The Philadelphia Chromosome in an Unusual Case of Myeloproliferative Disease

By CLARK W. HEATH, JR. AND WILLIAM C. MOLONEY

RECENT INVESTIGATIONS have shown that the Philadelphia (Ph1) chromosome is present in nearly all typical cases of chronic granulocytic leukemia (CGL).1-11 In a smaller number of cases, as a rule with unusual features, this minute chromosome has been absent.8-13 With the exception of a few atypical myeloproliferative disorders,8,17,18 the Ph1 chromosome has been found only in association with CGL.

Problems of disease classification arise from the present lack of specific criteria for the diagnosis of CGL and from the uncertain relationship of allied myeloproliferative disorders to CGL. In general, typical cases of CGL display enlargement of the spleen and a distinctive peripheral blood picture, characterized by marked leukocytosis, presence of immature granulocytes, basophilia, and low leukocyte alkaline phosphatase activity (LAP). The classical pathologic feature of CGL is invasion and proliferation of myeloid cells in various organs. However, changes following antileukemic therapy may obscure this picture and make it difficult to establish the diagnosis even on postmortem examination.

The relationship of the Ph1 chromosome to the leukemic process and its significance in the diagnosis of myeloproliferative disease are at present uncertain. One approach to clarifying such questions is through detailed observation of individual patients, and in particular, patients with atypical disease. The present report describes findings in an unusual case of myeloproliferative disease associated with greatly elevated LAP and the presence of the Ph1 chromosome.

CASE REPORT

S. B., a 74-year-old white male retired electrical engineer, was hospitalized in July, 1963, because of melena and congestive heart failure. Since 1921 the patient had suffered from ankylosing spondylitis associated with rheumatoid arthritis of the hands and ankles. He had had psoriasis since age 19 and recurrent episodes of uveitis since 1955. He had never received x-ray therapy, either to his spine or to his skin lesions.

Physical examination showed a pale, elderly man with a rigid kyphotic spine, signs of mild congestive heart failure and an extensive psoriatic rash over the trunk. The liver and spleen were not palpable, and there was no significant lymphadenopathy.

X-rays of the gastrointestinal tract showed sigmoid diverticulosis and a small para-
Table 1.—Peripheral Blood Findings and Leukocyte Alkaline Phosphatase (LAP) Values

<table>
<thead>
<tr>
<th>Date</th>
<th>Hemoglobin (Gm. Per Cent)</th>
<th>Platelets (per cu. mm.)</th>
<th>White Cell Count (per cu. mm.)</th>
<th>Neutrophil PMN</th>
<th>Band</th>
<th>Meta</th>
<th>Eos.</th>
<th>Baso.</th>
<th>Lymph.</th>
<th>Mono.</th>
<th>LAP*</th>
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<td>7/13/63</td>
<td>8.8</td>
<td>3,400,000</td>
<td>14,550</td>
<td>54.0</td>
<td>—</td>
<td>—</td>
<td>8.0</td>
<td>16.0</td>
<td>22.0</td>
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<td>—</td>
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<tr>
<td>7/24/63</td>
<td>10.9</td>
<td>2,500,000</td>
<td>18,550</td>
<td>64.0</td>
<td>—</td>
<td>—</td>
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<td>21.0</td>
<td>9.0</td>
<td>1.5</td>
<td>286.5</td>
</tr>
<tr>
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<td>3,925,000</td>
<td>11,000</td>
<td>62.5</td>
<td>—</td>
<td>—</td>
<td>6.5</td>
<td>15.5</td>
<td>12.5</td>
<td>3.0</td>
<td>—</td>
</tr>
<tr>
<td>9/9/63</td>
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<td>940,000</td>
<td>4900</td>
<td>58.0</td>
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<td>13.0</td>
<td>17.5</td>
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<td>1.0</td>
<td>—</td>
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<td>11.5</td>
<td>12.0</td>
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<td>3000</td>
<td>62.5</td>
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<td>—</td>
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<td>4250</td>
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<td>—</td>
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<td>3.0</td>
<td>314.0</td>
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<td>2700</td>
<td>47.5</td>
<td>2.0</td>
<td>—</td>
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<td>21.0</td>
<td>17.5</td>
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<td>1,250,000</td>
<td>3800</td>
<td>68.5</td>
<td>—</td>
<td>—</td>
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<td>13.5</td>
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<td>420,000</td>
<td>4600</td>
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<td>8.0</td>
<td>—</td>
<td>5.5</td>
<td>13.5</td>
<td>8.0</td>
<td>0.5</td>
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<tr>
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<td>17.0</td>
<td>2.5</td>
<td>—</td>
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<td>5200</td>
<td>71.0</td>
<td>—</td>
<td>—</td>
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<td>12.5</td>
<td>12.0</td>
<td>0.5</td>
<td>340.0</td>
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</tbody>
</table>

*Scoring on the basis of 0 to 400 (normal range 15-65).
†Normal range 140,000-440,000 per cu. mm. (direct method).

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esophageal hernia. Chest x-ray revealed diffuse cardiac enlargement, basilar pulmonary congestion and ankylosis of the spine. Stool guaiac test was strongly positive for occult blood. Bleeding, clotting and prothrombin tests were within normal limits. Urinalysis was unremarkable. BUN was 29 mg. per cent and uric acid 5.8 mg. per cent.

Hematologic studies (table 1) showed hemoglobin to be 8.8 Gm. per cent, hematocrit 29 per cent, white-blood cells 14,550 per cu. mm., platelets 3,400,000 per cu. mm. and reticulocytes 1.8 per cent. The differential revealed 54.0 per cent segmented neutrophils, 8.0 per cent eosinophils, 16.0 per cent basophils and 22.0 per cent lymphocytes. Morphologic changes in the red cells consisted of moderate anisocytosis, poikilocytosis and hypochromia. Slight polychromasia was noted together with occasional target and teardrop forms. The blood smear contained striking masses of platelets. Sternal marrow aspiration showed numerous megakaryocytes and marked myeloid activity with increased numbers of metamyelocytes and myelocytes and a striking basophilocytosis (table 2). Serum iron was 22 µg. per cent, serum vitamin B<sub>12</sub> 928 µg. per ml. and serum folate activity 16 µg. per ml.

The patient was treated with digitalis, diuretics, salt restriction, a bland diet and oral iron. The congestive failure was promptly relieved, stools became guaiac negative and the anemia gradually improved (table 1). Because of the greatly elevated platelet count, busulfan (4 mg. daily) was begun on July 26, 1963, at the time of hospital discharge. The busulfan dose was reduced to 2 mg. daily on August 19, to 1 mg. every other day on September 9 and was discontinued on September 30. During therapy the platelet count gradually fell and has remained around 500,000 to 1,000,000 per cu. mm. The white cell count declined to levels between 2000 and 5000 per cu. mm. However, basophilocytosis of 10 to 20 per cent and eosinophilocytosis of 5 to 10 per cent have persisted (table 1). Sternal marrow aspiration repeated in September, 1963, February, 1964, and August, 1964, showed greater maturity in the granulocytic population and increased erythroid activity (table 2). Whereas serum iron values have remained low, hemoglobin levels have been stable; except for a transient episode of arthritis and uveitis in October, 1963, the patient has remained free of symptoms.

**Leukocyte Alkaline Phosphatase Studies (LAP)**

Serial determinations of LAP activity were carried out using a modification of the histochemical methods of Comो<sub>14</sub> and Kaplow.<sup>15</sup> As shown in table 1, throughout the patient's course there has been a marked elevation of LAP activity.
Table 3—Cytogenetic Data

<table>
<thead>
<tr>
<th>Date</th>
<th>Ph' Chromosome</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date</td>
<td>Present</td>
</tr>
<tr>
<td>7/25/63</td>
<td>15</td>
</tr>
<tr>
<td>9/9/63</td>
<td>39</td>
</tr>
<tr>
<td>2/3/64</td>
<td>12</td>
</tr>
<tr>
<td>8/12/64</td>
<td>18</td>
</tr>
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</table>

*Each containing 46 chromosomes.

Cytogenetic Studies

On four separate occasions direct preparations for cytogenetic study were made from sternal marrow aspirations according to the method of Tjio and Whang. In each specimen, every metaphase counted contained a normal diploid number of 46 chromosomes. The Ph1 chromosome was clearly present in 84 of 120 metaphases counted (table 3). In no metaphase was the Ph1 chromosome clearly not present. Karyotypes constructed from two diploid metaphases in each specimen revealed no abnormalities other than the Ph1 chromosome.

In the preparation from September 9, which was technically superior to the other preparations, several polyploid metaphases were observed in which one or more Ph1 chromosomes could be identified (fig. 1). An estimate of polyploidy in this specimen was made by scanning under low-power magnification. Of 750 metaphases counted, 36 (4.8 per cent) were polyploid of which 23 (3.1 per cent) appeared tetraploid and 13 (1.7 per cent) octoploid. This estimate of polyploidy corresponded roughly with the proportion of megakaryocytes recorded in the bone marrow cell differential (4.0 per cent, table 2).

COMMENT

The difficulties of differentiating CGL from other myeloproliferative disorders is illustrated by the present case in which certain features are characteristic of CGL (Ph1 chromosome, basophiliccytosis) whereas others are not (elevated LAP, marked thrombocytosis, absence of splenomegaly). Similar diagnostic problems have been noted in recent reports dealing with cytogenetic aspects of myeloproliferative disease. While the Ph1 chromosome has come to be considered virtually pathognomonic of CGL, it is now apparent that this cytogenetic abnormality may occasionally be associated with unusual clinical findings or be absent from otherwise typical cases of CGL.

In a series of 27 cases considered to be typical CGL, Tough et al. encountered 2 lacking the Ph1 chromosome. Among 18 cases diagnosed as CGL Sandberg et al. found 4 in which the Ph1 chromosome was not present; each of these 4 cases was said to present atypical clinical features. Eight additional atypical Ph1-negative cases have recently been reported from this same laboratory. Block et al. mention without details 3 Ph1-negative CGL cases. Speed and Lawler record 1 Ph1-negative case said to be clinically and hematologically typical of CGL.

Tough et al. have described an unusual case of myeloproliferative disease in which the Ph1 chromosome was found. This case resembled the one presented here in that its principal feature was marked thrombocytosis (platelets 2,575,000 per cu. mm.). LAP values were not given for this patient.
Kemp, et al. have studied a patient with P32-treated polycythemia vera in whom the Ph1 chromosome was found several months before the appearance of frank CGL. Low LAP scores were recorded at the time of cytogenetic study. Bowen and Lee discuss in detail a Ph1-positive case with peripheral blood findings resembling myelofibrosis. LAP levels in this patient were described as low normal.

The most striking feature of the present case is the coexistence of the Ph1 chromosome with increased LAP activity. This finding is significant in view of speculation concerning the relationship of Group G chromosomes to the regulation of myelopoiesis. Since the discovery of the Ph1 chromosome in 1960, theories have been proposed to link the control of LAP activity directly to a locus on the long arm of one of these small acrocentric chromosomes. Such theories suggest that deletion of this locus from the minute Ph1 chromosome in CGL may cause decreased LAP activity in CGL, whereas trisomy of the locus in mongolism may lead to increased LAP. In view of the present case, and of the clinical evidence discussed below, such an interpretation of direct genetic control would seem oversimplified. If a relationship does exist between LAP and Group G chromosomes, it is more likely indirect, perhaps reflecting a more general and as yet ill-defined influence of Group G genetic material on myelopoiesis.

Various published observations support the concept that LAP and Group G chromosomes are not directly related. In the Ph1-negative cases of CGL described by Krauss et al. and Block et al., LAP levels were similar to those in Ph1-positive CGL. The latter authors also describe 3 Ph1-positive cases in which the proportion of Ph1-positive marrow metaphases before and
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during therapy remained unchanged whereas LAP activity rose to normal levels. Hammouda et al.23 observed a similar disparity between LAP levels and Ph'-positive metaphases among cases of CGL in blast-cell crisis. Crawford and Pegrun24 present a Ph'-negative case of CGL in which there was no LAP activity. Telfik et al.25 describe a boy with ulcerative colitis and typical Ph'-positive CGL in whom LAP scores ranged between normal and elevated levels.

Findings in the present case also support the concept that Ph'-positive cell lines are not limited to granulocytic elements but involve erythroid and megakaryocytic precursors as well.8,18,26,27 The observation of Ph1 chromosomes in polyploid metaphases in the present case is in accord with previous reports.8,18,26 That these polyploid cells represent megakaryocytes is suggested by the fact that their frequency in cytogenetic preparations roughly corresponded to the frequency of megakaryocytes in marrow smears. In addition, the finding in this case of 100 per cent Ph1-positive metaphases in the presence of considerable erythroid marrow activity implies the existence of the Ph1 chromosome in erythroid precursors.

The long-standing history of ankylosing spondylitis in the present case raises the question of a possible relationship between such rheumatic illness and myeloproliferative disease. The answer to this question remains uncertain. Abbott and Lea28 have presented data to suggest an increase in rheumatic diseases among patients with leukemia. Court Brown and Doll,29 however, in a small series of patients with ankylosing spondylitis not treated with x-ray, found no appreciable difference in leukemia incidence when compared with the general population.

SUMMARY

In an unusual case of myeloproliferative disease, the Ph1 chromosome was found in association with persistently elevated levels of LAP activity. Clinical findings in this case included marked thrombocytosis, basophilocytosis, absence of splenomegaly and a preceding history of untreated ankylosing spondylitis. Cytogenetic findings were compatible with the existence of the Ph1 chromosome in erythroid and megakaryocytic as well as granulocytic marrow precursors.

This case illustrates the difficulties currently encountered in the clinical differentiation of myeloproliferative disorders and in interpreting the diagnostic significance of the Ph1 chromosome. The co-existence in this case of the Ph1 chromosome and elevated LAP does not support the concept of a direct relationship between Group G chromosomes and LAP activity.

SUMMARIO IN INTERLINGUA

In un caso inusual de morbo myeloproliferative, le chromosomes Ph1 esseva trovate in association con persistentemente elevate nivello de activitate de phosphatase alcalin del leucocytes. Le ccnstatations clinic in iste caso includeva marcate grados de thrombocytosis, basophilocytosis, absentia de splenomegalia, e le antecedente de non-tractate spondylitis ankylosante. Le
constatationes cytogenetic esseva congrue con le existentia del chromosoma Ph in le precursors erythroide e megacaryocytic como etiam granulocytic del medulla.

Iste caso illustra le difficulatte currentemente incontrate in le differentiation clinic de disordines myeloproliferative e in le interpretation del signification diagnostic del chromosoma Ph. Le coexistencia notate in le presente caso del chromosoma Ph con elevate nivellos de phosphatase alcalin del leucocytos non supporta le conception de un relation directe inter chromosomas de Gruppo G e le activitate de phosphatase alcalin del leucocytos.

ACKNOWLEDGMENT

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REFERENCES

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