De Novo Appearance of the Ph\(^1\) Chromosome in a Previously Monosomic Bone Marrow (45,XX,-6): Conversion of a Myeloproliferative Disorder to Acute Myelogenous Leukemia

By Gertrude Kohn, Noga Manny, Amiram Eldor, and Maimon M. Cohen

Bone marrow examination of a patient with a myeloproliferative disorder revealed monosomy for chromosome No. 6 (45,XX,-6). Two months later, during blastic crisis, reinvestigation of the bone marrow showed the presence of the Ph\(^1\) chromosome in the previously aneuploid cell line (45,XX,-6,-22,+Ph\(^1\)). This case differs from those previously published in that the Ph\(^1\) chromosome appeared de novo during the development of frank acute myelogenous leukemia.

Many different numerical and structural chromosomal aberrations have been observed in a wide variety of neoplasias. With only a single exception (the Ph\(^1\) chromosome in chronic myelogenous leukemia), no apparent specificity exists between the cytogenetic findings and the particular type of neoplasm examined. Even such a relationship, however, is not absolute, since this marker chromosome has also been observed, albeit rarely, in patients suffering from other hematologic conditions. This paper describes a patient, originally investigated for a myeloproliferative disorder, whose bone marrow karyotype was monosomic for a group C chromosome (45,XX,-6) and who developed a Ph\(^1\)-positive clone in this original aneuploid cell line during blastic crisis.

**CASE REPORT**

NH, a 38-yr-old female, was first examined in December 1972, following referral for severe fatigue of 1 mo duration, bleeding tendency, anemia, and thrombocytopenia. On admission, physical examination revealed marked pallor and a mild purpuric rash of the extremities. The cardiorespiratory system, as well as the size of the liver, were normal; the spleen was palpable 2 cm below the left costal margin. The results of laboratory examinations during the course of her disease are given in Table I. The peripheral blood smear revealed microcytic, hypochromic red cells with anisocytosis, poikilocytosis, target cells, a few nucleated red cells, a shift to the left in the white cell series, and bizarre-looking thrombocytes. The bone marrow aspirate was hypercellular, with 29% myeloblasts, a diminished number of megakaryocytes, and mild megablastic changes in the erythroid series. The peroxidase stain of the bone marrow was negative; alkaline-resistant hemoglobin was 2% (normal, < 1.0%); hemoglobin electrophoresis revealed a normal pattern. The serum vitamin B\(_{12}\) was 1000 pg/ml. Leukocyte alkaline phosphatase (LAP) score was 72 (normal, 40–100).

These findings suggested a myeloproliferative disorder or smoldering type of acute myeloblastic leukemia. However, in the absence of conclusive evidence, further observation was warranted, and the only treatment given was repeated blood transfusions and 20–30 mg prednisone daily. The patient’s condition remained stable for 6 mo. In June 1973, she was readmitted because of a...
sudden deterioration of her condition manifested by fever, profuse bleeding tendency, marked anemia, and leukocytosis of 80,000/cu mm, with the majority being myeloblasts. The bone marrow aspirate consisted almost solely of myeloblasts, and the LAP score at this time dropped to values of 19 and 2. These findings were compatible with frank acute myeloblastic leukemia, and a combined regimen of cytosine arabinoside (150 mg/sq m/day), vincristine (1.5 mg/sq m), cyclophosphamide (150 mg/sq m/day), and prednisone (150 mg/sq m/day) was instituted. Remission was, however, not achieved despite the addition of rubidomycin (40 mg/sq m/day), and the patient expired 2 mo after her admission to the ward. Permission for autopsy was refused.

**Cytogenetic Studies**

Chromosomal preparations were obtained on several occasions both from bone marrow and peripheral blood cultures. Lymphocyte cultures were grown both with and without phytohemagglutinin (PHA) stimulation, while bone marrow cells were cultured without PHA. The cells from all cultures were harvested and slides prepared with a slight modification of the method of Moorhead et al.1 Air-dried slides were stained with Giemsa stain and examined microscopically. Chromosomal banding was demonstrated by the fluorescent staining of Lin, Uchida, and Byrnes.2

The results of the cytogenetic investigations are shown in Table 2. The first bone marrow sample showed a karyotype of 45 chromosomes lacking a member of group C, which upon fluorescent analysis was identified as chromosome 6 (Fig. 1, top row). PHA-stimulated peripheral blood lymphocytes obtained 5 days later revealed a normal female karyotype (46,XX). Two months later, with the onset of a blastic crisis, reinvestigation of the bone marrow revealed two stem lines of cells. Both lines had a modal number of 45 chromosomes and lacked the C-group chromosome; however, one possessed the Philadelphia chromosome (29%), while the other (71%) was similar to the original observation. The fluorescent pattern of the Ph¹-positive cells clearly demonstrated the deletion of a 22 chromosome with excess dull fluorescent material on the long arm of chromosome 9 (Fig. 1, bottom row). This observation is best explained by a translocation, as previously reported.3 Lymphocyte cultures stimulated with PHA also evidenced mosaicism but of a different type; 63% of the cells were normal female (46,XX), while the remaining 37%
Table 2. Results of Cytogenetic Examinations of Bone Marrow and Peripheral Blood Cultures*

<table>
<thead>
<tr>
<th>Date</th>
<th>Bone Marrow</th>
<th>Peripheral Blood</th>
</tr>
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<tbody>
<tr>
<td>April 19, 1973</td>
<td>45,XX,-6 (30)</td>
<td></td>
</tr>
<tr>
<td>April 24, 1973</td>
<td></td>
<td>+PHA 46,XX (30)</td>
</tr>
<tr>
<td>June 25, 1973</td>
<td>45,XX,-6 (20)</td>
<td>45,XX,-6,-22,+Ph(^1) (8)</td>
</tr>
<tr>
<td>June 29, 1973</td>
<td>+PHA 46,XX (16)</td>
<td>45,XX,-6,-22,+Ph(^1) (10)</td>
</tr>
<tr>
<td>July 6, 1973</td>
<td>-PHA (48 hr) 45,XX,-6,-22,+Ph(^1) (22)</td>
<td>-PHA (72 hr) 45,XX,-6,-22,+Ph(^1) (21)</td>
</tr>
</tbody>
</table>

*Figures in parentheses indicate number of cells studied.

had the karyotype of 45,XX,-6,-22,+Ph\(^1\). Non-PHA-stimulated lymphocytes, investigated 1 wk later, both after 48 and 72 hr of culture, indicated only the Ph\(^1\)-positive cells which lacked the C-group chromosome.

**DISCUSSION**

Preleukemic patients possessing any cytogenetic abnormality, be it aneuploidy or structural rearrangements, are more likely to develop frank leukemia within a few months than patients whose karyotypes are normal.\(^4\) With the exception of the Ph\(^1\) chromosome in CML, no cytogenetic finding has been consistently associated with any of the preleukemic or leukemic states, although the C group (6-12+X) has perhaps been most frequently involved.\(^5\) The application of chromosome-banding techniques has allowed the precise identification of the abnormal karyotypes in some of these conditions. The present case is the first to possess monosomy for chromosome 6. However, too few cases have been studied with banding procedures to allow definitive conclusions concern-
ing specific monosomic or trisomic states in the various myeloproliferative disorders.

Between 30% and 50% of the preleukemic cases demonstrate a variety of abnormal karyotypes in a varying percentage of bone marrow cells.4-6 On the other hand, approximately 90% of CML cases demonstrate the Ph' chromosome in their bone marrow. However, this finding has not been observed solely in CML and also occurs in rare cases of other leukemias,7-10 myeloproliferative disorders,8,11 thrombocytopenia,12 and even acute lymphatic leukemia.13

Recent work by Rowley3 has demonstrated that the Ph' chromosome is not merely a terminal deletion of 22q, as it had been considered prior to banding analysis, but in fact represents a translocation of 22q material to the long arm of chromosome 9. An additional type of translocation involving the Ph' chromosome to 2q has also been recently described.14 In our patient, the deleted portion of chromosome 22 has apparently been translocated to chromosome 9 (Fig. 1, lower line). The exact relationship of the Ph' chromosome to CML has never been satisfactorily elucidated, although its etiologic role has been presumed.

The Ph' chromosome is believed to be an acquired characteristic15,16 which is clonal in origin.17-19 To the best of our knowledge, the present case represents the first documentation of its de novo appearance in a previously Ph'-negative patient. During the initial examination of an undiagnosed hematologic problem, chromosome analysis of the bone marrow revealed cells with only 45 chromosomes and a normal G group. In all the cells, however, monosomy 6 was present. At the same time, chromosome analysis of PHA-stimulated peripheral blood lymphocytes yielded a normal female karyotype (46,XX). Such tissue mosaicism has been frequently reported in similar cases. Our patient is unique in that 2 mo after the initial Ph'-negative bone marrow was obtained, a repeat sample revealed 29% of metaphases containing a Ph' chromosome. This marker chromosome most likely evolved as a new mutation in the already aneuploid cell line (45,XX,-6) which, during the blastic crisis, was selected for and proliferated advantageously, ultimately reaching the peripheral blood. Thus, Ph'-positive 45,XX,-6 cells found in peripheral blood originated from the bone marrow, while the normal cells (46,XX) which responded to PHA were lymphocytes.

While most patients with a Ph'-positive bone marrow frequently develop aneuploidy during blastic crisis, the reverse occurred in our patient. Either a new mutation occurred, just prior to or concomitant with the onset of the blastic crisis, or the Ph' chromosome was already present at the time of the first bone marrow examination, but in too small a percentage of cells to be readily discerned. Therefore, this patient represents a case of myeloproliferative disease differing from all the other published cases in that the Ph' chromosome appeared de novo, in a previously aneuploid cell, during the appearance of frank acute myelocytic leukemia.

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