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Immunogenotypes of Lymphoid Malignancies; the Rearrangement of T Cell Receptor β Chain Gene Can Occur before the γ Chain Gene Rearrangement

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Immunoglobulin (Ig) and T cell receptor (TcR) gene rearrangements were analyzed in 101 cases of lymphoid malignancies in association with a surface phenotype study. In leukemias/lymphomas with mature phenotype, there is a good correlation between phenotypes and genotypes. However, in leukemias/lymphomas with immature phenotype, we found many discordances between phenotypes and genotypes, suggesting the stochastic nature of hematopoietic cell differentiation at the early stage. As for TcR β and γ chains, the rearrangement of γ chain gene is considered to occur slightly prior to that of β chain gene. However, we observed a mature T cell malignancy, adult T-cell leukemia, with rearranged β chain gene and germ line γ chain gene, showing the possible existence of another pathway of T cell differentiation.

Key words: Leukemia/lymphoma — Immunoglobulin — T cell receptor — Gene rearrangement

Recently, molecular genetical analysis using rearranging immune gene probes in conjunction with immunophenotyping of leukemia/lymphoma has made great contributions to the determination of clonality and cellular origin.

In B cell lineage, immunoglobulin (Ig) heavy (H) chain gene is recombined to produce IgH molecule, followed by Ig κ chain gene, Ig λ chain gene recombination and expression, successively. Such a gene rearrangement order was demonstrated by analyzing the Ig gene pattern of pre-B cell leukemia.[1] In T cell lineage, genes encoding Ti molecule, β chain and α chain of T cell receptor (TcR), are rearranged in the course of T cell differentiation. The β chain gene rearrangement occurs in an early stage of thymic ontogeny.[2] We previously demonstrated that leukemic T cells corresponding to stage I of thymic differentiation, even the cells without E-rosette formation, can rearrange the TcR β chain gene.[3] The rearrangement and expression of the α chain gene are believed to occur at a later stage than those of the β chain genes.[4]

A third gene that is rearranged in T cells, γ chain gene, has been identified[5] and revealed to be rearranged in the early T cell maturation stage in almost the same way as β chain gene is.[6] The product of this gene has recently been found to be expressed on some T cell populations as a recognition molecule distinct from αβ heterodimer of TcR that forms a complex with CD3 antigen.[7–9]

We analyzed 101 cases of leukemias/lymphomas by Southern blotting using DNA probes encoding IgH (JH), Igκ (Jκ), TcR β chain (cβ1) and γ chain (Jγ1) in conjunction with a surface marker study to investigate the precise cellular origin and to obtain insight into hematopoietic cell differentiation.

Materials and Methods

Patients We examined 101 cases of peroxidase-negative leukemias and lymphomas. According to phenotypic analysis, they were classified as follows: T-acute lymphoblastic leukemia (T-ALL)/lymphoblastic lymphoma (LBL) (21 cases), common ALL (c-ALL) (22 cases), acute unclassified leuke-

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mia (AUL) (16 cases), B-non Hodgkin's lymphoma (B-NHL) (16 cases), B-chronic lymphocytic leukemia (B-CLL) (10 cases), T-NHL (5 cases), T-CLL (3 cases) and adult T-cell leukemia (ATL) (8 cases).

T-ALL/LBL cases were subclassified into 3 stages according to the T-cell differentiation scheme proposed by Reinherz *et al.*[10]: stage I (10 cases), stage II (7 cases) and stage III (4 cases). We also classified c-ALL into 2 groups according to the criterion of pre-B cell stage proposed by Nadler *et al.*[11]: stage III (Ia, CD19, CD10; 8 cases) and stage IV (Ia, CD19, CD10, CD20; 14 cases). In this study, AUL cases, defined as peroxidase-negative, common ALL antigen (CALLA)-negative, non-T, non-B leukemia, were divided into myeloid associated antigen-positive (10 cases) and -negative (6 cases) groups. These were, in addition, subdivided by terminal deoxynucleotidyl transferase (TdT) reactivity.

Cell Characterization Mononuclear cell fractions of fresh materials were separated from the peripheral blood and bone marrow by Ficoll-Hypaque density gradient centrifugation, or obtained from lymph nodes by mincing and elimination of aggregates after sedimentation.

May-Giemsa, myeloperoxidase, PAS, nonspecific esterase and naphthol AS-D chloroacetate esterase stainings were applied for morphological and cytochemical analysis. TdT was examined by using rabbit anti-TdT antibody (kindly provided by Dr. Bollum, Uniformed Service University of Health Science, USA).

Surface marker analysis was performed by using immunofluorescence, as previously described.[12] CD2, CD5, and CD7 were tested by using OKT11 (Ortho Pharmaceutical), Leu1 (Becton Dickinson), and WT-1 (kindly provided by Dr. Tax, Sint Rabboudziekeuhuis, The Netherlands), respectively, as pan T-cell markers. CD1, CD3, CD4, and CD8 were checked by using OKT6, 3, 4, and 8 (Ortho) as T-cell differentiation antigens. Surface Ig and CD20 were tested by using fluorescein-conjugated rabbit anti-human Ig antibody (Hechst) and B1 (Coulter) as B-cell markers. CD10 was checked by using J5 (Coulter) as common ALL antigen (CALLA), and Ia antigens by using OKIa1 (Ortho). CD11 and CD13 were detected by using OKM1 (Ortho) and MCS2 (kindly provided by Dr. Tatsumi, Kobe Univ.) as myeloid associated antigens.

DNA Analysis High-molecular-weight DNAs were obtained from leukemia or lymphoma cells, digested with *Bam*HI or *Eco*RI, size-fractionated on 0.6% agarose gel, and transferred to nitrocellulose membrane as described by Southern.[13] The membrane was hybridized to nick-translated DNA probes, washed and autoradiographed.

DNA probes used in this study were as follows (sizes of the germ line genes and restriction enzymes used): JH; J region gene of Ig heavy chain[14] (19 kb, *Bam*HI), Jκ; J region gene of Ig κ chain[15] (10 kb, *Bam*HI), Cβ1; C region gene of TcR β chain[16] (23 kb, *Bam*HI; 10 kb, 4 kb, *Eco*RI) Jγ1; J region gene of TcR γ chain[17] (15 kb, 12.5 kb, *Bam*HI; 3.3 kb, 1.8 kb, *Eco*RI: kindly provided by Dr. Rabbitts, MRC Laboratory of Molecular Biology, England).

Results

T-ALL/LBL In T cell acute lymphoblastic leukemia (T-ALL) and T lymphoblastic lymphoma (T-LBL), our main question is whether or not the stages of TcR β chain and γ chain gene rearrangements are dissociated. As shown in Table I and Fig. 1, we found 1 case of stage I T-LBL whose TcR genotype is discrepant; γ chain gene rearranged, but β chain gene in germ line configuration. The other 3 stage I cases and all stage II and III cases with rearranged β chain gene have rearranged γ chain gene simultaneously. We found 2 cases which also have rearranged IgH gene in stage II.

Common ALL In 22 common ALL cases tested, 21 have rearranged IgH gene, but only 3 cases have rearranged Igκ gene simultaneously. Concerning TcR genes, 6 out of 19 tested cases have rearranged β chain gene, and 8 out of 18 tested cases have rearranged γ chain gene (Table II). No β chain gene re-

Table I. Genotypes of T-cell Malignancies

	TcR		Ig	
	γ	β	H	κ
Immature type				
T-ALL/LBL: stage I	4/10	3/10	0/10	
II	7/7	7/7	2/7	0/2[a]
III	4/4	4/4	0/4	
Mature type				
T-NHL	3/3	3/3	0/3	
T-CLL	5/5	5/5	0/5	
ATL	0/8	0/8	0/8	

Lymphoblastic lymphoma (LBL) is considered as an immature T-cell neoplasm because this disorder can not be distinguished from T-ALL clinically and phenotypically. Data are given as rearranged cases/tested cases.

a) The Igκ examined 2 cases in stage II had rearranged IgH genes.

arranged case with germ line γ chain gene could be observed. Interestingly, we found most of the TcR gene rearranged cases were stage IV (Ia, CD19, CD10, CD20), considered to be a more mature phenotype in the B lineage differentiation pathway.

AUL Peroxidase-negative, common ALL antigen (CALLA)-negative, non-T, non-B leukemias are subclassified into 2 groups as we previously reported[12]; myeloid antigen-positive and -negative ones. There is a tendency that myeloid antigen-positive cases have germ line structure (6/10), and myeloid antigen-negative cases do not have germ line configuration (1/6) (Table III). However, there is no strict correlation between myeloid antigen and rearrangement of immune genes, since one case with myeloid antigen has rearranged IgH, TcR β and γ. In this study, all AUL cases, which have one or more rearranged immune genes, have rearranged IgH. No case of sole rearrangement of TcR genes could be observed. Indeed, in 9 cases with rearranged immune genes, 6 cases have only IgH rearrangement (not TcRs rearrangement).

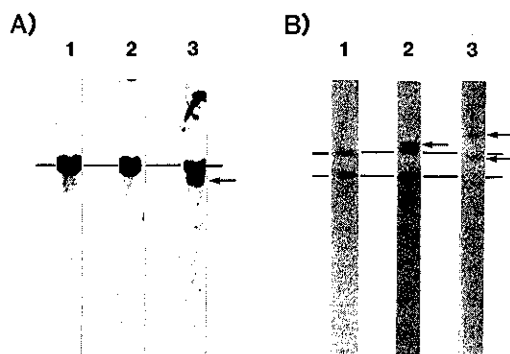

Fig. 1. Southern blots of T-ALL/LBL cases corresponding to stage I of intrathymic differentiation. Each DNA sample was digested with *Bam*HI and probed by Cβ1 (A) or Jγ1 (B). Case numbers in (A) are the same as in (B). Cells from all cases were positive for CD7 and/or CD2, but negative for CD1, 3, 4, and 8. Dashes indicate germ line sizes, and arrows indicate rearranged bands.

Leukemia/lymphoma with Mature Phenotype As shown in Tables I and II, there is a good

Table II. Genotypes of B-precursor and B-cell Malignancies

	Ig		TcR	
	H	κ	γ	β
Immature type				
c-ALL B1(−)	8/8	2/8	1/6	1/6
B1(+)	13/14	1/14	7/12	5/13
Mature type				
NHL	16/16		0/10	1/16
B-CLL	10/10		0/10	0/7

Data are given as rearranged cases/tested cases.

Table III. Genotypes of AUL

A)		IgH	Igκ	TcRγ	TcRβ
My-Ag(+) AUL:	TdT(−)	3/5	1/5	1/4	1/5
	TdT(+)	1/5	0/5	0/4	1/5
My-Ag(−) AUL:	TdT(−)	2/3	1/3	1/3	1/3
	TdT(−)	3/3	2/3	0/2	0/3

Data are given as rearranged cases/tested cases.

B)		IgH(G) TcRγ(G) TcRβ(G)	IgH(R) TcRγ(G) TcRβ(G)	IgH(R) TcRγ(G) TcRβ(R)	IgH(R) TcRγ(R) TcRβ(R)
My-Ag(+) AUL:	TdT(−)	2[a]	2		1
	TdT(+)	4		1	
My-Ag(−) AUL:	TdT(−)	1	1		1
	TdT(+)		3		

Abbreviations: G, germ line; R, rearranged; My-Ag, myeloid antigens; TdT, terminal deoxynucleotidyl transferase.
a) Number of cases.

Table IV. Relation between Genotype and Phenotype in Cases with Dual Genotype

No.	Dx.	CD2	CD7	CD3	CD4	CD1	CD8	CD20	cIg	CD11	CD13	CD10	Ia	TdT	IgH	Igκ	TcRγ	TcRβ
1.	T-ALL	−	+	−	+	−	−	−	−	−	−	+	−	+	R	G	R	R
2.	LBL	+	+	−	−	+	−	−	−	−	−	−	−	+	R	G	R	R
3.	c-ALL	−	−	−	−	−	−	−	−	−	−	+	+	+	R	G	R	R
4.	c-ALL	−	−	−	−	−	−	+	−	−	−	+	+	+	R	G	R	R
5.	c-ALL	−	−	−	−	−	−	+	−	−	−	+	+	+	R	G	R	R
6.	c-ALL	−	−	−	−	−	−	+	−	−	−	+	+	+	R	G	R	R
7.	c-ALL	−	−	−	−	−	−	+	−	−	−	+	+	+	R	G	R	R
8.	c-ALL	−	−	−	−	−	−	+	+	−	−	+	+	+	R	R	R	R
9.	AUL	−	−	−	−	−	−	−	−	−	−	−/+[a]	−	−	R	R	R	R
10.	AUL	−	−	−	−	−	−	−	−	+	−	−	+	−	R	G	R	R
11.	AUL	−	−	−	−	−	−	−	−	+	−	−	+	+	R	G	G	R
12.	B-NHL[b]	−	−	−	−	NT	−	NT	−	−	NT	−	+	NT	R	R	G	R

In this table, the dual-genotypic c-ALL cases without TcRβ rearrangement were not included. cIg, cytoplasmic IgM; −, negative; +, positive; R, rearranged; G, germ line; NT, not tested.

a) In this case, some blasts were positive for CD10, but negative for CD7, CD20, Ia, and TdT. Therefore, this case was diagnosed as AUL.

b) This case was negative for surface Ig and cytoplasmic Ig, but positive for Ia and IgG-Fc receptor with no T-cell-associated antigens. A diagnosis of so-called "defective B-cell lymphoma" was made phenotypically.

correlation between phenotypes and genotypes in leukemias/lymphomas with mature phenotype. All 26 of the B-NHL and B-CLL cases have rearranged IgH gene. Notably, one B-NHL case also has rearranged TcR β chain gene, but not γ chain gene. In T-NHL and T-CLL, all of the tested cases have rearranged β chain and γ chain of the TcR genes, and retained the germ line configuration of IgH gene. In 8 cases of ATL, one has germ line TcR γ chain gene with rearranged β chain gene. The other 7 cases have rearranged β chain and γ chain gene simultaneously with germ line IgH.

Dual Genotype Cases We usually use the words "dual genotype" when genes encoding both Ig and TcR are rearranged simultaneously. Precise phenotypes and genotypes of dual genotype cases are listed in Table IV, and Southern blots of representative cases are shown in Fig. 2. In general, there is no specific phenotypic marker associated with dual genotype which is common to all types of diseases, although dual genotypic cases of T-ALL/LBL and c-ALL are frequently observed at limited stages of differentiation.

Discussion

To elucidate the precise cellular origin of lymphoproliferative disorders, we employed a molecular genetical approach usig Ig and TcR genes as probes in conjunction with immunological cell characterization. The Ig genes and TcR β chain gene are well characterized and documented as regards their rearrangement and relation to their product expression.[1-4] A third gene rearranging in T cells, TcR γ chain gene, was recently identified,[5] and some T cell populations use γ chain gene product as a recognition molecule which is linked to the CD3 molecule.[7-9] Davey *et al.* reported that γ chain gene rearrangement and its transcription occur early in the T cell ontogeny,[18] and Born *et al.* found that most of their T cell leukemia/lymphomas have simultaneous rearrangement of β and γ chain genes.[6] From this point of view, our main interest is the relationship of the hematopoietic cell differentiation and immune gene rearrangements, especially that or γ chain gene.

First of all, in the study of T-ALL/LBL, there is very high concordancy in β and γ chain gene rearrangement. Only one case with stage I phenotype showed a discrepancy; γ chain gene rearranged, β chain gene in germ line configuration. So we can assume that the γ chain gene rearranges just prior to β chain gene. However, we might have picked up a special case because this case showed leukemic conversion with peroxidase-positive blasts whose γ chain gene has the same rearrangement pattern as the initial LBL

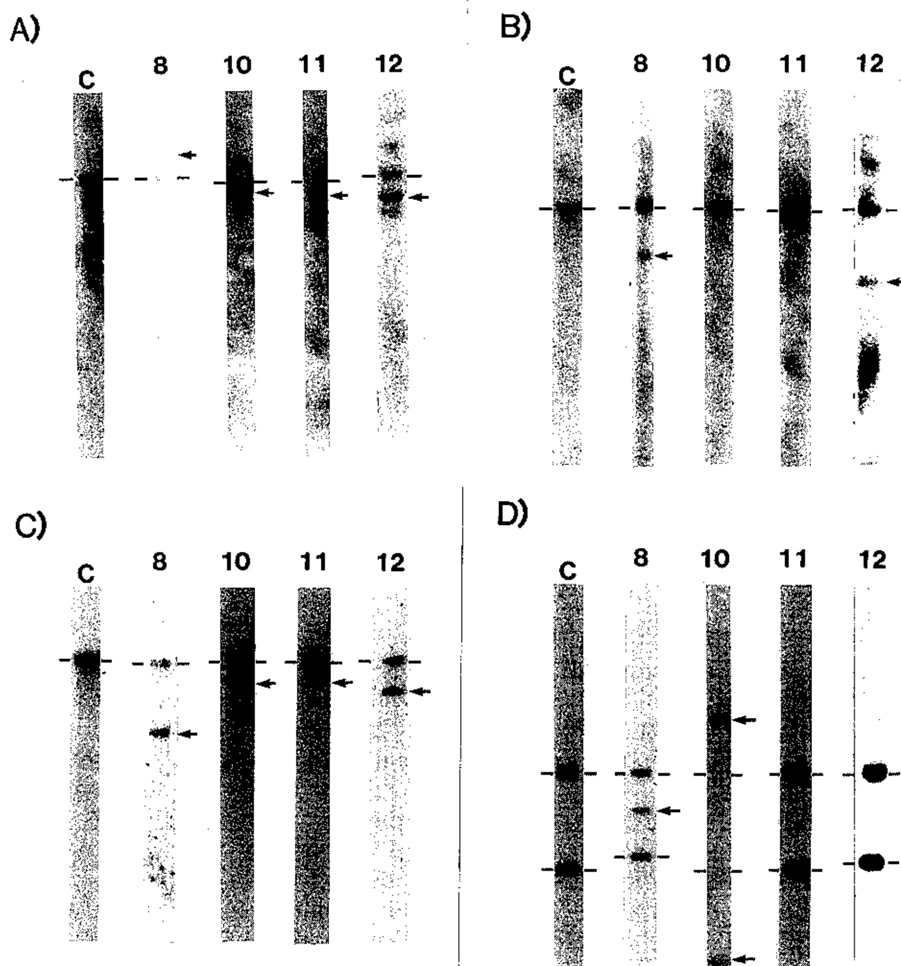

Fig. 2. Southern blots of dual genotype cases. Each DNA sample was digested with *Bam*HI and probed by JH (A), *Bam*HI and Jκ (B), *Bam*HI and Cβ1 (C), *Eco*RI and Jγ1 (D). Case numbers in (A), (B), (C), and (D) are the same as in Table IV. Dashes indicate germ line sizes, and arrows indicate rearranged bands.

phase (unpublished results). We experienced an HTLV-I-positive cell line of monocytic origin (as judged from cytochemical and immunological findings). The genotype of this cell line has only γ chain gene rearrangement (unpublished results). Therefore, there might be a cell population with rearranged γ chain gene which can express myelo-monocytic phenotype.

In addition, there are two cases of T-ALL/LBL with rearranged IgH genes. Of course, these cases have rearranged β and γ chain genes of TcR. Contrary to our expectation, the dual genotype (IgH and TcR) cases correspond to stage II phenotype of the T cell differentiation scheme, not stage I, a more primitive stage.

Common ALL cases, in accordance with previous reports,[1] have rearranged IgH genes (21/22), but only 3 cases have rearranged Igκ gene. Interestingly, there are many cases that have rearranged β chain (6/19) and γ chain (8/18) gene of TcR. Almost all of the dual genotype cases have rearranged β and γ chain genes, but two cases have only rearranged γ chain gene. No cases which have

only rearranged β chain gene were observed. These results support the possibility that the rearrangement of γ chain gene of TcR slightly precedes that of β chain gene, even if the rearrangement occurs as a sequential event of IgH rearrangement (probably because of common recombinase activity[19]). Unexpectedly, rearrangements of TcR genes were demonstrated more in stage IV cases than in stage III ones. Similar observations were reported recently.[20] This finding is thought to be analogous to the fact that IgH gene rearrangement is observed in stage II T-ALL/LBL. Since dual rearrangement is now considered to be due to common recombinase activity, we might assume that common recombinase is activated in a rather mature stage of differentiation. Thus, we might assume that the pressure that leads to dual rearrangement is stronger in rather mature stages of differentiation.

AUL is thought to be derived from the most primitive and least committed hematopoietic cells, based on immunophenotyping studies. The precise cellular origin of AUL is not well understood so far. Our aim in analyzing the immunogenotype of AUL cells is to get an insight into the nature of the cells. As shown in Table III, there are many phenotype- or genotype-discordant genotype cases, which means myeloid antigen-positive cases with rearranged immune gene(s) or dual rearrangement cases, and we could not find any strict correlation between phenotype and genotype in leukemia with immature characteristics. Therefore we assume that the rearrangement of immune genes occurs stochastically in cells at a very early stage of hematopoietic cell differentiation. However, the initial rearrangement event is likely to occur in the IgH gene, because all rearranged cases have rearranged IgH gene in the AUL cases tested. We found one AUL case with rearranged IgH and TcR β chain genes (γ chain gene; germ line). Such a genotype could not be seen in dual genotype cases of common ALL.

In lymphoproliferative disorders with mature phenotype, we observed a high concordancy between phenotypes and genotypes. The exceptions are one case of B-NHL with rearranged IgH and TcR β chain genes and one case of ATL with rearranged β chain gene and germ line γ chain gene. In this context, we can assume that the rearrangement of TcR γ chain gene slightly precedes that of TcR β chain gene from the study of T-ALL/LBL and common ALL. Though the fact that some T cell populations use γ chain gene product as a recognition molecule has been recently demonstrated,[7-9] most T cells use α and β chain but not γ chain as TcR. Thus, the role of rearranged γ chain gene product in the course of T cell differentiation remains to be explored.

On the other hand, we found three cases whose rearrangement pattern is opposite (β chain rearranged, γ chain in germ line): one ATL, one AUL and one B-NHL. The fact that one ATL case as a mature T cell phenotyped tumor has such a phenotype strongly suggests the presence of a T cell population without rearranged γ chain gene. However there is another possibility, that there may be another γ chain gene which cannot be detected by our DNA probe.

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