Immunoglobulin light chain gene rearrangements in precursor-B-acute lymphoblastic leukemia: characteristics and applicability for the detection of minimal residual disease

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We analyzed the frequency and characteristics of Vκ-Jκ and Vλ-Jλ rearrangements in patients with precursor-B-acute lymphoblastic leukemia (ALL) and evaluated the applicability of these rearrangements as targets for minimal residual disease (MRD) detection. Using the BIOMED-2 primer sets, Vκ-Jκ and Vλ-Jλ rearrangements were detected in 30% and 17% of patients, respectively. Vκ-Jκ rearrangements were particularly frequent in common-ALL, children between 5-10 years, and *TEL-AML1*-positive patients. Vκ-Jκ and Vλ-Jλ rearrangements showed a good stability between diagnosis and relapse and reached good sensitivities in real-time quantitative polymerase chain reaction analysis. Our data show that Vκ-Jκ and Vλ-Jλ rearrangements can be successfully applied for MRD detection in a subset of patients with precursor-B-ALL.

Key words: acute lymphoblastic leukemia, B-cell, immunoglobulin light chain, minimal residual disease, stability.

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Precursor B-acute lymphoblastic leukemias (ALL) can be considered as malignant counterparts of normal precursor B cells arrested in a particular stage of development. So far, most studies on immunoglobulin (Ig) light chain gene rearrangements have focused on *IGK*-Kde rearrangements,[1,2] and studies on Vκ-Jκ and Vλ-Jλ rearrangements mainly used Southern blot analysis and/or included only small series of precursor-B-ALL patients.[3-6] These studies have shown that also in precursor-B-ALL the Ig gene rearrangements display hierarchy, starting with *IGH* rearrangements, followed by *IGK* rearrangements, *IGK* deletions and/or *IGL* rearrangements.[3] However, little is known about the characteristics of Vκ-Jκ and Vλ-Jλ rearrangements and whether their frequency is related to age and/or the presence of fusion gene transcripts, as has been shown for other gene rearrangements.[7-9] The recent design of multiplex polymerase chain reaction (PCR) approaches for detection of Vκ-Jκ and Vλ-Jλ rearrangements[6] allows easy and rapid identification of Ig light chain gene rearrangements and may, therefore, provide more insight into the occurrence and regulation of these rearrangements in precursor-B-ALL. In addition, Vκ-Jκ and Vλ-Jλ rearrangements may be used as new targets for the detection of minimal residual disease (MRD) in precursor-B-ALL.

Design and Methods

Patients' samples

Bone marrow samples were obtained at diagnosis from 100 consecutive pediatric precursor-B-ALL patients enrolled into the DCOG-ALL9 protocol. From six patients, bone marrow samples were also obtained during follow-up. In addition, 56 relapsed precursor-B-ALL patients were included, based on the availability of sufficient DNA both at diagnosis and relapse.[10]

Detection and identification of Vκ-Jκ and Vλ-Jλ rearrangements

PCR analysis of Vκ-Jκ and Vλ-Jλ rearrangements was performed using the BIOMED-2 multiplex primer-sets (IVS Technologies, San Diego, CA, USA).[6] Detection of other Ig/TCR rearrangements and sequencing was performed as described previously.[1,6,7,11] To evaluate the stability of Ig/TCR gene rearrangements between diagnosis and relapse, mixed PCR-heteroduplex analyses were performed.[10] In a subset of patients (including all 56 relapsed patients), Southern blot analysis was performed using the IGKDE and/or IGKJ5 probe.[1] Part of these IGK-Kde data have been published previously.[1,10]

Real-time quantitative PCR (RQ-PCR) analysis

RQ-PCR analysis was performed using newly designed primer and probe sets (Figure 1A).[1] Data were interpreted according to the guidelines of the European Study Group on MRD in ALL (*manuscript in preparation*).[12]

Results and Discussion

Frequency of Vλ-Jλ and Vκ-Jκ rearrangements

Vκ-Jκ and/or Vλ-Jλ rearrangements were detected in 40 out of the 100 consecutive

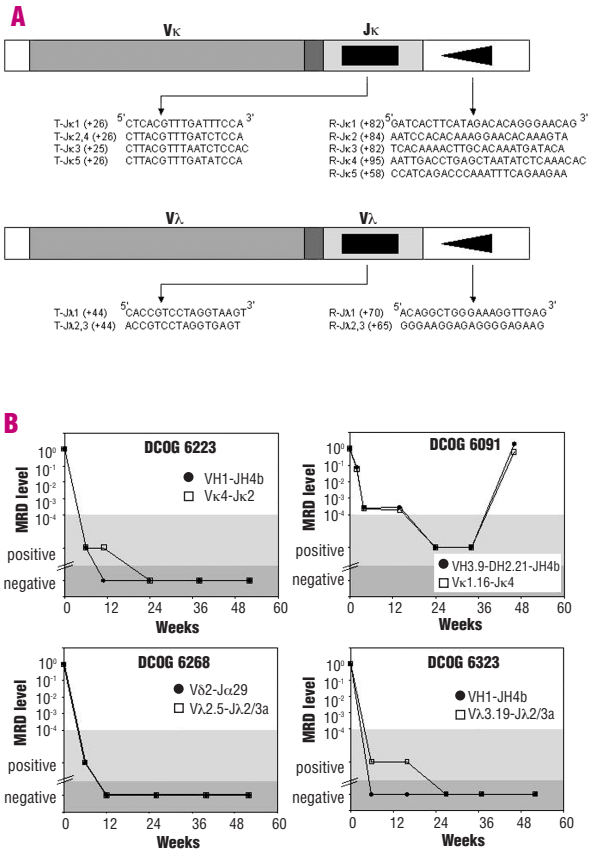

Figure 1. A. Primers and probes used for RQ-PCR analysis of Vκ-Jκ and Vλ-Jλ rearrangements. The position of the first 5' nucleotide of each primer/probe downstream (+) relative to the RSS of the gene segment is indicated as well as the oligonucleotide sequence of each primer and probe. **B.** RQ-PCR analysis of precursor-B-ALL patients using Vκ-Jκ or Vλ-Jλ rearrangements. For comparison, also MRD data obtained with another Ig/TCR gene rearrangement as the MRD-PCR target is shown. The light gray area indicates the non-reproducible range (below the quantitative range of the RQ-PCR assay), in which low MRD levels could be detected but not quantified. The dark gray area indicates MRD negativity (below the detection limit of the RQ-PCR method).

precursor-B-ALL patients. Vκ-Jκ rearrangements were observed in 30 patients and Vλ-Jλ rearrangements in 17 patients. Non-conventional Vκ-Jκ rearrangements, mainly involving the most downstream located and inversely oriented Vκ4.1 and Vκ5.2 gene segments, occurred at low frequency. The frequency of Vκ-Jκ, but not of Vλ-Jλ rearrangements, was significantly related to immunophenotype, age at diagnosis and the presence of *TEL-AML1* (Table 1).

Patients with Vκ-Jκ and/or Vλ-Jλ rearrangements showed higher frequencies of *IGK*-Kde, *TCRG*, Vδ2-Jα, and/or *TCRB* rearrangements than did the Vκ-Jκ/Vλ-Jλ-negative patients (Table 2). In contrast, incomplete *IGH* rearrangements were virtually absent. In all Vκ-Jκ and/or Vλ-Jλ-positive patients, at least two other Ig/TCR gene rearrangements were detected.

Characteristics of Vκ–Jκ and Vλ–Jλ rearrangements

Sequence analysis was successful for 27 Vκ-Jκ rearrangements and showed that VκI, VκII, VκIII and

Table 1. Frequency of Vκ-Jκ and Vλ-Jλ in 100 consecutive pediatric precursor-B-ALL patients.

	Vκ-Jκ		*Vλ-Jλ*
Overall (n=100)	30%		17%
Age at diagnosis			
Age at diagnosis 0-1.5 (n=8)	25%		25%
Age at diagnosis 1.5-5 (n=50)	28%	*	12%
Age at diagnosis 5-10 (n=25)	52%		24%
Age at diagnosis 10-15 (n=17)	6%		18%
Fusion gene transcripts			
Negative[a] (n=72)	26%	*	13%
TEL-AML1 (n=20)	55%		20%
BCR-ABL (n=4)	0%		25%
11q23 aberrations[b] (n=4)	0%		75%
Immunophenotype			
Common-ALL (n=67)	40%	*	16%
Pre-B-ALL (n=32)	9%		16%

[a]'Negative' refers to patients without specific chromosome aberrations (TEL-AML1, BCR-ABL, and MLL rearrangements) as determined by PCR analysis and/or routine cytogenetic analysis; [b]the four patients with 11q23 abnormalities included one patient with t(4;11), one patient with t(5;11), and two patients with an MLL rearrangements involving an unknown partner gene. *$p<0.05$ by the χ^2 test.

VκIV were used in 70%, 7%, 7% and 15% of Vκ-Jκ-positive patients, respectively. Interestingly, Vκ2.30 was never used, whereas this gene segment is frequently involved in Vκ-Kde rearrangements.[1,2] Jκ1, Jκ2, Jκ3, Jκ4 and Jκ5 were used in 19%, 30%, 7%, 33% and 11%, respectively. The mean number (range) of 3' deletions, insertions, and 3' deletions were 5.1 (0-17), 5.9 (0-20), and 3.0 (0-11), respectively. Vκ-Jκ rearrangements generally used the more proximally located Vκ segments, whereas more distally located Vκ segments were more frequently used in Vκ-Kde rearrangements (*data not shown*).

Sequence analysis of the 17 Vλ-Jλ rearrangements showed that Vλ1, Vλ2 and Vλ3 were used in 18%, 35% and 47% of cases, respectively. Jλ2 or Jλ3 was used in all sequences analyzed, confirming previous data.[4] Given the high homology between Jλ2 and Jλ3 and the position of the Jλ consensus primer, no distinction could be made between these two segments. The mean number (range) of 3' deletions, insertions, and 3' deletions were 4.8 (0-18), 5.0 (0-11), and 3.1 (0-9), respectively.

Stability of Vκ–Jκ and Vλ–Jλ rearrangements

At diagnosis, Vκ-Jκ rearrangements were identified in 25 out of 56 relapsed precursor-B-ALL patients (27 Vκ-Jκ rearrangements); Vλ-Jλ rearrangements were detected in 13 patients. At relapse, in 22 out of 25 Vκ-Jκ -positive patients (88%) all Vκ-Jκ rearrangements remained stable, whereas in three patients the mono-allelic Vκ-Jκ rearrangements were lost. Thus, 24 out of 27 Vκ-Jκ rearrangements (89%) were stable. Notably, all Vκ-Jκ rearrangements accompanied by an intron-Kde rearrangement on the same allele (n=12) remained stable. The three patients in whom the Vκ-Jκ rearrangement was lost at relapse, all appeared to have subclonal Vκ-Jκ rearrangements at diagnosis, as determined by combined Southern blot and PCR analysis. Ten out of 13 Vλ-Jλ rearrangements remained stable at relapse

Table 2. Ig/TCR rearrangements in precursor-B-ALL patients with or without Vκ-Jκ and/or Vλ-Jλ rearrangements.

	IGH		*IGK*			*TCRD/A*		*TCRG*	*TCRB*		*Total number of Ig/TCR rearrangements*[b]
	DH-JH	*VH-JH*	*Vκ-Jκ*	*intron-Kde*	*Vκ-Kde*	*TCRD*[a]	*Vδ2-Jα*		*Dβ-Jβ*	*Vβ-Jβ*	
Vκ-Jκ/Vλ-Jλ- (n=60)	30%	87%	0%	5%	38%	49%	35%	50%	10%	13%	4.4
Vκ-Jκ+[c] (n=30)	3%[#]	87%	100%	23%[#]	47%	48%	53%	80%[#]	20%	40%[#]	6.7[#]
Vλ-Jλ+[c] (n=17)	0%[#]	76%	41%	24%[#]	59%[#]	41%	65%[#]	65%	6%	47%[#]	6.8[#]

[a]*TCRD: Vδ2-Dδ3/Dδ2-Dδ3;* [b]*Total number of Ig/TCR gene rearrangements (mean);* [c]*Note: seven patients had both Vκ-Jκ and Vλ-Jλ rearrangements;* [#]$p<0.05$ *by the* χ^2 *test as compared to Vκ-Jκ/Vλ-Jλ-negative patients.*

(77%); three Vλ-Jλ rearrangements were lost, probably due to clonal selection (one patient) or secondary rearrangements (two patients).

Applicability of Vκ-Jκ and Vλ-Jλ rearrangements as targets in RQ-PCR analysis

RQ-PCR analysis of 11 Vκ-Jκ rearrangements resulted in a quantitative range of $\leq 10^{-4}$ in 45%; a sensitivity of $\leq 10^{-4}$ was reached in 82%. In the ten Vλ-Jλ rearrangements analyzed, a quantitative range of $\leq 10^{-4}$ was obtained in 50%; a sensitivity of $\leq 10^{-4}$ was reached in 80%. Non-specific amplification was observed in 6/11 (Vκ-Jκ) and 6/10 (Vλ-Jλ) cases. There was no straightforward relation between the obtained sensitivity and the number of inserted/deleted nucleotides. In six patients, MRD was evaluated using Vκ-Jκ or Vλ-Jλ RQ-PCR analysis. MRD results were comparable to MRD data obtained by other Ig/TCR gene rearrangements and only in the non-reproducible part of the assay ($<10^{-4}$) were some minor discrepancies observed (Figure 1B and *data not shown*).

Using the BIOMED-2 primers, Vκ-Jκ rearrangements were detected in 30% of childhood precursor-B-ALL patients, consistent with previously reported Southern blot-based data.[3,4,13] The frequency for Vλ-Jλ (17%) was slightly lower than previously reported,[4,14] probably because the BIOMED-2 primer set does not contain a primer for Jλ6[6] which is found in about 20% of Vλ-Jλ rearrangements in precursor-B-ALL.[4]

Like other Ig/TCR rearrangements, Vκ-Jκ rearrangements in precursor-B-ALL were influenced by age at diagnosis and the presence of fusion transcripts, particularly *TEL-AML1*.[7-9,15] Multivariate analysis indicated that the presence of *TEL-AML1* is the most important factor for the presence of Vκ-Jκ rearrangements and this is probably related to the latency period of the *TEL-AML1*-positive (pre)leukemic cell.[7,8] Vλ-Jλ rearrangements showed no significant relation with age or fusion transcripts. However, the presence of Vλ-Jλ rearrangements in three out of four patients with 11q23 rearrangements is surprising, as the presence of *MLL* rearrangements is generally associated with an immature Ig/TCR rearrangement pattern.[16] The presence of Vλ-Jλ rearrangements in a *BCR-ABL*-positive precursor-B-ALL patient also deserves further investigation, as recent reports suggested that *BCR-ABL* blocks B-cell differentiation at the pre-B-cell stage and hence also blocks rearrangements of the *IGK* and *IGL* loci.[17]

Virtually all patients with *IGK* or *IGL* rearrangements had VH-JH rearrangements and lacked DH-JH rearrangements, suggesting that, as in normal precursor-B-cell differentiation, the light chain genes are only rearranged if the *IGH* rearrangements are completed. Nevertheless, sequence analysis showed that in-frame *IGH* rearrangements are not necessarily present in all patients with Ig light chain gene rearrangements (*data not shown*). This may indicate that, under the high pressure of RAG activity, pre-B-cell receptor signaling is not a strict requirement for induction of Ig light chain gene rearrangements in precursor-B-ALL. Also, aberrant signaling molecules (such as truncated BTK in *BCR-ABL*-positive ALL) may mimic a constitutively active pre-B-cell receptor.[18] Alternatively, after successful rearrangement of the *IGH* locus, pre-B-cell receptor signaling, and induction of light chain gene rearrangements, ongoing rearrangements of the *IGH* loci may result in the loss of the functional *IGH* rearrangement. In accordance with Southern blot-based data from Van der Burg *et al.*,[3] no *IGK*-Kde deletions were observed in about 40% of patients with *IGL* rearrangements. Furthermore, *IGL* rearrangements were detected irrespectively of whether accompanying Vλ-Jλ rearrangements were in-frame or not (*data not shown*). Apparently, the hierarchy of *IGK* and *IGL* rearrangements is less strict than in normal B-cell development, which again may at least in part be explained by the high RAG activity in precursor-B-ALL.[19]

Finally, our data indicate that Vκ-Jκ and Vλ-Jλ rearrangements can be used for MRD detection. Addition of Vκ-Jκ and Vλ-Jλ tubes to the PCR panel used for target identification will not increase the number of patients with at least two Ig/TCR targets. However, Vκ-Jκ and Vλ-Jλ rearrangements show a high stability between diagnosis and relapse and do relatively well in RQ-PCR analyses. Therefore, Ig light chain gene rearrangements may replace *TCRG* gene rearrangements (which reach good sensitivities in only a minority of cases)[20] and may, besides complete *IGH* and complete *TCRB* gene rearrangements, be preferred targets for RQ-PCR-based MRD analysis in childhood precursor-B-ALL.

VHJvdV was responsible for the conception and design of this study, for analysis and interpretation of data, and for writing the manuscript. MdB performed the experiments, analyzed and interpreted the data and revised the manuscript critically for important intellectual content. ErvW and JJMvD analyzed and interpreted the data and revised the manuscript critically for important intellectual content. All authors gave final approval of the version to be published. The authors declare that they have no potential conflicts of interest. We gratefully acknowledge Menno van Zelm for designing the primers and probes, Bibi van Bodegom for secretarial assistance and Dr. A.W. Langerak for critically reading this manuscript. We thank the members of the Molecular Immunology Unit (department of Immunology, Erasmus MC) for helpful discussions and technical support. We acknowledge Dutch pediatricians for obtaining patients' samples and the DCOG for the fruitful and pleasant collaboration. This work was supported by the Dutch Cancer Society (SNWLK2000-2268).

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