Diversity of T-cell receptor Gene Rearrangements in South Indian Patients with Common Acute Lymphoblastic Leukemia

Natarajan Sudhakar[1,3], Nirmala Karunakaran Nancy[1*], Kamalalayam Raghavan Rajalekshmy[2], Thangarajan Rajkumar[1]

[1]Dept of Molecular Oncology, [2]Dept of Hematology and Immunology, Cancer Institute (WIA), 38, Sardar Patel Road, Chennai 600036, [3]Department of Biotechnology, Dr.MGR Educational and Research Institute University, Maduravoyal, Chennai 600 095,Tamil Nadu, India

ABSTRACT

Background: Precursor B-Acute Lymphoblastic Leukemia (precursor B-ALL) occurs due to the uncontrolled proliferation of B-lymphoid precursors arrested at a particular stage of B-cell development. Precursor-B-ALL is classified mainly into pro-B-ALL, common-ALL and pre-B-ALL. The Common Acute Lymphoblastic Antigen CD10 is the marker for common-ALL. **Objective:** This study was aimed to examine the diversity of T-cell receptor Gamma (*TCRG)* and T-cell receptor Delta (*TCRD)* gene rearrangements in South Indian Common-ALL patients. **Methods:** Clonality of *TCRG* and *TCRD* was studied in 52 cases (pediatric=41 and adolescents and young adults=11) of common-ALL. *TCRG* and *TCRD* gene rearrangements were amplified by PCR and the clonality was assessed by Heteroduplex analysis of amplified products. **Results:** In pediatric common-ALL, clonal *TCRG and TCRD* gene rearrangements were detected in 19 (46.3%) and 18 (43.9%) cases respectively. In adolescents and young adults (AYA), *TCRG* was rearranged in 8 (72.7%) cases and *TCRD* was rearranged in 4 (36.3%) cases. In the present study of common-ALL, the frequency of a *TCRG* rearrangement VγII-Jγ1.3/2.3 was significantly high in AYA compared to pediatric (36.3% vs 4.8%; $p<0.025$). Thus, VγII-Jγ1.3/2.3 was highly diverse in AYA compared to pediatric. That shows the difference in biology of the disease between pediatric and AYA in South Indian population. **Conclusion:** The reason for the high frequency of VγII-Jγ1.3/2.3 in AYA of common-ALL in South Indian population in connection with unknown infectious agents or environmental carcinogens needs to be evaluated further.

Keywords: TCR, ALL, Heteroduplex Analysis

*Corresponding author: Dr. Nirmala Karunakaran Nancy, Dept of Molecular Oncology, Cancer Institute (WIA), 38, Sardar Patel Road, Chennai 600036, Tamil Nadu, India. Tel: (+) 91 44 22350131; (+) 91 44 22350340, Fax: (+) 91 44 24912085, e-mail : nirc18@gmail.com

INTRODUCTION

Precursor B-Acute Lymphoblastic Leukemia (precursor B-ALL) occurs due to the uncontrolled proliferation of B-lymphoid precursors arrested at a particular stage of B-cell development. It is classified mainly into pro-B-ALL, common-ALL (C-ALL) and pre-B-ALL. The Common Acute Lymphoblastic Antigen CD10 is the marker for C-ALL (1). Initially the Immunoglobulin (Ig) and T-cell receptor (TCR) were regarded as lineage specific markers for B- cells and T- cells respectively. But later it was recognized that TCR is also rearranged in malignant precursor B-cells and similarly Ig in malignant T- cells by lineage promiscuity (2). During B- and T- cell differentiation the germ line encoded variable (V), diversity (D) and joining (J) gene segments of TCR gene complex and Ig rearrange by the process of recombination. Thereby each lymphocyte obtains a unique combination of V-(D)-J segment that code for the variable domain of TCR and Ig molecules. The rearranged V-(D)-J sequence represents a 'specific signature' of each lymphocyte. Due to the clonal origin of leukemic cells, each malignant lymphoid disease will represent the expansion of a clonal population with a specific Ig/TCR signature (3).

The pattern of *TCRG* and *TCRD* gene rearrangements and their junctional region characteristics in T-cell ALL in South Indian patients have been documented (4). But the pattern of cross-lineage TCR gene rearrangements in South Indian C-ALL has not been identified so far. In the current study we examined the diversity of *TCRG* and *TCRD* gene rearrangements in South Indian C-ALL patients.

MATERIALS AND METHODS

Patients. Bone marrow (BM) and peripheral blood (PB) samples were obtained from Acute Lymphoblastic Leukemia (ALL) patients prior to starting the treatment. Diagnosis of ALL was based on standard FAB classification and immunophenotypic criteria. The 52 cases of C-ALL include 41 pediatric and 11 adolescents and young adults (AYA). Age of the patients ranged from 2 yrs to 24 yrs. All the pediatric and AYA patients were enrolled for treatment under MCP 841 protocol (5,6) after obtaining a written informed consent.

Collection of BM and PB and Separation of Mononuclear Cells. Two milliliters of BM and 10 ml of PB was collected from the patients prior to treatment and mononuclear cells (MNC) were separated using Ficoll Hypaque^{TM} (density: 1.077g/ml, Amersham Biosciences, Uppsala, Swedan) density gradient centrifugation. Cells were washed twice with phosphate buffered saline and stored at -70°C until use.

Immunological Marker Analysis. Flow cytometric immunophenotyping was performed with FACSCaliber system (Becton Dickinson, San Jose, CA, USA). The MNC of the ALL patients were analyzed for the expression of cell surface markers using a panel of monoclonal antibodies (BD Pharmingen^{TM}, BD Biosciences, CA, USA) comprising HLA-DR, CD2, SmCD3, CD4, CD5, CD7, CD8, CD10, CD19, CD20, CD13, CD33 by direct immunofluorescence technique and indirect immunofluorescence technique was used with monoclonal antibodies for CyIgμ and SmIg. The leukemic cells were considered positive if the CD marker was expressed in >20% of the cells.

DNA Isolation from MNC and Amplification of *TCRG* and *TCRD* Gene Rearrangements. The MNC stored at –70°C was thawed at room temperature and the DNA

from BM/PB was isolated using QIAamp kit (Qiagen, Hilden, Germany) according to manufacturer's instructions. The quality of DNA was checked by agarose gel electrophoresis and also amplifying for the *C-ABL* housekeeping gene. A 50µl PCR reaction contained 10X PCR buffer, 1.5mM $MgCl_2$, 200µM of dNTP's (AB Gene, UK), 2U of Hotstart Taq Polymerase (AB Gene, UK) and 10 pmol of forward and reverse primers with 100ng of genomic DNA. PCR was performed in a Perkin-Elmer 480 thermal cycler (Applied Biosystems). PCR conditions included preactivation of the enzyme for 10 min at 94°C followed by 35 cycles at 94°C for 60s, 60°C for 90s and 72°C for 2 min and a final extension of 10 min at 72°C. The primers used for *TCRG* and *TCRD* gene rearrangements were as described (7).

The amplified products and expected size of *TCRG* rearrangement are VγI-Jγ1.3/2.3 (533 bp), VγII-Jγ1.3/2.3 (522 bp), VγIII-Jγ1.3/2.3 (522 bp), VγIV-Jγ1.3/2.3 (558 bp), VγI-Jγ1.1/2.1 (329 bp), VγII-Jγ1.1/2.1 (318 bp) and *TCRD* rearrangement are Vδ2-Dδ3 (501 bp), Dδ2-Dδ3 (608 bp), Dδ2-Jδ1 (550 bp). The patient sample which had already amplified for a rearrangement and confirmed by Heteroduplex analysis and sequencing was used as a positive control and water and normal lymphocytes were used as a negative control. Amplified products were visualized by electrophoresing on a 1.5% ethidium bromide containing agarose gel.

Heteroduplex Analysis. To distinguish the clonal gene rearrangements of malignant leukemic cells from polyclonal rearrangement present in normal cells, heteroduplex analysis was performed. For the analysis, 12µl of the amplified PCR product was denatured at 94°C for 5 min to obtain single stranded PCR products and then cooled to 4°C for 60 min to induce renaturation. After denaturation and renaturation of the amplified products, the sequences with identical junctional region produce homoduplex bands and sequences with non identical junctional region produce heteroduplex bands. The PCR products were then resolved on a 6% non-denaturing polyacrylamide gel in 0.5X Tris-Borate-EDTA buffer run at 45V overnight at room temperature. The gel was visualized after ethidium bromide staining (8).

Statistical Analysis. Two-tailed Fisher's exact test was performed to compare the frequency of TCR gene rearrangements between pediatric and AYA in C-ALL. Also the clinical features were compared between pediatric and AYA. A p value of <0.05 was considered as statistically significant.

RESULTS

In C-ALL, monoallelic rearrangement was observed in 32 of 52 (61.5) cases. In pediatric, *TCRG* rearrangement was detected in 19 of the 41 (46.3%) cases and incomplete *TCRD* (Vδ2-Dδ3 and Dδ2-Dδ3) rearrangement was detected in 18 (43.9) cases. The frequency of *TCRG* and *TCRD* rearrangements are presented in Table 1. VγIII-Jγ1.3/2.3 and VγIV-Jγ1.3/2.3 gene rearrangements were detected only in pediatric and not detected in AYA. Oligoclonal rearrangement was shown in 5 (9.6) cases and all the cases were of Vδ2-Dδ3 rearrangement. Bi-allelic rearrangement was shown in 6 (11.5) cases including 5 cases of pediatric and one case of AYA. Clonal rearrangement was not detected in 9 (17) cases. The clonal homoduplex bands, biallelic rearrangement and oligoclonal rearrangement are illustrated in Figure 1.

Table 1. Frequencies of TCRG and TCRD gene rearrangements in pediatric and AYA

TCR genes	Common-ALL (n=52)	
	Pediatric (n=41)	AYA (n=11)
TCRG	19 (46.3)	8 (72.7)
VγI-Jγ1.3/2.3	11 (26.8)	4 (36.3)
VγII-Jγ1.3/2.3	2 (4.8)	4 (36.3)*
VγIII-Jγ1.3/2.3	3 (7.3)	0 (0)
VγIV-Jγ1.3/2.3	1 (2.4)	0 (0)
VγI-Jγ1.1/2.1	4 (9.7)	3 (27.2)
VγII-Jγ1.1/2.1	1 (2.4)	1 (9)
TCRD	18 (43.9)	4 (36.3)
Vδ2-Dδ3	15 (36.5)	4 (36.3)
Dδ2-Dδ3	9 (21.9)	1 (9)
Dδ2-Jδ1	0 (0)	0 (0)

*p < 0.025 by two-tailed Fisher's exact test

Note:

- Monoallelic rearrangement was detected in 32/52 (61.5) cases
  Biallelic rearrangement in 6/52 (11.5) cases
  Oligoclonal rearrangement in 5/52 (9.6) cases
  No clonal rearrangement detected in 9/52 (17.3) cases
- In pediatric, more than one clonal rearrangement was detected in 3 cases of *TCRG* and 6 cases of *TCRD*. In AYA, 5 cases showed more than one clonal *TCRG* or *TCRD* rearrangement.

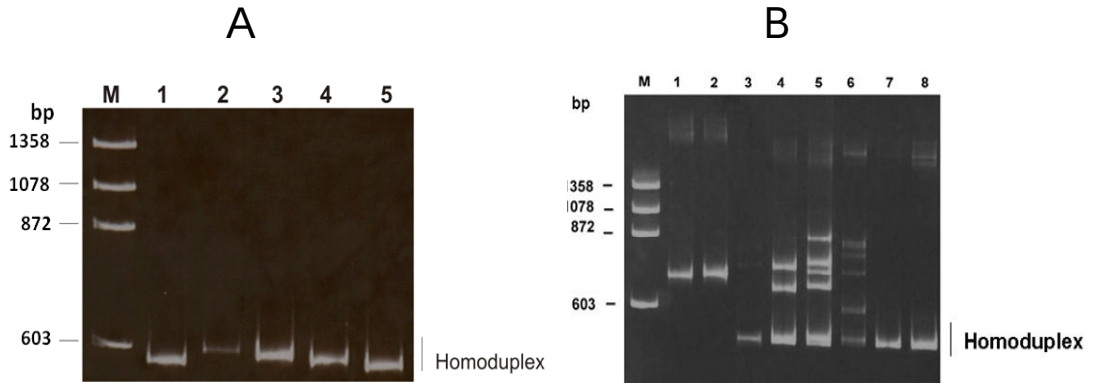

Figure 1. Heteroduplex analysis of TCRG and TCRD rearrangements. The amplified PCR product was denatured at 94°C for 5 min to obtain single stranded DNA and then cooled to 4°C for 60 min to induce renaturation. The samples were then loaded on a 6% non-denaturing polyacrylamide gel. A. Lane 1 to 5 – Homoduplex bands of VγII-Jγ1.3/2.3 (522bp), M – Molecular weight marker. B. Lane 1 and 2- homoduplex bands of Dδ2-Dδ3 (608 bp) ; Lane 3, 7 and 8- homoduplex bands of Vδ2-Dδ3 (501 bp); Lane 4 – biallelic rearrangement in Vδ2-Dδ3, Lane 5 and 6- oligoclonal pattern of Vδ2-Dδ3. M- Molecular weight marker

In AYA, *TCRG* was rearranged in 8 of the 11 (72.7) cases and *TCRD* in 4 of the 11 (36.3) cases. In the present study of C-ALL, the frequency of *TCRD* was comparable in pediatric and AYA whereas *TCRG* was high in AYA compared to pediatric. When the frequency of *TCRG* rearrangements was compared between pediatric and AYA, it was detected that VγII-Jγ1.3/2.3 (also known as Vγ9-Jγ1.3/2.3) rearrangement was significantly high in AYA compared to pediatric (36.3% vs 4.8%; p< 0.025). The clinical features of common-ALL were compared between pediatric and AYA (Table 2). The male to female ratio in common-ALL was high in AYA compared to pediatric but the difference was not statistically significant.

Table 2. Clinical features of common-ALL cases with pediatric and AYA

Clinical features	Pediatric (n=41)	AYA (n=11)
Median age	5 yrs	18.5 yrs
Range	2 yrs to 15 yrs	16 yrs to 24 yrs
Male	26 (63.4)	8 (72.7)
Female	15 (36.5)	3 (27.2)
Male to Female ratio	1.7:1.0	2.6:1.0
WBC count (10^9/L)		
<20	24 (58.5)	5 (45.4)
20-50	6 (14.6)	3 (27.2)
50-100	5 (12.1)	1 (9.0)
>100	6 (14.6)	2 (18.1)
Generalized lymphnode -enlargement	28 (68.2)	8 (72.7)
Hepatosplenomegaly	23 (56.0)	8 (72.7)
Mediastinal mass	2 (4.8)	1 (9)

DISCUSSION

Biology of ALL is distinct in different ethnic and geographic populations. ALL in South Indian patients was distinct in terms of paucity of common ALL and high relative incidence of T-ALL (9). But over the years there is a gradual reduction in T-ALL and a proportionate increase in common-ALL was observed (Rajalekshmy, K.R, unpublished observations).

In C-ALL, monoallelic rearrangement was observed in 61.5 of the cases and bi-allelic rearrangement in 11.5 of the cases. Brumpt et al. and Scrideli et al. independently reported biallelic rearrangements in 59.6% and 40.6% of B-ALL cases respectively (10,11). In contrast to those reports biallelic rearrangement was considerably less in the present study. In AYA, *TCRG* was rearranged in 8 of the 11 (72.7 %) cases and *TCRD* in 4 of the 11 (36.3 %) cases. In general, *TCRG* and *TCRD* gene rearrangements were studied more in childhood precursor-B-ALL but rarely studied in AYA or adults. In childhood B-ALL, Cave *et al* detected *TCRD* in 115 of 188 (61) cases and *TCRG* in 62 of 108 (57) cases (12). Szczepanski et al. observed rearrangement of *TCRG* in 59% (113 of 192 cases) and *TCRD* in 55% (112 of 202 cases) of precursor-B-ALL (13). Van der Velden et al. in 271 cases of C-ALL reported 72% of incomplete *TCRD* rearrangement and 58% of *TCRG* rearrangements in their study (14). Yao et al. studied 40 Chinese adult B-ALL patients and reported *TCRG* and *TCRD* rearrangements in 77% and 55% of the patients respectively (15). Thus, the rearrangement of both *TCRG* and *TCRD* in pediatric C-ALL is comparatively less than the frequency reported from other geographic populations.

A significant discrepancy in the frequency of Vγ II-Jγ1.3/2.3 between pediatric and AYA might be due to the difference in biology of the disease between pediatric and AYA. A recent review highlighted that the lack of progress in treating cancers in AYA is in part due to lack of study, which highlights the biology of the disease in AYA compared to that of children and adults (16). Hara et al. identified *TCRG* in 36 of 57 (63%) B-precursor ALL and detected Vγ II family in 24.5% of the cases (17). A study by Brumpt et al. showed a comparable frequency of *TCRG* (66% of children and 61% of AYA) in both children and AYA with B-cell precursor ALL. Also, Vγ II family was commonly rearranged in 20 of the 62 cases (32%) and notably high in adults (24%) compared to children (8%) (10). Scrideli et al. documented *TCRG* rearrangements in Brazilian children with B-ALL and detected a high frequency of *TCRG* in 54 of 79 (69%) cases with a high proportion (48.5%) of Vγ II rearrangement (11). In the present

study of C-ALL, a *TCRG* rearrangement VγII-Jγ1.3/2.3 was highly diverse in AYA compared to pediatric and we speculate that the high frequency may attribute to exposure of immune system to environmental carcinogens or an unknown infectious agent.
In conclusion in South Indian C-ALL patients, a *TCRG* rearrangement - VγII-Jγ1.3/2.3 was highly diverse in AYA compared to pediatric. That shows the difference in biology of the disease between pediatric and AYA. However, the study has to be done in more number of samples to confirm the difference. Again the reason for the high frequency of VγII-Jγ1.3/2.3 in AYA of C-ALL in South Indian population in connection with unknown infectious agents or environmental carcinogens needs to be evaluated further.

ACKNOWLEDGMENTS

We thank the Department of Science and Technology, Government. of India for funding the project (Grant number SP/SO/B12/2000) and Lady Tata Memorial Trust, Mumbai, India for the award of Senior Scholarship to N.S.

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