Chromosome Analysis of Spleen and/or Lymph Nodes of Patients With Chronic Myeloid Leukemia (CML)

By C. Stoll, F. Oberling, and E. Flori

Origin and spread of the chromosomally abnormal cells that appear in chronic myeloid leukemia (CML) after transformation are unknown. Spleen and lymph node may be involved. In 16 patients with CML splenectomy and/or adenectomy were performed before or during the blastic crisis of the disease, followed by a chromosomal analysis of the cells from the removed organ. At the same time, the chromosomes of the blood cell and of the bone marrow were also analyzed. Analyses were done with R banding. The results show that an extramedullary clonal development with duplication of the Ph1 chromosome and other features occurred. From a cytogenetic standpoint, acute blastic phase of CML is frequently characterized by an increased number of chromosomes owing to preferential gain of additional chromosomes. This, then, would clearly point to extramedullary acute transition in CML.

The Philadelphia chromosome (Ph1) is characteristic of the bone marrow of patients with chronic myeloid leukemia (CML) in the chronic phase of the disease.1 The transformation into the blastic phase is accompanied by additional chromosomal abnormalities in the bone marrow mitosis of two-thirds of the patients.2 In CML, before blastic transformation the spleen contains Ph1-positive cells.3,4 After transformation additional chromosomal changes appear.5 However, the origin and spread of the chromosomally abnormal cells are unknown. The appearance of the aberrant cells in the spleen or other sites of extramedullary hematopoiesis is not well known.6 Simultaneous cytogenetic analysis of bone marrow and extramedullary hematopoietic tissue may shed some light on the question. For this reason we studied chromosomes in the bone marrow and also in the spleen and/or lymph nodes of patients with CML.

MATERIALS AND METHODS

Sixteen patients with CML were studied (Table 1). Nine of them had their spleen removed during the chronic or blastic phase. Lymph nodes of six other patients were removed during the blastic phase. In one patient the lymph node and spleen were removed during the blastic phase. Chromosome analyses were performed on the removed organs, on bone marrow, and on blood. The blast phase was thought to exist when myeloblasts in the marrow exceeded 20%, and/or when the combined percentage of the myeloblasts and of the promyelocytes in the marrow was above 30%.

Cytogenetic technique. Blood and bone marrow cells were obtained through conventional venous or sternal puncture. Blood was put in a tube containing 0.1 ml stock heparin and centrifuged. The plasma and the buffy coat were aspirated, and 1 ml of this suspension was distributed into each of three tubes containing McCoy 5a medium. If the patient’s WBC count was greater than 10,000/mm³, less than 1 ml of the suspension was added to the medium.
The cultures were incubated at 37°C for 24, 48, or 72 hr; 0.1 ml PHA was added only to the 72-hr culture in order to obtain the constitutional karyotype of the patient. At 22, 46, or 70 hr 0.1 ml Velban (0.5 μg/ml) was added to the respective flasks and allowed to incubate for 45 min. After having been centrifuged the cells were incubated in hypotonic KCl (0.075 M) for 17 min. After centrifugation cells were resuspended in about 10 ml freshly made fixative of ethanol-glacial acetic acid-chloroform (6:1:3). Two subsequent changes were done with a 3:1 ethanol-glacial acetic acid fixative. Three to five drops of the suspension obtained after centrifugation were dropped onto ice-cooled slides. Slides were air-dried.

For bone marrow 0.5-2 ml bone marrow was collected in a conical centrifuge tube containing 5 ml Hanks' solution, 0.1 ml heparin, and 0.1 ml Velban. The procedure was then the same as for blood except that the cells were incubated in hypotonic KCl for 20 min.

For spleen and lymph node the procedure was as follows: Immediately after the organ was removed, a small piece of each organ was cut off and the cells were obtained by filing the tissue above a Petri dish containing basal medium (Eagle) with human blood cord serum (9:1) and 0.05 γ-colchicine/per ml. The cell suspension was allowed to stand for 2 hr at 37°C and centrifuged for 7 min at 1000 rpm; the supernatant was then removed and the cells were resuspended in 0.075 M prewarmed KCl for 15 min. The cells were then recentrifuged, the supernatant was poured off, and the cells were resuspended in fixative (ethanol:acetic acid 3:1) at room temperature for 45 min. The cell suspension was centrifuged again and the procedure repeated for 15 min. The slides were then made. Wet ice-cold slides were prepared by dropwise addition of the cell suspension.

R banding was performed by following the usual procedure. For every patient at least 100 mitoses were studied, except for patients 3 and 5, where only 49 and 42 mitoses, respectively, could be found.

RESULTS
See Table I and Figs. 1–7. In eight patients (1–4, 7–9, and 14 an initial bone marrow examination in the untreated chronic phase could be performed. All patients had 80%-100% Ph1-positive diploid cells without other chromosomal abnormalities. In three patients, two of them (patients 1 and 3) still in the chronic stage, no change was seen on subsequent examination of bone marrow (patients 1, 3, and 8) and of spleen or lymph node (patients 1 and 8). In patients 2–4, 7, 9, and 14, in spleen or lymph node additional abnormalities appeared. In these six patients a chromosomal difference was found between bone marrow and spleen or lymph node in the course of the disease.

Eight patients (5, 6, 10–13, 15, and 16) were examined for the first time during the blastic phase. In two of them (patients 5 and 6) no difference between bone marrow and spleen or lymph node was seen. In the other six a chromosomal discrepancy between bone marrow and spleen or lymph node was seen.

DISCUSSION
The same frequency of Ph1-positive cells in both bone marrow and spleen was found only in one patient (patient 1) in the chronic stage of the disease. In some patients (nos. 4–6, 9, and 16) a much higher frequency of Ph1-positive cells, with additional chromosomal changes, was found in the spleen or in the lymph nodes than in the bone marrow examined at the same time.

In 2 of the 8 patients seen for the first time during the blastic stage of the disease no chromosomal difference between bone marrow and spleen or lymph node was noted. In the other 6 patients, however, a divergence between these tissues appeared. A chromosomal divergence between bone marrow and spleen or lymph node was noted in 12 of our 16 patients. Clinically the course of the
<table>
<thead>
<tr>
<th>Patient No.</th>
<th>Phase</th>
<th>Blood (Without PHA)</th>
<th>Bone Marrow (Direct Preparation)</th>
<th>Tissue (Direct Preparation)</th>
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<td>C</td>
<td>ND</td>
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<td>C</td>
<td>46,XY,Ph&lt;sup&gt;1&lt;/sup&gt;</td>
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<td>16</td>
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<td>S:46,XY,inv(7),Ph&lt;sup&gt;1&lt;/sup&gt;</td>
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Percentage of cells not 46, XX or XY, Ph<sup>1</sup> in parentheses. C, chronic; B, blastic. ND, not done. S, spleen; L, lymph node.
Fig. 1. Patient 4, bone marrow: 49, XY, −15, +8, +13, Ph, +i(22q−), t(9/15).

disease had worsened from the moment when the abnormal clones appeared in the bone marrow; thus the appearance of the chromosomal aberrations preceded clinical and morphologic signs of blastic transformation in the bone marrow and in the peripheral blood.

Our results indicate that in CML the spleen and lymph nodes may play a
significant role in the transformation of the chronic phase into the blastic phase. Our material is so far quite limited, but results obtained by others support our conclusions. In some patients only Ph-positive cells were seen in the bone marrow, whereas in the spleen (in addition to Ph) all cells contained a large marker chromosome of C-group size. Serial chromosomal analyses of

**Fig. 2.** Patient 4, spleen: 51, XY, −15, +5, +8, +13, 2Ph, +(22q−), t(9/15).
aspirates from bone marrows and spleens of patients with CML followed from the chronic phase until blastic transformations were done. The results suggested that the spleen may be more important than the bone marrow for the further karyotypic evolution of Ph'-positive cells that announce the blastic crisis. Our data support this statement. Comparative cytologic studies of bone

Fig. 3. Patient 10, bone marrow: 47, XX, +8, i(17q), Ph'.

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marrow and extramedullary hematopoietic tissue in CML\textsuperscript{10} showed a predominance of cells in the spleen prior to blastic transformation in the bone marrow. Megaloblastoid changes are more common in the spleen than in the bone marrow, and basophilic leukocytes are more numerous in the spleen too. Kaur et al.\textsuperscript{11} reported an increase of T lymphocytes in the spleen in CML.

Fig. 4. Patient 10, spleen: 47, XX, -6, +7, +8, i(17q), Ph\textsuperscript{1}. 
whereas in normal spleens there is a predominance of B lymphocytes. These studies reinforce the cytogenetic data on the role of the spleen in the pathogenesis of CML. Hossfeld analyzed karyotypes of lymph nodes when the classical picture with only diploid Ph-positive mitoses of CML was seen in blood and bone marrow; he found hyperdiploidy in lymph nodes. Baccarani et
al. showed that liver myeloid cells in one case were more frequently aneuploid than marrow cells and carried a significantly higher number of the cytogenetic abnormalities commonly seen in late CML.

Very little is known about the role that other sites of extramedullary haematopoietic tissues play in the origin and spread of blastic crisis. Since 1974

![Image of chromosomes]

**Fig. 6.** Patient 16, bone marrow: 46, XY, Ph1.
it has been known that involvement of lymph nodes may precede cytologic signs of blastic crisis in the bone marrow. Our karyotype analyses of lymph nodes support this statement. Hossfeld's findings agreed with ours. Kilmann drew the same conclusions in wound tissue. Kwaan et al. showed that meningeal involvement may be the first manifestation of blastic crisis.

Fig. 7. Patient 16, spleen: 46, XY, inv(7), Ph1.
These findings suggest the possibility of a primary origin of the blastic crisis in extramedullary tissue, but it is also possible that the other extramedullary organs are colonized by already transformed bone marrow cells.

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