Predominance of Fetal Type DJ<sub>n</sub> Joining in Young Children with B Precursor Lymphoblastic Leukemia as Evidence for an In Utero Transforming Event

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Summary

The presence of N sequences in the complementarity determining region 3 (CDR3) of the rearranged immunoglobulin H chain is developmentally regulated: N regions are generally present in the DJ<sub>n</sub> joinings of adult B cells but are often absent in fetal B cells. Analysis of the CDR3 in 61 B precursor acute lymphoblastic leukemias indicated that 87.5% of the leukemias obtained from children ≤3 yr old lacked N regions at the DJ<sub>n</sub> junction. In contrast, in children >3 yr old, only 11.1% of the leukemias lacked N regions at this junction, a frequency similar to what we have observed in B cells from children and adults. These findings suggest that the majority of leukemias presenting within the first 3 yr of age arise from an in utero transforming event.

Hypervariability within the CDR3 of the human Ig H chain is initially generated at the time of VDJ joining by the recombination of multiple V<sub>m</sub>, D, and J<sub>m</sub> gene segments (1-3). This VDJ recombination process is dependent upon two recombinase enzymes, RAG1 and RAG2, since mice deficient in either gene fail to produce lymphocytes with rearrangements in their Ig or TCR loci (4-8). Exonucleolytic activity produces joinings in which germline nucleotides are lost from the ends of the joined segments (1-4). Variability is increased when nontemplate-derived nucleotides (N regions) are added between joined gene segments through the action of another enzyme, terminal deoxynucleotidyl transferase (Tdt)† (1-4). In both IgH and TCR rearrangements, palindromic (P) mono- or dinucleotides may be found adjacent to a recombined gene gene segment when the segment is present in its entirety. These germline-encoded nucleotides arise from a flip-over mechanism of the 5' end of one strand of the joining segment (9).

The developmental regulation of N region addition has been demonstrated in both mice and humans (10-13). DJ<sub>n</sub> joinings that lack N regions are found more frequently at the fetal stage of development. No more than 5% of the DJ<sub>n</sub> junction sequences of B lymphocytes present in murine fetal liver contained an N region, whereas in newborn mouse spleen and liver, 5-23% of the DJ<sub>n</sub> junctions had N regions (10-12). In contrast, a significantly higher percentage of DJ<sub>n</sub> junctions with N regions (64-73%) were found in B cells from adult (4-8 wk) murine spleen (10-12). A similar trend was found in human B cells; evaluation of >500 DJ<sub>n</sub> joining DNA sequences obtained from human fetal, neonatal, and adult lymphoid tissue revealed N regions at the DJ<sub>n</sub> junction at frequencies of 68%, 86%, and 91-100%, respectively (13, 14). Together, these observations suggest that CDR3 sequences lacking N regions at the DJ<sub>n</sub> junction are representative of a DJ<sub>n</sub> recombination event that occurred during the time of fetal development when TdT activity may have been absent. Indeed, in the murine system, TdT levels rise slowly in the developing thymus, which correlates with the absence of N regions in fetal TCR-γ/δ rearrangements (15, 16). Similarly, in the murine B lymphoid system, TdT was not detected in fetal liver but was demonstrated in adult bone marrow B lineage cells (16).

The functional significance of the absence of N regions in the early stage of development is not clear. Gu et al. (12) speculate that the absence of N regions implies predominant expression of germline-encoded specificities. Thus, idiotypic interactions in a germline-encoded network might play a functional role in the development of the antibody repertoire early in ontogeny. In newborns, Feeney (10) found a higher percentage of N regions in productive vs. nonproductive rearrangements and speculated that this increase suggests a preferential activation of B cells whose IgH sequences contain N regions by antigens or cellular interactions. Alternatively, IgH sequences with N regions might have an advan-

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† Abbreviation used in this paper: Tdt, terminal deoxynucleotidyl transferase.
tage in the transition from pre-B cell to B cell, perhaps through enhanced binding to surrogate L chains.

B lineage acute lymphoblastic leukemia (ALL) of childhood results from the transformation of B precursor cells and their clonal expansion (17–19). We reasoned that if lack of N regions at the DJ₆ junction was a marker for fetal-derived B cells, then leukemias arising from an in utero transforming event should show a bias for DJ₆ joinings that lack N regions. Furthermore, the age distribution of leukemias lacking N regions at their DJ₆ joinings might provide insight into the length of time required to develop clinical disease from the time of the transforming event.

### Materials and Methods

**Source of Cells.** Bone marrow samples with >70% lymphoblast replacement were obtained at diagnosis from 63 patients (6 mo

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**Table:** DNA sequence of the DJ₆ region from 61 cases of B lineage lymphoblastic leukemias. The first column indicates the sequence code (case number and the length of the CDR3, including the Va and J₆ primers). Each sequence is subdivided into D (and when applicable multiple D with enhanced binding to surrogate L chains. 

| PATIENT | HGC | CDR3 CODE (LENGTH) | DJ₆ | K | J |
|---------|-----|-------------------|-----|---|---|
| C16-111 | 2.5 | (L94) | Vα5-17 | (3) | 26 |
| C16-121 | 3.0 | (L94) | Vα5-17 | (3) | 26 |
| C16-131 | 3.5 | (L94) | Vα5-17 | (3) | 26 |
| C16-141 | 4.0 | (L94) | Vα5-17 | (3) | 26 |
| C16-151 | 4.5 | (L94) | Vα5-17 | (3) | 26 |
| C16-161 | 5.0 | (L94) | Vα5-17 | (3) | 26 |
| C16-171 | 5.5 | (L94) | Vα5-17 | (3) | 26 |
| C16-181 | 6.0 | (L94) | Vα5-17 | (3) | 26 |
| C16-191 | 6.5 | (L94) | Vα5-17 | (3) | 26 |
| C16-201 | 7.0 | (L94) | Vα5-17 | (3) | 26 |
| C16-211 | 7.5 | (L94) | Vα5-17 | (3) | 26 |
| C16-221 | 8.0 | (L94) | Vα5-17 | (3) | 26 |
| C16-231 | 8.5 | (L94) | Vα5-17 | (3) | 26 |
| C16-241 | 9.0 | (L94) | Vα5-17 | (3) | 26 |
| C16-251 | 9.5 | (L94) | Vα5-17 | (3) | 26 |
| C16-261 | 10.0 | (L94) | Vα5-17 | (3) | 26 |
| C16-271 | 10.5 | (L94) | Vα5-17 | (3) | 26 |
| C16-281 | 11.0 | (L94) | Vα5-17 | (3) | 26 |
| C16-291 | 11.5 | (L94) | Vα5-17 | (3) | 26 |
| C16-301 | 12.0 | (L94) | Vα5-17 | (3) | 26 |

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**Figure 1.** DNA sequence of the DJ₆ region from 61 cases of B lineage lymphoblastic leukemias. The first column indicates the sequence code (case number and the length of the CDR3, including the Va and J₆ primers). Each sequence is subdivided into D (and when applicable multiple D with enhanced binding to surrogate L chains. 

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1578  
Fetal Type DJ₆ Joining in Young Children with B Precursor Leukemia
DNA Sequence Analysis. DNA sequencing data were analyzed for utilization of D and J segments using the sequence analysis software pack (Release 5; Genetic Computer Corp., Madison, WI) at the University of Wisconsin and a Micro Vax II computer (Digital Equipment Corp., Marlboro, MA), according to described criteria (14).

Results and Discussion

80 DNA sequences of VDJ joinings from 61 B lineage ALLs were analyzed for the presence of N regions at the DJβ junction (Fig. 1). Overall, 31/80 (38.8%) DJβ junctions lacked N regions, a frequency higher than that reported for human adult tissues and similar to that found in human fetal tissue (13). When the percentage of leukemias lacking N regions was analyzed as a function of age at diagnosis, a striking pattern emerged: in patients ≤3 yr old, 87.5% (14/16) of the leukemias were comprised entirely of CDR3 sequences lacking N regions; whereas in children >3 yr old, only 11.1% (5/45) of the leukemias met this criterion.

The percentage of leukemias without N regions in children ≤3 yr old was much higher than expected even when compared with data reported for normal human fetal liver or neonatal cord blood (13), whereas the frequency observed in children >3 yr old was close to that observed in adults (13, 14). To exclude the possibility that the paucity of N regions in these young patients could be due to an inherent abnormality in TdT activity, we examined the DJβ joinings in lymphocytes obtained from the end of therapy marrows of three of these patients when residual leukemia was not detectable using PCR analysis (Fig. 2). N regions were present...
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