Emergence of a Cell Line With Extreme Hypodiploidy in Blast Crisis of Chronic Myelocytic Leukemia

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Cytogenetic studies in a patient with chronic myelocytic leukemia (CML) demonstrated the emergence of an extremely hypodiploid cell line at the time of blast crisis, a modal chromosome number of 35, with the modal karyotype 35,XY,-3,-4,-5,-7,-9,-11,-12,-13,-15,-16,-17,-19,-20,-22, +t(9;22) (q34;q11), +Mar1, +Mar2, +Mar3. Giemsa-banding confirmed complex chromosome rearrangements and demonstrated distinct banding patterns for the marker chromosomes. Cytologic characteristics of the leukemia blasts were predominantly myeloid. There was no important clinical response to chemotherapy, including vincristine and prednisone, or to radiotherapy.

KARYOTYPIC ABNORMALITIES associated with malignant cell lines have been frequently noted and have aroused a great deal of interest regarding the possible etiologic role of such changes in carcinogenesis. Such chromosomal changes are particularly well described for chronic myelocytic leukemia (CML), in which identification of the Philadelphia (Ph¹) chromosome has become an important criterion for the diagnosis. CML is a disease characterized by an initially stable and well-tolerated phase that eventually progresses to an accelerated, more highly malignant, and terminal phase referred to as blast crisis. New karyotypic abnormalities in addition to the Ph¹ chromosome are frequently detected in the malignant clone of cells that predominates in blast crisis. Although hyperdiploidy is common, hypodiploidy is an unusual finding in the cells of patients with CML blast crisis.¹ Severe degrees of hypodiploidy are quite rare. This may, in part, reflect the requirement for a minimum amount of chromosomal material and stability for cell survival. We recently encountered a patient with Ph¹-positive CML who was found to have an extensive chromosomal rearrangement, in addition to hypodiploidy, of a degree not previously reported for this disease. Details of these observations form the basis of this report. The biologic and clinical implications of the findings are considered.

MATERIALS AND METHODS

Bone marrow aspirates and peripheral blood samples obtained by venipuncture were collected in preservative-free heparin. Bone marrow and blood cells were prepared for metaphase chromosome analysis by 2-hr exposure to Colcemid in culture at 37°C.² Following a 20-min exposure to a hypotonic (0.075 M) KCl solution, the cells were fixed for examination. Trypsin-Giemsa chromosome banding analysis was performed as described by Seabright.³
CASE REPORT

A Caucasian male was 44 yr old when leukocytosis was noted on a routine blood test in October, 1973. Bone marrow examination revealed hypercellularity with myeloid preponderance consistent with CML. Karyotype analysis revealed 16 of 16 cells with the typical Ph′ chromosome. Peripheral blood counts returned to normal levels promptly with low-doseage busulfan therapy. The patient remained asymptomatic over the next 4 yr while receiving intermittent busulfan therapy.

In September, 1977, the patient developed right shoulder pain, and a lytic lesion in the proximal humerus was noted on x-ray examination. Biopsy of the lesion revealed necrotic tissue, and examination of a bone marrow sample obtained from a distant site revealed changes still consistent with chronic-phase CML. However, 2 mo later a repeat bone marrow examination revealed a marrow space packed with immature mononuclear cells containing 1–2 prominent nucleoli and a moderate amount of vacuolated basophilic cytoplasm. Mature elements were diminished, and a slight increase in reticulin fibers was present.

Peripheral blood examination demonstrated circulating immature cells similar to those seen in the bone marrow. Despite treatment with busulfan, hydroxyurea, vincristine, and prednisone, the patient lost weight rapidly and became progressively weaker. Because other treatment modalities had failed, a course of daunomycin and fractionated total body irradiation (20 rads daily for 3 days) was initiated. This produced pancytopenia. The patient developed sepsis and respiratory failure and died in January, 1978. Postmortem examination revealed lobar pneumonia and pulmonary hemorrhage. Leukemic infiltrates were present in the liver. Bone marrows of ribs, sternum, vertebrae, iliac crest, and femur were uniformly hypercellular with leukemic cells.

RESULTS

Initial cytogenic studies completed at the time of the first blood and bone marrow examinations demonstrated 46,XY,Ph′-positive cells (Table I). Studies repeated 3 yr later indicated karyotypic stability during this period. Aneuploidy was noted in a minority of metaphases and was attributed at the time to random chromosome loss and laboratory artifact. Retrospective review of this material failed to indicate a second cell line resembling the abnormal clone recognized in the subsequent examination. Peripheral blood cells were examined when the patient presented with clinical blast crisis. Bone marrow aspirates were unobtainable, although biopsies and touch preparations provided histologic confirmation of blast crisis. Peripheral blood cultured for 24 hr without mitogen stimulation exhibited marked hypodiploidy. A range of 33–39 chromosomes was found with a modal chromosome number of 35. The modal karyotype was 35,XY,−3,−4,−5,−7,−9,−11,−12,−13,−15,−16,−17,−19,−20,−22,+t(9;22)(q34;q11),+Mar1,+Mar2,+Mar3 (Fig. 1). Giemsa-banding of five cells demonstrated identical marker chromosomes in each. Mar1 appears to be a chromosome 12 with the deleted short arms replaced by the short arms and satellites of an acrocentric chromosome. Since three acrocentric chromosomes were lost, the satellite material

| Sample        | Date | 32 | 33 | 34 | 35 | 36 | 37 | 38 | 39 | 40 | 41 | 42 | 43 | 44 | 45 | 46 | Ph′ | Total Cells Examined |
|---------------|------|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|----|
| Bone marrow   | 6/27/74 | 16 |    |    | 16 |    |    |    |    |    |    |    |    |    |    | 16 |
| Bone marrow   | 1/14/76 | 15 |    |    | 15 |    |    |    |    |    |    |    |    |    |    | 15 |
| Bone marrow   | 9/21/76 | 1  | 1  | 3  | 1  | 5  | 2  | 3  | 2  | 4  | 10 | 51 | 1  | 84 | 84 |
| Bone marrow   | 10/8/76 | 1  |    |    | 1  | 2  | 3  | 1  | 5  | 21 | 21 |    |    |    |    |    |
| Blood         | 1/10/78 | 1  | 5  | 6  | 20 | 1  | 3  | 1  |    |    |    |    | 37 | 37 |

*Blast crisis was clinically evident by November, 1977.
Fig. 1. Karyotype for 35.XY, -3, -4, -6, -7, -9, -11, -12, -13, -15, -16, -17, -19, -20, -22, +t(9;22)(q34;ql 1), +Mar1, +Mar2, +Mar3. No normal chromosomes 9 or 22 are present; only the translocation products are identified. The 9q+ chromosome is indicated by the arrow.

of Mar1 presumably originated from one of the missing acrocentrics. Both Mar2 and Mar3 are similar in size to chromosome 17, but they have distinct banding patterns. Mar2 has the appearance of a chromosome 19, with partial deletion of the long arm and addition of two distinct bands. Mar3 was also found to have a distinct banding pattern, but we were unable to identify the derivative chromosome or chromosomes.

The cytologic characteristics of the immature cells found during blast crisis included a nuclear:cytoplasmic ratio of less than 0.8, moderate amounts of vacuolated basophilic cytoplasm, 1–2 nucleoli per nucleus, irregular nuclear borders, and occasional nuclear folding. Thus these cells exhibited morphologic characteristics of both myeloid and lymphoid blast types.

DISCUSSION

The development of aneuploidy in addition to the Ph1 chromosome is a manifestation of CML that is associated with a relatively poor prognosis. Although the incidence of aneuploidy in CML may be as high as 80%, hypodiploidy is distinctly unusual in CML. Hypodiploidy, when found in CML, is generally associated
with development of blast crisis.\cite{5} The degree of chromosome loss previously reported has been limited. In the large review by Whang-Peng et al.\cite{6} no case with less than 44 chromosomes was found. In the several reports by Sandberg and associates\cite{5,8,11,12} the lowest modal chromosome number found in CML was a single case of 43 chromosomes. Among the hypodiploid cell lines examined, 44 and 45 modal chromosomal numbers were more frequent. Thus, our case demonstrates an extraordinary degree of hypodiploidy not previously reported for CML. Two cases of acute leukemia have previously been reported with a cell line of 27 chromosomes.\cite{13,14} Although hypodiploidy is especially rare in lymphoid leukemias,\cite{15} both of these cases were acute lymphoblastic leukemia (ALL). Hypodiploidy may be more common in acute myelocytic leukemia (AML), but modal chromosome numbers of less than 42 are extremely rare.\cite{2,16-20}

The karyotype of the leukemic cells of our patient in CML blast crisis indicated a complex rearrangement in addition to extreme hypodiploidy. All five metaphases examined with Giemsa-banding technique contained the identical three markers. This suggests that the specific rearrangement and chromosome loss was also a relatively constant characteristic of the leukemic clone at that time. Deletion of chromosome 17 has been associated with hypodiploidy and blast crisis in CML.\cite{5} Other deletions described in CML include missing chromosomes 7, 16, and Y. Loss of Y occurs in 8% of chronic-phase CML patients and may merely reflect a nonspecific change associated with aging.\cite{2,21} Our patient demonstrated absence of chromosomes 7 and 16 as well as 17. The significance of cell lines in blast crisis with severe hypodiploidy may rest with the chromosomes that are not involved. Thus diploidy for chromosomes 10, 18, and 21 was preserved in our patient, and also in the two cases of near haploidy reported for ALL. It is conceivable that with extreme hypodiploidy, cell functions regulated by genes associated with these chromosomes, for example, must remain unaltered to permit neoplastic growth. It should be noted that despite a complex chromosome rearrangement and extensive chromosome loss, all leukemic cells examined from our patient still carried the Ph\textsuperscript{1} chromosome. The significance of specific chromosome changes other than the chromosome 22 translocation remains unclear.

Patients with blast crisis whose leukemic cells have lymphoid properties appear to have a somewhat better prognosis than those patients whose blasts retain myeloid characteristics.\cite{4} Comparison of the karyotypic pattern associated with each of these groups and outpatients may be useful. Hypodiploidy in blast crisis appears to occur more frequently in CML patients with lymphoid characteristics than in patients with myeloid characteristics.\cite{4,9} This is in contrast to the findings in acute leukemias. Although approximately 50% of cases of ALL are aneuploid, hypodiploidy occurs in less than 2% of these.\cite{15} In AML, on the other hand, a wide range of modal chromosome numbers is found in which the incidence of hypodiploidy may be 25%.\cite{16,21} Response to vincristine and prednisone occurred much more frequently in patients with hypodiploid leukemic clones in CML than in patients with diploid or hyperdiploid clones.\cite{4,7,9,10} Despite the hypodiploidy found in our patient, most cytologic criteria\cite{4} indicated a myeloid derivation of the leukemic clone in blast crisis for our patient, and he did not have a measurable response to vincristine and prednisone treatment.

Although aneuploidy is a common finding in the clonal evolution to blast crisis, associations between specific chromosomal changes and neoplastic growth cannot
be reliably made at this time. The karyotypic variability of CML may reflect a particular susceptibility to abnormal mitosis. Extensive chromosome loss (extreme hypodiploidy) may be compatible with the development of a selective growth advantage of a neoplastic cell population in blast crisis over competing hematopoietic cells.

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