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Oligoclonal Immunoglobulin Gene Rearrangements in Philadelphia Chromosome-positive Common Acute Lymphoblastic Leukemia

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The occurrence of more than two rearranged bands of immunoglobulin heavy chain (IgH) genes in B precursor acute lymphoblastic leukemia (ALL) has recently been documented. To elucidate the nature of such leukemias, we studied 30 patients with common ALL, including 6 patients with Philadelphia chromosome (Ph[1])-positive ALL, by immunophenotyping and genotyping. In 10 of the 30, Southern blotting showed oligoclonal patterns of IgH gene arrangements, which were frequently detected in Ph[1]-positive ALL. In one patient of the 10, three rearranged bands of Ig κ chain genes were detected. Ph[1] abnormality and co-expression of myeloid associated antigens were found in 5 and 5 of the 10, respectively. Detection of multiple fragments of IgH genes would be suggestive of multipotent progenitor origin of these ALL.

Key words: Immunoglobulin gene — Ph[1]-positive common acute lymphoblastic leukemia

Detection of immune gene rearrangements has been considered to be a sensitive marker for monoclonality as well as lineage commitment.[1-4] One allelic rearrangement and two allelic rearrangements in DNAs from single clonal cells should cause one and two rearranged bands on Southern blots, respectively, after appropriate restriction enzyme digestion. Recently, more than two immunoglobulin (Ig)[7] heavy chain gene rearrangements in acute leukemias have been reported, and their origin and clinical importance have been discussed.[5-10] To elucidate the nature and origin of such leukemias, we analyzed thirty Japanese patients with common acute lymphoblastic leukemia (ALL) for immunophenotype and immunogenotype.

MATERIALS AND METHODS

Thirty patients with common ALL were examined. Six of them were Philadelphia chromosome (Ph[1])-positive. Mononuclear cells were obtained from peripheral blood or bone marrow aspirates by Ficoll-Hypaque density gradient centrifugation after informed consent of the patients had been obtained. Analyses were carried out on freshly isolated cells or on samples cryopreserved in liquid nitrogen.

Immunophenotype The surface marker study was performed by the immunofluorescence method, as described previously.[11] CD2 was tested by using OKT11 (Ortho Diagnostic Systems Inc., Raritan, NJ) as a T-cell marker. CD19 and CD20 were tested by using B4 (Coulter Immunology, Hialeah, FL) and B1 (Coulter), respectively as B-cell markers. CD10 was checked by using J5 (Coulter) as common ALL antigen, and Ia antigens by using OKIal (Ortho). CD11b, CD13, CD15 and CD33 were detected by using OKM1 (Ortho), MCS2 (kindly provided by Dr. Tatsumi, Kobe University), LeuM1 (Becton-Dickinson Immunocytometry Systems, Mountain View, CA) and My9 (Coulter), respectively, as myeloid associated markers.

Immunogenotype High-molecular-weight DNAs were extracted from mononuclear cells, digested with an appropriate restriction enzyme (Takara Shuzo, Kyoto), electrophoresed on 0.6% agarose gel, and transferred to nitrocellulose membrane as described by Southern.[12] The membrane was hybridized to [α-^{32}P]dCTP-labeled DNA probes, washed and autoradiographed. Molecular probes used were as follows: JH, joining (J) region gene of Ig heavy chain (IgH) (*Eco*RI-*Hin*dIII, 3.4 kb)[13]; Jκ, J region gene of Ig κ chain (*Sac*I-*Sac*I, 1.9 kb)[14]; Jγ1, J region gene of T cell rearranging gene γ (TRGγ) (*Hin*dIII-*Eco*RI, 0.7 kb)[15]; Cβ1, constant region gene of T cell receptor β chain (TcRβ) (*Hin*dIII-*Eco*RI, 3.5 kb).[16]

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[7] Abbreviations: Ig, immunoglobulin; ALL, acute lymphoblastic leukemia; IgH, Ig heavy chain; Igκ, Ig κ chain; Ph[1], Philadelphia chromosome; TcRβ, T cell receptor β chain; TRGγ, T cell rearranging gene γ.

RESULTS

Table I shows the immunogenotypes of common ALL cells. All 30 patients had rearranged IgH genes, and 13 of them also had rearranged Igκ genes. Nineteen and 7 patients had rearranged TRGγ and TcRβ genes, respectively. In 10 patients, Southern blots of IgH genes showed interesting patterns as illustrated in Fig. 1. More than two rearranged bands of IgH genes were found in 5 patients (cases 2, 3, 6, 8 and 9). In 4 patients (cases 4, 5, 7 and 10), two rearranged bands in addition to a germ line band of significant intensity were detected. The germ line band was considered as the genotype of leukemic cells because the percentage of nonleukemic cells was less than the sensitivity of Southern blot analysis. In one patient (case 1), the Southern blot showed a rearranged band of markedly different intensity from the germ line band, which could not be due to a single population of cells with one rearranged allele and another germ line allele. Thus, it was considered that leukemic cells existed with the germ line IgH genes. Polysomy or translocation involving chromosome 14, where IgH genes are located, was not found. Because Southern blots of *Bgl*II-digested DNAs also showed oligoclonal patterns, restriction polymorphism could be ruled out. In these 10 patients, therefore, leukemic blasts were considered to consist of more than one population with respect to Ig gene arrangements. Southern blotting of Igκ genes in case 7 showed three rearranged bands and a germ line band (Fig. 2). Representative Southern blots of Igκ, TRGγ and TcRβ genes are shown in Fig. 3.

Phenotypic and karyotypic characteristics of ALL with oligoclonal IgH gene arrangements The phenotypes of the 10 patients with oligoclonal IgH gene arrangements are shown in Table II. Five of them (50%) reacted with one or more of CD11b, CD13 and CD33, whereas 5 out of 20 (25%) with monoclonal IgH rearrangements did. Five patients had Ph^1 chromosomal abnormalities. In the 6 patients with Ph^1-positive ALL, these oligoclonal arrangement patterns were detected with especially high frequency (83%).

DISCUSSION

More than two IgH gene rearrangements have been found in some infant and childhood acute leukemias.[7-9]

Table I. Immunogenotypes of common ALL Cells

	IgH	IgH[a)]	Igκ	TRGγ	TcRβ
Total cases	30/30	10/30(33%)	13/30 (43%)	19/30 (63%)	7/30 (23%)
Ph^1(+) cases	6/6	5/6 (83%)	2/6 (33%)	4/6 (67%)	1/6 (17%)

Data are given as rearranged/examined cases (% positive cases).
a) Oligoclonal pattern of IgH gene rearrangements was found.

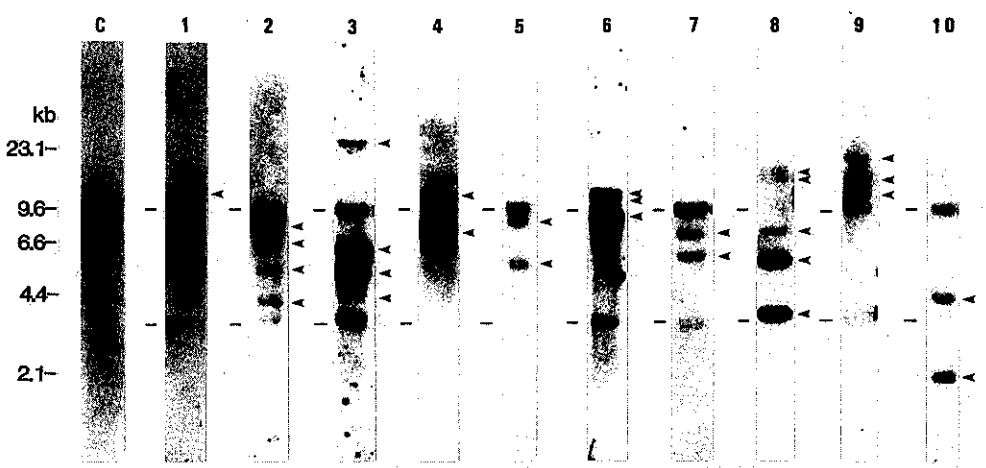

Fig. 1. Southern blot of DNAs from common ALL patients. DNAs were digested with *Hin*dIII and hybridized to the JH probe. Case numbers are the same as in Table II. C shows the germ line gene. The rearranged bands are indicated by the arrows.

Our results showed that about a third of Japanese patients with common ALL (including only one childhood ALL, case 2) had oligoclonally arranged IgH genes, and more than two Igκ gene rearrangements were found in one patient. This indicates that oligoclonal IgH gene arrangements are not rare in adult ALL as well, and that Igκ gene can yield two clonal rearrangements.

Substantial Ig gene rearrangements for functional Ig molecule production may cause multiple different IgH rearrangements in a single neoplastic clone during clonal evolution. Novel V_H to V_HDJ_H joining is known to occur in murine pre-B cell line transformed by Ableson murine leukemia virus during culture.[17] On the other hand, Bird *et al.*[10] sequenced IgH DNA from two patients with multiple rearranged bands, and showed that these rearrangements did not always share common DJ joinings. A possible explanation was that malignant transformation had occurred in a stem cell lacking rearranged Ig genes and continuing IgH gene rearrangements followed. Frequent TRGγ or TcRβ gene rearrangements might be induced in the process of IgH gene rearrangements.[18]

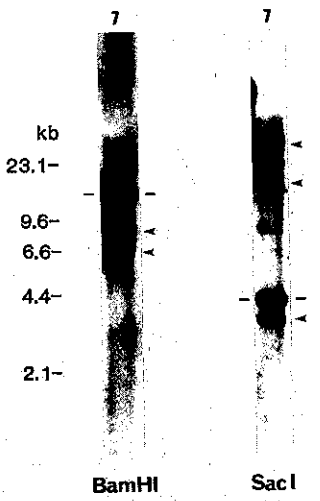

Fig. 2. Southern blot of DNAs from case 7. DNAs were digested with *Bam*HI and *Sac*I, and hybridized to Jκ probe. The germ line Igκ gene is indicated by the line. The rearranged bands are indicated by the arrows.

Recent reports have documented a high incidence of multiphenotype or multigenotype in Ph^1-positive acute leukemias, which supported the theory that pluripotent progenitors were involved in these leukemias[19,20] and that most Ph^1-positive ALL is acute mixed-lineage leukemia. In our study, oligoclonal arrangements of IgH genes were frequently found in adult patients with Ph^1-positive ALL. Further, even in the patients without Ph^1 abnormality, expression of myeloid associated antigens, which suggested that malignant transformation had occurred in lymphoid-myeloid common progenitor cells, was detected. These findings represent supportive evidence for pluripotent stem cell origin of common ALL cells with multiple arranged IgH gene. Adult ALLs with myeloid antigen expression are known to form a high risk group.[21] Detection of multiple arranged fragments of IgH genes would be as poor a prognostic factor in adult common ALL as Ph^1 abnormality or myeloid antigen expression.

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Table II. Phenotypes and Genotypes of Common ALL Cells with Oligoclonal IgH Gene Rearrangements

No.	Ia	CD19	CD10	CD20	CD2	CD11	CD13	CD15	CD33	Igκ	TRGγ	TcRβ	Ph^1
1	93	78	33	4	7	5	3	NT[a]	32	R	G	G	+
2	91	74	63	5	6	41	0	0	0	G	G	G	−
3	85	87	82	14	2	2	0	30	0	G	G	G	NT
4	79	82	77	16	19	0	8	5	1	G	R	R	+
5	87	90	39	16	8	5	36	NT	63	R	R	R	−
6	81	55	78	49	0	6	12	25	1	G	R	G	NT
7	71	77	77	66	8	2	0	2	NT	R[b]	G	G	+
8	98	95	97	68	0	NT	1	2	1	G	R	G	+
9	92	91	88	78	5	6	0	47	3	G	R	G	+
10	93	92	92	90	0	5	0	NT	13	G	R	R	−

Data are given as % positive cells.
a) NT, not tested.
b) Three rearranged fragments of Igκ genes were found.

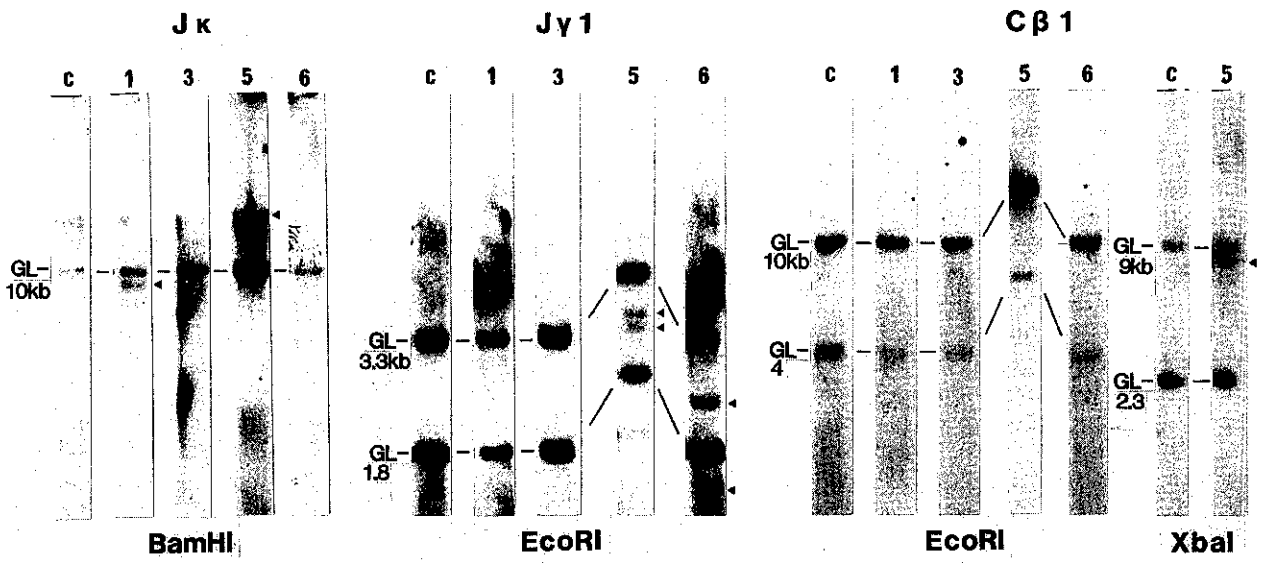

Fig. 3. The representative Southern blot of Igκ, TRGγ and TcRβ genes. Case numbers are the same as in Table II. C shows the germ line gene. The rearranged bands are indicated by the arrows.

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