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Phenotypic and Genotypic Lineage Switch of a Lymphoma with Shared Chromosome Translocation and T-Cell Receptor γ Gene Rearrangement

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A case of non-Hodgkin's lymphoma showed a phenotypic and genotypic cell lineage switch twice during nine years of his clinical history; first, T-cell type, pleomorphic small cell lymphoma developed, followed by B-cell type, diffuse centroblastic/centrocytic lymphoma, and finally T-zone lymphoma without follicles again developed, from which AST-1 cultured cell line was established. Karyotype analysis demonstrated a shared abnormal chromosome, der(1)t(1;?)(p36;?), among the first relapsed B-cell tumor, the second relapsed T-cell tumor and AST-1 cell line. Furthermore, T-cell receptor (TCR) γ gene rearrangement bands of the same size were observed in the first relapsed B-cell tumor and the second relapsed T-cell tumor as well as AST-1 cell line. These results suggested that both relapsed tumors of different cell lineages are derived from a common malignant clone, presumably a committed lymphoid stem cell. A unique translocation, t(2;14)(q37;q11.2), which may involve TCR δ/α gene complex, was observed in the second relapsed tumor and AST-1 cells. To attempt to isolate the breakpoint of this translocation, the configuration of TCR δ/α gene complex was studied. The result showed that two rearrangements of TCR α gene detected with J_α probes were the products of the normal TCR rearrangement process, and were not involved in the translocation at this region. This patient, together with the AST-1 cell line, provided us a unique opportunity to study the development and clonal evolution of malignant lymphoma.

Key words: Lymphoma — Chromosome translocation — Stem cell — T-cell — B-cell

Most neoplastic disorders have been believed to be derived from a clonal proliferation of a single malignant cell. Therefore, the detection of clonality is important for tumor diagnosis. Phenotypic studies with monoclonal antibodies (MoAbs) are very useful for estimating differentiation stage of lymphoid malignancies, but they are not competent to detect clonality.[1] Genotypic studies, however, are valuable for detecting a clonal proliferation, even if neoplastic cells are very immature or mixed with non-neoplastic populations of various types.[2,3] Recent study showed that a cytogenetic analysis may be more reliable, because certain chromosomal abnormalities are restricted to tumor cells and, more importantly, may be related to tumorigenesis.[4,5]

Unique lymphomas consisting of two different clones were reported, in which two distinct clones appeared simultaneously or sequentially. Biclonal lymphomas of morphologically distinct clones usually represent a change in the appearance and cell cycle, i.e., transformation of an original clone.[6] Recent phenotypic and genotypic studies also demonstrated the presence of biclonal B-cell lymphomas,[7] although genuine biclonal lymphomas may be rarer than has been suggested, because even these analyses may not always be reliable due to a class switch or somatic mutations of immunoglobulin (Ig) genes.[8,9]

Biclonal lymphomas consisting of T- and B-cell clones were also reported based on serological analysis.[10] In a few cases, the biclonality of T- and B-cell lineage was confirmed by genotypic analysis.[11–13] No data have been presented to date, however, to suggest the relation between these two different clones.

In this paper, we report a lymphoma case with a unique disease history; first, T-cell type, pleomorphic small cell lymphoma, next, B-cell type, diffuse centroblastic/centrocytic lymphoma, and subsequently, T-zone lymphoma without follicles developed, from which a T-cell line, AST-1 was established. Phenotypic and genotypic studies confirmed the lineage switch events. Cytogenetic and genotypic analyses, however, revealed that the first relapsed B-cell lymphoma and the second relapsed T-cell lymphoma had a shared chromosomal translocation and showed T-cell receptor (TCR) γ gene rearrangement bands of the same size, implying that these two lymphomas of different cell lineages were derived from a common malignant clone, presumably a committed lymphoid stem cell.

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AST-1 and its originating T-cell lymphoma showed a unique translocation, t (2; 14) (q 37; q 11.2). The significance of this translocation, which may involve the TCR δ/α gene complex, was studied, but it was found that the TCR δ/α region was not involved in this translocation.

MATERIALS AND METHODS

Case history and histopathology On September, 1980, a 55-year-old male patient with non-Hodgkin's lymphoma was admitted to Aichi Cancer Center Hospital because of a generalized lymphadenopathy. On the basis of lymph node biopsy and staging working up, he was diagnosed as T-cell type, pleomorphic small cell lymphoma (low grade lymphoma, Fig. 1A) of clinical stage III. Complete remission (CR) was obtained on March, 1983, after cytoreductive chemotherapy. On September, 1987, generalized subcutaneous tumors were recognized, and tumor biopsy revealed B cell type, diffuse centroblastic/centrocytic lymphoma (Fig. 1B). Chemotherapy showed CR in January, 1989, but on February, 1989, he complained of generalized subcutaneous tumors and high fever. The biopsy from the right axillar lymph node revealed T-zone lymphoma without follicles (low grade lymphoma, Fig. 1C). Cytology of the second relapsed lymphoma cells in the peripheral blood is shown in Fig. 1D. At this second relapse, AST-1 cell line was established. Combination chemotherapy showed no response. He died of cardiac failure on June, 1989. Autopsy was not performed.

Histology and immunohistology Sections were prepared from formalin-fixed and paraffin-embedded tissues, stained with hematoxylin and eosin, and histologically examined. Diagnosis was made according to the updated Kiel classification.[14] Some of the sections were stained by using the avidin-biotin peroxidase complex (ABC) method[15] with a Vectastain ABC kit (Vector Lab., Burlingame, CA) after deparaffinization in xylene.

Cell culture At the second relapse (June, 1989), heparinized peripheral blood was obtained with the patient's informed consent. Blood smear showed that 80% of nucleated cells were atypical lymphoblasts. Mononuclear cells were separated by Ficoll (Pharmacia, Uppsala, Sweden) gradient centrifugation, and seeded into T-30 Falcon plastic flasks (Oxnard, CA) at 1×10^6 cells/ml in 8 ml of RPMI 1640 medium supplemented with 20% fetal calf serum (FCS), aqueous penicillin G (100 U/ml) and streptomycin (50 μg/ml), together with 1×10^6 human T cell leukemia virus type 1 (HTLV-1)-positive HUT-102 cells preirradiated with a dose of 30 Gy. Cultures were then incubated at 37°C in a humidified atmosphere of 5% CO_2, being fed once a week by partial replacement of spent medium with fresh medium. Neither conditioned medium nor feeder cells were used.

Cell surface and intracellular markers The fresh lymphoma and AST-1 cells were stained with MoAbs directed to differentiation antigens of hematopoietic cells (source of MoAbs, see refs. 9, 16, 17), and fluorescein isothiocyanate (FITC)-labeled monospecific goat anti-human IgG, IgA, IgM, IgD, Igκ or Igλ and analyzed by flow cytometry (FACStar, Immunocytometry Systems, Mountain View, CA), as described previously.[16]

EB virus nuclear antigen (EBNA) was tested by the method of Reedman and Klein with a slight modification.[18] Terminal deoxynucleotidyl transferase (TdT) was assayed by an indirect immunofluorescence technique using a kit (Bethesda Research Laboratory, Rockville, MD).

Lysozyme activity was examined by the immunoperoxidase method (Bethesda Research Laboratory). Phagocytic activity was determined by light microscopy on May-Grunwald-Giemsa stained slides after incubation of cells with opsonized zymosan at 37°C for 30 min.

Chromosome analysis The minced tumor cells obtained from a piece of tumor, about $5\times5\times1$ mm in size, and AST-1 cells were cultured in RPMI 1640 supplemented with 20% FCS. Chromosome preparations were made on the third day of culture. Peripheral blood cells at the second relapse were cultured for 24 h without mitogen and processed for chromosome analysis. The cells were incubated with colcemid at a final concentration of 0.02 μg/ml for 1.5 h at 37°C. Cells were then treated with 50 m*M* KCl solution for 20 min at room temperature, and fixed with methanol-acetic acid (3:1). Chromosomes were banded by the trypsin-Giemsa methods.[19]

Southern blot study High-molecular-weight DNA was extracted from fresh lymphoma and AST-1 cells, and digested with appropriate restriction endonucleases. A panel of somatic cell hybrids between 2301 human and B82 mouse cells for chromosome mapping was provided by Dr. M. C. Yoshida (Hokkaido University School of Basic Science) through the Committee for Research Resources, funded by a Grant-in-Aid for Cancer Research from the Ministry of Education, Science and Culture, Japan. The conditions of Southern blotting were as described previously.[20, 21] The probes used were human IgH joining region probe, J_H (provided by Dr. J. V. Ravetch),[22] human TCR β chain gene probes, $C_T\beta$[23] and $J_{\beta2}$[24] (provided by Dr. T. W. Mak and Dr. Kurosawa, respectively), human TCR α chain gene probes, J_αRR, J_αBB, J_αSS, J_αHE[25] (provided by Dr. T. H. Rabbitts), pMI5, pMI3, pMI2, and pMI1 (provided by Dr. M. Isobe), TCR δ chain gene probe, pMI19[26] (provided by Dr. C. M. Croce), and TCRγ chain gene probe, Jγ1[27] (provided by Dr. T. H. Rabbitts).

Isolation of phage clones containing rearranged regions A genomic library of AST-1 cell line was constructed with partially digested genomic DNA with *Sau*3AI and

λ Dash phage vector (Stratagene, La Jolla, CA). The library was screened with $J_\alpha BB$ and pMI3 probes. Five positive clones were isolated from 3×10^5 individual clones. For sequencing, the modified dideoxy method with T7 DNA polymerase (Sequenase™, USB, Cleveland, OH) was used.

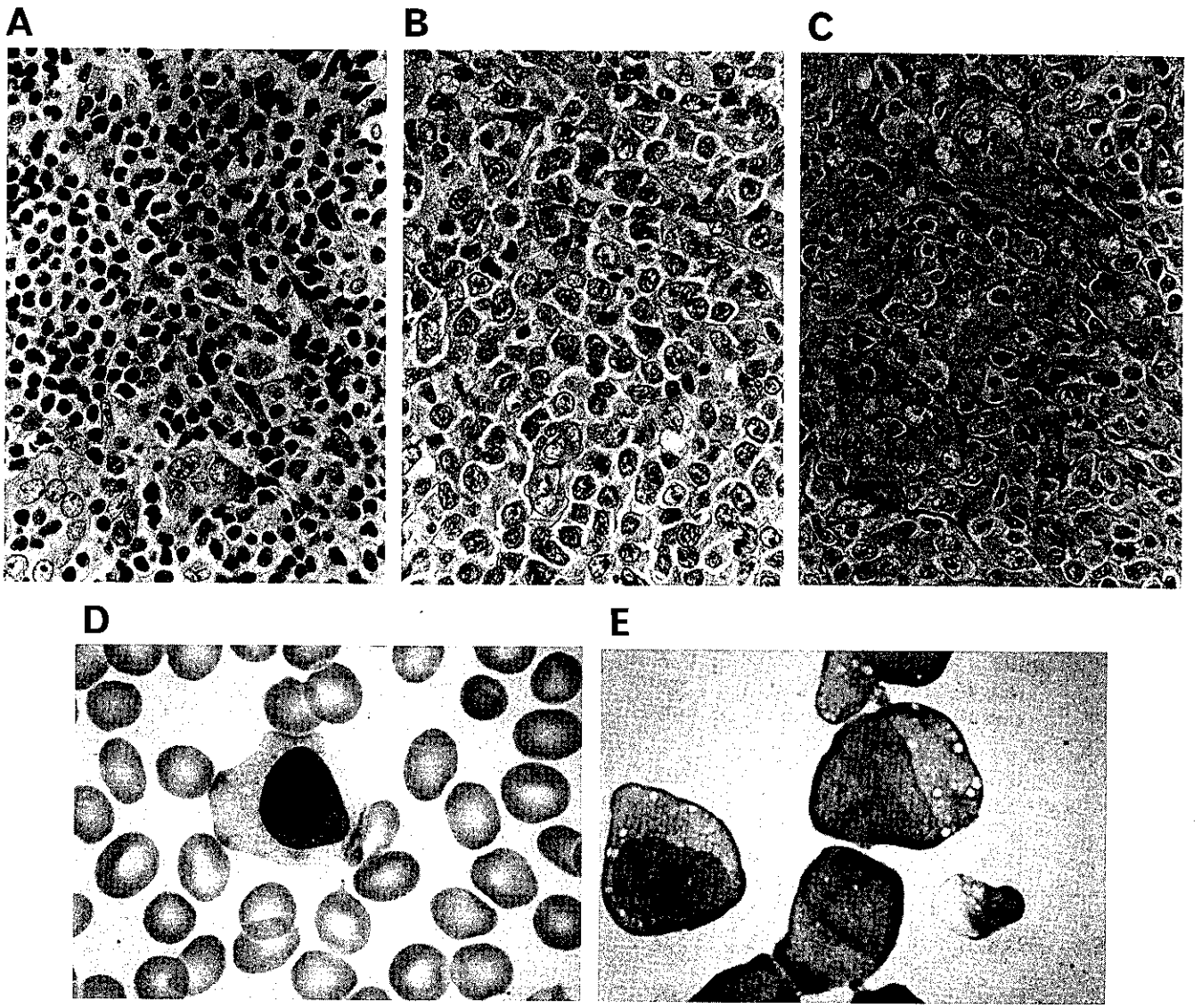

Fig. 1. Histopathology, and cytology of patient's tumors and AST-1 cells. Hematoxylin-eosin staining of the primary (A), the first relapsed (B), and the second relapsed tumors (C). (Magnification, 400×). (A) The primary and second relapsed tumors were diagnosed as pleomorphic small cell lymphoma and T-zone lymphoma without follicles, respectively, while the first relapsed tumor was diagnosed as diffuse centroblastic/centrocytic (B-cell type) lymphoma. The primary lymphoma is predominantly composed of small to intermediate sized cells. The neoplastic cells vary only slightly in size, but considerably in shape. The nuclei are moderately irregular, and mitotic figures are rare. (B) The first relapsed skin tumor is composed mainly of large centroblasts and centrocytes, having large round nuclei and several medium-sized, often peripheral nucleoli. Mitotic figures range from 4 to 8 per high-power field. (C) The second relapsed lymphoma shows a moderate number of large cells including immunoblasts with small to intermediate sized cells. An admixture of eosinophils is also seen. Mitotic figures range from 7 to 10 per high-power field. May-Giemsa staining of a leukemic lymphoma cell at the second relapse (D) and AST-1 cells (E). (Magnification 600×). Cells were mostly round to ovoid with a modal diameter of about 15 to 20 μm, and had large round to ovoid nuclei with fine chromatins and few prominent nucleoli. The cytoplasm is relatively basophilic and contains a few vacuoles. Some larger cells possess cytoplasmic azurophilic granules.

Table I. Phenotypic and Genotypic Analyses of Initial and Two Relapsed Lymphomas and AST-1 Cell Line

Phenotype / Genotype		Fresh lymphoma cells			AST-1 cell line
		Primary	1st relapse	2nd relapse	
Antigen	MoAb				
CD1	OKT6	ND	−	−	−
CD2	OKT11	ND	23	94	76
CD3	Leu4	ND	−	93	86
CD4	Leu3	ND	18	15	−
CD5	Leu1	ND	10	94	99
CD6	Tp120	ND	11	95	97
CD7	Tp40	ND	−	88	96
CD8	Leu2	ND	19	−	−
CD9	BA-2	ND	−	−	39
CD10	NL-1	ND	−	−	−
CD11	OKM1	ND	−	−	−
CD12	MCS2	ND	−	−	−
CD14	My4	ND	−	−	−
CD15	LeuM1	ND	−	−	63
CD16	Leu11	ND	−	−	−
CD19	Leu12	ND	23	−	−
CD20	Leu16	ND	57	−	−
CD21	B2	ND	−	−	−
CD24	BA1	ND	−	−	−
CD25	Tac	ND	−	−	67
CD33	My9	ND	−	−	17
CD45RO	UCHL-1	+	−	+	ND
T cell	MT-1	+	−	+	ND
B cell	L26	−	+	−	ND
MHC					
class II	OKIa1	ND	70	28	97
class II	OKDR	ND	85	43	99
Ig					
IgG		ND	65	−	−
IgA		ND	37	−	−
IgD		ND	−	−	−
IgM		ND	−	−	−
Igκ		ND	69	−	−
Igλ		ND	−	−	−
HTLV-1	ATV19	ND	−	−	98
Gene					
IgH		ND	R/R	G/G	G/G
TCRβ		ND	G/G	R/R	R/R
TCRγ		ND	G/R or R/D	ND	R/D

Abbreviations: CD, cluster of differentiation; MHC, major histocompatibility complex; Ig, immunoglobulin; H, heavy chain; R, rearrangement; G, germline; D, deletion; ND, not done. Phenotype was studied in general by flow cytometry with MoAbs as described in ref. 9, 16 and 17. Results are expressed as the percentage of cells expressing each antigen. −; <10%. Immunostaining was also conducted with UCHL-1, MT-1 and L26 MoAbs as described in "Materials and Methods," when formalin-fixed paraffin-embedded specimens were examined. +, >50%, −, <10%. HTLV-1 was tested with acetone-fixed smears by immunofluorescence. Genotype was studied by Southern blotting with IgH, and TCRβ and TCRγ probes as described in "Materials and Methods" (see Figs. 3 and 4).

RESULTS

Establishment of AST-1 cell line Approximately 3 weeks after the start of coculture with irradiated HTLV-1$^+$ HUT-102 cells, cells proliferated in suspension culture forming loosely attached cell clumps with a doubling time of 36 h. The cells have been in culture for more than one year and were designated as AST-1. Cells were negative for EBNA and were free of mycoplasma infection. Anti-HTLV-1 antibody stained most of the cultured cells (Table I). A smear of the cultured cells (Fig. 1E) showed similar morphological characteristics to those of the original lymphoma cells in peripheral blood (Fig. 1D).

Phenotypic study of lymphoma cells and AST-1 cells (Table I) Since no lymphoma cells were available for flow cytometric analysis from the primary lymphoma (September, 1980), immunohistological examination of the formalin-fixed, paraffin-embedded lymphoma tissue was performed using UCHL-1 [cluster of differentiation (CD) 45RO] and MT-1 anti-T cell and L26 anti-B cell antibodies, all of which are known to be useful for staining sections prepared from routinely fixed tissues.[28-30] UCHL-1 and MT-1 antibodies stained a significant proportion of lymphoma cells, whereas only a few cells were positive with L26 antibody, showing that the initial lymphoma has a T-cell phenotype (data not shown).

The first relapsed lymphoma (September, 1987): Flow cytometric analysis showed that the prodominant cell population in the lesion was CD9$^-$, CD10$^-$, CD19^{+-}, CD20$^+$, major histocompatibility complex (MHC)-class II$^+$ and surface(s) IgG$^+$ mature B cells. Immunohis-

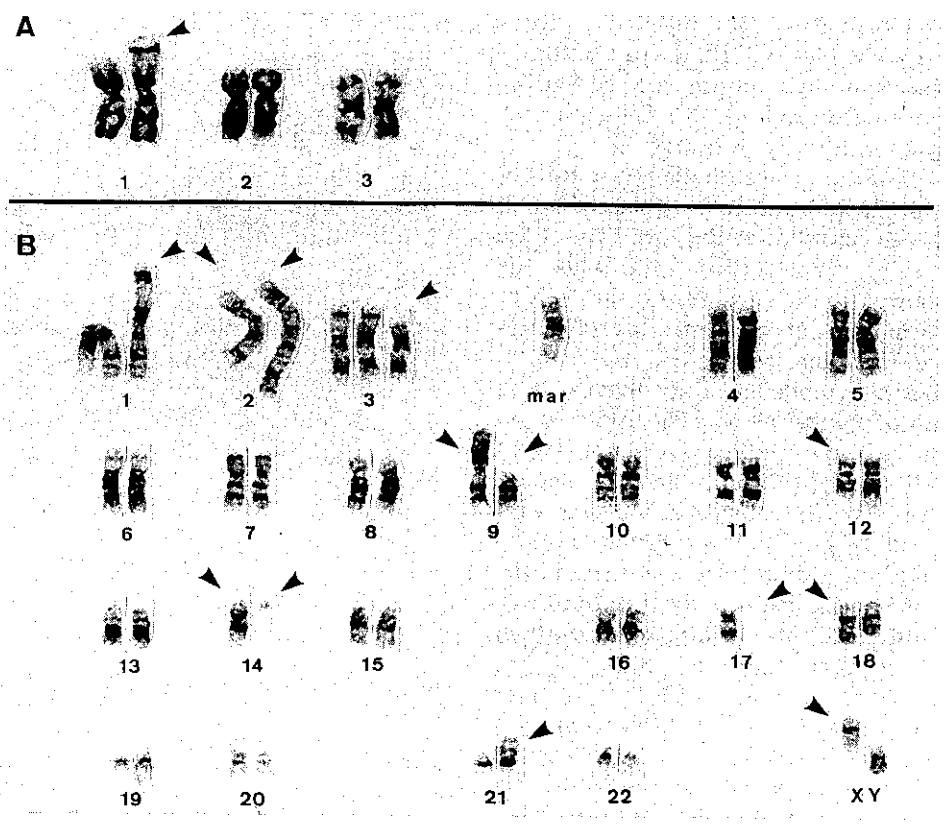

Fig. 2. Karyotypes of the first relapsed tumor and AST-1 cells. (A) Partial karyotype of skin tumor at the first relapse showing der(1)t(1;?)(p36;?), (an arrowhead). See the text for details. (B) Karyotype of AST-1 cells. Chromosome aberrations observed are as follows; $-1, -2, -2, -9, -9, -12, -14, -14, -17, -18, -21, -X$, + der(1)t(1;?)(p36;?), + der(2)t(2;?)(q31;?), + der(2)t(2;14)(q37;q11.2), + del(3)(p21), + del(9)(p13), + der(9)(X;9)(q13;p24), + der(12)t(12;14)(q15;q24), + der(14)t(2;14)(q37;q11.2), + der(14)t(12;14)(q15;q24), + der(18)t(18;?)(q21;?), + der(21)t(21;?)(p13;?), + der(X)t(X;9)(q13;p24), + mar. Arrowheads indicate derivative chromosomes or deleted chromosomes. The karyotype of the second relapse was identical to this karyotype (not shown).

tological staining of paraffin sections with UCHL-1 and MT-1 antibodies was almost entirely negative, while L26 antibody stained a significant population.

The second relapsed lymphoma (March, 1989): The predominant cell population in the lesion showed T-cell phenotype, $CD2^+$, $CD3^+$, $CD5^+$, $CD7^+$, $CD4^-$, and $CD8^-$. Immunohistological staining of paraffin sections with UCHL-1 and MT-1 was also positive. B-cell antigens such as CD19, CD20, and sIg, which were positive in the first relapsed lesion, were uniformly negative. TdT was negative. Peripheral blood lymphoma cells, from which AST-1 was established, showed the identical T-cell phenotype to tumor cells in the lymph node.

AST-1 cells: AST-1 cells showed almost the same T-cell phenotype as the second relapsed lymphoma cells, $CD2^+$, $CD3^+$, $CD5^+$, $CD7^+$, $CD4^-$, $CD8^-$, suggesting that AST-1 cell line was derived from the second relapsed lymphoma clone. AST-1 cells, however, strongly expressed MHC-class II and CD25 antigens as well, which may be induced by HTLV-1 infection[31] or activation through culture. Neither B-cell nor myeloid antigens were expressed except CD9 and CD15, the expression of which may be induced through culture as well. Neither EBNA nor TdT was detected in AST-1 cells. Neither lysozyme nor phagocytic activity was observed.

Chromosome study The first relapsed lymphoma: Fifteen dividing cells were available from the first relapsed lesion (October, 1987). Seven cells showed the karyotype of 46, XY, $-1, +$der(1)t(1;?)(p36;?) (Fig. 2A), while the remaining eight showed the normal karyotype, 46,XY. Therefore, this abnormal chromosome, der(1)t(1;?)(p36;?) is specific to the tumor cells.

The second relapsed lymphoma: Two dividing cells were available from peripheral blood cells at the second relapse (March, 1989). The karyotype was 47,XY,der(1)t(1;?)(p36:?), der(2)t(2;14)(q37;q11.2) and 11 other abnormal chromosomes. This karyotype was identical with that of AST-1 cell line (Fig. 2B). The aberrant chromosome, der(1)t(1;?)(p36;?), common to the first and the second relapsed tumor cells, had the identical chromosomal fragment translocated to 1p36, although the origin of the fragment has not been identified.

AST-1: Fifty-six karyotypes of AST-1 cells were analyzed and the modal number of the chromosome was 47 (61%). AST-1 cells showed identical chromosomal abnormalities to those in the second relapsed lymphoma cells, including der(1)t(1;?)(p36;?), and der(2)t(2;14)(q37;q11.2) (Fig. 2B).

Southern blot study A Southern blot study with J_H probe showed IgH gene rearrangement in the first relapsed tumor, but not in the second relapsed tumor or AST-1 line (Fig. 3A, AST-1; data not shown). On the other hand, two rearrangement bands of TCR β gene were observed in both the second relapsed tumor and AST-1 line (Fig. 3B). The rearrangement patterns were identical, implying that AST-1 was derived from the second relapsed tumor clone. These results, together with phenotypic analysis, demonstrated that the first relapsed tumor was of B-cell lineage, while the second relapsed tumor and AST-1 were of T-cell lineage. Interestingly, TCRγ rearrangement bands with the same size were demonstrated in the first relapsed B-cell tumor as well as in AST-1 cell line (Fig. 4B). By densitometric analysis of the IgH germline band and the rearranged one detected with J_H probe, monoclonal proliferation of malignant B cells was estimated to be about 20% in the first relapsed subcutaneous tumor (Fig. 3A, lane 2 and 4A, lane 1). Almost the same intensity ratio was observed between TCRγ germline band and the rearranged one (Fig. 4B, lane 1), suggesting that the first relapsed B-cell tumor had TCRγ rearrangement in addition to IgH rearrangement.

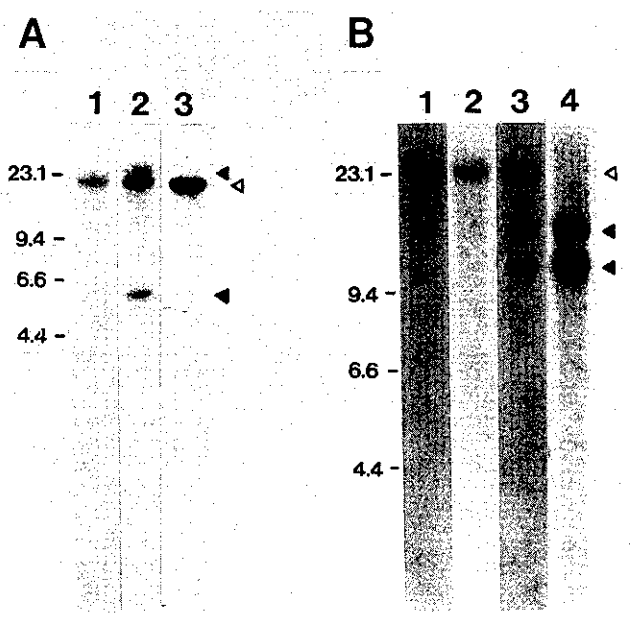

Fig. 3. Southern blot of the first and the second relapsed lymphomas and AST-1 cell line with IgH and TCR β probes. (A) IgH gene rearrangement is observed in the first relapsed tumor (lane 2), but not in the second relapsed tumor (lane 3). Human placenta DNA is in lane 1. DNA was digested with *Eco*RI, and hybridized with J_H probe. (B) The second relapsed tumor (lane 3) and AST-1 cells (lane 4) show two rearrangement bands of TCR β gene, their sizes being identical with each other. The first relapsed tumor (lane 2) shows only a germline band. DNA from fibroblasts of this patient is in lane 1. DNA was digested with *Bam*HI, and hybridized with $J_{\beta 2}$. Open arrowheads indicate the germline bands, while solid arrowheads show rearrangement bands. Dashes indicate the positions of the size markers (23.1, 9.4, 6.6, and 4.4 kb).

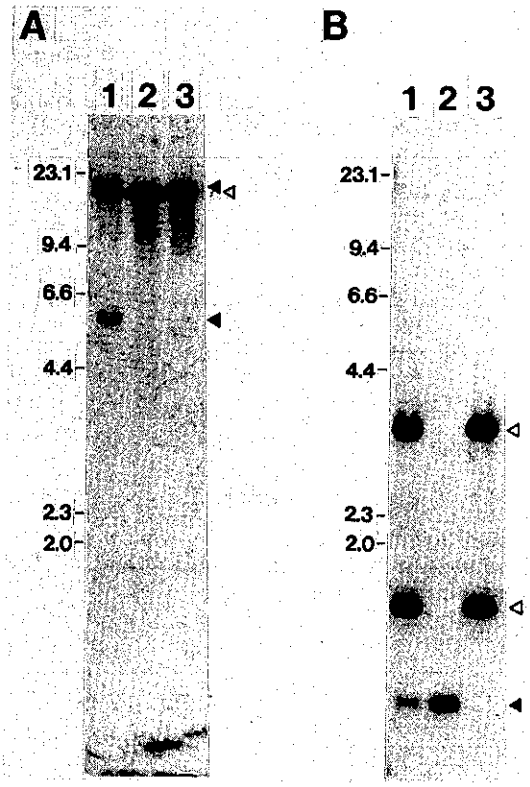

Fig. 4. Southern blot of the first relapsed B-cell lymphoma and AST-1 cell line with J_H and TCR γ probes. DNA from the first relapsed tumor (lane 1), AST-1 line (lane 2) and human placenta (lane 3) was digested with *Eco*RI. The blot was first hybridized with J_H probe (A), and then with $J_{\gamma 1}$ probe (B). (A) IgH gene rearrangement is observed in the first relapsed tumor (lane 1). The intensity ratio between the rearrangement and germline bands was quite similar to that observed with $J_{\gamma 1}$ probe (Fig. B, lane 1). (B) Rearrangement bands of the same size are observed with TCR $J_{\gamma 1}$ probe in the first relapsed tumor (lane 1) and AST-1 line (lane 2). See the legend to Fig. 3.

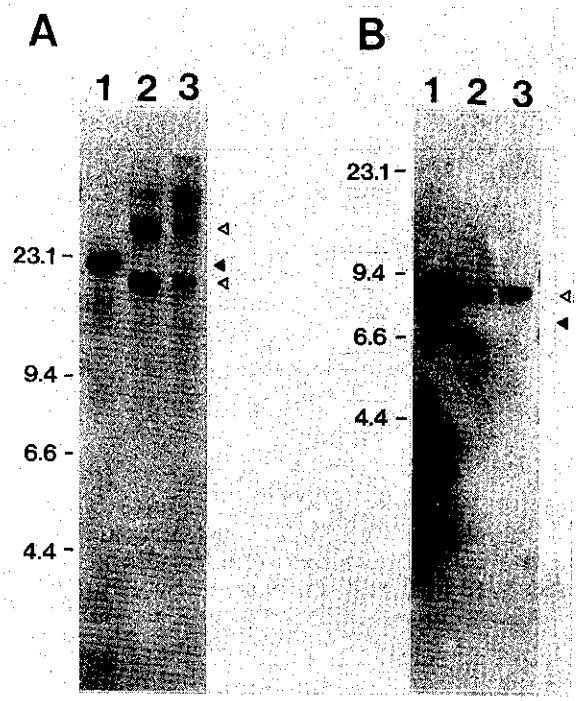

Fig. 5. Southern blot of AST-1 cell line with J_αBB and pMI3 probes of TCR δ/α complex. (A) J_αBB probe (Fig. 6) shows rearrangement of one allele and deletion of the other in AST-1 cells (lane 1). The fibroblast culture derived from the patient (lane 2) and human placenta (lane 3) showed the germline bands (open arrowheads). A solid arrowhead indicates a rearrangement band. DNA was digested with *Xho*I. (B) The pMI3 probe (Fig. 6) demonstrates a rearrangement band, in addition to the germline band, in AST-1 cell (lane 1). DNA was digested with *Sac*I. See the legend to Fig. 3.

Isolation and characterization of rearranged regions of TCR α chain gene The unique reciprocal translocation t(2;14)(q37;q11.4) was observed in the second relapsed tumor and AST-1 cells, but not in the first relapsed tumor. TCR δ/α gene complex is present in the 14q11.2 region, and is frequently involved in development of T-cell malignancy. Although 2q37 has not been reported to be associated with the common chromosome abnormality of hematopoietic malignancy, it is possible that the gene at 2q37 may play a role in tumor progression or cell lineage switch of lymphoma. Accordingly, we have tried to isolate the breakpoint at 2q37 using TCR δ/α region probes.

First, the configuration of TCR δ/α locus was studied. TCR δ gene probe, pMI19 (Fig. 6), showed the deletion of both alleles, implying that both alleles of TCR α gene were rearranged in AST-1 cells (data not shown). The configuration of the joining region of TCR α gene was studied from the 5′ side to the 3′ side (Fig. 6). The 5′-most probe J_αRR showed deletion of both alleles (data not shown). The next probe, J_αBB, detected one rearrangement with *Xho*I digestion (Fig. 5A). Probes 3′ to it, J_αSS, J_αHE, and pMI5, showed only germline bands (data not shown). The next probe, pMI3, demonstrated the other rearrangement (Fig. 5B). Probes 3′ to it, pMI2 and pMI1, showed only germline patterns. These results implied that two alleles of TCR α gene were rearranged at different sites (Fig. 5). Since one of these two rearrangements was assumed to be involved in the translocation, an attempt was made to isolate both rearranged regions.

A genomic library of AST-1 line was constructed using λ Dash phage vector, and was screened with both J_αBB and pMI3 probes. Five positive clones (#1, 2, 3, 5, and 9)

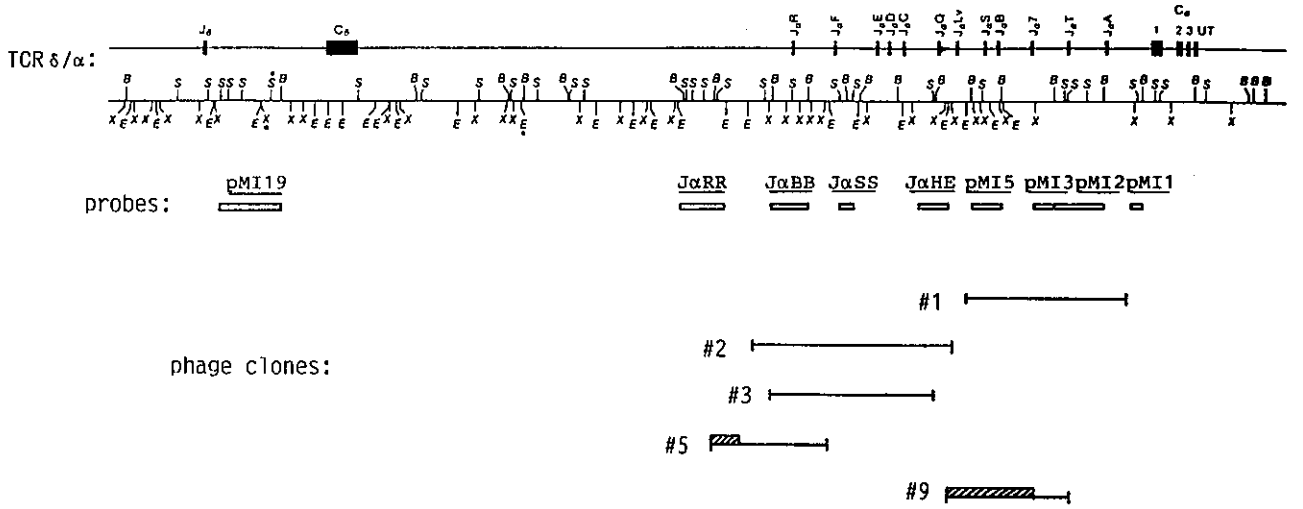

Fig. 6. Structure of TCR δ/α gene and localization of probes and phage clones. Solid boxes indicate exons, while open boxes show probes. The insert of each phage clone is indicated with a solid bar. Hatched boxes indicate the rearranged regions. E, *Eco*RI; B, *Bam*HI; S, *Sac*I; X, *Xba*I. The figure by Isobe *et al.* (Ref. 26) is shown with some modification.

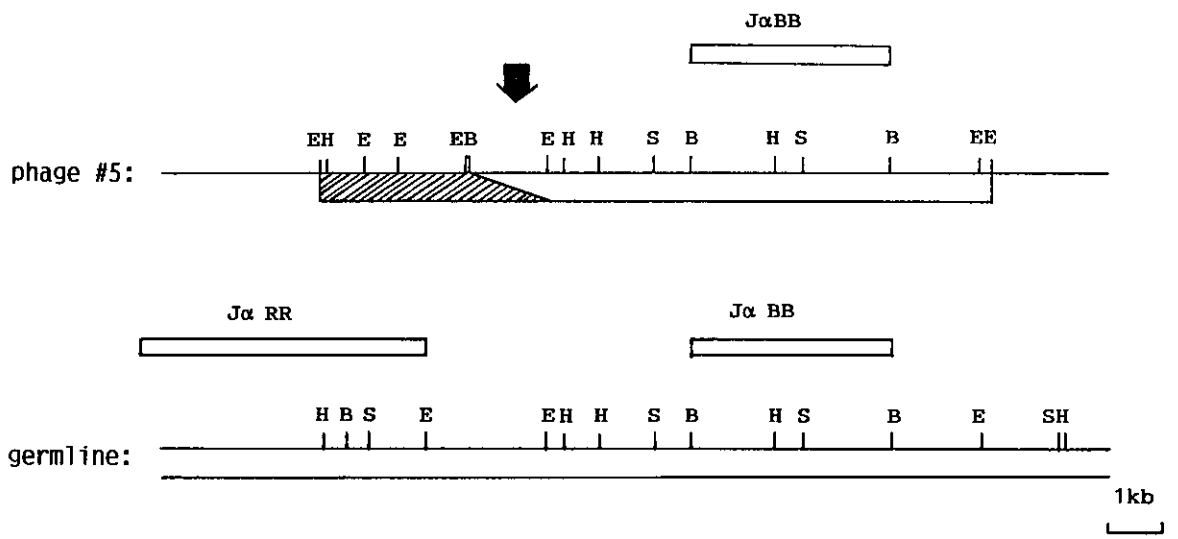

Fig. 7. Restriction enzyme map of phage #5. The solid arrow indicates the rearranged site. See the legend to Fig. 6.

were isolated (Fig. 6). The restriction maps of these clones showed that three clones (#1, 2, and 3) had the germline sequence, while two clones (#5 and 9) had rearranged regions (Fig. 6). Fig. 7 showed the restriction enzyme maps of the insert of clone #5 and germline restriction map. The 5′ portion of the insert (5′ side to the arrow) was different from the germline configuration, while the 3′ portion (3′ side to the arrow) was identical. Therefore, the arrow indicates the breakpoint of this rearrangement, the 5′ portion being the rearranged region. Fig. 8 showed the maps of clone #9 and the germline configuration. Two-thirds (5′ side to the arrow) of the insert was different, suggesting that this portion was the rearranged region.

To determine which of these two regions was involved in the translocation, the breakpoint region of clone #9 (*Xba*I/*Xba*I 0.6 kb fragment, Fig. 8) was sequenced. The sequence showed a significant homology with human $V_\alpha 8$

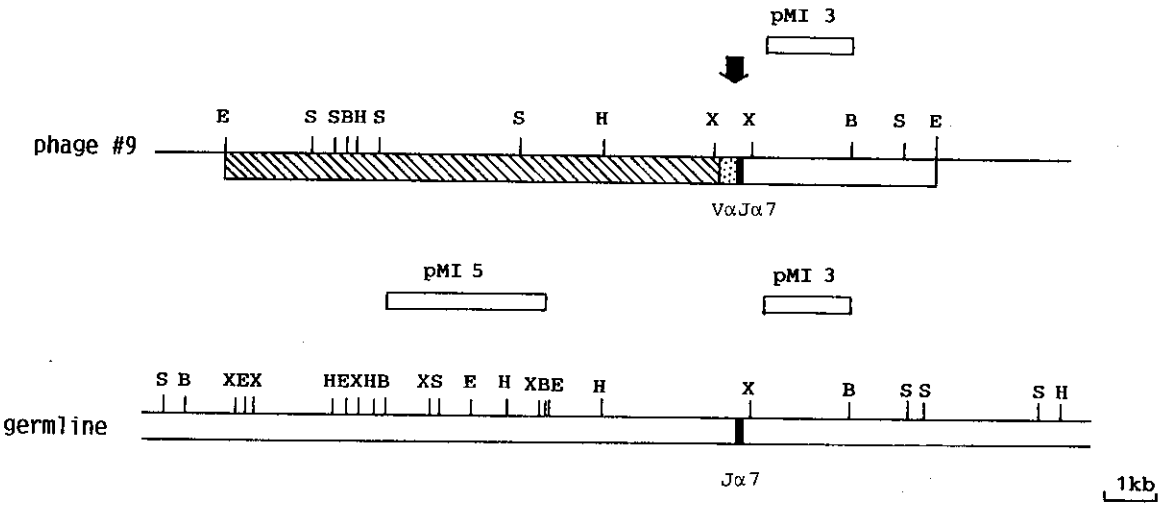

Fig. 8. Restriction enzyme map of phage #9. Dotted and solid boxes indicate V_α and J_α segments, respectively. See the legends to Figs. 6 and 7.

family members. A Southern blot of the 1.5 kb *Bam*HI/*Hin*dIII fragment containing the rearranged site of clone #5 (Fig. 7, indicated by the arrow) demonstrated the gene rearrangement with various restriction endonucleases corresponding to the rearrangements detected with a probe mixture of V3, V4 and V5, suggesting that the fragment of clone #5 contains one of these Vα family members (data not shown). The most 5′ fragment probe of each rearranged region was analyzed by Southern blotting with a panel of somatic cell hybrids. Both fragments of clones #5 and #9 showed the hybridization pattern consistent with chromosome 14, suggesting that the rearrangements at the α locus do not contain chromosome 2 element (data not shown). These results suggested that the two rearrangements at α were the results of normal V-J rearrangement processes.

DISCUSSION

The patient studied showed a unique disease history; first, T-cell type, pleomorphic small cell lymphoma, second, B-cell type, diffuse centroblastic/centrocytic lymphoma, and subsequently, T-zone lymphoma without follicles. The first relapsed B-cell lymphoma developed five years after complete remission of the primary T-cell lymphoma, the interval being longer than that between the first and the second relapses. This may suggest that the relapsed lymphomas are secondary malignancies induced by chemotherapy, since secondary tumors usually develop 3–10 years after chemotherapy.[32,33] It is also possible, however, that the primary and the second relapsed T-cell lymphomas, both belonging to the low-grade group, were derived from the same malignant clone. Since no materials of the primary lymphoma were available for precise studies, it was not possible to determine the relation to both relapsed lymphomas.

Phenotypic and genotypic studies on the two relapsed tumors demonstrated the lineage switch from B-cell to T-cell lymphoma, suggesting that these lymphomas were derived from different clones. Karyotypic study, however, showed that the same abnormal chromosome der(1) t(1;?)(p36;?) was shared by the first relapsed B-cell lymphoma and the second relapsed T-cell lymphoma. Furthermore, by TCRγ gene analysis, rearrangement bands of the same size were demonstrated in the first relapsed B-cell tumor and AST-1 line from the second relapsed T-cell tumor. These results suggested that both relapsed tumors are derived from a common clone, although this may be coincidental, since the V gene repertoire of TCRγ gene is rather limited.[34] The intensity ratio between rearrangement band and germline band was similar in the former B-cell tumor, when either J_H probe or TCRγ probe was used, suggesting that malignant B-cell clone shows not only IgH, but also TCRγ gene rearrangement, as reported with a certain fraction of B cell tumors.[35] Cytogenetic and genotypic analyses altogether suggested that these relapsed tumors of different cell lineage have developed from a committed lymphoid stem cell, and may be regarded as a kind of stem cell disease. Several cases with biclonal T-cell and B-cell lymphoma have been reported to date, but genotypic study was performed in only a few cases to show the biclonality.[11–13] No reports included both cytogenetic and genotypic data to suggest the relation between the two clones. Does this result

suggest that malignant lymphoma is usually a stem cell disease? Recently, several hematological malignancies have come to be recognized as a pluripotent stem cell disease, which includes chronic myelogenous leukemia (CML), myelodysplastic disease, and hybrid acute leukemia.[36] It is unlikely, however, that malignant lymphoma in general is a stem cell disorder, because a lymphoma clone is usually more differentiated than a hybrid acute leukemia or blastic crisis of CML. Even in this case, the possibility of biclonal origin of this lymphoma can not be ruled out, but the results suggest that lymphoma may, in some cases at least, involve clonal genetic events at an earlier stage such as committed lymphoid stem cells, although the characterization of the V region and demonstration of a clonal N-segment in two relapsed tumors may be necessary. A similar idea has been proposed by Hu *et al.*[11] and Shimizu *et al.*[12] by studying bioclonal T- and B-cell lymphomas, and also by Greaves.[36]

The 1p36 locus is frequently involved in hematological malignancies.[37, 38] Reciprocal translocations, t(1; 17)(p36;q21) and t(1;3)(p36;q21) were reported to be associated with acute nonlymphocytic leukemia and myelodysplastic disease.[38, 39] Besides der(1)t(1;?)(p36;?), other additional chromosome abnormalities were observed in the second relapsed tumor and AST-1 cells, but not in the first relapsed tumor. These abnormalities may play a role in either lineage switch or clonal evolution. One of these additional chromosome abnormalities, t(2;14)(q37;q11.2), was interesting, because it may involve TCR δ/α gene complex. We tried to isolate the breakpoint of the 2q37 locus with TCR δ/α gene probes in order to clearify the significance of this translocation. Two clones containing two different rearranged regions were obtained from a genomic phage library of AST-1 cell. Analysis of these two clones elucidated that both the rearranged regions were the products of normal TCR α gene rearrangement processes, suggesting that this translocation does not involve the J segment region of the TCR α gene. Another candidate for the breakpoint may be a V_α segment, because Bernard *et al.* reported a T-cell leukemia with a translocation joining the 3′ region of c-*myc* and a V_α segment.[40] Therefore, it is possible that t(2;14)(q37;q11.2) translocation involves a V segment other than those involved in the normal V-J rearrangements. On the basis of this hypothesis, we are conducting Southern blot analysis with V_α family probes[41] (provided by Dr. Y. Yoshikai), using conventional electrophoresis and pulse field gel electrophoresis. We are also producing hybrid cells between AST-1 cells and a mouse T cell line to isolate hybrid clones containing either $2q^+$ or $14q^-$, which may allow us to elucidate the possible involvement of a V_α segment in this translocation.

In conclusion, cytogenetic and genetic analysis of our unique lymphoma case showed that two relapsed lymphomas with different phenotypes, T cell and B cell, may be derived from the same clone, suggesting that a certain fraction of lymphomas may involve clonal genetic events of an earlier stage, although this may be exceptional. The unique case as such is very valuable for studying the development of hematopoietic tumors.

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