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

Karin Fasching, Simon Panzer, Oskar A. Haas, Arndt Borkhardt, Rolf Marschalek, Frank Griesinger, and E. Renate Panzer-Grümayr

Childhood acute lymphoblastic leukemia (ALL) is frequently initiated in utero at a time of developmentally regulated insertion of N regions into the DJH rearrangements of immunoglobulin heavy-chain (IgH) genes. Here it is shown that N regions are present in the clonotypic DJH rearrangements in 11 of 12 infant ALLs with t(4;11). These data are compared with the 122 previously published DJH 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)

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 and, thus, their different biologic functions.

Greaves5 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,3 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 week10—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 IgH genes. The time period of these rearrangement processes during fetal development can be determined by the presence or absence of N regions between the DJH 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

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We therefore used the leukemia clone-specific junctional regions of DJH 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 DJH 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 IgH 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 IgH rearrangements were determined by V_H-family–specific and J_H consensus primers for the amplification of all incomplete DJH rearrangements, as described previously.16-17 Amplified products were sequenced directly, and involved gene segments were identified by BLAST sequence similarity

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Submitted March 2, 2001; accepted May 29, 2001.

Supported in part by the Österreichische Kinderkrebshilfe and by a grant from the Deutsche Krebshilfe (Bo 10-124.3).

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BCP ALL and their ages at diagnosis

Figure 1. Nucleotide sequence of IgH 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.

Results and discussion

We identified 16 IgH 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 DJH regions. We excluded the DJH rearrangement in patient 1 because the sequence between DH and JH 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 DH gene segment most proximal to the JH was included in the analysis. As depicted in Figure 1, only 1 of the 13 unique sequences lacked N nucleotides at the DJH 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 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 DJH junctions and thus have a longer latency period than the t(4;11) infant leukemias. There are 2 groups of BCP ALL that have different clinical behavior: those with N regions in their DJH 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.

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

<table>
<thead>
<tr>
<th>Age at diagnosis (y)</th>
<th>No. of ALLs/study</th>
<th>N(+) n</th>
<th>N(−) n</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Younger than 1 year</td>
<td>12</td>
<td>11</td>
<td>1</td>
<td>This study</td>
</tr>
<tr>
<td>Younger than 3 years</td>
<td>16</td>
<td>2</td>
<td>16</td>
<td>Wassermann et al16</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>5</td>
<td>5</td>
<td>Steenbergen et al18</td>
</tr>
<tr>
<td></td>
<td>14</td>
<td>4</td>
<td>12</td>
<td>Schneider et al15</td>
</tr>
<tr>
<td>Older than 3 years</td>
<td>46</td>
<td>41</td>
<td>10</td>
<td>Wassermann et al16</td>
</tr>
<tr>
<td></td>
<td>39</td>
<td>35</td>
<td>12</td>
<td>Steenbergen et al18</td>
</tr>
</tbody>
</table>

Leukemias are considered N+ if one DJH junction has N nucleotides inserted. They are considered N− if one DJH 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, 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. This assumption is supported by the findings that most IgH rearrangements in ALL are either incomplete or not potentially productive, underlying 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.

Acknowledgment

This article is dedicated to Helmut Gadner for his 60th birthday.

References

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