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Original Article

Precise discrimination of lymphoproliferative disorders with combined *TCRG* and *TCRB*: Japanese series

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Abstract

TCR gene rearrangement detection has proven to be a useful diagnostic method. In this work, 88 T-cell lymphoproliferative disorders and 45 non-T-cell proliferation in Japanese patients were studied for rearrangement of *TCRG* and *TCRB* using PCR and multiplex PCR. The sensitivity and specificity of *TCRB* assay in mature T-cell proliferation were 98.2% and 90.9%, respectively. The sensitivity and specificity of *TCRG* were 84.0% and 73.3%, respectively. The combination of rearranged *TCRG* and *TCRB* analysis provides an extremely high sensitivity for the diagnosis of mature T-cell proliferative disorders. All patients in this group exhibited rearrangement of either *TCRG* or *TCRB* or both *TCRG* and *TCRB*. All cases of B-lymphoid malignancies and reactive lymphoid cases tested for *TCRB*-DJ gave complete negative results, thus providing an alternative discrimination from CD3(+) and T-cell leukemia/lymphoma. Although 25% of B-cell non-Hodgkin lymphoma (B-NHL) cases revealed *TCRG* rearrangement, all B-NHL cases gave negative *TCRB* rearrangement. In summary, PCR-based combination analysis of *TCRG* and *TCRB* rearrangement is a powerful diagnostic tool which also provides accurate discrimination for each type of lymphoproliferative disorder.

Keywords: *TCRG*, *TCRB*, ATLL, mature T-cell disorder, lymphoproliferation

1. Introduction

The diagnosis of different groups of lymphoproliferative disorders including lymphoid malignancies needs accurate clinical, morphological, immunophenotypic, and sometimes genotypic investigations. Most of the cases can be diagnosed based on their morphology and immunophenotype. However, 10–15% of cases remain suspicious and need clonality assessment to confirm the diagnosis. To enhance the diagnostic accuracy and increase the understanding of T- and B-lymphoid malignancies, the studies of molecular clonality defining *TCR* and *IG* gene rearrangement profiles are especially useful (Foucar, 2007; Gazzola *et al.*, 2014; Nicot, 2005). Since the frequencies of some T-lymphoid disorders are geographically different, reports of *TCR* clonality in patients from different areas are necessary to complete the knowledge of these disorders.

T-cell receptor (TCR) on the surface of T-lymphocytes recognizes antigens that bind to the major histocompatibility complex (MHC) molecules (Garcia *et al.*, 1996; Matsui, Boniface, Steffner, Reay, & Davis, 1994; Racioppi, Ronchese, Matis, & Germain, 1993). Recently, it

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has been shown that the MHC class I-like molecules, CD1 and MR1, also involve TCR antigen recognition. CD1 binds lipid-based antigens while MR1 binds small molecule metabolites. These antigen-bound molecules are subsequently recognized by TCR (Rossjohn *et al.,* 2015). TCR consists of two protein chains, either the α and β chains (95% of T cells) or the γ and δ chains (5%). These TCR chains are encoded from different chromosomes. *TCRB* or *TCRG* genes localize on chromosome 7q34 or 7p14 while *TCRA* and *TCRD* genes localize on chromosome 14q11 (Brenner, Strominger, & Krangel, 1988; Davis, 1985; Raulet, 1989; Yoshikai *et al.*, 1985). The *TCR* loci contain V, D, and J segments (or only V and J segments in α and γ chain locus) which are assembled by site-specific recombination. The process directed by the lymphoid-specific RAG proteins during T-cell development in the thymus. Dysregulation of RAG activity was found in aberrant V(D)J recombination events which are associated with many lymphomas. (McBlane *et al.,* 1995; Mombaerts *et al.,* 1992; Teng & Schatz, 2015).

Some lymphoid leukemias and lymphomas have abnormal rearrangement involving these *TCR* genes. In lymphomagenesis, the process may also require expression of pre-rearranged *TCRA* and *TCRB* genes since the relationship of TCRA/TCRB co-expression and lymphomagenesis has been found, indicating a critical role of TCR signaling as an important component of the malignant transformation (Dubé *et al.,* 1991; Fasseu *et al.,* 2007; Fisch *et al.,* 1992). The misregulated TCR signaling could be the first step in lymphomagenesis and leukemogenesis whereby the abnormal cells use the TCR signaling for proliferative advantage. Accordingly, activation of TCR signaling components drives malignant cell growth, whereas inhibitors of TCR signaling block the malignant growth. Targeting the TCR signaling pathway might be a productive strategy to inhibit growth of leukemia and lymphoma (Aifantis, Raetz, & Buonamici, 2008).

Since the abnormal rearrangement of *TCR* genes is involved in the initiation of abnormal transcription and cell differentiation of T-cell malignancies, detection of *TCR* gene rearrangements is therefore useful in the diagnosis of T-cell malignancies and it could serve as powerful molecular markers for demonstrating the presence or absence of clonality rearrangement in lymphoid populations (Langerak, Wolvers-Tettero, & van Dongen, 1999). In the diagnosis of T-cell malignancies, it is important to distinguish the clonal malignant T-cell proliferations from reactive disorders. These two conditions are difficult to discriminate by histological and immunological examinations and the application of *TCR* genetic testing is needed. Southern hybridization (SH) with TCR Cβ probe was the previous routine for clonal TCR detection but it was time-consuming. Therefore, a rapid and accurate diagnostic setting for *TCRB* and *TCRG* rearrangement was needed. Nowadays, PCR-based *TCR* rearrangement analysis is gradually replacing SH. The TCR assays are not only used for diagnosis but also for minimal residual disease assessment as well as immunotherapeutic indications (Mahe, Pugh, & Kamel-Reid, 2018).

TCRG is an early rearranged gene, and therefore *TCRG* rearrangement is detectable in the majority of T-cell neoplasms (Blom *et al.,* 1999; de Villartay *et al.,* 1989). Since the total number of *TCRG* variable regions is less than those of the *TCRB*, a *TCRG* clonality analysis is easier than the *TCRB* (Theodorou *et al.,* 1996). However, some amplified products of the *TCRG* fragments (VJ fragment) appeared to have similar size (indistinctive clonal bands) because of the limited number of diversity segments within the *TCRG* region (Blom *et al.,* 1999). In this regard, the multiplex PCR of *TCRB* for an effective diagnosis of lymphoproliferation was proposed (Thériault *et al.,* 2000).

This work reports the accurate molecular testing for discriminating T-cell malignancies from non-malignant T-cell proliferation in Japanese patients. The results of the combined *TCRG* and *TCRB* rearrangement profiles not only provide a better understanding of each subset of lymphoproliferative disorders but will also be greatly useful for hematological laboratory applications. Cumulative data and comparison of *TCR* rearranged frequencies from different populations and geographical areas are the important genetic background records, which might be especially useful for future therapeutic adjustments for different populations.

2. Materials and Methods

2.1 Patients and specimens

A total of 133 clinical samples were analyzed. The clinical diagnoses of non-Hodgkin lymphoma (NHL) and leukemia were based on histopathology, immunophenotype, karyotype, and clinical features (Swerdlow *et al.,* 2008). We retrospectively reviewed the medical records between 1998 and 2008 in Tenri Institute of Medical Research of 88 patients who were newly diagnosed as T-cell lymphoproliferative disorders. These included 15 unspecified peripheral T-cell lymphoma (PTCL), 18 angioimmunoblastic lymphoma (AITL), 26 adult T-cell leukemia/lymphoma (ATLL), 7 T-large granular leukemia, 13 precursor T-acute lymphoblastic leukemia/lymphoma (ALL/LBL), 3 cutaneous T-cell lympho-ma, and 6 other disorders. In addition, 45 non-T-cell proliferation cases were included. They were 7 dermatopathic lymphoadenitis, 9 B-ALL, 16 B-NHL, 7 healthy adults, and 6 carriers of human T-cell leukemia retrovirus (HTLV) type-I.

Among the 88 T-cell disorder samples, 25 were previously analyzed for *TCRB* gene rearrangement by means of the Southern hybridization method with Cβ probe (Ikuta, Ogura, Shimizu, & Honjo, 1986). All patients provided their informed consent before the initiation of any medical procedure.

2.2 Multiplex PCR for *TCRB* gene amplification

Multiplex PCR was performed according to the BIOMED-2 protocol (van Dongen *et al.,* 2003). Twenty-three consensus Vβ, 2 specific Dβ, and 13 specific Jβ primers were used in this analysis (Table 1). They were combined in three multiplex reaction tubes A, B, and C (Table 2). Reactions were performed in a total volume of 25 µL containing 50 to 100 ng DNA, 200 µM dNTPs, 1.5 mM $MgCl_2$, 5 pM of each primer, and 1.0 U AmpliTaq Gold DNA polymerase (Applied Biosystems, CA, USA). The PCR conditions were initial denaturation at 95 °C for 7 min followed by 35 cycles of denaturation at 94 °C for 30s, primer annealing at 60 °C for 30s, extension at 72 °C for 30s and the final extension step at 72 °C for 10 min. After amplification, the specific PCR products were detected by heteroduplex analysis.

Table 1. Sequences of 38 primers used for multiplex PCR-based *TCRB* rearrangement analysis.

V segments	Sequence (5'-3')
Vβ2	AACTATGTTTTGGTATCGTCA
Vβ4	CACGATGTTCTGGTACCGTCAGCA
Vβ5/1	CAGTGTGTCCTGGTACCAACAG
Vβ6a/11	AACCCTTTATGGTACCGACA
Vβ6b/25	ATCCCTTTTTTGGTACCAACAG
Vβ6c	AACCCTTTATTGGTACAACAG
Vβ7	CGCTATGTATTGGTACAAGCA
Vβ8a	CTCCCGTTTTCTGGTACAGACAGAC
Vβ9	CGCTATGTATTGGTATAAACAG
Vβ10	TTATGTTTACTGGTATCGTAAGAAGC
Vβ11	CAAAATGTACTGGTACAACAA
Vβ12a/3/13a/15	ATACATGTACTGGTATCGACAAGAC
Vβ13b	GGCCATGTACTGGTATA
Vβ13c/12b/14	GTATATGTCCTGGTATCGACAAGA
Vβ16	TAACCTTTATTGGTATCGACGTGT
Vβ17	GGCCATGTACTGGTACCGACA
Vβ18	TCATGTTACTGGTATCGGCAG
Vβ19	TTATGTTTATTGGTATCAACAGAATCA
Vβ20	CAACCTATACTGGTACCGACA
Vβ21	TACCCTTTACTGGTACCGGCAG
Vβ22	ATACTTCTATTGGTACAGACAAATCT
Vβ23/8b	CACGGTCTACTGGTACCAGCA
Vβ24	CGTCATGTACTGGTACCAGCA
Jβ1.1	CTTACCTACAACTFTFAATCTGGTG
Jβ1.2	CTTACCTACAACGGTTAAACCTGGTC
Jβ1.3	CTTACCTACAACAGTGAGCCAACTT
Jβ1.4	CATACCCAAGACAGAGAGCTGGGTTC
Jβ1.5	CATACCTGTCACAGTGAGCCTG
Jβ1.6	CATACCTGTGACAGTGAGCCTG
Jβ2.2	CTTACCCAGTACGGTCAGCCT
Jβ2.6	CTCGCCCAGCACGGTCAGCCT
Jβ2.7	CTTACCTGTAACCGTGAGCCTG
Jβ2.1	CCTTCTTACCTAGCACGGTGA
Jβ2.3	CCCGCTTACCGAGCACTGTCA
Jβ2.4	CCAGCTTACCCAGCAGTGAGA
Jβ2.5	CGCGCACACCGAGCAC
Dβ1	GCCAAACAGCCTTACAAAGAC
Dβ2	TTTCCAAGCCCCACACAGTG

2.3 Detection of *TCRG* gene monoclonality by PCR

The PCR protocols used for detection of *TCRG* gene rearrangements were modified from those published by BenhattarBenhattar, Delacretaz, Martin, Chaubert, and Costa (1995). Each reaction contained 100 to 200 ng DNA, 200 µM dNTPs, 2.0 mM $MgCl_2$, 1 µM of each 3' and 5' primer, and 1.0 U Taq DNA polymerase (Toyobo, Japan) in a final volume of 25 µL. The PCR conditions were denaturation at 94 °C for 5 min with the addition of Taq polymerase at 85 °C for hot start, 35 cycles of denaturation at 94 °C for 42s, primer annealing at 62 °C for 42s, extension at 72°C 42s, and further extension at 72 °C for 5 min. Aliquots of the PCR mixtures were analyzed by 8% non-denaturing polyacrylamide gel electrophoresis and visualized under UV illumination after ethidium bromide staining.

2.4 Detection limit of PCR

The detection limit of *TCRG* PCR was determined using DNA obtained from a serial ratio of SKW-1 T cells (Saji & Tanigaki, 1982) in NALM-6 pre B-cells (T-cells in the B-cell background) (Drexler, Gaedicke, & Minowada, 1986). In the same way as *TCRB*-VDJ and *TCRB*-DJ were determined by serial dilution of SMZ-1 T-cell DNA (Miyanishi & Ohno, 1992) in polyclonal DNA mixtures (T-cell line DNA in 100 ng of mononuclear DNA).

2.5 Statistical analysis

The χ^2 test was used to compare the frequency difference of rearranged or germ line results between the groups. The sensitivity was calculated as TP/TP+FN (TP: true positive cases; FN: false negative cases), specificity as TN/TN+FP (TN: true negative cases; FP: false positive cases) and positive predictive value as TP/TP+FP.

3. Results and Discussion

3.1 Detection limit of the test

The PCR-based *TCRG* rearrangement analysis could detect at least 0.1% ($1/10^3$) clonally rearranged T-cells in the B-cell background. Multiplex PCR of *TCRB*-VDJ (included in tubes A and B) and *TCRB*-DJ (tube C) could clearly detect 3% clonal SMZ-1 DNA in polyclonal DNA mixtures (3 ng T-cell line DNA in 100 ng of mononuclear DNA). Hence, the detection limit of multiplex PCR in *TCRB* was less than *TCRG* by one order of magnitude.

3.2 Comparison of *TCR* rearrangement detection by PCR and Southern hybridization

To compare between PCR-based *TCR* rearrangement and Southern hybridization method, 25 specimens were selected as reference samples for malignant T cell lymphoproliferations in which they showed a discrete rearranged band of $C\beta_1$ probe by SH. Among these 25 reference samples, three cases showed germ line (no rearrangement) of *TCRG* and two cases had no rearrangement of *TCRB* gene by PCR as indicated by '*' in Table 3. The sensitivity to detect T-cell proliferations of *TCRB* PCR from

Table 2. Combination of primers for multiplex PCR-based rearrangement analysis of *TCRB*-VDJ and DJ segments.

A tube (VDJ)		B tube (VDJ)		C tube (DJ)	
V primers	JββA primers	V primers	JββB primers	D primers	JβAB primers
Vβ2	Jβ1.1	Vβ2	Jβ2.1	Dβ1	Jβ1.1
Vβ4	Jβ1.2	Vβ4	Jβ2.3	Dβ2	Jβ1.2
Vβ5/1	Jβ1.3	Vβ5/1	Jβ2.4		Jβ1.3
Vβ6a/11	Jβ1.4	Vβ6a/11	Jβ2.5		Jβ1.4
Vβ6b/25	Jβ1.5	Vβ6b/25			Jβ1.5
Vβ6c	Jβ1.6	Vβ6c			Jβ1.6
Vβ7	Jβ2.2	Vβ7			Jβ2.2
Vβ8a	Jβ2.6	Vβ8a			Jβ2.6
Vβ9	Jβ2.7	Vβ9			Jβ2.7
Vβ10		Vβ10			
Vβ11		Vβ11			Jβ2.1
Vβ12a/3/13a/15		Vβ12a/3/13a/15			Jβ2.3
Vβ13b		Vβ13b			Jβ2.4
Vβ13c/12b/14		Vβ13c/12b/14			Jβ2.5
Vβ16		Vβ16			
Vβ17		Vβ17			
Vβ18		Vβ18			
Vβ19		Vβ19			
Vβ20		Vβ20			
Vβ21		Vβ21			
Vβ22		Vβ22			
Vβ23/8b		Vβ23/8b			
Vβ24		Vβ24			
23	9	23	4	2	13

Table 3. *TCR* rearrangement: Comparison between Southern hybridization and PCR in T cell proliferation.

Southern	PCR				No. of samples	Diagnosis
TCRB (Cβ1probe)	*TCRB*	*TCRB*	*TCRB*	*TCRG*		
	VDJ	DJ	VDJ+DJ			
R	R	R	R	R	11	3 ATLL, 3 AITL, 1 LGL, 3 PTCL, 1 LBL
R	R	G	R	R	9	1 ATLL, 2 LGL, 2 PTCL, 1 LBL, 1 T-ALL, 2 CTCL
R	G	G	G	R	2*	1 ATLL, 1 AITL
R	R	R	R	G	1	1 AITL
R	R	G	R	G	2	2 ATLL
Total					25	

ATLL, adult T-cell leukemia/lymphoma; AITL, angioimmunoblastic T-cell lymphoma; LGL, large granular lymphocyte leukemia; PTCL, peripheral T-cell lymphoma, not otherwise specified; LBL, lymphoblastic lymphoma; T-ALL, T-Acute lymphoblastic leukemia; CTCL, cutaneous T-cell lymphoma (Mycosis fungoides, Sezary syndrome); R, rearrangement; G, germ line *, results showing germ line in the PCR for *TCRB*

these 25 samples was 92.0% (23/25) and for *TCRG* was 88.0% (22/25). Perfect sensitivity was observed with the combined PCR results of *TCRG* and *TCRB*. It could detect all 100% rearranged cases of T-cell lymphoproliferations among these 25 reference cases.

3.3 *TCRG* and *TCRB* rearrangement in T- and non-T-cell lymphoproliferations

Rearrangement of the *TCR* genes was analyzed in 133 cases: 88 were T-cell lymphoproliferative disorders, 38 were non-T-cell proliferative disorders and 7 healthy subjects.

The samples of the patients were divided into 3 groups: mature-T, immature-T, and non-T-cell proliferation. The rearrangement results of *TCRG* and *TCRB* are shown in

Tables 4 to 6. In the mature T-cell proliferations (Table 4), the frequency of ATLL was the highest and *TCRG* rearrangement was seen in 21 of 26 (80.8%) samples, while 17/17 samples showed rearranged *TCRB* gene. The 5 cases included in these 17 samples were the cases showing no rearrangement of *TCRG*. Among AITL, 14 out of 18 (77.8%) samples were positive for *TCRG* rearrangement, whereas the 4 samples without *TCRG* rearrangement exhibited rearrangement of the *TCRB* gene.

In PTCL, *TCRG* clonal rearrangement was detected in 12 out of 15 samples. One of the three samples with no rearrangement of *TCRG* exhibited rearrangement of *TCRB-DJ* segment, and the remaining two samples rearranged in both *TCRB-VDJ* and *DJ* segments. Clonal rearrangement of combined *TCRG* and *TCRB* gene could be completely observed in all 75 cases (100%) of mature T-cell lymphoproliferative disorders (Table 4).

Table 4. *TCR* rearrangement of mature T cell lymphoproliferation.

Category	*TCRG*			*TCRB*							*TCRG+TCRB*
	N			N	VDJ		DJ		VDJ+DJ		
		R	G		R	G	R	G	R	G	
AITL	18	14	4	13	13	0	6	7	13	0	18/18
ATLL	26	21	5	17	17	0	7	8	17	0	26/26
PTCL	15	12	3	13	11	2	7	5	13	0	15/15
T-LGL	7	7	0	5	5	0	3	2	5	0	7/7
T-CLL	3	3	0	2	1	1	0	2	1	1	3/3
MF	1	1	0	1	1	0	0	1	1	0	1/1
Sézary	2	2	0	2	2	0	0	2	2	0	2/2
ALCL	3	3	0	2	1	1	2	0	2	0	3/3
Total	75	63	12	55	51	4	25	27	54	1	75/75
		(84.0%)	(16.0%)		(92.7%)	(7.8%)	(48.1%)	(51.9%)	(98.2%)	(1.8%)	(100%)

AITL, angioimmunoblastic T-cell lymphoma; ATLL, adult T-cell leukemia/lymphoma; PTCL, peripheral T-cell lymphoma, not otherwise specified; LGL, large granular lymphocyte leukemia; CLL, chronic lymphocytic leukemia; MF, mycosis fungoides; Sézary, Sézary syndrome; ALCL, anaplastic large cell lymphoma; R, rearrangement; G, germline.

Table 5. *TCR* rearrangement of T cell lymphoblastic leukemia/lymphoma.

Category	*TCRG*			*TCRB*							*TCRG+TCRB*	
	N			N	VDJ		DJ		VDJ+DJ			
		R	G		R	G	R	G	R	G	R	%
T-ALL	8	3	5	8	2	6	2	6	4	4	4/8	50
CD3(-)	5	1	4	5	1	4	0	5	1	4	1/5	20
CD3(-/+)	1	0	1	1	0	1	1	0	1	0	1/1	100
CD3(+)	2	2	0	2	1	1	1	1	2	0	2/2	100
LBL,CD3(+)	5	5	0	4	4	0	3	1	4	0	5/5	100
Total	13	8	5	12	6	6	5	7	8	4	9/13	69.2

T-ALL, T-Acute lymphoblastic leukemia; LBL, lymphoblastic lymphoma; R, rearrangement; G, germ line.

Table 6. Non-neoplastic lymphoid disorders and non T cell malignancies.

Category	*TCRG*					*TCRB*												*TCRG+TCRB*	
	N					N	VDJ				DJ				VDJ+DJ					
		R	%	G	%		R	%	G	%	R	%	G	%	R	%	G	%	R	%
Healthy adults	7	0	0	7	100	7	0	0	7	100	0	0	7	100	0	0	7	100	0/7	0
Dermatopathic ly[1]	7	0	0	7	100	7	0	0	7	100	0	0	7	100	0	0	7	100	0/7	0
HTLV-I carrier	6	0	0	6	100	6	0	0	6	100	0	0	6	100	0	0	6	100	0/6	0
B-ALL	9	8	88.9	1	11.1	9	4	44.4	5	55.6	0	0	7[2]	100	4	44.4	5	55.6	9/9	100
B-NHL	16	4	25.0	12	75.0	15	0	0	15	100	0	0	15	100	0	0	15	100	4/16	25
Total	45	12	26.7	33	73.3	44	4	9.1	40	91.0	0	0.0	42	100	4	9.1	40	91.0	13/45	(28.9%)

[1] Dermatopathic lymphadenitis; NHL, non-Hodgkin lymphoma; HTLV-I, human T-cell leukemia virus type I; R, rearrangement; G, germline
[2] Only 7 samples were sufficient for *TCRB*-DJ PCR of B-ALL.

Table 5 details the clonal *TCR* rearrangement in immature T-cell proliferative disorders. Rearrangement of *TCRB* and *TCRG* was related with CD3(+) T-cell lymphoblastic leukemia or lymphoma while no rearrangement of these genes was associated with the CD3(-) immature T-leukemia/lymphoma. Rearranged *TCRG* and *TCRB* were seen in all 5 and 4 cases, respectively, of the CD3(+) LBL. Both cases of CD3(+) T-ALL showed *TCRG* and *TCRB* rearrangement whereas 4 out of 5 cases of CD3(-) T-ALL did not. All positive TCR rearrangement in T-ALL leukemia cases were either CD3(+) or CD3(+/-). Rearranged *TCRG* could be found in 8/13 specimens and rearranged *TCRB* was observed in 8/12 cases of this T-cell LBL group. Thus, this multiplex PCR procedure is a useful and reliable discriminating test for immature T-cell CD3(+) from T-cell CD3(-) LBL.

In the group of non-T-cell and non-neoplastic lymphoid samples (Table 6), all of the 23 samples exhibited negative for the clonal *TCRB* or *TCRG* rearrangement by healthy adults (all 7), cases of reactive dermatopathic lymphadenitis (all 7) and carriers of HTLV-I (all 6).

For the cases of B-NHL, all 15 cases tested for *TCRB* showed negative for *TCRB* rearrangement although 4 out of 16 B-NHL cases tested for *TCRG* showed positive rearrangement, suggesting that rearrangement of *TCRB* (rather than *TCRG*) can correctly discriminate B-NHL from various T cell lymphomas. In B-ALL, although the majority of the cases (8/9) showed *TCRG* rearranged positive and some of them showed *TCRB*-VDJ rearranged positive, all 7 B-ALL cases available for *TCRB*-DJ testing exhibited negative for the DJ rearrangement. This finding implied that multiplex PCR of the *TCRB*-VDJ and DJ segment could be suitable to discriminate B-ALL from CD3(+) T-ALL. Of note, all of the B malignancies studied and non-neoplastic reactive lymphoid cases tested for *TCRB*-DJ gave complete negative results. This suggested that the best specificity of analyzing the *TCRB*-DJ together with VDJ provides an alternative way to rule out immature CD3(+) T-ALL and LBL, CD3(+) (Tables 5 and 6). Moreover, although B-ALL (Table 6) and T-ALL, CD3(-) (Table 5) exhibited negative for *TCRB*-DJ rearrangement, their *TCRG* rearranged results were obviously different. Only 1/5 of T-ALL, CD3(-), compared to 8/9 of B-ALL, gave positive results for *TCRG* rearrangement. However, only 25% of B-NHL cases showed positive rearrangement of combined *TCRG* and *TCRB* and all 15 available cases in this group gave negative *TCRB* rearrangement. Most of the mature T-cell leukemia/lymphoma exhibited positive of the combined *TCR* rearrangement (Table 4).

In the mature T-cell proliferative disorders, single step *TCRG* could detect rearrangement of 84.0%, whereas in multiplex PCR for *TCRB*, the rearrangement frequencies were *TCRB*-VDJ 92.7%, DJ 48.1%, and the combined VDJ+DJ 98.2% in which the rearrangement positivity occurred with either VDJ or DJ segment. When the results of *TCRG* and *TCRB* were combined, rearrangement was perfectly detected in all 75 samples. In mature T-cell proliferation, the sensitivity, specificity, and positive predictive values of the tests for *TCRB* PCR were 98.2%, 90.9%, and 93.1%, respectively, while for the *TCRG* PCR they were 84.0%, 73.3%, and 84.0%, respectively. For immature T-cell proliferation, the sensitivity, specificity, and positive predictive values of the tests for *TCRB* PCR were 66.6%, 90.9%, and 61.5%, respectively, while they were 61.5%, 73.3%, and 40.0% respectively for *TCRG* (Table 7). Statistically, the discriminated value of *TCR* rearrangement is the best for testing between healthy or non-T-cell disorder samples versus the mature T-lymphoid malignancies (P<0.0001) (Table 8).

4. Conclusions

ATLL, which is predominant in the southwestern region of Japan, appeared to have epidemiologic features (Takatsuki, Matsuoka, & Yamaguchi, 1996). The HTLV-1 was proven to be the etiologic agent for this mature T-cell malignancy (Hinuma, 1987). Genetically, each of *TCRB* or *TCRG* solely could not completely detect clonal expansion of T-cell malignancies. More advantages were found when using *TCRB* PCR to detect monoclonality of ATLL than the *TCRG* PCR. For example, rearrangement of *TCRB* was seen in all 17 ATLL cases whereas non-rearrangement of *TCRG* was seen in 5 out of 26 ATLL cases (Table 4). Also in the SH-reference

Table 7. Sensitivity and specificity of the test.

Statistical	Mature		Immature	
	TCRG (%)	*TCRB* (%)	*TCRG* (%)	*TCRB* (%)
Sensitivity	63/75 (84.0)	54/55 (98.2)	8/13 (61.5)	8/12 (66.6)
Specificity	33/45 (73.3)	40/44 (90.9)	33/45 (73.3)	40/44 (90.9)
Positive predictive value	63/75 (84.0)	54/58 (93.1)	8/20 (40.0)	8/13 (61.5)

Table 8. Summary of Chi-square statistical results of *TCRB* and *TCRG* rearrangement analysis in T-cell malignancies vs. non-T lymphoid disorders and healthy individuals.

Genes	Rearranged segments	Statistical test group	P-value
TCRB	VDJ+DJ	Healthy plus Non-T disorders vs. Mature T-lymphoid malignancies	P<0.0001
TCRB	DJ	Healthy plus Non-T disorders vs. Mature T-lymphoid malignancies	P<0.0001
TCRB	VDJ+DJ	Healthy plus Non-T disorders vs. Immature T-lymphoid malignancies	P=0.0002
TCRB	DJ	Healthy plus Non-T disorders vs, Immature T-lymphoid malignancies	P=0.0001
TCRG		Healthy plus Non-T disorders vs. Mature T-lymphoid malignancies	P<0.0001
TCRG		Healthy plus Non-T disorders vs. Immature T-lymphoid malignancies	P=0.0595

specimens, non-rearrangement was seen in 1 case of ATLL by *TCRB* PCR while non-rearrangement seen in 2 cases by *TCRG* PCR (Table 3). These samples consisted of more than 10% atypical lymphocytes. Tsukasaki *et al.* (1997) reported that HTLV-I gene integration could change during progression of the disease; therefore, the rearrangement of the *TCRB* or *TCRG* or both genes could be concomitantly altered. Also, *TCR* Vβ segments were used in this work where the Vβ12 contained in BioMed 2 primer set was highly rearranged in CD4(+) T cells of HTLV-1 infected individuals (Saito *et al.,* 2006). Since a variety of histological records of this disorder existed, some difficulties occurred in the diagnosis of certain cases of AITL malignancy based solely on histological examination. Detection of *TCRB* clonality is therefore recommended for a diagnosis of AITL (Ferry, 2002; Shah, Harris, Smith, & Hodges, 2009). By using TCRB primers which consisted of Vβ 19 segment, we could detect rearrangement in a higher number of cases compared to using other Vβ segments in AITL.

Among the 13 cases of immature T-lymphoid neoplasm, *TCRD* and *TCRG* gene rearrangements were observed in the CD44(+) and CD25(+) population (data not shown). Although Blom, Res, and Spits (1998) reported that *TCRB* rearrangement was seen in the minority population of T-cell precursor cells, we found *TCRB* rearrangement (VDJ+DJ) in 8 out of 12 tested cases. Of these, all cases of LBL, CD3(+) showed rearrangement of both *TCRB* and *TCRG*. For the immature T-ALL, our results agree with the other reports that only a small proportion of the CD3(-) immature T-ALL samples exhibited rearrangement of the *TCRB* gene (Langerak *et al.,* 1999; Taghon & Rothenberg, 2008). Although only a few cases were available, *TCRG* rearrangement was also detected in only a small proportion of CD3(-) immature T-ALL compared to CD3(+) T-ALL.

However, some of B-ALL and B-NHL also exhibited rearrangement of the *TCRG* or *TCRB*-VDJ. Of note, all available samples of 7 B-ALL and 15 B-NHL cases showed negative rearrangements using the TCRB DJ primer which was the same finding as seen in the non-neoplastic lymphoid samples (Table 6). As mentioned above, CD3(-) T-ALL could have negative rearrangement for the *TCRB*-DJ. However, the majority of CD3(-) T-ALL cases (4/5) also showed no rearrangement for *TCRG* gene, while the majority of B-ALL cases (8/9) exhibited rearrangement of *TCRG*. With the combined *TCRG* and *TCRB*-VDJ PCR results, rearrangement was seen in all cases of B-ALL (9/9) although they were mostly negative at the *TCRB*-DJ segment. Previous reports revealed that 60% of pre-B-ALL had clonal *TCRG* rearrangement whereas only 35% of cases showed *TCRB* rearrangement (Alatrakchi *et al.,* 1998; Szczepanski *et al.,* 1999). In this work, children were diagnosed either as common ALL or pre-B ALL by immunological characteristics that exhibited *TCRG* and *TCRB* rearrangements in 88.9% and 44.4%, respectively (Table 6). These informative data could be useful for the diagnosis of B- and T-ALL and further understanding of biological characteristics of these leukemic phenotypes.

The genotypic profiles of mature B-cell malignancy have been previously reported as positive *TCRB* rearrangement in 16% and *TCRG* rearrangement in 11% (Evans *et al.,* 2007). In this work, we found slightly different results. In NHL, *TCRB* rearrangement of both VDJ and DJ was not observed in all 15 samples tested for B-cell NHL, whereas rearranged *TCRG* was found in 25% of samples (4/16).

The diagnosis of lymphoproliferative diseases always refers to clinical, morphological, immunophenotypical, cytogenetic, and genetic analysis. Even though most of the cases were confidently diagnosed by morphology and immunophenotype, some difficult cases needed a genetic study for accurate determination. The genetic data from this work should be useful in this leukemia/lymphoma area. *TCRB* multiplex PCR detection was shown in this study to have discriminating capability of mature and immature monoclonality for not only the T- but also the B-lymphoproliferations. This genetic profiling analysis could perfectly replace SH (Sandberg *et al.,* 2005), thereby giving more informative details for each sample. T-cell clonality assessment is important for the differential diagnosis of T-cell lymphoproliferative disorders. *TCRG* and *TCRB* rearrangements are favorite targets of T-cell clonality detection. SH is the gold standard for clonality testing but usually requires a large amount of high-quality DNA, and the method is time-consuming and expensive. Moreover, since radioisotopes are used in SH, careful handling by the technician is required. Currently, PCR-based T-cell clonality detection is widely applicable due to its reliability, sensitivity, and specificity. PCR provides rapid and accurate detection and requires less DNA template than other techniques (Gazzola *et al.,* 2014; Spagnolo *et al.,* 2004). The combination of *TCRG* and *TCRB* PCR is a powerful strategy for the diagnosis of T- and B-lymphoid malignancies. We clearly show that mature T-cell lymphoid malignancies in Japanese patients have unique 100% rearrangement with combined *TCR* analysis (*TCRG*+ *TCRB*). In contrast, the very young immature T-ALL with CD3(-) showed positive rearrangement in only 25% of cases when tested with combined *TCR*, whereas none of them were positive with the DJ rearrangement analysis. Although PCR-based rearrangement for *TCRG* and *TCRB* could not be detected in the majority of T-ALL, CD3(-) cases, the results of *TCRG* and *TCRB* in this cell type agree with each other, which makes this T-ALL, CD3(-) different from B-ALL from a DNA standpoint. In B-ALL, the majority of cases were *TCRG* positive although all of them (7/7) showed positive for DJ. In addition, it would be interesting to study the differences in the prognosis and therapeutic outcome or survival of each T-ALL, CD3(-), and B-ALL patient whose *TCR* rearrangement results are different.

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