei, as observed after May-Grunwald-Giemsa staining (Fig. 3A and 3B). KH-4 were small lymphoid cells with spicules and contained densely stained round nuclei and some vacuoles in the cytoplasm (Fig. 3C).

Ultrastructural studies revealed that all cells were immature blasts with large nuclei and many polysomes. In KH-4, many myelin figures were observed in the cytoplasm.

The results of cytochemical staining are shown in Table I. Both fresh bone marrow cells and cultured leukemic cells of KH-3A, 3B and KH-4 were positive for an acid-phosphatase, and they were negative for other staining for myeloid cells.

Surface Markers Cell surface receptors and other immunological markers of the patients' fresh bone marrow leukemic cells and the established lines and clones are shown in Table II. None of the cells reacted with ERFC, C3b, c-Ig and OKM1, but all were

Leu1	10.2	T11	OKT9	OKT10	J5	TdT	c-Ig
NT[b)]	NT	0	21.1	64.9	19.2	20.0	NT
9.0	3.0	2.0	90.0>	90.0>	22.0	0	0
2.0	2.0	1.6	80.9	30.8	6.0	10.0	0
35.6	69.3	2.0	90.0>	90.0>	10.0	0	0
90.0>	90.0>	5.0	90.0>	4.0	5.0	50.0	0
2.0	2.0	0	2.0	90.0>	11.0	0	0
1.5	0	0	1.5	90.0>	1.0	0	0
NT	NT	1.0	24.0	90.0>	81.7	60.0	NT
0	2.0	3.5	7.0	90.0>	37.5	10.0	0
0	4.0	0	90.0>	5.0	0	0	0
1.3	NT	7.5	0.8	49.0	17.1	25.0	NT
0	0	NT	0	90.0>	15.2	50.0	0
0	0	0	0	90.0>	90.0>	10.0	0

Table III. Chromosome Analysis before and after Cell Culture

Patient	Time of sample collection	No. of chromosomes[a]				Karyotype
		44	45	46	47	
Case 1	Diagnosis	8	14	11	0	46XX; 45XX, −E
	1st relapse	3	8	23	0	46XX, 45XX, −E
	Culture KH-3A	6	6	13	2	46XX; 46XX, +2q; 45XX, −E; 47XX, +13
	KH-3A-2	0	0	20	0	46XX, +2q; 46XX
	KH-3A-3	0	0	20	0	46XX
	2nd relapse	0	3	27	0	46XX; 45XX, −E
	Culture KH-3B	7	14	6	0	46XX; 45XX, −E
Case 2	Diagnosis	5	9	27	0	46XY; 45XY, −E
	Culture KH-4	5	9	18	0	46XY; 45XY, −E

a) Number of metaphases counted.

strongly positive for OKIa1. In Case 1, 19.2% of cells were positive for J5 at first relapse, and 81% of cells at second relapse. Since other T and B cell markers were negative, Case 1 was diagnosed as cALL, especially at second relapse. After the establishment of cultured lines, KH-3A cells were positive at low percentages for B1, Leu1 and J5; a low percentage of KH-3B reacted with J5. These two lines showed no surface marker change after TPA treatment.

Five clones isolated from KH-3A by agarose cloning showed cell properties different from those of their parental KH-3A line. KH-3A-2 and -3 showed different properties upon reaction with B1, Leu1 and 10.2 and their percentage reaction after TPA treatment increased. KH-3A-1 and KH-3A-5 gave almost the same results as KH-3A-2 (data not shown), and these 3 clones were characterized by the expression of the T-cell lineage marker. On the other hand, KH-3A-3 and KH-3A-4 showed almost the same surface marker as that which was observed after induction of differentiation into the B-cell lineage.

KH-4 cells were positive at low percentages for B1 and J5, but after TPA treatment, they became highly positive for them wihtout the expression of c-Ig. Five clones from KH-3B and 10 clones from KH-4, isolated by methylcellulose cloning, showed the same phenotype as their parental lines. EBNA was negative in the three cell lines.

Chromosome Analyses Chromosome analyses before and after cell culture are shown in Table III. Although at the diagnosis of Case 1, chromosomes were distributed from numbers 44 to 46, most of the cells at the first and second relapse had 46 chromosomes, showing a normal karyotype. In the cultured KH-3A line, the distribution of chromosomes was from numbers 44 to 47 and its karyotypes consisted of populations which were cytogenetically different, one normal, 46XX, and the others abnormal, 45XX, −E/46XX, +2q (Fig. 4A)/47XX, +13 (Fig. 4B). The marker chromosome, 2q+, represented a joining of the long arm of chromosomes No. 2 and No. 13. KH-3A-2 cells had 46 chromosomes and the 2q+ chromosome were observed in 80% of the cells. KH-3A-3 cells all had normal karyotype.

In Case 2, the distribution pattern of bone marrow and cultured cells (KH-4) showed mosaicism of 46XY and 45XY, −E.

TCR and Ig Gene Rearrangements As shown in Table IV, both TCR β-chain and Ig (H_J and C_μ) gene rearrangements were observed in KH-3A, KH-3A-2, KH-3A-3, and KH-3B cell lines. Both directional gene rearrangements suggest that these cells originated from an early stage of lymphoid stem cell, and KH-3A-2 and KH-3A-3, which showed different phenotype, such as early differentiation for T or B cell lineage on TPA treatment, had the same genotype.

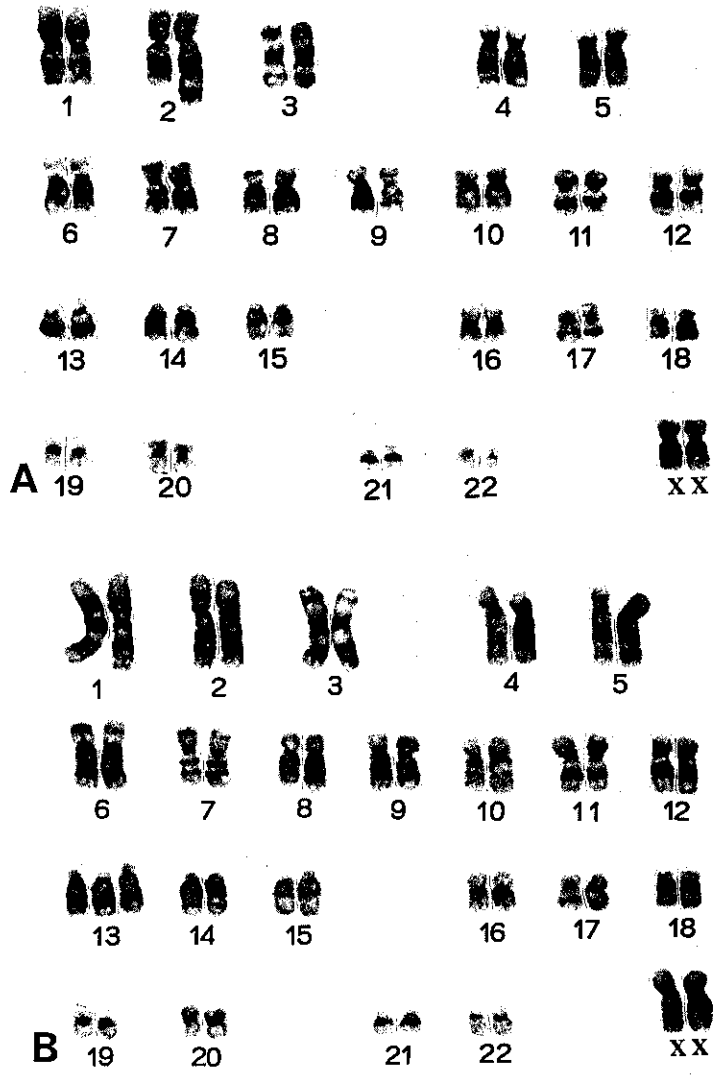

Fig. 4. G-banded karyotype of KH-3A cells with 46XX, +2q (A) and 47XX, +13 (B).

Discussion

Three cell lines were established from two adolescents with non-T,non-B ALL. Although at the beginning of cultivation, leukemic round cells were attached to the BMS cells at the bottom of dishes, they started to grow as a single or aggregated cell suspension culture after 2–4 weeks (in Case 1) or after four months (in Case 2). Though the growth rates of the three cultured lines after their establishment were not stimulated by the addition of conditioned medium of adherent fibroblast-like cells (data not shown), these adherent cells might play some role as a feeder layer in stimulating the growth of leukemic cells, as previously shown for the establishment or the cloning of lymphoma/leukemia cell lines using cells of heterologous origin.[24, 25] At the same time, the unique

growth characteristics of cultured cells may be important in relation to the use of the feeder layer, because the present three lines continued to grow for up to seven days at a high saturation density without an additional medium supplement, as previously shown in NALM-1.[3]

Surface marker analyses revealed almost the same phenotype in KH-3A and KH-3B but a different one in KH-4. KH-4 cells grew in clusters of hundreds of cells, as usually found in the lymphoblastoid B-cell lines presumably derived from normal B-cells. In the case of KH-4, however, cells had no projection in their cell surfaces and did not react with B-cell markers or EBNA. KH-4 cells were induced to differentiate into B-cell lineage by TPA treatment. It has been reported that TPA induces fresh cells obtained from CALLA$^+$ ALL patients to differentiate into B-cells.[26] Korsmeyer *et al.* suggested that, judging from the immunoglobulin gene rearrangement, cALL cells may be very immature B-cells.[27]

On the other hand, in Case 1, we observed a change of cell population (it has been reported that the expression of CALLA changes during treatment and relapse[28]): 19.2% of cells were positive for J5 at first relapse, and 81.7% at second relapse. Although Case 1 was diagnosed as cALL, the established cell line (KH-3A) might be composed of mixed cell populations because B1, Leu1 and 10.2 monoclonal antibodies were expressed at low percentages. Five clones isolated from KH-3A by agarose cloning showed cell properties different from those of their parental KH-3A line. The loss of J5, and expression of T and B cell markers after TPA treatment showed that cells were induced to differentiate, since J5 is said to be a marker of early lymphoid cells which is lost as the cells differentiate into either T or B cells.[29] This could well be the first successful attempt at isolating several clones (KH-3A-1-5) which show T and B cell properties from a non-T, non-B ALL cell line (KH-3A).

In our cell lines, chromosome analyses also revealed heterogeneity of cALL. KH-3A had various karyotypes, such as 46XX/46XX, +2q/45XX, −E/47XX, +13. KH-3A-2 cells, which differentiated into T-cell lineage, showed 46XX, +2q; all KH-3A-3 cells, which differentiated into B-cell lineage, showed 46 XX. Judging from the gene rearrangement, both TCR and Ig genes were rearranged in KH-3A,[30] KH-3B and 2 sublines isolated from KH-3A (KH-3A-2 and -3). Although it might be expected that KH-3A-2 and KH-3A-3 would have different gene rearrangements, both cell lines were the same from the viewpoint of TCR and Ig gene rearrangements. The TCR and Ig gene rearrangements are thought to occur early during T- or B-cell differentiation, and some cALL patients were reported to show rearrangement different from their phenotype.[22, 31] The discrepancy between the cell surface marker examination and the gene analysis seems to be an unexpected event[23] and further systematic experiments on chromosome analyses, induction of cell differentiation and gene rearrangement analyses in more cases may allow an accurate classification of cALL. Our cell lines may be helpful in studies on the origin of non-T, non-B leukemia.

(Received May 12, 1987/Accepted Oct. 27, 1987)

REFERENCES

1) Dexter, T. M., Allen, T. D. and Lajtha, L. G. Conditions controlling the projection of hematopoietic stem cells *in vitro*. *J. Cell. Physiol.*, **91**, 335–344 (1977).
2) Gartner, S. and Kaplan, H. S. Long term culture of human bone marrow cells. *Proc. Natl. Acad. Sci. USA*, **77**, 4756–4759 (1980).
3) Minowada, J., Janossy, G., Greaves, M. F. and Walters, T. R. A non-T,non-B human cell line (NALM-1): establishment of the cell line and presence of leukemia-associated antigen. *J. Natl. Cancer Inst.*, **59**, 83–87 (1977).
4) Minowada, J., Janossy, G., Greaves, M. F., Tsubota, T., Srivastava, B. I. S., Morikawa, S. and Eili, T. Expression of an antigen associated with acute lymphoblastic leukemia in human leukemia-lymphoma cell lines. *J. Natl. Cancer Inst.*, **60**, 1269–1277 (1978).
5) Schneider, U., Schwenk, H. U. and Bornkamm, G. Characterization of EBV-negative "null" and "T" cell lines derived from children with acute lymphoblastic leukemia and leukemic transformed non-Hodgkin lymphoma. *Int. J. Cancer*, **19**, 621–626 (1977).
6) Nakazawa, S. Kaneko, K., Sasaki, M.,

Tsunematsu, Y., Koide, T., Enomoto, Y. and Watanabe, S. "Null" leukemic cell line (KOPN). *Clin. Hematol.*, **20**, 189 (1978) (in Japanese).

7) Rosenfeld, C., Goutner, A., Choquet, C., Venaut, A. M., Kayibanda, B., Pico, J. L. and Greaves, M. F. Phenotypic characterization of a unique non-T,non-B acute lymphoblastic leukemia cell line. *Nature*, **267**, 841–843 (1977).

8) Miyoshi, I., Hiraki, S., Tsubota, T., Kubonishi, I., Matsuda, Y., Nakayama, T., Kishimoto, H., Kimura, I. and Matsui, H. Human B cell, T cell and null cell leukemic cell lines derived from acute lymphoblastic leukemias. *Nature*, **267**, 843–844 (1977).

9) Lazarus, H., Barell, E. F., Krishan, A., Livingston, D. M., Harris, K., Schlossman, S. F. and Chess, L. Characterization of a unique cell line (Laz 221) from human acute lymphocytic ("null" cell) leukemia. *Cancer Res.*, **38**, 1362–1367 (1978).

10) Tatsumi, E., Takiuchi, K., Domae, H., Shirakawa, S. and Uchino, N. Established cell line (HPB-Null) derived from old man with null cell ALL. *Clin. Hematol.*, **20** (Suppl.), 189 (1978) (in Japanese).

11) Hirose, M., Tobinai, K., Minato, K., Ohira, M., Ise, T., Watanabe, S., Shimoyama, M., Taniwaki, M. and Abe, T. A novel cell line (P30/Ohkubo) of pre-T cell (C/T hybrid) phenotype derived from an acute lymphoblastic leukemia with some phenotypic change during clinical course. *Acta Heamatol. Jpn.*, **46**, 729–736 (1983).

12) Okamura, J., Okano, H., Ikuno, Y., Tasaka, H., Motomura, S., Letarte, M. and Gelfand, E. W. Characterization of new non-T,non-B acute lymphoblastic leukemia cell lines: analysis of surface antigens by quantitative cellular radioimmunoassay and flow cytometry. *Leuk. Res.*, **8**, 97–104 (1984).

13) Greaves, M. and Janossy, G. Patterns of gene expression and the cellular origins of human leukemias. *Biochem. Biophys. Acta*, **516**, 193–230 (1978).

14) Minowada, J., Sagawa, K., Trowbridge, I. S., Kung, P. C. and Goldstein, G. Marker profiles of 55 human leukemia-lymphoma cell lines. *In* "Malignant Lymphoma," ed. S. A. Rosenberg and H. S. Kaplan, pp. 53–74 (1982). Academic Press, London.

15) Law, B., Jger, G., Thiel, E., Pachman, K., Podt, T., Huhn, D., Thierfelder, S. and Dormer, P. Phenotypic changes in acute lymphoblastic leukemia cells of common type in diffusion chambers. *Leuk. Res.*, **4**, 561–569 (1980).

16) Nadler, L. M., Ritz, J., Reinherz, E. L. and Schlossman, S. F. Cellular origins of human leukemias and lymphomas. *In* "Leukemia Markers," ed. W. Knapp, pp. 3–17 (1981). Academic Press, London.

17) Seabright, M. A rapid banding technique for human chromosomes. *Lancet*, **2**, 971–972 (1971).

18) Bladley, T. R. and Metcalf, D. The growth of mouse bone marrow cells *in vitro*. *Aust. J. Exp. Biol. Med. Sci.*, **44**, 287–299 (1966).

19) Chervenik, P. A. and Boggs, D. R. *In vitro* growth of granulocytic and mononuclear cell colonies from the blood of normal individuals. *Blood*, **37**, 131–135 (1971).

20) Ayres, K. N. High cloning efficiency of human lymphoid cells in agarose without feeder layers. *J. Natl. Cancer Inst.*, **68**, 919–923 (1980).

21) Reedman, B. M. and Klein, G. Cellular localization of an Epstein-Barr-virus (EBV)-associated complement-fixing antigen in producer and non-producer lymphoblastoid cell lines. *Int. J. Cancer*, **11**, 499–520 (1973).

22) Kobayashi, Y., Hirai, H., Okabe, T., Urabe, A and Takaku, F. T-cell receptor gene rearrangement in a patient with common acute lymphocytic leukemia. *Jpn. J. Cancer Res. (Gann)*, **76**, 796–798 (1985).

23) Geoffrey, R. K., Ugo, R., Alvin, M. M., Susan, M., Sharon, B. M. and Sanford, S. Rearrangement of immunoglobulin heavy chain genes in T cell acute lymphoblastic leukemia. *Blool*, **65**, 725–729 (1985).

24) Epstein, A. L. and Kaplan, H. S. Feeder layer and nutritional requirement for the establishment and cloning of human malignant lymphoma cell lines. *Cancer Res.*, **39**, 1748–1759 (1979).

25) Seshadri, R., Matthews, C., Gardiaks, C. and Morley, A. A. The effect of bone marrow stromal cells on the cloning of human leukemia/lymphoma cell lines. *Int. J. Cell Cloning*, **2**, 227–285 (1984).

26) Nadler, L. M., Ritz, J., Bates, M. P. and Park, E. K. Induction of human B cell antigens in non-T cell acute lymphoblastic leukemia. *J. Clin. Invest.*, **70**, 433–442 (1982).

27) Korsmeyer, S. J., Arnold, A. and Bakhski, A. Immunoglobulin gene rearrangement and cell surface antigenic expression in acute lymphoblastic of T cell and B cell precursor origins. *J. Clin. Invest.*, **71**, 301–313 (1983).

28) Greaves, M., Paxton, A., Janossy, G., Pain, C., Jhonson, S. and Andrew, T. A. Acute lymphoblastic leukemia associated antigen. III Alteration in expression during treatment

and in relapse. *Leuk. Res.*, **4**, 1–14 (1980).
29) Magrath, I. T. Lymphocytic differentiation: an essential basis for the comprehension of lymphoid neoplasia. *J. Natl. Cancer Inst.*, **67**, 501–514 (1977).
30) Kobayashi, Y., Hirai, H., Okabe, T., Urabe, A. and Takaku, F. T-cell receptor and immunoglobulin gene rearrangement and expression in blasts of common ALL cases. *Proc. Jpn. Cancer Assoc., 45th Annu. Meet.*, 757 (1986).
31) Hara, J., Kawa-Ha, K., Yumura, K., Ishihara, S., Doi, S. and Yabuuchi, H. Further heterogeneity of childhood common acute lymphoblastic leukemia. *Jpn. J. Cancer Res. (Gann)*, **77**, 28–31 (1986).