Cytogenetic Characterization of Putative Human Myeloblastic Leukemia Cell Lines (ML-1, -2, and -3): Origin of the Cells

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ABSTRACT

Cytogenetic studies were performed on ML cell lines (ML-1, -2, and -3), as well as on the leukemic cells of a patient from whom the ML cells were derived. The ML-1 cell line showed numerical and structural cytogenetic changes, i.e., 48,X,-Y,lp-,6q-,11q-,+12,+13q+,14q-, and 17q-. The ML-2 cell line had two copies of the 13q+, whereas the ML-3 cells contained three clones, i.e., 47.X,-Y,lp-,-6q-,11q-,+12,+13q+, 48.X,-Y,lp-,-6q-,11q-,+12,+13q+, and 49.X,-Y,lp-,-6q-,+6q-,11q-,+12,+13q+, +13q+. The neoplastic cells, when the patient was diagnosed as having T-cell malignant lymphoma (Stage IV), had the 11q- and 13q+. The leukemic cells in a subsequent acute myeloid leukemia phase of this patient contained structural (1p- and 6q-) and numerical (+12, -Y and +2D-group chromosomes: two 13q+) changes in addition to the 11q-. These findings suggest that the acute myeloid leukemic cells of this patient probably originated from the neoplastic cells of the preceding T-cell lymphoma, and that the chromosome changes originally seen in the lymphoma cells were preserved in the established ML cell lines, though the cells of these lines had myeloid characteristics.

INTRODUCTION

The establishment of nonrandom cytogenetic changes in human neoplastic states, especially leukemias, has afforded not only a correlation between these changes and the diagnostic aspects of these leukemias, but also their prognostic and clinical implications (1, 2). In human leukemias, a relationship has been shown to exist between specific cytogenetic changes and the phenotypes of some of the leukemic cells, based on the findings with recent techniques for demonstrating surface markers, including the use of monoclonal antibodies (3, 4). Thus, cytogenetic studies can be of help in determining the origin of leukemic cells.

Though a relatively large number of human leukemia cell lines derived from the cells of patients with leukemia and lymphoma have been established (5, 6), lines with specific chromosome changes characteristic of the types of conditions from which they originated are scarce. Recent interest has been concentrated on an association between cytogenetic changes in human leukemia cell lines and expression of oncogenes mapped to affected chromosomal bands (7–9). Thus, a comparative cytogenetic study of the leukemic cells in vivo with the derivative cell lines is necessary in order to distinguish between the original chromosome changes present in vivo and additional karyotypic aberrations, if any, appearing or acquired in vitro.

We have performed cytogenetic studies on the human myeloblastic ML cell lines (ML-1, -2, and -3), as well as on the leukemic cells of the patient from whom the cells of the lines originated and who originally presented with T-cell lymphoma, and discuss the relationship between the chromosome changes and the origin of the neoplastic cells, since the ML-1 cells have capability to differentiate into granulocytes or monocytes (10, 11). The results should be of value to those who have studied these cell lines or intend to use them in future investigations and in the interpretation of results obtained in studies involving these cell lines.

MATERIALS AND METHODS

Cells. The ML cell lines (ML-1, -2, and -3) were provided by Dr. Jun Minowada, Hines Veteran Medical Center, IL, and Dr. John Pauly, Roswell Park Memorial Institute, NY. These cell lines were established from the peripheral blood cells obtained on July 13, 1978, from a patient with acute myeloid leukemia; clinical and detailed hematological information on this patient has been reported elsewhere (12).

The patient was diagnosed as having T-cell malignant lymphoma (Stage IV) terminating in acute myeloid leukemia. He noticed a growing mass in his neck in October 1977 and was diagnosed as having T-cell malignant lymphoma on October 19, 1977. The bone marrow was shown to be heavily infiltrated by cells resembling lymphoblasts. The patient was treated with cyclophosphamide and prednisolone with no response; methotrexate and vincristine were then instituted. Further chemotherapy consisted of Adriamycin and 1-beta-arabinofuranosylcytosine, with which complete remission was achieved in April 1978. Eight weeks later, he was readmitted with headaches and diagnosed by hematological examinations as having acute myeloid leukemia on June 9, 1978 (12). The blasts at the acute myeloid leukemia phase had a myelo-monoblastoid appearance with differentiation to promyelocytes. Cytochemical examination of the blasts revealed them to have a positive reaction for peroxidase, Sudan black, acid phosphatase, and specific and non-specific esterases (12). The blasts also contained Ia-like antigen but not common acute lymphoblastic leukemia antigen or surface immunoglobulins (12).

The cells of the ML lines show a myeloblastic morphology and have a cytochemical pattern similar to that of the blasts in vivo. They also react with various anti-myeloid cell monoclonal antibodies (13).

Cytogenetic Studies. Cytogenetic examinations of bone marrow cells from the patient were performed on 6 occasions: October 19, 1977, January 24, 1978, January 31, 1978, June 9, 1978, July 13, 1978, and July 28, 1978. The first 3 specimens were obtained when the patient was diagnosed as having T-cell lymphoma and the marrow was infiltrated with lymphoblasts, the latter 3 when he suffered from an acute myeloid leukemia. Chromosome analysis of the ML-1 cells was first made on June 9, 1979 and of ML-2 and -3 cells on August 23, 1985.

For chromosome study, the cells were arrested in mitosis by exposure to colcemid (Grand Island Biological Co., Grand Island, NY), at a final concentration of 0.01 μg/ml for 1–2 h before termination of the cultures, and treated with a 0.075 M KCl hypotonic solution for 30 min. After 3 changes of fixative solution (methanohacetic acid, 3:1 vol/vol), chromosomes were made with an air drying or flaming-drying method. Chromosome banding of the bone marrow cells was performed by a Giemsa (G)-banding method (14) and by a sequential quinacrine (Q)-banding method (15) after destaining the slides with acridine orange was also performed on the ML cells (16). The description of the chromosome findings used the International System for Human Cytogenetic Nomenclature (ISCN, 1985) (17).
RESULTS

Cell Lines

ML-1. The 20 ML-1 cells analyzed on June 9, 1979 contained numerical and structural changes with a modal number of 47 chromosomes. The Q-banding pattern revealed trisomy of chromosome no. 12 and a missing Y-chromosome. The deleted portion of the short arm of 1p appeared to have a banding pattern similar to that of the long arm of chromosome no. 14, possibly resulting from a translocation. Furthermore, a marker chromosome derived from no. 17, del(17)(q23), and another marker derived from chromosome no. 14, der(14)(14;?)(q13;?), appeared in the cells.

Chromosome analysis of this line after more than its 100th passage revealed a chromosome number ranging from 59 to 97 with a modal number of 91. Banding analysis showed that the structural and numerical changes observed above were duplicated. The long arm of chromosome no. 6 was deleted at band q23; the deleted part could not be detected in the karyotype. A terminal portion of chromosome no. 11 was deleted at band 11q23, resulting in the 11q-. Furthermore, Q-banding showed an extra pale band on the terminal portion of chromosome no. 13 (Fig. 1). This negative pale band (11q23->qter) seemed to be translocated onto the terminal region of a chromosome no. 13, though it was difficult to confirm this with certainty. In addition to 4 normal no. 14 chromosomes, 4 markers affecting chromosome no. 14 seen in the cells on June 9, 1979 were again observed.

Marker 1: (1p-): der(1)t(1;14)(p22;q13)
Marker 2: (6q-): del(6)(q23)
Marker 3: (11q-): der(11)t(11;13)(q23;q32?) or del (11)(q23)
Marker 4: (13q+): der(13)t(13;11)(q32?q23) or der(13)t(13;?)(q32;?)
Marker 5: (14q-): der(14)t(14;?)q13;?)
Marker 6: (17q-): del(17)(q23)
Marker 7: del(1)(p22)
Marker 8: der(16)t(16;1)(p13;p35)

The structural and numerical changes of the fresh leukemic cells (see below) were present in the cells of the cell line after more than 100 passages, though new markers (Markers 7 and 8) appeared.

ML-2. The ML-2 cells analyzed on August 23, 1985 had a chromosome number ranging from 41 to 48 with a modal number of 48. Banding analysis revealed that the structural changes (Markers 1-4) and the +12 and -Y seen in the ML-1 cells were preserved (Fig. 2). Furthermore, two copies of Marker 4 (13q+) were present. Thus the ML-2 cells had a 48,X,-Y,1p-,.6q-,11q-,+12,+13q+ karyotype. R-
Fig. 2. Q-banded karyotype of an ML-2 cell obtained on August 23, 1985 showing two copies of 13q+ (long arrows) in addition to structural and numerical changes which were observed in the ML-1 cells. A short arrow points to the 11q-.

Fig. 3. Q-banded (Q) and R-banded (R) partial karyotypes of the ML-2 cell line, showing a terminal deletion of the long arm of chromosome no. 11 and extra chromosomal material on the long arms of two of the chromosome no. 13 (the chromosome 13 on the left is the only normal one). Arrows point to possible breakpoints on these chromosomes.

ML-2. Though the marrow cells at the T-cell lymphoma stage of this patient were reported as having a normal male karyotype (12), reexamination of slides on cells obtained on October 19, 1977 and January 24, 1978 revealed the 11q- and 13q+ observed in the ML cells (Fig. 5). No metaphases were obtained from the January 31, 1978 sample. Cytogenetic study on June 9, 1978 revealed 2 of 23 cells to contain 48 chromosomes; the remaining 21 cells had a normal karyotype. The 5th chromosome examination revealed 1 of 19 cells to have a normal karyotype; the remaining 18 cells had 47 or 48 chromosomes.

ML-3. The ML-3 cells showed a chromosome number ranging from 43 to 49 with a modal number of 49. Banded karyotypes revealed that the structural changes of 1p- (Marker 1), 6q- (Marker 2), and Markers 3 and 4 were preserved. The cell line consisted of three clones cytogenetically: one had one copy of Marker 2 (6q-) and one copy of Marker 4 (13q+) (6 of the 20 cells analyzed), another one showed one copy of Marker 4 (13q+) and two copies of Marker 2 (7 of the 20 cells) and the remaining 7 cells had two copies of Marker 2 and two copies of marker 4.

The karyotypes of the clones were:

Clone 1: 47,X,-Y,1p-,6q-,11q-,+12,+13q+

Clone 2: 48,X,-Y,1p-,6q-,+6q-,11q-,+12,+13q+

Clone 3: 49,X,-Y,1p-,6q-,+6q-,11q-,+12,+13q+,+13q+ (Fig. 4).

Bone Marrow Cells

Though the marrow cells at the T-cell lymphoma stage of this patient were reported as having a normal male karyotype (12), reexamination of slides on cells obtained on October 19, 1977 and January 24, 1978 revealed the 11q- and 13q+ observed in the ML cells (Fig. 5). No metaphases were obtained from the January 31, 1978 sample. Cytogenetic study on June 9, 1978 revealed 2 of 23 cells to contain 48 chromosomes; the remaining 21 cells had a normal karyotype. The 5th chromosome examination revealed 1 of 19 cells to have a normal karyotype; the remaining 18 cells had 47 or 48 chromosomes.
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Fig. 4. Q-banded karyotype of an ML-3 cell showing an extra copy of 6q− (m2). Chromosome nos. 16, 18, and 21 were missing from this metaphase. A short arrow points to the 11q−, and long arrows point to the 13q+ chromosomes.

As reported previously (12), conventional Giemsa staining showed the chromosome changes to consist of an extra C-group and two extra D-group chromosomes and a missing G-group chromosome, i.e., 48,+C,+2D,−G. Further analysis with Giemsa-banding showed that the extra C-group chromosome was a result of trisomy of chromosome no. 12, and that the two extra D-group chromosomes were possibly due to two extra no. 13q+s. The missing G-group chromosome was, in fact, due to a missing Y-chromosome. Moreover, structural cytogenetic changes were present i.e., 1p−, 6q−, and 11q− (Fig. 6). Thus, the chromosome changes of the cells in the acute myeloid leukemic phase of this patient were interpreted as 48,X,−Y,1p−,6q−,11q−,+12,+13q+,+13q+. The breakpoints of the 1p− and 6q− were difficult to identify, since the banding quality of the metaphases was not optimal.

DISCUSSION

The patient from whose cells the ML cell lines were derived developed acute myeloid leukemia within 2 months after an initial remission of a T-cell lymphoma. This, taken in conjunction with the cytogenetic findings, which were not characteristic of secondary leukemia, makes it difficult to consider the myeloid leukemia of this patient occurring as a secondary event following therapy for the lymphoma. In fact, it was this consideration which made us reanalyze the initial bone marrow specimens using improved banding techniques. Cytogenetically, the marrow cells when the patient was diagnosed as having T-cell lymphoma showed the 11q− and 13q+; the leukemic cells in the acute myeloid leukemia phase contained structural and numerical changes in addition to the 11q− and 13q+. Though it was difficult to identify clearly the extra chromosomal material on the terminal portion of the long arm of chromosomes no. 13 in the ML cells, it was present in the leukemic cells of the T-cell lymphoma phase and of the acute myeloid phase. Since we reexamined old slides, the chromosome conditions were not optimal for banding analyses. However, the findings indicate that the numerical (−Y and +12) and structural (1p− and 6q−) changes in the myeloid leukemic cells occurred additionally; the neoplastic cells in the T-cell lymphoma and in the acute myeloid leukemia phases unquestionably originated from the same clone containing the 11q−. Thus, the myeloid leukemic cells of this patient probably developed from the T-cells, though with the appearance of additional cytogenetic changes.

Chromosome changes in ML-1 and -3 cell lines have been reported previously by Pelicci et al. (8). Their assignment of the karyotypic changes was slightly different from ours, excluding the partial deletion of the long arm of chromosome no. 6. They interpreted the 1p− marker chromosome (Marker 1 in our material) as a centromeric recombination between chromosomes nos. 1 and 14; they did not describe the changes of chromosome nos. 11 and 13, and the 14q− (Marker 5). Moreover, our cytogenetic study revealed that the ML-2 cells had two extra copies of Marker 4 (13q+) and that the ML-3 cells showed a further cytogenetic change, i.e., +6q−. The ML lines were established from cells of the patient when he was in the acute myeloid leukemia phase and have a capability to differentiate into granulocytes or monocytes (10, 11). Recently, Palumbo et al. (18) have cited a possible relationship between the rearrangement of the JH immunoglobulin chain locus in ML cells and the patient's T-cell lymphoma. As mentioned previously, on a cytogenetic basis, the cells in both the T-cell lym-
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Fig. 5. Conventionally (Giemsa) stained marrow metaphase (A) of patient obtained on October 19, 1977, at which time he was diagnosed as having a T-cell malignant lymphoma. The arrows point to an 11q− chromosome and to a 13q+ (top left). Top right, Q-banded karyotype of the same metaphase after destaining with 25% acetic acid. The arrows point to an 11q− (breakpoint may be at 11q23) and to a 13q+. Below the metaphases are shown partial Giemsa and Q-banded karyotypes (four rows under B) of the same metaphase, showing a terminal deletion of the long arm of chromosome no. 11 and a 13q+ (middle). C, chromosomes no. 11 with (bottom left) and without the 11q− (bottom right).

Phoma and in the myeloid leukemia phases appeared to have originated from the same clone.

Another interesting feature of the cytogenetic findings in the leukemic cells of this patient and in the ML cells is the 6q− change, which is not a common chromosome change in acute nonlymphocytic leukemia (2). In fact, 6q−, especially the deletion at bands 6q21−q23, is usually seen in lymphoid neoplasias (19−21). Interestingly, a marker chromosome originating from chromosome no. 6 with a deletion of the long arm has been described in the MOLT-4 cell line (22), which was derived from a T-cell malignant lymphoma and whose cells express a high level of c-myb mRNA (23). Recently, the c-myb oncogene has been mapped to band 6q23 (24), suggesting an association between the cytogenetic change of 6q−, lymphoid malignancy, and amplification of c-myb. The ML cells have been also shown to express a high level of c-myb mRNA (8, 25); the ML lines were established from the patient’s blood cells when he was diagnosed as having acute myeloid leukemia, and the 6q− change appeared de novo during that phase. However, the neoplastic cells probably originated from T-cells, as mentioned above, and the 6q− change in this case might be conceivably part of a karyotypic evolution in prior T-cell lymphoma cells. This finding might suggest that further studies are needed to resolve a possible relationship between the 6q−, lymphoid neoplasia (especially T-cell neoplasia), and the expression of the c-myb oncogene.
REFERENCES

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