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Immunoglobulin (Ig) and T-Cell Receptor (TCR) Gene Rearrangement Pattern in Adult Acute Lymphoblastic Leukemia in India

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Abstract: ALL in adults is an entity distinct from that in children, as the two have different prognostic factors. Amongst factors affecting prognosis in adult ALL, literature on molecular rearrangement is limited, we therefore analysed the Ig and TCR gene rearrangements in adult ALL and looked for their correlation with the disease outcome. In 52 cases of ALL (median age 23 years), IgH chain gene was rearranged in 97% B-Cell Precursor (BCP) ALLs and 40% T-ALLs. One or the other TCR locus was rearranged not only in all T-ALLs, but also in 86% of BCP-ALLs. Though the overall survival in patients with TCR γ rearrangement was least, both in BCP-ALL (34.3 ± 19.5% vs. 72.2 ± 11.9%, p=0.16) and T-ALL (25.0 ± 21.7% vs. 75.0 ± 21.7%, p=0.23), but it was not statistically significant. Moreover, in BCP-ALL, IgH locus with more than 2 bands rearranged was associated with a lower Hb at presentation (mean 4.8 Vs 7.4 g/dL, p=0.01). The pattern of rearrangement of these genes in our study appeared to be different from the West, viz TCR-β rearrangement was seen in a higher proportion f BCP-ALLs (68%) and rearrangement for TCR-γ genes were invariable deletions of Cγ1 and TCR-δ locus had only monoallelic rearrangement.

Key words: Adult ALL · ALL in India · immunophenotype · Ig gene rearrangements · TCR gene rearrangements

NTRODUCTION

Acute lymphoblastic leukemia in adults has a worse prognosis than that in children [1, 2]. Some factors responsible for this difference include advanced age, hyperleukocytosis at presentation, higher frequency of more immature B or T lineage phenotypes, lack of TEL gene rearrangements, higher proportion of patients with the t(9;22) Ph+ translocation [1, 3]. The analysis of Ig and TCR genes is a tool to characterize the disease and detect clonal assessment at the molecular level [4]. Unlike childhood ALL, limited studies on the biology of this disease in adults are available [5-7]. Although very few studies have reported the rearrangement pattern in adult ALL [8-10], the clinical significance of these rearrangements is limited. In the present study, we attempted to elucidate the clinical significance of the above rearrangements in adult ALL.

MATERIALS AND METHODS

Patients: Fifty two cases of adult ALL (age >16 years) presenting to the Medical Oncology Clinic of All India Institute of Medical Sciences, New Delhi, India, from 1991 to 1999 were included in the present study. Of these 37 were classified to have B-lineage and 15 T-lineage origins. The median age of the patients was 23 years (range 16-55 years), 42 of them were males and 10 were females. The median WBC count was $55x10^9\ l^{-1}$ (range $5.7x10^9$ - $440x10^9\ l^{-1}$). Patients were treated with MCP-841 protocol [11]. Briefly, the protcol uses three induction cycles of 28 days each (prednisolone, vincristine, L-asparaginase, daunorubicin, 6-mercaptopurine, cyclophosphamide), followed by consolidation of remission (cyclophosphamide, vincristine, 6-mercaptopurine, cytosine arabinoside and prednisolone). Subsequently, 6 maintenance cycles of 3 months each

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(oral methotrexate and 6-mercaptopurine) are used. Cranial irradiation (2000 CGy during second induction) and intrathecal methotrexate (12, weekly during the 3 induction cycles and the consolidation cycle) are used for CNS prophylaxis. In all cases immunophenotypic studies were performed at diagnosis using a wide panel of monoclonal antibodies [12]. The samples were taken either from peripheral blood or bone marrow depending on the total leukocyte count and the percentage of blasts at the time of presentation. After concentration on Ficoll Hypaque, the mononuclear cells comprising more than 90% blasts were stored at -70°C till further use.

The overall survival for 29 patients (23 B-lineage, 6 T-lineage) available for evaluation of response and subsequent followup on protocol MCP 841 was 49.3±12.1 at 48 months.

Genomic blot hybridization: High molecular weight DNA was extracted from leukemic cells according to standard procedures [13]. Ten µg of DNA was digested with the appropriate restriction enzyme (Hind III and Eco RI). Size separated in 0.8% agarose gel and transfered to S&S Nytran (Schleicher and Schuell) membrane according to the standard Southern blotting techniques [4]. Overnight hybridization was done with DNA probes labeled with ^{32}P-dCTP (Dupont, NEN Research, Boston, MA, USA) using a random primer DNA labelling kit (Stratagene Prime-It; Stratagene, La Jolla, CA, USA).

The configuration of Ig and TCR genes was analysed using the following probes: a 5.5 kb Bam HI-Hind III fragment specific for the IgJH region, a 400 bp Bgl II fragment for the $C\beta_2$ region that also recognises $C\beta_1$, a 200 bp Bam HI fragment for the Cγ1 region, a 900 bp Sst1-Xba1 fragment for the Jδ2 region [12].

Statistical analysis: Comparison of qualitative clinical and laboratory features between cases with and without IgH and TCR rearrangements was performed using Fisher's exact test. Differences in quantitative variables between two groups were compared using Wilcoxon rank sum test. All calculations were performed using EPI-INFO (Ver 6.0) software. The Kaplan Meier method was used to estimate survival rates and differences between two groups compared by using log-rank test.

Interpretation: The IgH chain gene / T cell receptor genes were classified as rearranged, based on the detection of DNA fragments distinct from germline, or deletions, in any of the two restriction digests used (Eco RI and Hind III).

RESULTS

Ig and TCR gene rearrangement patterns in BCP ALL: Ig heavy chain gene was rearranged in thirty six of 37 cases of B-lineage while one case revealed a germline configuration. In 6/36 patients (17%) more than two rearranged bands were found. Of the remaining cases, 9 (25%) showed only one rearranged band and 21 (58%) had two rearranged bands. Nearly all samples retained a germline band of variable intensity on at least one digest.

The configuration of TCRβ was rearranged in 25/37 (68%) cases (Table 1); Cβ2 in 36% (9/25) and Cβ1 in 20% (5/25) cases. Rearrangement of both Cβ1 and Cβ2 was seen in 44% (11/25) cases. In addition, the second constant region of TCR-β (a 4 kb fragment readily identified in Eco RI digest) was rearranged in 6 cases.

TCR-γ gene rearrangements were present in 25% (9/36) patients and were invariable deletions of the Cγ1 band.

Rearrangements of the TCRδ locus with one or the other digest were seen in 22/36 (61%) cases. These rearrangements were predominantly (10/22 rearranged, 45%) monoallelic rearrangements with one band retained in germline configuration. However 7/22 (32%) cases had one rearranged band with deletion of the germline band. Two rearranged bands were seen in 5/22 (23%) cases.

Lineage inappropriate rearrangements for one or the other TCR loci studied was seen in 31/36 (86%) BCP ALLs (Table 2). All the 3 loci were rearranged in 5 (15%) patients and 2 of the 3 loci were rearranged in 13 (38%)

Table 1: IgH and TCR gene patterns in BCP ALL

Ig heavy chain	
Rearranged IgH gene	36/37 (97%)
Single rearranged band	9/36 (25%)
Two rearranged bands	21/36 (58%)
Three rearranged bands	4/36 (11%)
Four rearranged bands	2/36 (6%)
TCR-γ	
Rearranged TCR-γ (deleted Cγ1)	9/36 (25%)
TCR - δ	
Rearranged TCR-δ	22/36 (61%)
Rearranged one band/ deleted one	7/22 (32%)
Rearranged one band/ germline one	10/22 (45%)
Rearranged two with/without germline band	5/22 (23%)
TCR - β	
Rearranged TCR-β	25/37 (68%)
Rearranged Cβ1	5/25 (20%)
Rearranged Cβ2	9/25 (36%)
Rearranged both Cβ1 and Cβ2	11/25 (44%)

Table 2: Frequency of IgH and TCR gene rearrangements in BCP ALL

IgH gene rearranged, TCR genes germline	5/36 (14%)
IgH gene rearranged, TCR genes rearranged	31/36 (86%)
All 3 TCR loci rearranged	5/34 (15%)
Two TCR loci rearranged	13/34(38%)
Rearranged TCR-β+TCR-δ	10/36 (27%)
Rearranged TCR-γ+TCR-δ	2/35 (6%)
Rearranged TCR-β+TCR-γ	2/36 (6%)
Only one TCR gene rearranged	12/34 (35%)
Rearranged TCR-δ	5/36 (14%)
Rearranged TCR-β	7/37 (19%)
Rearranged TCR-γ	0

Table 3**:** IgH and TCR gene patterns in T-ALL

IgH gene	
Rearranged IgH gene	6/15 (40%)
Rearranged one band	5/6 (83%)
Rearranged both bands	1/6 (17%)
Rearranged one band/ delete one	0
TCR - γ	
Rearranged TCR γ (Deleted Cγ1)	6/15 (40%)
TCR - δ	
Rearranged TCR-δ	10/15 (66%)
Rearranged one band/ deleted one	2/10 (20%)
Rearranged one/Germline one	5/10 (50%)
Rearranged two bands with/without germline band	3/10 (30%)
Rearranged three bands	0
TCR - β	
Rearranged TCR-β	9/15 (60%)
Rearranged Cβ1	3/9 (33%)
Rearranged Cβ2	0
Rearranged C 1 and C 2	6/9 (67%)

Table 4: Frequency of IgH and TCR gene rearrangements in T-ALL

TCR genes rearranged, Ig genes germline	9/15 (60%)
TCR genes rearranged, Ig genes rearranged	5/15 (33%)
TCR genes germline, Ig genes rearranged	1/15 (6%)
All three TCR rearranged	2/15 (13%)
Two TCR loci rearranged	7/15 (46%)
Rearranged TCR-β+TCR-γ	3/15 (20%)
Rearranged TCR-β+TCR-δ	3/15 (20%)
Rearranged TCR-γ+TCR-δ	1/15 (6%)
Only one TCR gene rearranged	5/15 (33%)
Rearranged TCR-β	1/15 (6%)
Rearranged TCR-	4/15 (26%)

cases. TCRβ + TCR δ was most common combination (10/36, 27% patients) followed by TCRγ + δ (2/35, 6% patients) and TCRβ+γ (2/36, 6% patients). Rearrangement of TCRδ alone was seen in 5 cases. Seven cases rearranged only the TCRβ locus. The distribution of age, gender, hemoglobin, WBC count, platelet count and overall survival in relation to the pattern of IgH and TCR genes is shown in Table 5. Patients with multiple rearranged bands for the IgH gene presented with significantly lower Hb (p=0.01) than patients with 2 rearranged bands. Patients with TCRγ rearrangement had lower overall survival than those with germline pattern but was not statistically significant (34.3 ±19.5% vs 72.2 ± 11.9%, p=0.16).

Ig and TCR gene rearrangement patterns in T-ALL: Fifteen ALL samples immunophenotyped as T-ALL, showed rearrangement of one or the other TCR locus. Majority of the patients possessed TCR δ rearrangement (10/15 (66%)). Two patients rearranged one allele and deleted the other. Most of the patients 5 of the 10, retained one allele in the germline configuration while rearranging the other. Three cases had two rearranged bands with or without the presence of the germline band.

Nine of 15 (60%) patients showed rearrangement at the TCRβ locus (Table 3). In contrast to B-lineage leukemia, Cβ1 was predominantly rearranged in 3/9 (33%) cases. No rearrangement was detected in TCRβ2 region. Rearrangements at the TCRγ locus were exclusively deletional; 6/15 (40%) cases showed deletion of the Cγ1 region.

Rearrangements of multiple TCR loci was seen in a majority of the leukemias assigned to the T-lineage (Table 4). Thus, 2/15 (13%) cases rearranged all the three loci investigated, 7 had rearrangements of 2 of the 3 loci studied and atleast one TCR locus was rearranged in 5/15 (33%) cases. TCRβ + TCRγ was the combination observed in 3, TCRβ + δ in 3 and TCRγ + δ in 1 sample. Rearrangement of TCRβ alone was seen in 1 case. TCRδ was the only TCR rearranged in 4 cases.

Lineage inappropriate rearrangements were not as frequent as seen in the B-lineage leukemias. IgH chain gene was rearranged in 6/15 (40%) cases investigated (Table 3). Two rearranged bands were seen in 1 case and one rearranged band in presence of germline band was seen in 5 cases. Patients with TCRγ rearrangement had higher TLC, lower Hb and showed lower survival (25.0 ± 21.7% vs 75.0 ± 21.7%, p=0.23) at presentation than the ones in germline configuration but was not statistically relevant.

DISCUSSION

In the present study Southern blot analysis revealed IgH gene rearrangement in 97% of BCP-ALL, most of them

Table 5: Clinical variables and overall survival in relation to IgH and TCR genes in BCP ALL

		AGE	SEX	HB	WBC	PLT	Overall survival
	(No. of patients)	(Mean, yrs)	(M/F)	(Mean, g dl^{-1})	(x 10^9 l^{-1})	(x 10^9 l^{-1})	% (n)
IgH	2 Bands (30)	27.00	23/7.00	7.40	92.00	88.80	67.3±16.0 (12)
	>2 Bands (6)	19.80	5/1.00	4.80	58.80	33.80	50.0±35.4 (2)
	p(Mann-Whitney)	0.11	1.00	0.01	0.32	0.11	0.54
TCR-δ	Germline (14)	28.70	11/3.00	7.60	82.90	93.00	80.0±17.9 (5)
	Rearranged (22)	24.10	18/4.00	6.40	84.50	77.80	57.1±18.7 (9)
	p (Mann-Whitney)	0.14	1.00	0.21	0.86	0.66	0.91
TCR-β	Germline (12)	28.10	11/1.00	7.70	96.50	83.90	75.0±21.7 (6)
	Rearranged (25)	24.40	18/7.00	6.50	78.00	81.30	60.0±18.2 (9)
	p (Mann-Whitney)	0.44	0.23	0.32	0.92	0.89	0.65
TCR-γ	Germline (27)	25.10	23/4.00	6.90	90.10	85.80	72.2±11.9 (5)
	Rearranged (9)	27.20	5/4.00	6.90	67.90	70.90	34.3±19.5 (8)
	p (Mann-Whitney)	0.84	0.09	0.85	0.75	0.77	0.16

n: number of patients

Table 6: Clinical variables and overall survival in relation to IgH and TCR genes in T-ALL

		AGE	SEX	HB	WBC	PLT	Overall survival
	(No. of patients)	(Mean, yrs)	(M/F)	(Mean, g dl^{-1})	(x 10^9 l^{-1})	(x 10^9 l^{-1})	% (n)
IgH	Germline (9)	24.10	8/1.00	9.20	126.90	101.70	50.0±25.0 (4)
	Rearranged (6)	25.50	5/1.00	8.30	68.70	62.60	50.0±25.0 (2)
	p(Mann-Whitney)	0.48	1.00	0.48	0.48	0.85	0.67
TCR-δ	Germline (5)	23.40	5/0.00	9.80	142.10	65.30	25.0±21.7 (2)
	Rearranged (10)	26.20	8/2.00	8.40	84.30	90.90	75.0±21.7 (4)
	p(Mann-Whitney)	0.90	0.52	0.35	0.71	0.68	0.37
TCR-β	Germline (6)	29.50	5/1.00	9.40	55.50	138.40	100 (2)
	Rearranged (9)	22.40	8/1.00	8.40	135.60	38.50	38.1±19.9 (4)
	p(Mann-Whitney)	0.34	1.00	0.31	0.56	0.12	0.35
TCR-γ	Germline (9)	28.30	7/2.00	9.50	89.00	108.60	75.0±21.7 (4)
	Rearranged (6)	20.70	6/0.00	7.60	125.40	40.80	25.0±21.7 (2)
	p(Mann-Whitney)	0.07	0.49	0.14	0.19	0.16	0.23

n : number of patients

(~58%) having two rearranged bands, which is lower than that reported by others (80%) (8). Germline IgH gene was found in one patient, probably reflecting malignant transformation of a very early B cell precursor. In the present study multiple rearranged bands were found in 17% of cases which is similar to earlier studies (15%) [8] but is lower than the 30-45% observed in childhood ALL [14, 15]. In T-lineage ALL, IgH gene rearrangement was observed in 40% cases. There were no cases with multiple rearranged bands.

The overall frequency of lineage inappropriate rearrangements (86%) for TCR genes in adult BCP ALL revealed configurations comparable to those previously discovered for childhood ALL patients [12, 16-18]. TCR-β rearrangement was seen in 68% of our adult patients classified as B lineage leukemias which is much higher than 27-38% involvement reported by investigators from Europe [8, 10]. In T-lineage ALL it was observed in 60% of cases which is lower than (82%) reported in the literature [8].

The nature of rearrangement of TCR- β gene observed both in BCP-ALL and T-ALL was different in our study. TCR β1 locus was predominantly rearranged in T-ALL's (33% vs 20% in BCP-ALLs) and Cβ2 locus in BCP-ALLs (36% vs 0 in T-ALL). However, in the West TCR-β2 was preferentially used both in T-ALL and BCP-ALL. No rearrangement of C β2 were detected in BCP-ALL patients [8]. TCR β was the only TCR gene

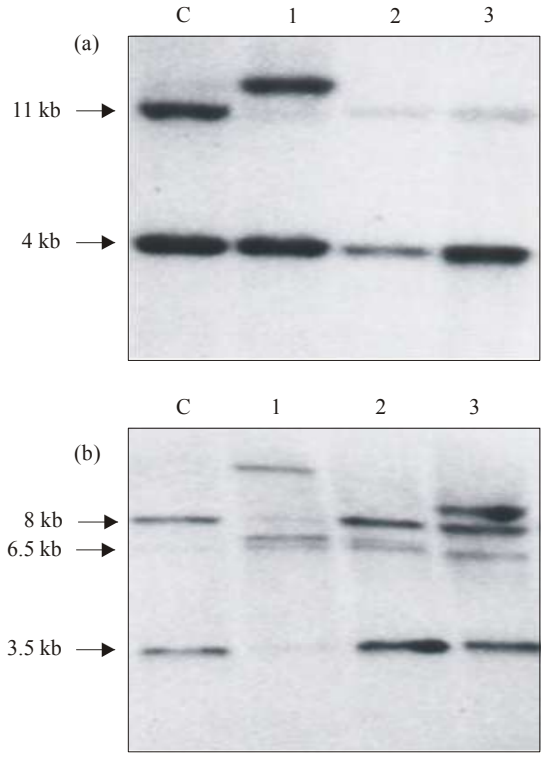

Fig. 1: a) Rearrangement pattern of TCR β gene examined by Southern blot analysis with a Cβ2 probe. DNA digested with EcoRI restriction enzyme detects Cβ1 region which occupies an 11 kb fragment and Cβ2 region which occupies 4 kb Cβ2 fragment. Lanes 1, 2, 3 are patient samples and lane C represents a germline control. In our series, lane number 2 shows rearrangement of Cβ2 region and lane 3, 4 are in germline configuration. b) DNA digested with Hind III restriction enzyme detects Cβ1 region at 3.5 kb, Cβ2 region at 8 kb and 6.5 kb. Lanes 1and 3 (patient samples) show rearrangement lane 2 (patient sample) in germline configuration and lane C represents a germline control

rearranged in 19% of our adult B-lineage leukemias and in one case of T-lineage leukemia thus suggesting a disturbed hierarchy of TCR gene rearrangements.

TCR-δ rearrangement in T-ALL's is similar to earlier studies [8]. However, the frequency of cross lineage TCR-δ gene rearrangement in BCP-ALL is much higher in our study (61%) than the frequencies reported in the literature. Most of the rearranged bands were seen with a retained germline band and deletions of both the alleles was not observed in any of cases.

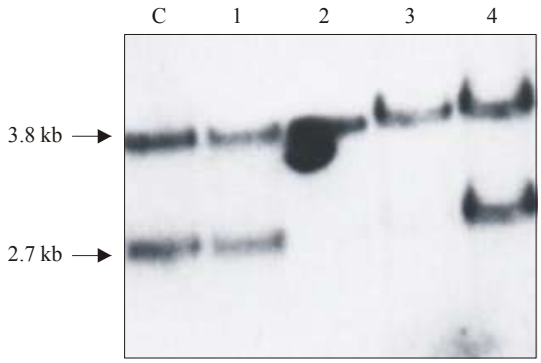

Fig. 2: Southern blot analysis with a Cγ probe on DNA digested with Hind III restriction enzyme. Lanes 1, 2, 3, 4 are patient samples and lane C represents the germline DNA control. The position of germline DNA fragments is shown, a 3.8 kb Cγ2 fragment and a 2.7 kb Cγ1 fragment. Restriction sites for EcoRI and HindIII detected deletions of the Cγ1 region as depicted in Lane 2 and 3

TCRγ rearrangement was observed in only 25% of BCP-ALL and 40% of T-ALLs. This is much lower than the reported figures of 58 and 86% in BCP-ALLs and T-ALL, respectively [8]. All the rearrangements of TCRγ gene were deletions of the Cγ1 region with Cγ2 retained in germline configuration. This pattern of rearrangement is in concordance to our earlier study reported on childhood BCP-ALL [12].

The observation of this study reveals that patients with multiple rearranged bands for the IgH gene presented with significantly lower Hb ($p=0.01$) than patients with 2 rearranged bands. No such association was found in childhood BCP reported earlier from this region [12]. Overall survival was evaluated in 23 of 52 cases studied (Table 5). The higher overall survival in BCP-ALL patients with germline TCR genes (TCRδ, TCR β TCRγ) is not statistically relevant. In T-ALL no statistically relevant correlations were found (Table 6).

In conclusion, our data indicate comparable pattern between adult and childhood ALL patients in terms of higher proportion of TCRβ rearrangements and invariable deletions of Cγ1 and only monoalellic rearrangement for TCRδ locus. However, there was no association found between the rearrangement pattern and the survival as was observed in childhood ALL. Thus, there is need for larger number of patients and longer follow up to reach any conclusion.

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