Translocations Involving Chromosome 12p11–13, Methotrexate Metabolism, and Outcome in Childhood B-Progenitor Cell Acute Lymphoblastic Leukemia: A Pediatric Oncology Group Study


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ABSTRACT

Children with B-progenitor cell acute lymphoblastic leukemia whose lymphoblasts at diagnosis accumulate high levels of methotrexate (MTX) and MTX polyglutamates (MTXPGs) appear to have a good prognosis. This has been attributed to increased sensitivity of their blast cells to MTX. However, the proportion of children who are cured of B-progenitor cell acute lymphoblastic leukemia exceeds the number whose lymphoblasts accumulate high MTXPG levels. We report that lymphoblasts from patients with <50 chromosomes who have translocations that involve the short arm of chromosome 12 accumulate low levels of MTXPGs. These patients appear to have an excellent survival because none of 14 patients with translocations affecting 12p has relapsed, 26–79 months following diagnosis.

INTRODUCTION

Contemporary multiagent chemotherapy regimens cure substantially more than half of all children over 1 year of age with pro-B ALL3 (1). The antifol MTX is a mainstay of most regimens (1–9). It is administered intrathecally, alone or with other agents, to prevent central nervous system leukemia; as a 24-h infusion during intensification therapy; and i.m. or p.o., once a week, during consolidation treatment. Several other agents are commonly used to treat childhood ALL, including vincristine, prednisone, L-asparaginase, and 6-mercaptopurine. For patients at higher risk of treatment failure, cyclophosphamide, anthracyclines, 1-β-d-arabinofuranosylcytosine, and epipodophyllotoxins have been added in an attempt to increase the cure rate (2, 3, 5, 10).

MTX is converted to MTXPGs, containing up to seven γ-linked glutamyl residues, by the enzyme FPGS (11). Accumulation of MTXPGs in lymphoblasts in vitro correlates with cell ploidy and is highest in hyperdiploid lymphoblasts, measured either as >50 chromosomes or as a DI of >1.16 (12–14). Hyperdiploidy is associated with a favorable outcome in patients who are treated with regimens that emphasize MTX (4),

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2 The abbreviations used are: pro-B ALL, B-progenitor cell acute lymphoblastic leukemia; MTX, methotrexate; MTXPG, MTX polyglutamate, containing 2–6 total glutamates; FPGS, folate polyglutamate synthetase; DI, DNA index; EFS, event-free survival; tr12p11–13, chromosome 12p11–13 translocation; POG, Pediatric Oncology Group; del(12p), chromosome 12p deletion.

suggesting that the extent of MTXPG accumulation in lymphoblasts may be important to the effectiveness of MTX treatment. Indeed, children with pro-B ALL, whose lymphoblasts at diagnosis accumulated high levels of both MTX and MTXPGs in vitro, experienced a superior EFS, compared with children whose lymphoblasts did not (15). Moreover, the accumulation of higher levels of MTXPGs in lymphoblasts in vivo was associated with greater antileukemic effects (15).

The proportion of children with pro-B ALL whose lymphoblasts accumulate high levels of MTXPGs is less than the number expected to be cured with contemporary chemotherapy. In reviewing a series of patients whose lymphoblasts had <50 chromosomes but whose age and WBC count suggested a favorable prognosis, we noted that lymphoblasts from many of them failed to accumulate high levels of MTXPGs. Here, we describe a group of patients with tr12p11-13s who had lymphoblasts that accumulated low levels of MTXPGs. There has been no recurrence of leukemia in these patients after a considerable follow-up, suggesting that they have a good prognosis.

MATERIALS AND METHODS

Between April 1989 and September 1994, children from designated POG institutions were registered on POG 8901, a limited-institution POG study that was undertaken to measure the accumulation of MTXPGs in lymphoblasts in vitro. This analysis involves a subgroup of 95 of these children with pro-B ALL who had both a lymphoblast MTXPG level determined at diagnosis and numerical or structural cytogenetic abnormalities. There were 50 boys and 45 girls. Their median age at diagnosis was 4.4 years (range, 1.3–18.6 years), and their median white cell count at diagnosis was 13.0 × 10⁹/liter (range, 0.7–315.2 × 10⁹/liter). Forty patients were hyperdiploid, with >50 chromosomes, and 55 were nonhyperdiploid, with <50 chromosomes. Patients were treated on one of several successive contemporary front-line POG therapeutic protocols (POG 8602, POG 8698, POG 8699, POG 9005, or POG 9006). Written informed consent was obtained both for the laboratory studies and for the treatment protocols according to Institutional Review Board requirements. Data on patients 3, 7, 8, 11, and 12 (Table 1) have been published previously (12).

Pro-B ALL (early pre-B and pre-B cell) was diagnosed by standard POG criteria (16). Karyotype analysis was carried out in the POG Cytogenetics Reference Laboratory at the University of Alabama (Birmingham, AL), as well as in the Baylor Cancer Cytogenetics Laboratory (Houston, TX), the Cytogenetics Laboratory at the University of Texas Southwestern Medical Center (Dallas, TX), and the Cytogenetics Laboratory at the Medical College of Wisconsin (Milwaukee, WI). The DI was determined at St. Jude Children’s Research Hospital (Memphis, TN; Ref. 17).

Lymphoblasts were separated by Ficoll-Paque sedimentation from a 1–2-ml bone marrow aspirate sample obtained at diagnosis. Samples were judged satisfactory if the percentage blasts was 75% or more or the initial white cell count was 25.0 × 10⁹/liter or greater. Cells were washed three times with HBSS. Five million lymphoblasts were incubated with 1.0 μM [³H]MTX in 2.0 ml of RPMI 1640 containing 10% fetal bovine serum in a P-35 culture dish in 5% CO₂, 95% O₂, for 24 h at 37°C. Following incubation, cells were washed three or four times with HBSS to remove extracellular [³H]MTX. Extracts were prepared by addition of trichloroacetic acid or by boiling for 5 min. MTX and MTXPGs were separated using high-performance liquid chromatography. These techniques are described in more detail elsewhere (12). Results were judged valid if the lymphoblast MTXPG level was >100 pmol/10⁹ cells or the predominant MTXPG had five glutamyl residues. A lymphoblast MTXPG level of >500 pmol/10⁹ cells was defined as high, based on biological advantage (14, 15), whereas a level >1500 pmol/10⁹ cells was arbitrarily defined as very high. This level yields a better patient distribution than the previous “very high” level of >2000 pmol/10⁹ cells used (12). Results are means of triplicate determinations.

The translocation is reported as involving one or more of the bands 12p11, 12p12, and 12p13 in different patients and may represent different break sites. They have been grouped together for this analysis. There were seven boys and seven girls. Their median age was 4.4 years (range, 2.1–10.6 years), and their median initial white cell count was 20.3 × 10⁹/liter (range, 3.8–260.1 × 10⁹/liter). One (2.5%) of the 40 hyperdiploid patients had 61 chromosomes, a DI of 1.34, and tr12p11-13s (Table 1, patient 14). His lymphoblast MTXPG level was 1727 pmol/10⁹ cells, falling in the very high range. Thirteen (24%) of the 55 nonhyperdiploid patients had 45–47 chromosomes, a DI of 1.00, and tr12p11-13s (Table 1). All had MTXPG levels of <500 pmol/10⁹ cells, that is, in the low range. The median value was 167 pmol/10⁹ cells (range, 41–468 pmol/10⁹ cells; Table 1 and Fig. 1).

RESULTS

tr12p11-13s. A total of 14 (15%) of the 95 patients had tr12p11-13 as a feature of the principal cytogenetically abnormal clone (Table 1). Translocations are reported as involving one or more of the bands 12p11, 12p12, and 12p13 in different patients and may represent different break sites. They have been grouped together for this analysis. There were seven boys and seven girls. Their median age was 4.4 years (range, 2.1–10.6 years), and their median initial white cell count was 20.3 × 10⁹/liter (range, 3.8–260.1 × 10⁹/liter). One (2.5%) of the 40 hyperdiploid patients had 61 chromosomes, a DI of 1.34, and tr12p11-13s (Table 1, patient 14). His lymphoblast MTXPG level was 1727 pmol/10⁹ cells, falling in the very high range. Thirteen (24%) of the 55 nonhyperdiploid patients had 45–47 chromosomes, a DI of 1.00, and tr12p11-13s (Table 1). All had MTXPG levels of <500 pmol/10⁹ cells, that is, in the low range. The median value was 167 pmol/10⁹ cells (range, 41–468 pmol/10⁹ cells; Table 1 and Fig. 1).

del(12p). There were three patients with del(12p). They had <50 chromosomes and a DI of 1.00 (Table 1, patients 15–17). They had lymphoblast MTXPG levels of 804, 597, and 396 pmol/10⁹ cells. In addition to del(12p), patient 16 had a complex four-way translocation involving chromosome 12, but this involved 12q rather than 12p. Patient 17 had a minor clone with a t(12;15)(p11;q13).

Translocations Not Involving 12p11-13. Twenty-three (42%) of the 55 patients with <50 chromosomes had one or more translocations, none of which involved 12p11-13 (Fig. 1). The median MTXPG level in these patients was 261 pmol/10⁹ cells, with a range of 31–1832 pmol/10⁹ cells. Seven (30%) of these 23 patients had high lymphoblast MTXPG levels, including two with very high levels. Comparing patients with translocations, there was a trend toward higher MTXPG levels in those without versus those with tr12p11-13s (P = 0.093).

Translocations not involving 12p11-13 were present in lymphoblasts that accumulated low levels of MTXPGs, suggesting that they have a good prognosis.
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<th>Karyotype</th>
<th>MTXPGs (pmol/10^9 cells)</th>
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* %S, percentage of cells in S phase.

* R, relapse.
phoblasts from 8 (20%) of the 40 patients with >50 chromosomes. The median lymphoblast MTXPG level was 2069 pmol/10⁹ cells (range, 320-4437 pmol/10⁹ cells). Seven (88%) of the 8 patients had a high MTXPG level, including four with a very high level.

**Structural and Numerical Changes Not Affecting 12p.**
Sixteen (29%) of the 55 patients with <50 chromosomes with structural and numerical chromosomal abnormalities not affecting 12p did not have translocations (Fig. 1). Eight (50%) of these patients had high MTXPG levels, including two with very high levels. The median MTXPG level was 529 pmol/10⁹ cells (range, 203-2460 pmol/10⁹ cells). MTXPG levels were significantly higher in this patient group than they were in the group with translocations involving 12p11-13 (P < 0.001).

When lymphoblast MTXPG levels in the 39 nonhyperdiploid patients whose chromosomal abnormalities did not involve 12p were compared to the 13 with tr12p11-13, their MTXPG levels were significantly higher (median, 326 pmol/10⁹ cells; range, 31-2460 pmol/10⁹ cells; P = 0.006; Fig. 1).

**Response to Therapy.** The 4-year EFS for all 95 patients is 77% (SE = 6%). All 14 patients with tr12p11-13 remain in complete clinical remission after a median of 40 months (range, 26-79 months; Table 1). Six of the patients have been in remission for more than 4 years. One of the three patients with del(12p) relapsed 15 months after diagnosis (Table 1, patient 17).

**DISCUSSION**
Good prognosis for children with pro-B ALL has been associated with young age and low initial white cell count (19, 20). To these has been added the presence of hyperdiploidy, measured as a DI of >1.16 (4, 21, 22), which defines a population with an even better outcome. Recently, children with trisomies of both chromosomes 4 and 10 have been found to have a prognosis even better than that associated with hyperdiploidy (23).

Several studies have established an association between clinical outcome and the extent of MTX metabolism. Success of treatment has been linked to pharmacokinetic differences in MTX metabolism (24-26). Patients treated in the early 1980s, whose lymphoblasts accumulated high levels of MTX and MTXPGs in vitro, had a superior 5-year EFS compared to those whose lymphoblasts accumulated low MTX and MTXPG levels (15). The finding that hyperdiploid lymphoblasts accumulated high levels of MTXPGs, both in vitro and in vivo (12-14), suggests that MTX probably plays an important role in the cure of patients with hyperdiploid pro-B ALL.

In children with pro-B ALL and <50 chromosomes, those with translocations have a poorer prognosis than do those who do not (27, 28). We were not surprised, therefore, to find low levels of MTXPGs in lymphoblasts in many patients with translocations. However, all of the 13 patients with <50 chromosomes and tr12p11-13 had low MTXPG levels, and the levels were significantly lower than lymphoblast MTXPG levels in patients with <50 chromosomes with chromosomal abnormalities that did not affect 12p. These findings suggest that the inability to accumulate high levels of MTXPGs in vitro is a feature of lymphoblasts with tr12p11-13. The single patient with hyperdiploidy and tr12p11-13 had a very high lymphoblast MTXPG level, consistent with the hyperdiploid state (12). This suggests that factors other than those associated with tr12p11-13 may affect lymphoblast accumulation of MTXPGs and, therefore, that tr12p11-13 may not be associated with low MTXPG levels in every patient.

Data from the three patients with del(12p) are reported here, but they are not part of this analysis. The high levels of MTXPGs in two of them, and the failure of treatment in the third suggests that they may differ from patients with tr12p11-13 in their metabolism of MTX. Data on larger numbers of patients with del(12p) will be required to substantiate this impression.

All 14 patients with tr12p11-13 remain in first remission after considerable follow-up, suggesting that their response to treatment may be at least as good as that of the entire patient population studied. Some of these patients are likely to have been cured, despite the inability of their lymphoblasts to accumulate high levels of MTXPGs. This suggests that the lymphoblasts in such patients are either more sensitive than usual to low levels of intracellular MTXPGs or are more sensitive to another antileukemic agent.

The mechanism linking low MTXPG levels to tr12p11-13 is unknown. It is possible that the translocation inactivates a gene involved in regulating the expression of the genes for FPGS (29) or for the reduced folate carrier that transports MTX into cells (30, 31) because neither of these genes is located on 12p. Alternately, the translocation may result in activation of one of the genes for γ-glutamyl hydrolase (32, 33), the products of which hydrolyze MTXPGs to MTX. A decrease in FPGS or reduced folate carrier expression or an increase in γ-glutamyl hydrolase expression could contribute to decreased accumulation of MTXPGs in lymphoblasts.

Translocations involving 12p11-13, like those involving the MLL gene at 11q23 (34), are promiscuous, consistent with
the gene in question being located on 12p. A candidate gene on 12p might be \( p2^{N_{\text{p1}}} \) (35-37). This gene encodes a cell cycle inhibitor that interferes with the activity of the cyclin-cyclin-dependent kinase complexes and, thereby, the orderly progression of cells through the G1-S transition (38). Changes in expression of \( p2^{N_{\text{p1}}} \) might predispose the cell to undergo apoptosis, contributing to increased lymphoblast cytotoxicity to low intracellular levels of MTXPGs.

A more likely candidate is the TEL gene (39) because translocations involving TEL were present in three of the patients. The t(6;12) and the t(5;12) create TEL-STL (6-12 leukemia) and TEL-PDGF\( \beta \) gene fusion products, respectively (39, 40). A third patient has a t(12;21), detected cytogenetically, that creates a TEL-AMLJ fusion product (Table 1, patients 3, 11, and 9, respectively). The TEL gene is an ets-like gene, the function of which is currently unknown (39).

The TEL gene has assumed increased importance with the identification of a cryptic t(12;21)(p13;q22) in childhood pro-B ALL (41, 42). This translocation is difficult to recognize by light microscopy because the translocated DNA regions resemble each other morphologically. It can be demonstrated by fluorescent in situ hybridization (43). Rearrangement of the TEL gene and loss of the normal allele occur in association with this translocation (44, 45). The translocation involves fusion of the 5’ helix-loop-helix domain of the TEL gene on chromosome 12p13 with almost the entire AMLJ gene on chromosome 21q22 (41, 42). The TEL-AMLJ gene product can be demonstrated using reverse transcriptase-PCR, as can the antisense AMLJ-TEL product (44).

The TEL-AMLJ translocation occurs in about 25% of children with pro-B ALL (44, 46). Such children appear to have an excellent prognosis (44, 47). Studies are planned to determine whether children with pro-B ALL and the TEL-AMLJ translocation accumulate low levels of MTXPGs in their lymphoblasts.

REFERENCES


Translocations involving chromosome 12p11-13, methotrexate metabolism, and outcome in childhood B-progenitor cell acute lymphoblastic leukemia: a Pediatric Oncology Group study.


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