Abnormalities of the Long Arm of Chromosome 6 in Childhood Acute Lymphoblastic Leukemia

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To determine the biologic significance of the structural rearrangements of the long arm of chromosome 6(q) in acute lymphoblastic leukemia (ALL) at diagnosis, we studied 412 consecutive children whose leukemic cell chromosome had been completely banded and identified 45 (11%) children with this abnormality. The 45 cases were divided into del(6q) only (n = 11), del(6q) and numerical abnormalities (n = 4), del(6q) and structural abnormalities (n = 23), and 6q translocations (n = 7). The breakpoints of del(6q) were subdivided: del(6)(q15q21) in 11 cases, del(6)(q15q21) in six, del(6)(q21q23) in four, del(6)(q15) in four, and del(6)(q15q23) in three, and other deletions in 10 cases. Notably, all these deletions encompassed the 6q21 band, suggesting that this might be the locus of a recessive tumor suppressor gene, the absence of which contributes to malignant transformation or proliferation. Among the seven children with 6q translocations, a previously unidentified nonrandom translocation, t(6;12)(q21;p13) was noted in two cases with an early pre-B immunophenotype. Clinical features and event-free survival were similar among children with or without 6q abnormalities. Overall, children with 6q abnormalities were less likely than those without the abnormality to have a pre-B immunophenotype (P = .03). T-cell immunophenotypes were equally represented in cases with or without 6q abnormalities. However, all four children with del(6q) and a 12p abnormality had early pre-B ALL and all three children with del(6q) and a 9p abnormality had a T-cell immunophenotype. The lack of specificity for a particular immunophenotype may imply that the gene or genes affected by 6q abnormalities are broadly active in the multistep process of lymphoid leuke- monogenesis. The relatively high frequency of microscopically visible del(6q) indicates the need for molecular studies to identify cases with submicroscopic deletions.

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reactivity, lymphoblasts were classified as T (CD5+, CD7+, whether or not they were E rosette positive), B (sIg+), pre-B (cIg+), or early pre-B (CD19+, CD5-, CD7-, cIg-, sIg-, and CD10+) as previously described.26

Southern blot analysis. Probing of high-molecular-weight DNA was performed as described previously.27-28 BamHI, EcoRI, and HindIII digestions were used for analyzing Ig heavy-chain (IgH) genes; the probe consisted of a 3.4-kilobase (kb) EcoRI/HindIII fragment of the joining region (Ij). BamHI digestions were also probed by using a 2.5-kb light-chain gene fragment. T-Cell receptor β-chain (TCR-β) gene rearrangements were analyzed after BamHI, EcoRI, or XbaI digestion by probing with pB400, a 0.4-kb cDNA-containing sequence for Cβ. DNAs were labeled by the oligolabeling procedure. The filters were exposed to Kodak XAR...
film in the presence of intensifying screens. All experiments included control DNA containing germline arrangements of the immunoglobulin and TCR genes.

Statistical analysis. Differences in the distribution of clinical and biologic features among subgroups of patients were tested by Fisher's two-tailed exact test. Event-free survival curves were constructed by the Kaplan-Meier method with differences in times to failure analyzed by the log-rank test.  

RESULTS

Cytogenetic findings. Thirty-five (8.5%) of the 412 children with successful studies had normal chromosomes, and 45 (11%) had 6q abnormalities: del(6q) only (n = 11); del(6q) and numerical abnormalities (n = 4); del(6q) and structural abnormalities (n = 23); and 6q translocations (n = 7) (Table 1). The 6q deletion breakpoints were subgrouped as: del(6)(q15q21) (n = 11), del(6)(q13q21) (n = 6), del(6)(q21q23) (n = 4), del(6)(q15q23) (n = 3), and various other 6q deletion breakpoints (n = 10). Deletions in all these 38 cases involved the 6q21 band. A schematic representation of the extent of 6q deletions is shown in Fig 1: The deleted region was between 6q13 and 6q21 in nine of 11 cases with del(6q) as the sole abnormality, whereas the deleted region involved distal 6q (6q23→ter) in 9 of 23 children with structural abnormalities.

Among the seven children with 6q translocations, two children with early pre-B ALL had the same newly identified translocation, t(6;12)(q21;p13), one child with T-cell ALL had a t(6;7)(q24q36), and one child with early pre-B-ALL had a t(2;6)(p21;q15). The remaining two children had unbalanced translocations. Only one child (case 39) had an unbalanced chromosomal translocation resulting in monosomy of a fragment of chromosome 6qter. The breakpoints of translocations involved 6q15 in two cases, 6q21 in two cases, and other bands in three cases. Additional complex abnormalities were noted in cells from most children who had a 6q translocation.

Twenty-six (58%) of the 45 cases were pseudodiploid, four (9%) were hypodiploid, eight (18%) were hyperdiploid 47 to 50, six (13%) were hyperdiploid >50, and one (2%) was near-tetraploid. More than two related cell lines were observed in 18 cases. Numerical abnormalities consisted of trisomy or tetrasomy of chromosome 21 in nine cases, +18 in four, and +6, +14, +16 in three cases each.

None of the children in this study had either the t(9;22) or 11q23 translocations. The nonrandom translocation, t(1;19), in addition to del(6q), was identified in one child. Additional chromosomal deletions including unbalanced translocations resulting in monosomy of a part of the chromosome were present in 16 cases: 12p− in four, 9p− in three, 7q− in two, and 8p−, 9q−, 10q−, 13q−, 18q−, and Xq− in one case each. Each of the four cases (18, 19, 23, and 27) with rearrangement involving the 12p12 region had an early pre-B-cell immunophenotype, and the three cases with rearrangements involving 9p had T-cell immunophenotype.

Immunophenotyping and genotyping. Immunophenotype results for the 45 children with 6q abnormalities are shown in Table 2. Children with 6q abnormalities were significantly less likely to have pre-B-ALL as compared with those without the chromosomal changes: 4 of 44 cases vs 93 of 347 children tested (P = .03). Moreover, none of the children with 6q abnormalities in this study had mixed-lineage expression or a CALLA− early pre-B-cell immunophenotype. In children with 6q abnormalities, IgH gene and TCR-β gene rearrangements were noted in 17 of 21 and 11 of 18 children, respectively.
**Clinical and laboratory data and treatment outcome.**

Initial clinical and laboratory features of ALL children (age, sex, race, hemoglobin, white blood cell and platelet count, liver and spleen size, and CNS involvement) were similar for the 45 children with 6q abnormalities and the other 367 children lacking this abnormality (data not shown). None of the children with 6q abnormalities were infants. There were no differences in remission induction rates or event-free survival of ALL children for the groups with or without any 6q abnormality ($P = .98$).

**DISCUSSION**

Chromosomal abnormalities involving the 6q region were identified in 11% of children with ALL in this study. In our experience, the frequency of 6q abnormalities in childhood ALL is similar to that of 12p abnormalities (10%) or 9p abnormalities (10%). Although 6q abnormalities are observed in childhood ANLL, we have noted a very low frequency (0.9%). A nonrandom translocation, t(6;12)(q21;p13) was newly identified in two children who had an early pre-B immunophenotype. Of interest is the finding of +21 in eight children, an abnormality that occurs often in hyperdiploid ALL. A pre-B-cell immunophenotype and myeloid-antigen expression were observed less frequently in children with 6q abnormalities as compared with children lacking 6q abnormalities. Notably, all four children with concurrent del(12p) had an early pre-B-cell immunophenotype as was previously reported. All three children with concurrent del(9p) had T-cell immunophenotype, an association that has been noted by other researchers but is controversial. The karyotype of patient 24 had a stem line with del(9p) and a side line with del(9p) and del(6q). These findings suggest that 6q loss may develop later in a multistep process of malignant transformation, as previously suggested by Vogelstein et al for colon cancer. Nonetheless, 11 children with del(6q) as the sole abnormality were identified in our study.

Patients with del(6q) were reported to have a good prognosis by the Third International Workshop on Chromosomes in Leukemia. In contrast to that report, in our study the presenting clinical and laboratory features and the treatment outcomes in children with 6q abnormalities were not significantly different from those of children lacking 6q abnormalities ($P = .98$). The apparent discrepancy in the prognostic significance of 6q abnormalities could be related to differences in therapy used.

A tumor suppressor gene involved in 6q deletion? Recently, Diaz et al studied 21 leukemia cell lines by Southern blot and dot blot analysis and found homozygous deletions of $\alpha$- and $\beta$-interferon genes in region 9p21-22 in six cases and hemizygous deletions in three. There have been no reports of molecular analysis of homozygous or hemizygous deletion in ALL cases with 6q, however. Barletta et al have reported that six cell lines with del(6q) retained both copies of the c-myc protooncogene and expressed higher levels of c-myc messenger RNA, indicating possible structural and functional alterations of the c-myc locus. Ohyashiki et al, however, described no association of c-myc with del(6q) in two T-cell lines with a del(6q). Molecular studies are underway to clarify the region encompassing 6q21, the chromosomal region that appears to be uniformly deleted in cases with del(6q).

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the human chromosome
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