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Non-T, Non-B Acute Lymphocytic Leukemias: Cellular Origin Based on Molecular Analyses of Immunoglobulin and T-Cell α- and β-Chain Receptor Gene Rearrangements

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OKABE, M., MATSUSHIMA, S., FUKUHARA, T., TANAKA, M., SAKURADA, K., KAKINUMA, M., MAEKAWA, I. and MIYAZAKI, T. *Non-T, Non-B Acute Lymphocytic Leukemias: Cellular Origin Based on Molecular Analyses of Immunoglobulin and T-Cell α- and β-Chain Receptor Gene Rearrangements.* Tohoku J. exp. Med., 1987, **152** (2), 197-207 —— Fifteen non-T, non-B acute lymphocytic leukemia (ALL) cases were investigated for determining cellular origin based on molecular (immunoglobulin and T-cell α-receptor (TcRα) and T-cell β-receptor (TcRβ) genes) and immunophenotypical analyses. As defined by monoclonal antibodies, they were classified into 2 groups; 12 cases as common ALL antigen (CALLA)-positive ALL and 3 cases as CALLA-negative ALL. Southern blot analysis revealed that 11 CALLA-positive ALL cases contained rearranged J_H gene and 2 of them contained rearranged J_κ genes, similar to recent views that most CALLA-positive leukemic cells are neoplastic B-cell precursors. One CALLA-positive ALL case, whose leukemic cells were also Leu-1 positive, showed no rearrangement of J_H and TcRβ genes. On the other hand, non-T, non-B CALLA-negative ALL, so called null ALL, consisted of heterogenous groups with regard to lymphocyte differentiation and lineage; one out of 3 null ALL cases may be truely undifferentiated as shown neither J_H nor TcRβ gene rearrangement, but other 2 cases showed either J_H or TcRβ gene rearrangement. Dual rearrangements of Ig and TcRβ genes occur frequently in 3 out of 15 non-T, non-B ALL cases, but all cases of bigenotype showed no doubly marked profile and retained a completely fidelous immunophenotypic pattern. We further investigated the possibility that analysis of TcRα gene may be useful for determining cellular origin of non-T, non-B ALL leukemic cells. ———— non-T, non-B ALL; Ig genes; T-cell α- and β-receptor genes; monoclonal antibodies

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The detection of cell surface antigens by means of monocolonal antibodies has contributed to increase current knowledge on lymphocyte differentiation and origin of leukemic cell populations (Foon et al. 1982). Acute lymphocytic leukemia (ALL) have been classified into 4 major subtypes: null ALL, common ALLA-positive ALL (CALL), B-cell ALL (B-ALL) and T-cell ALL (T-ALL) (Sobol et al. 1985).

The discovery that immunologlobulin loci undergo rearrangement during B cell differentiation provided a new means of identifying tumors of B cell lineage and of demonstrating monoclonality (Arnold et al. 1983; Korsmeyer et al. 1983). More recently, isolation and characterization of cDNA clones corresponding to human T-cell receptor genes has confirmed that at the genetic level, Ig and T cell receptor genes share structural and functional features (Hedrick et al. 1984; Yanagi et al. 1984). Therefore, the analysis of T-cell receptor gene rearrangement offers a powerful means to identify tumors of T-cell lineage (Waldman et al. 1985; Okabe et al. 1987a). The presence of Ig gene rearrangements in CALLA-positive non-T, non-B ALL investigated thus far gave a strong support for the hypothesis that the leukemia cells are neoplastic B cell precursors (Korsmeyer et al. 1983). However, the cellular origin of null ALL remains to be elucidated. Recent reports have shown that Ig and TcRβ gene rearrangements may not be restricted to leukemias of B lineage and those of T lineage, respectively (Pelici et al. 1985). In fact, several authors have described that rearrangements of both Ig and TcRβ genes occur frequently in non-T, non-B ALL (Pelici et al. 1985; Tawa et al. 1985). In order to clarify the cellular origin of non-T, non-B ALL, we tried to combine analyses of Ig genes and TcRα- and β-receptor gene rearrangements with immunophenotypical analysis.

Patients and Methods

Patients

Fifteen adult patients with non-T, non-B ALL were subjected to investigation. The leukemic cells obtained from bone marrows and/or peripheral blood were examined by May-Grünwald-Giemsa stain and were classified by the FAB system (Bennett et al. 1976, 1981). Staining with periodic acid-Schiff (PAS), myeloperoxidase (MPO), Sudan Black B (SBB), and non-specific esterase were carried out. Null ALLs were diagnosed according to the following criteria: positive staining for PAS but negative staining for MPO, SBB and non-specific esterase, lack of reactivity with anti-T cell antibodies (Leu-1, OKT 3, OKT 11) and CALLA (J-5), and no expression of cytoplasmic Ig. Non-T, non-B common ALLs were diagnosed according to the following criteria: positive reactivity with CALLA, no expression of surface immunoglobulin and lack of reactivity with anti-T cell antibodies.

Immunological phenotyping

Surface Ig and cytoplasmic Ig were analyzed by direct immunofluorescence methods. FITC-labeled goat $F(ab)'_2$ anti-human immunoglobulin was purchased from Cappel Laboratories (Cochranville, PA, USA). Subset and/or stage specific surface markers were analyzed by Epics C flow cytometer (Coulter Electrics, Hialeath, FL, USA) using commercially available monoclonal antibodies and FITC-labeled $F(ab)'_2$ fragment of goat anti-mouse Ig

antibody (Cappel Lab.). The monoclonal antibodies, J-5, OKIal, OKB1, OKB2, OKT3 and OKT11 were purchased from Ortho Pharmaceutical Corporation (Raritan, NJ, USA). The monoclonal antibody, Leu 1 was purchased from Becton Dickinson (Sunnyvale, CA, USA).

Southern blot analysis

High molecular weight DNA was extracted from the cells and was digested to completion with *Eco* RI, *Hind* III or *Bam* HI. The digests were electrophoresed through 0.75% agarose gels, transferred to nitrocellulose membranes, and hybridized with a ^{32}P-labeled cDNA probe, as previously described in detail (Okabe et al. 1987b). The plasmids containing human TcRβ cDNAs were kindly provided by Dr. T. W. Mak ; α chain cDNA (pY 14) (Yanagi et al. 1985) and constant region β chain cDNA (JUR-2) (Yoshikai et al. 1984). J_H probe was a 3.3 kb *Eco* RI-*Hind* III fragment of p (Ch4HIgμ24) (Takahashi et al. 1980) and J_κ probe was a *Sac* I-*Sac* I fragment of embryonic J_κ region gene (Hieter et al. 1982), both obtained by the courtesy of Dr. T. Honjo.

Results

Cytochemical and immunological analyses

The clinical data and the phenotypes of the leukemic cells of 15 patients investigated were summerized in Table 1. The leukemic cells of 14 out of 15 patients showed no cytochemical nature of myelopoietc lineage since the blast cells were positive for PAS staining but negative for MPO, SBB and non-specific esterase staining, but those of one case (case 6) were interpreted as mixed lineages of largely lymphoid cells with a few myeloid cells because they contained a few MPO-positive cells. Three cases were classified as null ALL, as leukemic cells did not express CALLA, pan-B (B1 and/or B2), pan-T (Leu-1 and/or OKT 3), and OKT 11. Eleven out of them were classified as non-T, non-B common ALL, since CALLA, Ia antigen and B1 and/or B2 antigens were positive but, pan-T antigens and cytoplasmic and surface Ig were negative. One case (case 15) of common ALL showed positivity of Leu-1 antigen without immunophenotypes of B-cell lineage.

Analyses of Ig and TcRβ gene configurations

Each leukemic cells of 11 non-T, non-B common ALL patients showed J_H gene rearrangements and 2 of them also showed J_κ gene rearrangements (Fig. 1 A and B). Although case 7 showed one rearranged allele with one germ-line allele of H-chain genes, all other case of non-T, non-B common ALL contained either the rearrangements of both alleles (cases 5, 6, 9, 10, 11, 12 and 13) or one rearranged allele with deletion of the other allele (cases 4, 8 and 14). Faint hybridization bans on germ-line position in these cases may resulted from contamination of nonleukemic cells. Non-T, non-B common ALL case 10 showed 2 rearranged bands of J_H genes in addition to intense band of germ-line, probably because the percentage of comtamining non-leukemic cells was high.

TcRβ gene configurations in non-T, non-B common ALL are shown in Figs. 2A and 2B. A 0.85 kb *Pst* I-fragment of JUR-β 2 cDNA, which hybridizes with

TABLE 1. *Phenotypical and genotypical analyses in non-T, non-B ALL cases*

Case No.	Age	Sex	FAB classi-fication	Phenotypical analyses									Ig genes		TcR β gene		TcR α gene
				PAS	MPO	J-5	Ia	Pan-B	Pan-T	cIg	sIg	OKT10	JH	Jκ	Cβ1	Cβ2	
Null ALL																	
1	37	F	L2	+	−	−	−	−	−	−	−	−	G/G	G/G	G/G	G/G	D
2	57	M	L1	+	−	−	−	−	−	−	−	+	G/G	G/G	D/G	R/G	G
3	20	F	L2	+	−	−	+	−	−	−	−	−	R/R	G/G	G/G	G/G	G
Common ALL																	
4	60	F	L2	+	−	+	+	+	+	−	−	−	R/D	G/G	G/G	G/G	G
5	41	M	L1	+	−	+	+	+	−	−	−	−	R/R	G/G	G/G	G/G	NT
6	58	F	L2	±	+	+	+	+	−	−	−	−	R/R	G/G	G/G	G/G	G
7	70	F	L2	+	−	+	+	+	−	−	−	−	G/R	G/G	G/G	G/G	G
8	46	F	L2	+	−	+	+	+	−	NT	NT	NT	D/R	G/G	G/G	G/G	G
9	19	F	L2	+	−	+	+	+	−	−	−	−	R/R	G/G	G/G	G/G	G
10	17	M	L1	+	−	+	+	+	−	NT	NT	NT	R/R	NT	D/G	R/G	G
11	35	M	L2	+	−	+	+	+	−	−	−	NT	R/R	G/G	D/D	R/R	NT
12	54	M	L2	+	−	+	+	+	−	−	−	NT	R/R	G/G	D/D	R/R	D
13	19	F	L2	+	−	+	+	+	−	−	−	+	R/R	R/R	G/G	G/G	D
14	37	F	L2	+	−	+	+	+	−	NT	NT	NT	R/D	R/G	G/G	G/G	NT
15	18	M	L2	+	−	+	+	−	+	−	−	+	G/G	NT	G/G	G/G	NT

NT; not tested, G; germ-line, R; rearranged, D; deletion.

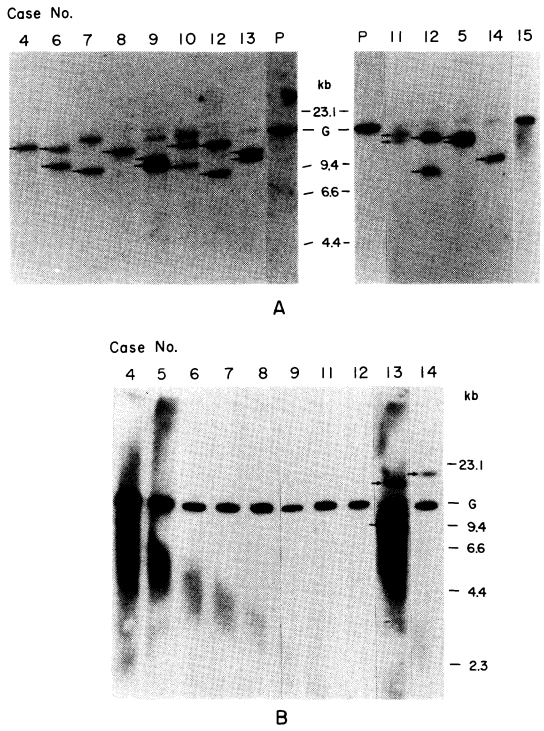

Fig. 1. Immunoglobuin gene configurations in non-T, non-B common ALL cases. (A) J_H gene configurations ; Each leukemic cells of 11 non-T, non-B common ALL cases contained rearranged J_H genes (Cases 4 through 14). One CALLA-positive ALL case, whose leukemic cells were Leu-1-positive, showed no rearrangement of J_H gene (Case 15). (B) J_x gene configurations ; Two out of CALLA-positive ALL cases contained J_x genes (Cases 13 and 14). High-molecular weight DNAs were digested with *Eco* RI. Rearranged genes are indicated by arrows (→). G, germ-line : P, human placenta DNAs.

both Cβ 1 and Cβ 2 (Yoshikai et al. 1984), was used. *Eco* RI-digested germ-line Tβ region genes gave 2 hybridization signals, 11 kb for Cβ 1 and 4 kb for Cβ 2, and *Hind* III-digested germ-line Tβ region genes gave 3 hybridization signals of 8 and 6 kb for Cβ 2 and 3 kb for Cβ 1 (Waldman et al. 1985). Eight cases of non-T, non-B common ALL showed germ-line patterns of TcRβ genes, but 3 cases showed dual rearrangements of J_H and TcRβ genes (Cases 10 through 12, Fig. 2), but case 15 contained neither rearranged J_H nor TcRβ gene, whereas the leukemic cells were positive for CALLA and Leu-1 antiges. In null ALL cases, case 1 showed germ-line patterns of both Ig and TcRβ genes (Fig. 3, lane 1). Case 2, whose leukemic cells possesed only OKT 10 antigen, showed germ-line arrangements of J_H and J_x genes, but showed the rearrangement of TcRβ gene ; *Eco* RI-digested DNA of the leukemic cells showed germ-line configuration but *Hind* III-digest showed the rearrangement of one allele with other germ-line allele in Cβ 2 genes and germ-line arrangements of Cβ 1 genes (Fig. 3, lane 2). Case 3, whose

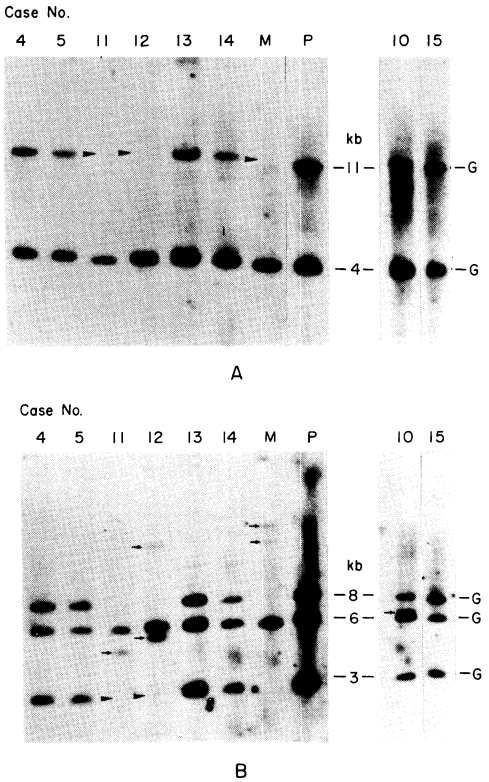

Fig. 2. TcRβ gene configurations in non-T, non-B common ALL cases. (A) Southern blot analysis in *Eco* RI-digested DNAs. (B) Southern blot analysis in *Hind* III-digested DNAs. *Eco* RI-digests give 2 hybridization signals of the constant regions of TcRβ gene, 11 kb for Cβ 1 and 4 kb for Cβ 2. *Hind* III-digests give 3 hybridization signals of 8 kb and 6 kb for Cβ 2 and 3 kb for Cβ 1. Eight cases of non-T, non-B common ALL contained germ-line TcRβ genes. Three cases (Cases 10 through 12) and Molt-4F cells, T-cell leukemia cell line showed the rearrangements of TcRβ genes. Case 15 showed no rearrangement of TcRβ genes, whereas the leukemic cells were positive for Leu-1 antigen. Rearranged genes are indicated by arrows (→). Deletions are indicated by marks (▶). M, Molt-4F ; P, human placenta DNAs.

leukemic cells were only Ia-positive, contained the rearranged J_H genes and germ-line arrangement of TcRβ genes (lane 3).

Analysis of TcRα gene configurations

TcRα gene configurations of the leukemic cells were investigated in 11 non-T, non-B ALL cases and were compared with those of TcRβ genes (Fig. 4). The germ-line organization of the gene corresponding to pY 14 showed 7 germ-line bands in *Eco* RI-digested DNA, that is consistent to the result of Yanagi et al. (1985). Eight out of 11 cases showed the germ-line arrangements. In three cases, only 6 germ-line bands were observed without rearranged band after *Eco* RI-

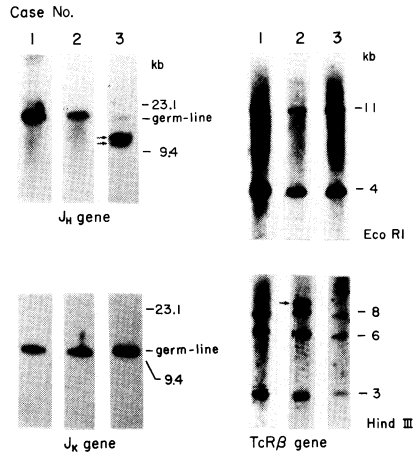

Fig. 3. Configurations of Ig and TcRβ genes in null ALL cases. Case 1 showed germ-line patterns of both Ig and TcRβ genes. Cases 2 and 3 contained either rearranged TcRβ genes of J_H genes, respectively.

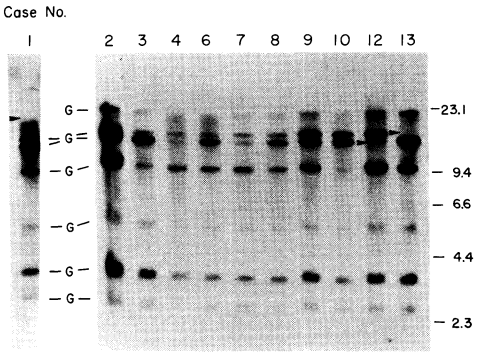

Fig. 4. TcRα genes configurations in non-T, non-B ALL. The germ-line organization of the gene corresponding to pY14, showed 7 germ-line bands in *Eco* RI-digests. In three cases, deletions of one band were observed but rearranged band was not found (Cases 1, 12 and 13). The deletions are indicated by marks (▶). G, germ-line.

digestion. Obvious relationship was not seen between the deletion of TcRα genes and the rearrangements of TcRβ genes in non-T, non-B ALL cases in the present study (Table 1).

Discussion

The leukemic cells obtained from 15 patients with non-T, non-B ALL were evaluated by phenotypical and genotypical (Ig and TcR gene rearrangements) analyses in the present study. Fourteen cases of non-T, non-B ALL were divided into two groups, 11 cases of CALLA-positive ALL and 3 cases of CALLA-negative ALL, as defined by monoclonal antibodies. Each leukemic cells of CALLA-

positive non-T, non-B ALL cases showed Ig gene rearrangements ; 9 cases showed only rearrangement of J_H genes and 2 cases showed rearrangements of both J_H and J_κ genes. These results support recent views that CALLA-positive leukemic cells are neoplastic B cell precursors (Korsmeyer et al. 1983), and further suggest that most CALLA-positive non-T, non-B ALL may be classified into two subgroups according to the stage of B-cell differentiation, namely one group contains only rearranged H chain gene and the other contains rearranged H and L chain genes. In the present study, we found that the leukemic cells of a case of common ALL possessed Leu-1 antigen but showed germ-line patterns of J_H and TcRβ genes, thus suggesting that CALLA- and Leu-1 -positive ALL cells may not be simply interpreted to be comitted as T-cell lineage along differentiation pathway.

The cellular origin of null ALL is still a unresolved matter when analyzed by monoclonal antibodies. The detection of Ig heavy-chain gene rearrangement in one out of the present cases of null ALL, who showed the phenotype as Ia-positive, CALLA-negative, B-cell marker-negative and T-cell marker-negative, is in agreement with the view that the early B cell precursors initiating Ig H gene rearrangements bear surface HLA-DR and later acquire CALLA and B-cell markers (Korsmeyer et al. 1983). In the leukemic cells of case 2 that were negative for CALLA, Ia, B1, OKT 11, Leu-1, cIg, smIg, but only positive for OKT 10, TcRβ gene was rearranged and J_H gene rearrangement was not detected, thus suggesting that the leukemic cells of this case may reside as immature thymocytes along the T cell pathway of differentiation. However, case 1 whose leukemic cells were null phenotype as Ia-, CALLA-, T-marker- and B-marker-negative, showed neither J_H nor TcRβ gene rearrangements, and therefore may be truely undifferentiated. Thus, these findings suggest that CALLA-negative non-T, non-B ALL consists of heterogenous subgroups with regard to lymphocytes differentiation and lineage.

Analysis of Ig genes is a powerful means for clarifying monoclonality in B-cell proliferations (Arnold et al. 1983). More recently, Kitchingman et al. (1986) and Raghavachar et al. (1986) have reported that in some cases of acute lymphoblastic leukemia which contained more than 2 hybridization signals of gene, more than one abnormal cell clone or subclone may exist. In the present study among 14 cases of non-T, non-B ALL, we found only one case which contained 3 hybridization signals of μ gene, but it is not elucidated whether or not this case may have more than one leukemic cell clone, because this case showed lower percentage of leukemic cells than other cases when studied in molecular analysis. Case 6 showed cytochemical future of mixed AL, that a few of the leukemic cells were MPO-positive in addition to most population of lymphoid cells, but did not contain more than 2 hybridization signals of μ gene. This finding suggest that the leukemic cells of this case may not be biclonal but monoclonal.

It is an important issue whether or not Ig and TcRβ gene rearrangements are

lineage-specific in lymphoid leukemias, since one third of non-T, non-B CALLA-positive ALL cases showed the dual rearrangements of Ig and TcRβ genes. Pelici et al. described that bigenotypic, namely both Ig and TcRβ genes are rearranged, lymphoid tumors are not uncommon (1985). Recently, Tawa et al. also described that dual rearrangements of Ig and TcRβ genes occur frequently in about 25% of non-T, non-B ALL (1985), that is consistent with the result of the present study. We further investigated whether or not 3 bigenotypic leukemias expressed both B- and T-cell differentiation markers. All cases of bigenotype displayed no doubly marked profile and, thus they retained a completely fidelous immunophenotypic pattern, since they expressed no pan-T surface antigen (Table 1). At present, it is not known whether combined Ig and TcRβ gene rearangements represents a consequence of neoplasmic transformation, or occur frequently during initial step of normal lymphoid differentiation. Dual rearrangements of Ig and TcRβ genes might be not restricted to neoplasmic transformation, since we found dual re-arrangement in a nonleukemic EB-virus transformant B-cell line (data not shown).

We also investigated the possibility that the analysis of T-cell α-receptor gene may be useful for determining cellular origin of non-T, non-B ALL. Three out of 11 cases of non-T, non-B ALL showed deletion of bands of TcRα genes without rearranged band, and there is no obvious relationship between the deletion of TcRα genes and the rearrangements of TcRβ genes in non-T, non-B ALL cases. Similary, TcRα gene rearrangement was rarely detected in T-cell leukemias (data not shown). Difficulty of detecting rearrangement of TcRα genes depends on the inherent structure of the α-chain gene. The β-chain gene differs from the α-chain gene in that there is only one constant region and there are more than 20 J segments covered over more than 50 kb upstream of the constant region (Harday et al. 1985 ; Winoto et al. 1985 ; Yoshikai et al. 1985). As rearrangement occurs into any one of the J segments over a region of more than 50 kb, the use of probes to the constant region generally do not detect rearrangement. Therefore, TcRα gene still awaits clinical application untill isolation of useful genomic probes to the region of the J-segments.

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