The Distribution of the Philadelphia Chromosome in Patients with Chronic Myelogenous Leukemia

By JACQUELINE WHANG, EMIL FREI III, J. H. Tjio, PAUL P. CARBONE AND GEORGE BRECHER

THE ASSOCIATION of a variety of chromosome abnormalities with human leukemias has been well established. The discovery of the Philadelphia chromosome is of particular significance because it is the first instance in which there is an association of a specific chromosome abnormality with a particular type of leukemia. The Philadelphia (Ph¹) chromosome is present in the marrow of most patients with chronic myelogenous leukemia, but has not been found in conditions resembling chronic myelogenous leukemia such as leukemoid reactions, polycythemia vera, agnogenic myeloid metaplasia or myelofibrosis.1⁵

It has been presumed that the Ph¹ chromosome occurs uniquely in leukemic cells, since it has not been found in cultured skin from patients with chronic myelogenous leukemia.⁴ Two recent preliminary reports suggest, however, that it may occur in erythroid precursors and megakaryocytes of such patients.⁵⁻⁷ The present report confirms and extends these findings.

MATERIAL AND METHODS

Patients selected for the present study were drawn from 24 patients with chronic myelogenous leukemia (CML) treated at the Clinical Center between May, 1961 and December, 1962. Eighty per cent of 32 drug trials in these 24 patients resulted in good clinical remissions with reduction in spleen size and return of the peripheral white blood cell count to normal.⁸ In 13 of these patients, erythroid precursors in the marrow which had represented less than five per cent of nucleated cells prior to treatment, increased to between 25 and 60 per cent and these patients form the basis of the present report.

Chromosomes of marrow cells were studied without prior in vitro culture in air dried preparations.⁹ Peripheral blood was cultured for 72 hours and preparations made according to the technic of Moorhead et al.¹⁰ The proportion of metaphases containing the Ph¹ chromosomes in each sample was scored independently by two of us (J. W. and J. H. T.). The percentage of erythroid precursors among nucleated marrow cells and the relative frequency of erythrocytic and granulocytic mitoses were determined in direct smears of marrow aspirates stained with Giemsa. Mitoses were classified as granulocytic if promyelocytic or myelocytic granules were present in the cytoplasm and as erythrocytic if the cytoplasm showed the homogeneous appearance and the deeply basophilic, polychromatic or orthochromatic staining characteristic of nucleated erythroid cells. Eighty per cent of cells in mitoses could usually be classified on this basis. Because of the importance of the correct identification of cells in mitoses, bone marrow aspirates in three patients were also studied under the ultraviolet microscope to determine presence of hemoglobin in dividing cells by absorption in the Soret band. A mercury arc monochromator was used for illumination at 2650 A and 4100 A. A Bausch and Lomb UV converter which utilized the RCA conversion tube was used for searching of the unfixed and unstained marrow preparation and

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for focusing. Mitoses were identified at 2650 Å where DNA absorption is pronounced. The monochromator was then turned to 4100 Å to determine the presence or absence of hemoglobin in the particular cells. Photographs were taken directly on Eastman Spectral Analysis film No. 1 (figs. 1 and 2).

RESULTS

Data concerning the 15 bone marrow studies on the 13 patients selected are presented in table 1. The number of metaphases scored ranged from 31 to 143 (median, 96). In 11 of the 14 marrows studied, 100 per cent of the metaphases scored contained the Ph¹ chromosome. Since 25 to 60 per cent of the nucleated cells were of the erythroid series and, more particularly, since 30 to 80 per cent of the cells in mitoses were of the erythroid series in the 11 marrow samples from 10 of the 13 patients, it is concluded that nucleated erythroid cells contain the Ph¹ chromosome. The identification of erythroid precursors in mitosis by measurement of cytoplasmic absorption due to hemoglobin in the Soret band (4100 Å) in three instances (M. S. 12-6-62, M. B. 12-17-62, and H. A. 2-27-63) resulted in mitotic differentials which were similar to those obtained from Giemsa-stained smears. With the UV spectrophotometer, 22 of 53 (42 per cent) of the mitoses in H. A. were erythroid, that is, cytoplasmic absorption occurred at 4100 Å. In the Giemsa-stained preparation, 18 of 50 (36 per cent) of the mitoses were considered erythroid, 29 myeloid, and 3 unclassified. In the other two cases (M. S. and M. B.), over 60 per cent of the marrow cells in mitosis absorbed at 4100 Å (fig. 1), which is in agreement with the mitotic differential on Giemsa-stained material (table 1).

In a number of chromosome preparations, polyploid cells in mitoses contained the Philadelphia chromosome. In each case the number of Philadelphia chromosomes corresponded to the ploidy of the cells, i.e., 2, 4, and 8 Ph¹ chromosomes were present in 4n, 8n, and 16n cells (figs. 3–5). These polyploid cells are apparently the result of endoreduplication. Polyploid mitoses suggest origin from megakaryocytes, but additional studies will be necessary to exclude the possibility that polyploid cells of the granulocytic series or multinucleated cells such as osteoclasts may be responsible for the finding.

It has been our experience, as has been well documented by Sandberg et al.,¹ that the incidence of Ph¹ chromosomes in peripheral blood cultures is significantly lower than in direct marrow preparations. Since it is now recognized that lymphocytes are normally the major source of proliferating cells in the peripheral blood under conditions of culture,¹¹ the presence of normal mitoses in the culture of peripheral blood might be ascribed to the absence of Ph¹ chromosomes from the lymphocytes in these patients. To explore this possibility, parallel chromosome determinations were done in marrow and peripheral blood cultures in patients undergoing treatment (table 2). In six patients in relapse with 40,000 to 187,000 white blood cells per cu. mm., the median incidence of Ph¹ chromosomes in culture was 78 per cent and patients with a high per cent of immature forms had the higher incidence of Ph¹ chromosomes. In four analyses of three treated patients in whom the WBC count was below 8,000 and immature granulocytes below 1 per cent, the in-
Table 1.—Distribution of the Philadelphia Chromosome (Ph') in Bone Marrow Cells

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sex</th>
<th>Date of Marrow</th>
<th>Nucleated RBC/Total Nucleated Cells (per cent)</th>
<th>Metaphases Scored</th>
<th>Mitotic Differential</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>Total</td>
<td>No. with Ph' Chromosome (per cent)</td>
<td>No. mitoses analyzed</td>
</tr>
<tr>
<td>G. H.</td>
<td>F</td>
<td>6-17-62</td>
<td>60</td>
<td>107</td>
<td>107 (100%)</td>
</tr>
<tr>
<td>R. C.</td>
<td>M</td>
<td>6-8-62</td>
<td>40</td>
<td>110</td>
<td>110 (100%)</td>
</tr>
<tr>
<td>M. S.</td>
<td>M</td>
<td>6-10-62</td>
<td>55</td>
<td>63</td>
<td>63 (100%)</td>
</tr>
<tr>
<td>M. S.</td>
<td>M</td>
<td>12-6-62</td>
<td>40</td>
<td>130</td>
<td>106 (88%)</td>
</tr>
<tr>
<td>B. F.</td>
<td>M</td>
<td>6-12-62</td>
<td>28</td>
<td>62</td>
<td>62 (100%)</td>
</tr>
<tr>
<td>M. O.</td>
<td>M</td>
<td>9-12-62</td>
<td>20</td>
<td>107</td>
<td>106 (99%)</td>
</tr>
<tr>
<td>R. B.</td>
<td>M</td>
<td>8-17-62</td>
<td>22</td>
<td>64</td>
<td>64 (100%)</td>
</tr>
<tr>
<td>L. M.</td>
<td>M</td>
<td>7-3-62</td>
<td>37</td>
<td>31</td>
<td>31 (100%)</td>
</tr>
<tr>
<td>W. B.</td>
<td>M</td>
<td>9-13-62</td>
<td>25</td>
<td>37</td>
<td>37 (100%)</td>
</tr>
<tr>
<td>M. B.</td>
<td>F</td>
<td>9-24-62</td>
<td>48</td>
<td>85</td>
<td>85 (100%)</td>
</tr>
<tr>
<td>M. B.</td>
<td>F</td>
<td>12-17-62</td>
<td>33</td>
<td>110</td>
<td>110 (100%)</td>
</tr>
<tr>
<td>E. D.</td>
<td>M</td>
<td>8-20-62</td>
<td>52</td>
<td>143</td>
<td>141 (98%)</td>
</tr>
<tr>
<td>W. R.</td>
<td>M</td>
<td>6-19-62</td>
<td>25</td>
<td>129</td>
<td>129 (100%)</td>
</tr>
<tr>
<td>F. S.</td>
<td>M</td>
<td>7-17-62</td>
<td>38</td>
<td>86</td>
<td>84 (98%)</td>
</tr>
<tr>
<td>H. A.</td>
<td>M</td>
<td>2-27-63</td>
<td>28</td>
<td>74</td>
<td>74 (100%)</td>
</tr>
</tbody>
</table>

*Per cent of mitoses analyzed.
Incidence of Ph1 chromosomes in blood cultures was between 0 and 2 per cent, although 80-100 per cent of mitoses in direct marrow preparations had the Ph1 chromosome.

DISCUSSION

The evidence, although indirect, appears conclusive that the Ph1 chromosome occurs in the erythroid as well as granulocytic cells of patients with chronic myelogenous leukemia. The observation previously reported5,7 and
confirmed here that polyploid cells often contain the Ph\(^1\) chromosome suggests but does not prove that it may also be present in megakaryocytes. It appears unlikely that the Ph\(^1\) chromosome arises simultaneously in different cell types. The present findings, therefore, suggest that the Ph\(^1\) chromosome usually arises in a stem cell common to the erythrocytic and granulocytic, and perhaps also the megakaryocytic, series and that this abnormality in genetic material of one of the small No. 21–22 chromosomes imparts some proliferative advantage to these cells.

It has been suggested that erythroid cells, particularly normoblasts, frequently may not present scoreable metaphases because their chromosomes do not spread well.\(^{11}\) In our experience the proportion of metaphases which were not scoreable was 15–30 per cent and did not vary with the proportion of erythroid cells. This was further substantiated by an incidental observation in a patient with CML without the Ph\(^1\) chromosome. During a brief period this
Fig. 4.—Metaphase of a tetraploid bone marrow cell from a CML patient, prepared without prior in vitro culture. There are two Ph' chromosomes.

patient's peripheral blood contained 600 normoblasts per cu. mm. with a high incidence of mitosis. Since there were no immature dividing granulocytes present in the peripheral blood, this afforded an opportunity to analyze a purely erythroid population of normal karyotype. Again the non-scoreable metaphases were of the order of 20 per cent. It is concluded that the nucleated erythroid cell, whether it carried the Ph' chromosome or not, was appropriately represented in the category of “metaphases scored.”

The discrepancy between the consistently high incidence of Ph' chromosomes in direct marrow smears both in relapse and remission noted in this series of cases and the much more variable percentages reported by others may be more apparent than real. In many reported cases the total number of mitoses counted was small, and the number of unclassified mitoses high, so that the relative incidence of normal and abnormal karyotypes cannot be estimated with precision. It is also possible, however, that we have accidentally observed a consecutive series of cases in which the Ph' chromosome origi-
Fig. 5.—Metaphase of an octoploid bone marrow cell from a CML patient, prepared without prior in vitro culture. There are four Ph\(^1\) chromosomes.

nated in a common precursor and replaced all the other marrow cells. We cannot exclude the existence of cases in which the cells with Ph\(^1\) chromosomes may fail to outgrow all other marrow cells, so that the percentage of cells with Ph\(^1\) chromosomes may vary in both the granulocytic and erythroid series. Alternatively, the Ph\(^1\) chromosome may originate in a precursor already differentiated toward the granulocytic series. In such a case, an increase in erythroid cells during remission should lead to a corresponding reduction in the incidence of Ph\(^1\) chromosomes in the marrow. Such a sequence has indeed been claimed for one of the 12 cases recently reported by Fitzgerald et al.\(^{11}\) Our data, however, would indicate that such cases are infrequent.

With the exception of the above mentioned case of Fitzgerald et al., published data are in accord with our observations, that presently used treatment schedules do not affect the incidence of Ph\(^1\) chromosomes in the marrow significantly.\(^9,12\) It is only when peripheral blood cultures are used that
such an effect may be discernable. As already emphasized by Fitzgerald et al., this effect is not dependent on treatment per se, but rather on the lowering of the WBC count and the disappearance from the blood of immature granulocytes capable of division. Differences in the apparent effect of treatment and disparate interpretation in the literature appear due to inclusion of treated cases in which the WBC count remained high and immature granulocytes persisted or reappeared after treatment.

The virtual absence of the Ph' chromosome from cultures of peripheral blood which do not contain immature myeloid cells supports the concept that proliferating cells in cultures of the peripheral blood are normally derived from lymphocytes and that lymphocytes do not carry the Ph' chromosome in patients with chronic myelogenous leukemia. The presence of Ph' chromosomes in cultures is presumably due to mitoses in promyelocytes or myelocytes, which appear in numbers in the peripheral blood during relapse. As noted by others, the incidence of Ph' chromosome in the blood cultures is frequently lower than in the marrow, even during relapse, which is in accord with the continued presence of a small percentage of lymphocytes in such blood.

**SUMMARY**

Thirteen patients with chronic myelogenous leukemia continued to have the Ph' chromosomes in 90-100 per cent dividing marrow cells during drug-induced clinical remissions. The Ph' chromosome was present in erythroid as well as granulocytic marrow cells, and possibly in megakaryocytes.

The presence of Ph' chromosomes was also studied in cultures of peripheral blood. In six patients in relapse, 40 per cent of metaphases contained the Ph' chromosome, and the percentage of these cells corresponded roughly to the relative frequency of immature granulocytes in the blood. In contrast, during remission, few or no Ph' chromosomes were found in peripheral blood cultures, presumably because in the absence of immature granulocytes the dividing cells in the cultures originate from lymphocytes, as they do in normal blood.

It is suggested that the Ph' chromosome usually arises in a precursor cell common to the erythroid, granulocytic, and megakaryocytic, but not the lymphoid series of hematopoietic cells.
Summario in Interlingua

Dece-tres patientes con chronic leucemia myelogene continuava monstrar chromosemas Ph1 in 90 a 100 pro cento de lor medullari cellulas in division durante periodos de pharmacogenic remissiones clinic. Le chromosoma Ph1 esseva presente in cellulas medullari tanto erythroide como etiam granulocytic e possibilemente etiam in megacaryocytos.

Le presentia de chromosomas Ph1 esseva etiam studiate in culturas de sanguine peripheric. In sex patientes in recidiva, 40 pro cento del metaphases contineva le chromosoma Ph1, e le procentage de iste cellulas correspondeva plus o minus al relative frequentia de immatur granulocytos in le sanguine. Del altere latere, durante remissions, pauc o nulle chromosomas Ph1 esseva trovate in culturas de sanguine peripheric, presumitemente proque in le absentia de immatur granulocytos le cellulas in division in le culturas veni ex inter le lymphocytes, como il es etiam le casco in sanguine normal.

Es postulate le these que le chromosoma Ph1 se presenta usualmente in un cellula precursori commun al serie erythroide, granulocytic, e megacaryocytic sed non al serie lymphoide de cellulas hematopoietic.

Addendum

Since the submission of this manuscript for publication, Tough et at. have reported in the April 20, 1963 issue of Lancet independent observations that the Ph1 chromosome is present in the erythroid and megakaryocytic, as well as the granulocytic, cells of patients with CML.

References


11. Fitzgerald, P. H., Adams, A., and Gunz,
THE PHILADELPHIA CHROMOSOME IN CML


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