Somatic Shift to Homozygosity for a Chromosomal Polymorphism in a Child With Acute Lymphoblastic Leukemia

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A 12-year-old girl with acute lymphoblastic leukemia (ALL) had two types of acquired cytogenetic abnormalities in her pretreatment peripheral blood and bone marrow: hyperdiploidy due to tetrasomy 8, 10, and 21; and, in the hyperdiploid cells, a shift from heterozygosity to homozygosity for a polymorphic variant on chromosome 15. Both abnormalities disappeared after chemotherapy, when the patient entered clinical remission. It has recently been found that shifts to homozygosity occur in retinoblastoma and Wilms’ tumor. Our observation extends this finding to leukemia and indicates that such shifts may have general importance in tumorigenesis.

IT HAS BECOME clear in recent years that malignant clones often exhibit chromosome abnormalities, and that these acquired abnormalities are often highly specific for a particular type of tumor. Translocations, as well as deletions of entire chromosomes or of specific regions, are well-known findings in chronic myelogenous leukemia, meningioma, and retinoblastoma, for example. Disturbances of chromosome segregation leading to loss or gain of apparently random chromosomes, and consequently to hypodiploidy or hyperdiploidy, are frequently found in acute myeloblastic leukemia (AML) and acute lymphoblastic leukemia (ALL). Other chromosomal mechanisms, leading to genetic homozygosity, have very recently been shown to be operating in retinoblastomas and Wilms’ tumors. The use of cloned DNA probes homologous to regions of chromosome 13q and chromosome 11p, respectively, has shown that regions that are heterozygous in unaffected cells of individuals with tumors are homozygous in the tumor cells themselves. Mitotic non-disjunction and duplication (leading to loss of an entire chromosome, duplication of its homolog, and consequent homozygosity for the entire chromosome) as well as mitotic recombination (leading to homozygosity for only that area of the chromosome distal to the recombination event) may both play a role in this shift from genetic heterozygosity to homozygosity.

We report here on a young girl with ALL who had two types of chromosome abnormalities in her malignant clone: hyperdiploidy due to tetrasomy 8, 10, and 21; and a shift from heterozygosity to homozygosity for a large chromosome polymorphism on chromosome 15p. To our knowledge this type of cytogenetic event has not been previously reported.

MATERIALS AND METHODS

Case Report. A 12-year-old white girl was seen for complaints of fever, lethargy, anorexia, abdominal pain, and pallor. Her past history and family history were unremarkable. On physical examination, she was found to have hepatosplenomegaly and petechiae on her palate. A complete blood count showed a hemoglobin of 9.1 g/dL; hematocrit of 26%; platelet count of 12,000/μL; WBC count of 55,700/μL with differential count of leukemic blast cells 88%; promyelocytes 2%; stabs 1%; segmented neutrophils 2%; and lymphocytes 7%. Most of the leukemic blasts had predominantly cytoplasmic vacuolation; however, some degree of nuclear vacuolation was observed in some of the cells. Cytoplasm was abundant with light-blue to dark-blue staining with Wright’s stain. Most of the blast cells were devoid of cytoplasmic granules; however, a minority of the blast cells (25% of the total number of blast cells) contained cytoplasmic granules. No Auer rods were seen. Nuclear chromatin was immature and the nuclei contained one to three prominent nucleoli.

Cytochemical staining of leukemic blasts showed: myeloperoxidase-negative; periodic-acid Schiff (PAS)-positive in 79% of blast cells and negative in 21% of blast cells; Sudan black-positive in 11% of blast cells and negative in 89% of blast cells; chloracetate esterase-negative; nonspecific esterase-negative; and acid phosphatase-negative. Immunological surface marker analysis of blast cells from bone marrow and blood showed HLA-DR (Ia) and B4 antigen positivity and other lymphoid lineage markers (common ALL antigens, B1, B2, SLgM, Leu-1, Leu-4, T1, and AET rosettes) negativity. Myeloid lineage antigens (MO1 and MO2) were also negative. Terminal deoxynucleotidyl transferase was present in only 50% of blast cells.

Although blast cells of this patient were observed to contain both PAS-positive and Sudan black-positive populations, the diagnosis of AML was excluded on the basis of negative myeloperoxidase and negative specific and nonspecific esterase staining. Thus, the final diagnosis of ALL with positivity for HLA-DR (Ia) and B4 antigens and negativity for common ALL antigen (CALLA) and other lymphoid lineage antigens was made. The patient was placed on a modified Berlin-Frankfurt-Muenster protocol,1 which uses vincristine, daunomycin, l-asparaginase, prednisone, and intrathecal ara C and methotrexate during induction phase. She began treatment four days after her initial evaluation and completed the induction phase of treatment 27 days later. She attained remission successfully and continues to be in complete remission twelve months later.

Cytogenetic Studies. Samples of blood or bone marrow were received on five occasions during the six-week period from the patient’s first referral until after remission was achieved. In accordance with institutional guidelines, informed consent for this and other standard diagnostic procedures was obtained at the time of referral. Blood samples were split into parallel cultures, with and without mitogen stimulation, and were grown for 72 hours. However, the unstimulated preparations yielded no mitotic cells. Bone marrow was studied in direct preparations without mitogen stimulation. Standard cytogenetic techniques2 were used, followed by trypsin-Giemsa banding.

Samples 1 and 2 were peripheral blood and bone marrow, respectively, received prior to the start of therapy. Two populations were found in both tissues. Sixteen of 20 cells (80%) in blood, and 19 of 27 cells (70%) in marrow, had a normal female karyotype. A very large, prominent p arm on one No. 15 chromosome was noted in

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Submitted June 11, 1985; accepted Aug 8, 1985.

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0006-4971/86/6702-0015$03.00/0
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Fig 1. D-group chromosomes (Nos. 13, 14, and 15) from normal and hyperdiploid cell lines. Top row, G-banded; middle row, C-banded; bottom row, Q-banded. Left side, normal (46, XX) cell line; right side, hyperdiploid cell line. Q-banded row includes No. 21 chromosome pair for comparison of its banding properties with those of the 15p+ region. Arrows indicate polymorphic variant 15p+.

every cell; this arm was dark on G-banding, pale on Q-banding, and dark on C-banding (Fig 1). On the basis of these staining properties, it was interpreted as a polymorphic variant. The second population in both tissues was hyperdiploid, with tetrasomy for chromosomes 8, 10, and 21 (Fig 2). In these cells, both No. 15 chromosomes had the large, dark-staining p arm (Fig 1). The karyotype was described as: 46, XX, var(15) (p11.2, QFQ42, CBG40)/52, XX, +8, +8, +10, +10, +21, +21, var(15 = 2) (p11.2, QFQ42, CBG40).

Sample 3 was peripheral blood taken ten days after the start of chemotherapy. Only five metaphases could be found; all had a normal female karyotype with the polymorphic variant 15 found once per cell.

Sample 4, a bone marrow specimen taken 17 days after the start of therapy, had no mitotic activity.

Sample 5, another bone marrow taken 35 days after the start of therapy and 8 days after its conclusion, showed a normal female karyotype, with the variant 15 found once per cell, in 19 of 20 cells examined. One cell was tetraploid.

DISCUSSION

The presence of many chromosomally normal cells along with a chromosomally abnormal, malignant clone is a common finding in leukemic disorders. Both populations may appear in mitogen-stimulated blood as well as in marrow; the malignant clone will disappear after successful therapy. The abnormal clone found in the pretreatment blood and bone marrow of our patient had two types of cytogenetic abnormalities. This clone disappeared from both blood and marrow after chemotherapy. One abnormality, the finding of hyperdiploidy, is not surprising insofar as it has often been reported in patients with acute leukemia, both ALL and AML. Gains of chromosomes 8 and 21 are common in AML. Gain of 21 is also common in childhood ALL; gains of 8 and 10 are less common in that disease but have been observed.

The second type of cytogenetic abnormality observed in our patient is an unusual structural change: A polymorphic variant, present on one No. 15 chromosome in the normal cell line, was found on both No. 15 chromosomes in the malignant hyperdiploid clone. Several mechanisms could account for the change from heterozygosity to homozygosity for this region.

First, the change could be due to simultaneous loss of the nonvariant 15 and duplication of the variant 15, leading to homozygosity for both arms, ie, for the entire chromosome. This could be caused by double nondisjunction of chromatids at a mitotic division.

Somatic recombination is a second mechanism that could lead to homozygosity for 15p+. Crossing over just above the centromere, for example, could lead, in the next cell generation, to a cell line in which both No. 15s have the large polymorphism. However, in this case, the remainder of the chromosomes (the 15q arms) would remain unchanged and any genetic heterozygosity present there originally would be retained. Somatic, or mitotic, recombination is a rare but well-documented phenomenon that has been known for years in Drosophila and in yeast and other fungi. Cancer geneticists have recently turned their attention to this phenomenon because of the discovery that retinoblastoma tissue often shows homozygosity for markers on chromosome 13 that were heterozygous in the patient's unaffected cells.
Fig 2. Hyperdiploid karyotype found in pretreatment blood and bone marrow: 52, XX, +8, +8, +10, +10, +21, +21. Arrows indicate polymorphic variant on short arm of both No. 15 chromosomes.

Wilms' tumor tissue often exhibits a similar shift to homozygosity for at least a part of chromosome 11. In retinoblastoma, the shift is apparently due to mitotic nondisjunction in some cases and to mitotic recombination in others; in Wilms' tumor, the mechanism is not yet known. However, it now seems likely that one or both of these genetic events is often crucial to the development of tumors.

In the case of retinoblastoma and Wilms' tumor, deletions of the chromosomal regions where homozygosity has been demonstrated (13q14 and 11p13, respectively) are known to be associated with development of the tumors. In the present instance, our patient was diagnosed as having ALL, and homozygosity at least for 15p (and possibly all of chromosome 15) was demonstrated cytogenetically. The relation between these two phenomena is not clear and need not be causal. Deletions of a region of chromosome 15, or of the entire chromosome, have been described in some cases of ALL, but it is not an especially frequent finding. In addition, the area of chromosome 15 where homozygosity is cytogenetically demonstrable (15p) is an area where deletions and duplications are tolerated and have no clinical consequence; the definition of the 15p+ as a polymorphic variant bears this out. Band 15q22, on the long arm, is notable for its consistent involvement in a 15;17 translocation in acute nonlymphocytic leukemia type M3; in addition, the feline sarcoma (fes) oncogene is located on band 15q24–25 on the long arm of chromosome 15. The shift to homozygosity that we observed in 15p may be the visible manifestation of a shift to homozygosity for the entire No. 15 chromosome. If the two No. 15s had different alleles for fes, the shift would have eliminated one allele and doubled the other. Fes is known to be highly expressed in both ALL and AML, whereas there is no evidence of fes transcripts in normal cells.

Alternatively, it may be that random mitotic nondisjunction and/or mitotic recombination, both leading to homozygosity for at least part of a chromosome, are not uncommon events in malignant cells. Just as apparently random gain or loss of chromosomes in AML and ALL indicates a general disturbance of normal segregation mechanisms, so may the processes leading to homozygosity of apparently random chromosomes indicate a general genetic instability in these cells. Observation of chance chromosomal polymorphisms, as in this case, combined with the use of DNA polymorphisms, should provide insight into these events.

ACKNOWLEDGMENT

We thank Ralph Ehrenpreis for expert technical assistance.

REFERENCES


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