Brief report

Presence of N regions in the clonotypic DJ rearrangements of the immunoglobulin heavy-chain genes indicates an exquisitely short latency in t(4;11)-positive infant acute lymphoblastic leukemia

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Childhood acute lymphoblastic leukemia (ALL) is frequently initiated in utero at a time of developmentally regulated insertion of N regions into the DJ_H rearrangements of immunoglobulin heavy-chain (Ig_H) genes. Here it is shown that N regions are present in the clonotypic DJ_H rearrangements in 11 of 12 infant ALLs with t(4;11). These data are compared with the 122 previously published DJ_H sequences and were found to have a pattern similar to that of ALL in children older than 3 years at diagnosis but were unlike that in children younger than 3 years who predominantly lack N regions. These findings, therefore, indicate that t(4;11)-positive infant ALL is initiated later in fetal development than most B-cell precursor ALL from children younger than 3 years and that they have a shorter latency period already in utero. (Blood. 2001;98:2272-2274)

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Introduction

Childhood acute lymphoblastic leukemia (ALL) is a heterogeneous group of leukemias with a predominance of the B-cell precursor (BCP) phenotype. A minority of these leukemias is associated with a translocation involving the mixed-lineage leukemia (*MLL*) gene on chromosome 11q23 that is fused, in 50% of patients, to the *AF4* gene on chromosome 4q21.[1] The chromosomal translocation t(4;11)(q21;q23) occurs mostly in infant ALL and confers a dismal prognosis in this age group, whereas in older children and adults the prognosis does not differ from t(4;11)-negative cases.[2,3] Differences in the chromosomal breakpoints of the *MLL* gene between infants and children or adults with t(4;11) ALL suggest different mechanisms for the development of these instances of ALL[4] and, thus, their different biologic functions.

Greaves[5] proposed a 2-step model for the development of ALL with an initiating event in utero, followed by a second mutation leading to overt leukemia. Indeed, leukemia-specific chromosomal translocations and clonotypic antigen-receptor gene rearrangements at birth recently confirmed the initiation of childhood ALL in utero.[6-9] Thus, depending on the time of clinical manifestation, the latency period varies among the different types of leukemia. In contrast to leukemias with long latency periods for which a chromosomal translocation and additional postnatal mutations are required,[9] the extremely short latency periods in infant ALL (with *MLL* rearrangements) suggest only limited further mutagenic requirements.[1] It seems likely that not only postnatal but also prenatal development of the disease is rapid.

Assuming that the gene fusion resulting from t(4;11) in infant ALL is indeed an initiating event, its origin must be restricted to a period between the beginning of B lymphopoiesis in the fetal liver—ie, the 6th gestational week[10]—and possibly a few months before the diagnosis of leukemia.[11] This translocation is assumed to occur in a cell that has already started to rearrange its Ig_H genes. The time period of these rearrangement processes during fetal development can be determined by the presence or absence of N regions between the DJ_H joinings. The addition of N nucleotides requires terminal deoxynucleotidyl transferase (TdT) that is not initially present in fetal lymphopoiesis but that has been observed by the end of the first trimester of gestation.[12-14]

We therefore used the leukemia clone-specific junctional regions of DJ_H rearrangements to determine the time point of a first mutation in utero in t(4;11) ALL in infancy. We show the presence of N regions between the DJ_H joinings in 11 of 12 infant ALLs with t(4;11) indicating their initiation at a later time during fetal development than most other leukemias that become apparent during the first 3 years of life.

Study design

The occurrence of t(4;11) ALL was analyzed in 13 infants. Inclusion criteria were patient age younger than 1 year at diagnosis and the presence of at least one clonotypic Ig_H rearrangement (Figure 1). All leukemias had a pro B phenotype with frequent coexpression of myeloid markers. The local institutional ethical committee approved the study, and informed consent was obtained from the parents.

DNA was extracted from cells by standard procedures.[15] Amplification of the ret-oncogen confirmed the integrity of DNA in all samples. Clonal Ig_H rearrangements were determined by V_H-family–specific and J_H consensus primers for the amplification of $V(D)J_H$ rearrangements and of primer sets for the amplification of all incomplete DJ_H rearrangements, as described previously.[16,17] Amplified products were sequenced directly, and involved gene segments were identified by BLAST sequence similarity

Figure 1. Nucleotide sequence of Ig_H rearrangements in t(4;11) infant ALL. Trimming of the rearranged segments is indicated by the numbers of nucleotides. N nucleotides between DD segments are shown in italics; shaded areas indicate sequence homology between 2 rearrangements.

Pat.no	DH		N		JH	VDJH gene segments
1	ctaactgggga	-0	J1-pseudogene	-0	J1	DH7-27/J1
2	aggatattgtactaatggtgtatgct	-5	gttcc	-2	J5	DH2-8/J5
3	gtattacgatattttgactggttattataa	-1	ag	-2	J5	DH3-9/J5
4a	aggatattgtagtggtggtagctg	-7		-4	J6	DH2-15/J6
4b	*gcttccct*tattgtagtggtggtagctg	-7		-4	J6	DH3-9/DH2-15/J6
5	aggatattgtactaatggtgtatgc	-6	cccgatttcccccctaagcccttc	-4	J4	DH2-8/J4
6	gtattacgatattttgactggttat	-6	gt	-0	J4	DH3-9/J4
7	gtattacgatattttgactggttat	-5	cctt	-5	J6	DH3-9/J6
8a	ggtattacgattttggagtggttattat	-3	gggt	-8	J6	DH3-3/J6
8b	atattgtagtagtaccagctgctata	-2	gttggatgg	-4	J5	VH3/DH2-2/J5
9	gtattacgatattttgactggttattataac	-0	cctcg	-2	J5	VH4/DH3-9/J5
10a	gatattgtagtagtaccagctgctat	-0	tgaggggg	-5	J6	VH4/DH2-2/J6
10b	atattgtagtagtaccagctgctat	-0	tgaggggg	-5	J6	VH6/DH2-2/J6
11	tagcagtggctggtac	-0	ggagg	-6	J4	VH3/DH6-19/J4
12	tagcagctcgtc	-1	gccccg	-7	J4	VH3/DH6-6/J4
13			gcgggc	-4	J5	VH3/J5

searches (http://www.ncbi.nlm.nih.gov/BLAST/) and by comparison with published sequences of all known human immunoglobulin genes (http://www.ebi.acc.uk), allowing the assignment of nucleotides to either the V, D, and J regions or to the P and N regions by exclusion.

Results and discussion

We identified 16 Ig_H rearrangements in 13 infants with t(4;11) ALL (Figure 1A). Ten leukemias had 1 rearrangement, and 3 leukemias (patients 4, 8, 10) had 2 rearrangements. We considered only 1 of the 2 rearrangements (in patients 4 and 10) because they had identical DJ_H regions. We excluded the DJ_H rearrangement in patient 1 because the sequence between D_H and J_H was homologous to the J1 pseudogene. Thus, 13 unique rearrangements were analyzed for the inclusion of N regions. We observed in patient 4 a D-D fusion, but only the D_H gene segment most proximal to the J_H segment was included in the analysis. As depicted in Figure 1, only 1 of the 13 unique sequences lacked N regions at the DJ_H junction. The results from this study are compared with those from 122 previously published cases (Table 1). It appears that the lack of N regions in t(4;11) infant ALL is less common than in children with ALL who are younger than 3 years at diagnosis but that they are about as common as in children older than 3 years.[15,18,19] Figure 1 illustrates the use of D_H and J_H families, similar to that reported previously.[16,18,19]

The data from this study indicate that most DJ_H regions from infant ALL with t(4;11) contain N nucleotides that developed at a time of TdT activity; hence, they were more mature than those without N regions. Interestingly, other leukemias, diagnosed in children before the age of 3, do not have N regions in their DJ_H junctions[15,18,19] and thus have a longer latency period than the

t(4;11) infant leukemias. There are 2 groups of BCP ALL that have N regions in their clonotypic DJ_H junctions, namely t(4;11) infant ALL with a manifestation mostly in the first year of life and other leukemias with a clinical manifestation after the 3rd year of life.[18,19] Both rearrange their DJ_H segments at a similar time during gestational development. It is obvious, however, that t(4;11) ALL has a remarkably shorter latency than the others.

We propose a model for the relation between the time of initiation of the leukemia, characterized by the clonotypic DJ_H rearrangements, and the age of the children at clinical manifestation of BCP ALL (Figure 2). Ig_H rearrangements that lack N regions occur during a narrow time window—the first weeks of B lymphopoiesis in fetal liver that is TdT negative. Transformed cells with such rearrangements most likely acquire additional mutations, leading to leukemias during the first 3 years of life. Ig_H rearrangements with the addition of N nucleotides in the DJ_H junction occur later in gestation, when TdT has already been activated. These leukemias become clinically apparent during the first year of life if a t(4;11) chromosomal translocation started leukemogenesis or, in its absence, after the 3rd year of life. Alternatively, the t(4;11) translocation arises in a TdT-negative primitive cell without Ig_H rearrangements. This target cell may represent a B-plus myeloid lymphoid stem cell, as described by Cumano et al[20] in mouse fetal liver, which would be unique in specific stages of in utero hematopoiesis. The N region–positive DJ_H rearrangement may be a later addition during progression to leukemia. Then, unrelated rearrangements are expected, such as in t(9;22) B lymphoid blast crisis of chronic myeloid leukemia.[16] However, in our series, no leukemia had multiple unrelated Ig_H rearrangements, but 2 leukemias had related rearrangements. In addition, the target cell for the t(4;11) translocation may be a rare progenitor with TdT expression at earlier stages of fetal lymphopoiesis than common B precursor cells. No such cells have been identified thus far in humans.

Table 1. Occurrence of N regions between the DJ_H junction of children with BCP ALL and their ages at diagnosis

Age at diagnosis (y)	No. of ALL/study	N + n	N − n	Reference
Younger than 1 year	12	11	1	This study
Younger than 3 years	16	2	16	Wassermann et al[18]
	8	5	5	Steenbergen et al[19]
	14	4	12	Schneider et al[15]
Older than 3 years	46	41	10	Wassermann et al[18]
	39	35	12	Steenbergen et al[19]

Leukemias are considered N+ if one DJ_H junction has N nucleotides inserted. They are considered N− if one DJ_H junction lacks N nucleotides. Leukemias are included in both groups if one rearrangement contains N nucleotides and the other does not.
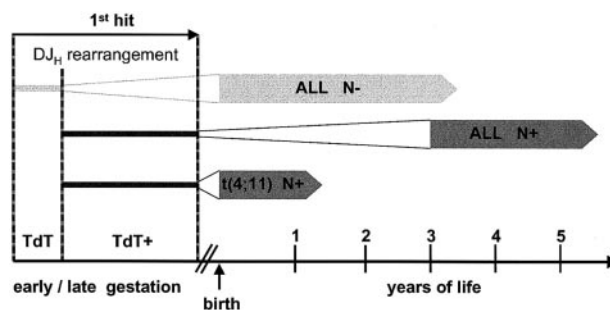

Figure 2. Time frame. Development of N− and N+ BCP leukemias in childhood.

It is assumed that a chromosomal translocation is an initiating event in leukemogenesis,[9,21] which can be induced by apoptotic stimuli that lead to the generation of gene fusions in B precursor cells, thus rescuing a cell programmed to die.[22] This assumption is supported by the findings that most Ig$_H$ rearrangements in ALL are either incomplete or not potentially productive,[16] underlining the immaturity of these cells, which would not survive without a transformation. It is further hypothesized that additional mutations are required for the development of leukemia. However, our findings support the hypothesis that the t(4;11) is either sufficient for leukemogenesis or provokes efficiently further changes that lead eventually to leukemia in infancy.[1,11]

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