Late-Appearing Philadelphia Chromosome in Two Patients With Chronic Myeloogenous Leukemia

By R. Lisker, L. Casas, O. Mutchinick, F. Pérez-Chávez, and J. Labardini

We describe two patients with typical chronic myeloogenous leukemia, who at the beginning of the disease lacked the Philadelphia chromosome in bone marrow cells, and 90 and 42 days later, respectively, its presence was shown in all cells analyzed from that tissue. These findings are compatible with the possibility that at least occasionally Ph' occurs secondarily in already leukemic cells. The rapid change from Ph'- to Ph' + CML in one of the patients (42 days), suggests the possibility that in addition to Ph' + cells enjoying marked selective advantage, this change is induced simultaneously in multiple bone marrow cells.

FiALKOW\textsuperscript{1} has recently suggested that at least in some cases of chronic myeloogenous leukemia (CML) the Philadelphia chromosome (Ph') is not a sufficient cause of the leukemia. This hypothesis was based on the finding that Ph'-negative non-T-lymphocytes of three patients with this disease were derived from the CML clone. Two possibilities were advanced: (A) at least two steps are needed for the development of CML, the first step producing a clonal proliferation of pluripotent stem cells, and the second producing the Ph' chromosome in descendents of the pluripotent stem cell clone; or (B) the Ph' occurs secondarily in already leukemic cells. Findings in three reported patients\textsuperscript{2,3} who were Ph'-negative at the time when the diagnosis of CML was established and who subsequently developed this chromosome anomaly, are compatible with these possibilities. This article describes two similar patients.

CASE REPORTS

Case 1

A 53-yr-old male was first seen by us on March 6, 1978 with a 6-mo history of malaise and asthenia. Moderate splenomegaly was found on physical examination. The hemoglobin was 10.9 g/dl; reticulocyte count 3.0%; WBC 420,000/cu mm, with 1% blasts, 25% immature granulocytes, 63% granulocytes, 3% basophils, 4% eosinophils, and 3% lymphocytes. Three normoblasts/100 leukocytes were seen, and the platelet count was 242,000/cu mm. Bone marrow examination showed increased cellularity 3\textsuperscript{'}; 46% of immature granulocytes, 40% of adult granulocytes, 6% eosinophils, 2% lymphocytes, 5% normoblasts, and 1% pronormoblasts. The mega-karyocytes were slightly increased in number. Serum vitamin B\textsubscript{12} was 3300 pg/ml (normal 150–1500 pg/ml) and the leukocyte alkaline phosphatase was 6 (normal 30–90). The patient was started on busulfan and received a total of 498 mg in 103 days. He then received 3 doses of vincristine (1.4 mg/sq m each) during a 15-day period (June 23 to July 6, 1976) and daily prednisone (60 mg/sq m). He has taken 1 mg daily of busulfan as maintenance therapy since July 7, 1978, and at present (May 21, 1980), has normal clinical and hematologic findings.

Bone marrow cytogenetic studies were done by the technique of Tjo and Wang.\textsuperscript{4} The cells were studied directly without culture after they had been exposed to colchicine for 90 min. The results are given in Table 1. Ph' was not seen in any cells from the first two studies but was found in all subsequently tested cells. Figures 1 and 2 show representative karyotypes of the February and May studies.

G-banding (Fig. 2) showed that the missing portion of the 22 chromosome was translocated to the long arm of one member of the no. 9 pair.

Case 2

A 42-yr-old female was first seen by us on July 12, 1978, with a 3-yr history of a left upper quadrant abdominal mass that had increased in size and become painful during the last year. She also had progressive anemia, exertional dyspnea, and moderate weight loss. Physical examination revealed generalized pallor and marked splenomegaly. Hemoglobin was 10.2 g/dl; reticulocyte count 5.6%; WBC 230,000/cu mm with 3% blasts, 28% immature granulocytes, 65% adult granulocytes, and 4% basophils. One normoblast/100 leukocytes was seen, and the platelet count was 250,000/cu mm. Bone marrow examination showed cellularity increased 3\textsuperscript{'}; 32% of immature granulocytes, 52% of adult granulocytes, 1% lymphocytes, and 4% normoblasts. Megakaryocytes were slightly increased. Serum vitamin B\textsubscript{12} was 7250 pg/ml and leukocyte alkaline phosphatase was 1. She received 301 mg of busulfan over a 95-day period and experienced a complete clinical remission (normal clinical and hematologic findings) until March 1980 when she relapsed with 86,500 WBCs and an enlarged spleen. Response to busulfan treatment has been good, WBC being 24,650 on May 17, 1980.

Bone marrow cytogenetic studies were done with the same technique as in case 1, and the results are given in Table 1. Ph' was absent in cells from the first two investigations, and a G-banded karyotype from the February study is shown in Fig. 3. Figure 4 shows a karyotype (conventional stain) representative of the cells from the third study, clearly showing the Ph' chromosome. In the left lower side of the figure, a partial G-banded karyotype of another cell shows the 9:22 translocation is added.

DISCUSSION

The clinical and laboratory findings and the courses in the patients reported here are typical of those found in the Ph'-positive type of CML.\textsuperscript{5,6} However, there is good evidence in both patients that during the initial phase of the disease, Ph' was absent in at least the
TWO PATIENTS WITH LATE-APPEARING Ph'

Table 1.

<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Date</th>
<th>Marrow Cell Karyotypes (No. Cells)</th>
<th>Stain</th>
<th>Clinical and Hematologic Status</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>10/16/1978</td>
<td>46,XY (11)</td>
<td>Conventional</td>
<td>Clinical remission</td>
</tr>
<tr>
<td></td>
<td>2/23/1979</td>
<td>46,XY (11)</td>
<td>Conventional</td>
<td>Complete remission</td>
</tr>
<tr>
<td></td>
<td>5/23/1979</td>
<td>46,XY,Ph' (8)</td>
<td>G-bands</td>
<td>Complete remission</td>
</tr>
<tr>
<td></td>
<td>7/3/1979</td>
<td>46,XY,Ph' (11)</td>
<td>Conventional</td>
<td>Complete remission</td>
</tr>
<tr>
<td></td>
<td>8/10/1979</td>
<td>46,XY,Ph' (8)</td>
<td>G-bands</td>
<td>Complete remission</td>
</tr>
<tr>
<td></td>
<td>5/23/1979</td>
<td>45,XY,–20 Ph' (1)</td>
<td>Conventional</td>
<td>Complete remission</td>
</tr>
<tr>
<td>2</td>
<td>2/22/1979</td>
<td>46,XX (25)</td>
<td>Conventional</td>
<td>Complete remission</td>
</tr>
<tr>
<td></td>
<td>3/16/1979</td>
<td>46,XX (8)</td>
<td>G-bands</td>
<td>Complete remission</td>
</tr>
<tr>
<td></td>
<td>4/27/1979</td>
<td>46,XX,Ph' (11)</td>
<td>Conventional</td>
<td>Complete remission</td>
</tr>
<tr>
<td></td>
<td>46,XX,Ph' (2)</td>
<td></td>
<td>G-bands</td>
<td>Complete remission</td>
</tr>
</tbody>
</table>

*One cell had lost a C group chromosome and the other lacked an F group chromosome.
†Only 10 of the 25 cells were karyotyped, the rest were carefully analyzed under the microscope.
‡Several cells had random loss of different chromosomes, but all showed Ph'.

The majority of dividing marrow cells. In fact, taking into consideration the number of cells analyzed in the first two studies, we can exclude at the 95% confidence level possible mosaicism for a Ph'-positive cell line of 12% and 4% of the mitosis in each patient, respectively. In case 1, we could not obtain adequate G-banded metaphases at this stage of the disease, but conventional stains shows the G group autosomes to be of equal size (Fig. 1). The G-banding studies performed in the second case leave little doubt that Ph' is not present initially (Fig. 3).

Similarly, there is little doubt that Ph' appeared subsequently in both patients. It was first noted in case 1 on May 23 (Fig. 2), and we have seen it in each of the 28 metaphases analyzed since that time. Although the chromosome preparations in case 2 were technically not optimal, there is no question that Ph' is present.

The transition time from Ph'-negative to Ph'+ positive CML in case 1 occurred in 90 days, and in case 2 in 42 days. In two of the previously reported similar cases, the time between when the marrow was last noted to be Ph'— and first noted to be Ph'+ was 4 yr, but there were no intermediate studies. This information was not available for the third case. The rapid change from Ph'— to Ph'+ marrow cell types (42 days) suggests that Ph'+ cells have marked selective advantage, although it is also possible that Ph' occurs initially in multiple cells simultaneously. It should be pointed out that, assuming a Ph'+ cell doubling time of 24 hr, no cell death, and absence for whatever reason of Ph'— cell growth 42 days after the appearance of a single Ph'+ cell, the number of such cells would be of approximately $4 \times 10^{12}$. Considering that the normal number of hematopoietic marrow cells is between 14 and 18 x $10^9$/kg of body weight a 70-kg man should have around $3 \times 10^4$ cells. Thus, the

![Fig. 1. Normal bone marrow karyotype of patient 1 obtained in February 1979.](image1)

![Fig. 2. G-banded bone marrow karyotype of patient 1 obtained in May 1979. Arrows indicate the Ph chromosome and the translocated no. 9 chromosome.](image2)
findings suggest that in this patient, Ph' was induced in multiple cells simultaneously.

An alternative possibility to explain why both patients had a late-appearing Ph' chromosome would be that the disease started in the spleen and produced peripheral blood changes and clinical disease before extensive bone marrow involvement. We believe this is an unlikely possibility in view of the fact that since the initial bone marrow studies, both patients had clearly abnormal cytologic findings and absence of the Ph' (Table 1).

REFERENCES


Fig. 3. Normal G-banded bone marrow karyotype of patient 2 obtained in February 1979.

Fig. 4. Bone marrow karyotype of patient 2 obtained in April 1979, showing the Ph chromosome with conventional stain. The last line is a partial G-banded karyotype of pairs 9 and 22, confirming the above finding.
Late-appearing Philadelphia chromosome in two patients with chronic myelogenous leukemia

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