Chromosomes and Causation of Human Cancer and Leukemia. XXI. Cytogenetically Unusual Cases of Leukemia

By Shin-ichi Sonta, Mitsuo Oshimura, and Avery A. Sandberg

Three male patients with leukemia were found with banding techniques to have unusual cytogenetic pictures in the cells of their marrow, spleen or blood. Case No. 1 (78 yr old) was that of a Ph'-negative CML with a missing Y in the blood (cultured without PHA) and marrow cells. The patient is still alive and responding to therapy. Case No. 2 (54 yr old) was considered prior to admission to have either CML or AML, but was shown, in fact, to be in the blastic phase of CML; all the cells in his marrow and spleen were Ph'-positive, but with no evidence of a translocation. Case No. 3 (50 yr old) with AML was shown to have a Ph' resulting from a standard translocation, i.e., [t(9;22)(q34;q11)], in a substantial number of the cells in the marrow and blood (cultured without PHA). The implications of these unusual findings are discussed in relation to the chromosomal pictures usually encountered in these states.

Knowledge regarding the origin and genesis of the Philadelphia chromosome (Ph') in chronic myelocytic leukemia (CML) has been substantially augmented by the demonstration with various banding techniques that the Ph' results from the deletion of chromosome 22, with the deleted segment usually being translocated to the long arm of chromosome 9, i.e., t(9;22)(q34;q11 or 12). However, exceptions to this translocation occur in CML and their significance remains to be determined. Furthermore, even though a Ph' has been described in conditions other than CML, the nature of such a chromosome has not been established with the certainty shown in CML.

Much has been written about the missing Y chromosome in Ph'-positive CML and other conditions. There are those who believe that the presence of a missing Y carries a more favorable prognosis in the disease, whereas others disagree. The occurrence of a missing Y in Ph'-negative CML appears to be very rare.

In the present paper are described three cytogenetically unusual cases of leukemia, the karyotypic findings being of sufficient interest and uniqueness possibly to shed light on the significance of the Ph' and the missing Y in human leukemic states.

Case Reports

Case No. 1

The patient was a 78-yr-old white male (R.F.), retired tool and die worker, who was essentially well until December 1974, when he noticed the onset of weakness, easy fatigue, generalized itch-
ing, and a gradual onset of weight loss totaling about 35 lb over a period of several months. In June 1975 his physician found a definite anemia and after examining peripheral blood and bone marrow smears, made the provisional diagnosis of CML and referred the patient to Roswell Park Memorial Institute (RPMI) for further evaluation and treatment. The patient gave no history of radiation exposure or ingestion of medications except for iron supplement. When first seen at RPMI on July 10, 1975, the liver was found to be considerably enlarged (18 cm below the costal margin) and the spleen palpable 2 cm below the costal margin. A complete blood count on July 8, 1975 revealed hemoglobin 8.7 g/100 ml, hematocrit 28%, platelet count 295,000/cu mm, WBC 75,000/cu mm with 74% neutrophils, 5% lymphocytes, 3% monocytes, 15% juveniles, 1% blasts, and 2% myelocytes. The leukocyte alkaline phosphatase reaction was negative. Examination of the bone marrow revealed an increased cellularity and a greatly elevated myeloid/erythroid (M:E) ratio due to the large number of granulocytic cells and a decreased number of nucleated red blood cells. The number of myeloblasts was within normal limits, but that of megakaryocytes was greatly decreased. The patient was started on busulfan, 6-mercaptopurine and transfused in July 1975. At this writing, the patient is doing well on maintenance chemotherapy and is being followed by his physician. When last contacted, the patient had no complaints.

Case No. 2

This 54-yr-old white male (D.R.) foundry worker was diagnosed as having either CML in the blastic phase or acute myeloblastic leukemia (AML) in July 1975, at which time he was found to have a WBC of 180,000/cu mm. He had a 6-mo history of anorexia with weight loss of about 50 lb and progressive weakness over the 2-wk period prior to his admission to RPMI. His physician found his liver 12 cm and spleen 8 cm below the costal margin. When the patient was admitted to RPMI on July 22, 1975, examination of his marrow revealed it to be very cellular in nature and to have a greatly elevated M:E ratio (40:1). The latter was due to the presence of increased numbers of myeloblastic (33%) and promyelocytic (42%) cells. No megakaryocytes were seen. At that time his WBC was 260,000/cu mm, hematocrit 21%, hemoglobin 7 g/100 ml, and platelet count 70,000/cu mm. Many myeloblasts were seen in the peripheral blood smear. The leukocyte alkaline phosphatase (LAP) activity of the cells in the marrow was higher than normal on July 28, 1975. The patient was started on cytosine arabinoside (Ara-C) and adriamycin on July 23, 1975, and did well, including an essentially normal bone marrow examination, through August 1975. However, on September 2, 1975, he was found to have a hemoglobin of 8 g/100 ml, hematocrit of 24%, platelet count of 2500/cu mm and a WBC ranging from 4900 to 6200/cu mm. Because of the severe thrombocytopenia and evident bleeding, the patient underwent splenectomy (spleen weight: 850 g) on September 30, 1975. The patient died of respiratory insufficiency on October 5, 1975. Autopsy examination revealed: markedly enlarged lymph nodes in mediastinum, paraaortic, and periportal areas (largest 6 cm in diameter), hypercellular marrow with more than 90% of the cells being immature granulocytic elements, and infiltration of all tissues by leukemic cells.

Case No. 3

This 50-yr-old white male (S.U.), owner of a moving company, first noticed symptoms in the summer of 1975, which consisted of fatigue, weakness, anorexia and some weight loss. In October 1975, the patient was found to have a pancytopenia and the diagnosis of aplastic anemia was made. The patient was then put on oxymetholone, prednisone, folic acid, and transfused with blood and platelets. No enlargement of either the liver or spleen was found. At that time, his hemoglobin was 7 g/100 ml, hematocrit 20%, WBC 4200/cu mm (85 lymphocytes, 13 segmented cells, 2 bands), platelet count 22,500/cu mm, and reticulocytes 0.9%. The patient had used insecticides in the summer of 1975 on two different occasions. Because of an increased number of immature cells in the marrow and the appearance of blasts in the blood, the patient was referred to RPMI. Except for generalized pallor, petechiae (particularly on the lower extremities) and lack of liver and spleen enlargement, the physical examination was not remarkable. Upon admission on December 31, 1975, the patient's hemoglobin was 10.1 g/100 ml, hematocrit 30%, WBC 7600/cu mm, and platelet count 7500/cu mm. Examination of a bone marrow specimen revealed it to be very hypocellular and not to contain any megakaryocytes; 28% of the cells were myeloblasts, 64% lymphocytes and the M:E ratio was 5.9:1. Shortly following admission, the patient was put on daunorubicin.
and Ara-C, and some improvement in his condition and anemia occurred, though the marrow, which was examined on three different occasions, was always very hypocellular with the myeloblasts not exceeding 4%, during the first 3 wk of treatment. On February 3, 1976, 21% of the cells in the marrow were myeloblasts. The therapy at that time was increased, and a month later the patient was found to have a hemoglobin of 7.6 g/100 ml, hematocrit of 23%, platelets 15,000/cu mm, WBC 3200/cu mm (11 segmented cells, 83 lymphocytes, 1 eosinophil, 4 myelocytes, 1 blast, and 4 nucleated red blood cells), and the patient continued to have petechiae on his lower extremities. The patient was transfused with 4.83 x 10^11 blood leukocytes from a female patient with Ph1-positive CML on January 17, 1976.

**CHROMOSOME DATA**

The cytogenetic techniques used have been described in detail previously, including the various banding methods, nomenclature of chromosomes, and photographic procedures.

**Case No. 1**

The chromosomal data of the bone marrow and peripheral blood cells are summarized in Table 1. The marrow cells contained a mode of 45 chromosomes (77%). The Q- and G-banding techniques revealed that 73 of 75 metaphases with 45 chromosomes had a missing Y, without any other chromosomal abnormality (Fig. 1). No evidence for a Ph1 was found. Of 98 cells, 17 had a normal karyotype, with a typical Y chromosome having brilliant fluorescence on its long arm following Q banding. Of the metaphases derived from the blood cultures without phytohemagglutinin (PHA), regardless of the time of culture (0, 24, and 48 hr), 85%-95% showed a 45,X pattern without a Ph1. Some cells with a normal karyotype were also observed in these slides. On the other hand, in the blood cells cultured with PHA for 72 hr, almost all metaphases were normal, but with a scatter of numbers which was considered as random loss.

Interestingly, the chromosomes in most of the cells with a missing Y were somewhat fuzzy, a finding not uncommon in leukemic metaphases, but not in cells with a normal karyotype. Moreover, almost all of the more than 50 polyploid cells observed directly in the marrow and peripheral blood showed loss of the Y chromosomes. A Y body was found in 2.7% of 600 peripheral nucleated blood cells, and in 4.6% of 500 bone marrow cells.

**Case No. 2**

Bone marrow and splenic aspirates were examined for their chromosomal constitution (Table 2). The first examination of the bone marrow was done on July 23, 1975, when the patient was admitted to RPM I. The chromosome number of these cells ranged from 43 to 102, with a flat mode at 50-51 chromosomes. Karyotypic analysis of 4 of the cells which had 50 chromosomes revealed them to be 50,XY,Ph1,+8,+11,+21,+21 and 2 cells with 51 chromosomes to be 51,XY,Ph1,+8,
Fig. 1. Karyotype of Q-banded chromosomes of a marrow cell of patient No. 1, showing the absence of the Y.

+11, +13, +21, +21 (Fig. 2). In all of these cells, one normal chromosome 22 was present, whereas the other chromosome 22 was replaced with one in which the distal part of its long arm had been deleted (Figs. 2 and 3). However, no sign of translocation of the missing part of this 22 could be found on any chromosome, including 9. An examination of the bone marrow obtained on July 28, 1975, revealed the chromosome number to range from 41 to 60; a Ph¹ was present in all of the cells.

Examination of a splenic aspirate obtained on September 30, 1975, revealed all the cells to have a Ph¹ -chromosome and the chromosome number to vary from 46 to 61, with the modal number being 51. There was a clone of cells with a common karyotype of 51,XY,Ph¹,+8,+11,+13,+21,+21. In this specimen, again, the missing segment from the abbreviated chromosome 22, i.e., the Ph¹, was not observed on any chromosome. On both July 23 and July 28, 1975, occasional marrow cells with two Ph¹ chromosomes were found (not shown in Table 2).

Table 2. Summary of Chromosomal Analyses (Patient No. 2)

<table>
<thead>
<tr>
<th>Material</th>
<th>No. of Cells Counted</th>
<th>Chromosome No.</th>
<th>≤45</th>
<th>46</th>
<th>47</th>
<th>48</th>
<th>49</th>
<th>50</th>
<th>51</th>
<th>52</th>
<th>53</th>
<th>54 ≤</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bone marrow (July 23, 1975)</td>
<td>25</td>
<td></td>
<td>2</td>
<td>1</td>
<td></td>
<td>3</td>
<td>2</td>
<td>7</td>
<td>7</td>
<td>2</td>
<td>1</td>
<td></td>
</tr>
<tr>
<td>Bone marrow (July 28, 1975)</td>
<td>26</td>
<td></td>
<td>1</td>
<td>3</td>
<td>4</td>
<td>2</td>
<td>6</td>
<td>2</td>
<td>4</td>
<td>4</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Splenic aspirate (Sept. 30, 1975)</td>
<td>68</td>
<td></td>
<td>1</td>
<td>5</td>
<td>1</td>
<td>2</td>
<td>7</td>
<td>18</td>
<td>24</td>
<td>5</td>
<td>2</td>
<td>3</td>
</tr>
</tbody>
</table>
Fig. 2. A Q-banded karyotype of a marrow cell from patient No. 2 showing the following features: \( \text{Ph}^1 \), no translocation evident on chromosome 9 or any other chromosome, extra chromosomes 8, 11, 13, 21 (tetrasomy). Note the brilliant fluorescence of the Y chromosome.

**Case No. 3**

A bone marrow obtained upon admission to RPMI on December 31, 1975, before any chemotherapy was given to the patient, was shown to have a mixture of diploid cells and cells that contained the \( \text{Ph}^1 \) chromosome (Table 3). The \( \text{Ph}^1 \) chromosome was shown with Q and G banding to be due to a deletion of chromosome 22 with a translocation to the long arm of chromosome 9, i.e., \( t(9:22)(q34;q11) \) (Fig. 4). Incidentally, of the seven \( \text{Ph}^1 \)-positive cells with 46 chromosomes, three cells had a missing Y and probably an extra 8, i.e., 46,\( X, \text{Ph}^1, 9q+, +8 \). On January 13, 1976, when the marrow was still very hypocellular but contained only 2\(^{-} \), myeloblasts and no nucleated red blood cells or megakaryocytes, the five metaphases examined were all diploid. An

<table>
<thead>
<tr>
<th>Material</th>
<th>No. of Cells Observed</th>
<th>Chromosome No.</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>43</td>
</tr>
<tr>
<td>Bone marrow (Dec. 31, 1975)</td>
<td>14</td>
<td>1</td>
</tr>
<tr>
<td>Bone marrow (Jan. 13, 1976)</td>
<td>5</td>
<td></td>
</tr>
<tr>
<td>Blood (no PHA) (Jan. 20, 1976)</td>
<td>40(17)†</td>
<td>1</td>
</tr>
<tr>
<td>Bone marrow (Feb. 3, 1976)</td>
<td>1</td>
<td></td>
</tr>
</tbody>
</table>

*Cells without a \( \text{Ph}^1 \) chromosome shown in parentheses.

†Chromosome constitution of the 17 cells analyzed with Q banding was revealed as six cells with 46,\( X, \text{Ph}^1, 9q+ \) (probably belonging to the donor of WBC), seven cells with 46,\( X, \text{Ph}^1, 9q+, +8 \), two cells with 45 chromosomes.
Fig. 3. Partial G-banded karyotypes of two marrow cells from patient No. 2 (Ph\(^1\)-positive CML) showing the Ph\(^1\)-chromosome without a translocation to chromosome 9.

Fig. 4. Partial karyotypes from two marrow cells of patient No. 3, demonstrating the Ph\(^1\) (9–22) translocation, extra 8 chromosomes, and the missing Y.
examination of blood cells cultured without PHA done on January 20, 1976, at which time the bone marrow continued to be extremely hypocellular with only 4% myeloblasts, revealed an interesting mixture of cells. Of the 17 cells karyotyped with Q banding, six had a normal male karyotype. However, of the remaining 11 cells, seven were pseud diploid, i.e., 46,X,Ph',9q+,+8, two were 46,XX,Ph',9q+, and two cells had 45 chromosomes. The two cells with the female chromosome constitution probably belonged to the subject with Ph'-positive CML whose WBC were given to the patient. G-banding analysis also revealed the seven abnormal cells with 46 chromosomes to have the following karyotype: 46,X,Ph',9q+,+8.

**DISCUSSION**

**Ph' Without Evidence of Translocation**

In the preponderant number of cases of Ph'-positive CML, the genesis of the Ph' has been shown to occur through deletion of the distal part of the long arm of a chromosome 22 and a translocation of this part to the distal end of the long arm of chromosome 9, i.e., t(9;22)(q34;q11 or 12). In about 10% of the cases of Ph'-positive CML, the translocation occurs to chromosomes other than 9, e.g., 2, 10, 13, 17, 19, 21, and 22. These findings appear to indicate that the deletion of 22 is the crucial event related to CML, rather than the site of the translocation. Only one case of Ph'-positive CML, that of a 55-yr-old female “with typical CML” and a prominent basophilia, in whom the translocation could not be found, has been published previously, our case appears to be the second one, though karyotypically and clinically it was more complicated. Our patient was definitely in the blastic phase of CML and his clinical features, course, and the presence of substantial splenomegaly pointed to the strong possibility that he was not a patient with AML. The additional chromosomal changes observed in the marrow and spleen of this patient, i.e., +8,+11,+13,+21 are not infrequent accompaniments of the blastic phase of CML. However, all the cells contained the Ph'-chromosome without evidence of translocation of the deleted part. Obviously, since the number of cases of Ph'-positive CML without evidence of translocation is extremely small, it is impossible to ascertain the role this finding plays in the course of the disease. As stated above, our patient was in the blastic phase of his disease when diagnosed, whereas the patient described by Mitelman was not. However, the latter patient developed the blastic phase in June 1974, (28 mo after diagnosis) and died in August 1974 (Mitelman, F., personal communication). Thus, the rather short survival of these two patients with CML and the early appearance of the blastic phase may possibly be related to the missing DNA.

**Ph'-Negative CML With A Missing Y**

An increased frequency of a missing Y in varying proportions of cells in the marrows of elderly males, with or without disease, particularly after the age of 70, has been described by several groups. The same karyotypic change occurs in 5%–10% of male patients with Ph'-positive CML, the missing Y being observed at a significantly earlier age than in nonleukemic subjects. A missing Y may endow the involved cases of CML with a better prognosis than that of patients without a lost sex chromosome, though this conclusion has not been universally accepted.

Of the three reported cases of Ph'-negative CML with a missing Y, the first reported case was that of a 69-yr-old male patient first diagnosed in April 1972,
and in whom no 46,XY cells were found in the marrow on the two occasions on which it was examined. The patient is still alive. The second published case was that of a 61-yr-old male patient who died 2 yr after the diagnosis. At the time of the diagnosis, the marrow contained some 46,XY cells, but the preponderant number was 45,X. On three subsequent occasions, including one just before death, the bone marrow contained exclusively 45,X cells. The third case described was of the juvenile form of CML, and whether or not the same criteria of evaluation and significance can be applied to this case in comparison with those of the adult form is uncertain. This 3½-yr-old boy died 12½ mo after being diagnosed. The bone marrow was examined on three occasions; on one, a few 46,XY cells were seen (2/13), but in the others only 45,X cells were observed. In all of the patients reported, cultured blood lymphocytes and/or fibroblasts revealed a 46,XY chromosome constitution, indicating that the 45,X picture was an acquired and not a congenital one.

Even though the authors of one case state that the short course of their patient is in contrast to the hypothesis that absence of the Y chromosome in CML is compatible with long survival, it should be pointed out that their patient lived 2 yr after diagnosis, a figure significantly higher than the median survival of 10 mo for patients with Ph'-negative CML. The course of the 3½-yr-old patient with the juvenile form of CML is difficult to interpret, since no comparative data are available for this form of the disease.

We have postulated that the missing Y as the sole karyotypic anomaly may prevent the cells from becoming involved in an acute leukemic process, citing the extremely rare occurrence (if ever) of male patients with acute leukemia and a missing Y as the only karyotypic anomaly in the leukemic cells. It is interesting to note that the present case of Ph'-negative CML with a missing Y and the ones previously reported had features that were closer to those of “classical” Ph'-positive CML than those of the Ph'-negative variety. At this writing, two of the patients are alive, and it will be of interest to establish whether their therapeutic and clinical courses will be relatively favorable, as would be predicted on the basis of our postulate regarding the protective role of the missing Y. So far, neither patient has entered the blastic phase, and both patients are doing well clinically. Obviously, it is impossible to generalize on the missing Y and the course of Ph'-negative CML on the basis of a few rare cases. Only through the reporting of other such cases will it be possible to collect a number sufficient for clinical evaluation of the missing Y in this type of CML.

**Ph' Translocation in AML**

The presence of a Ph'-like chromosome has been described in a number of different conditions, usually hematologic disorders other than CML, with one of the most common being AML. To date, the exact identity of such Ph'-chromosomes has not been established with certainty, and banding techniques have offered an opportunity to determine reliably their origin. Even though in the past a Ph' has been described in AML and its significance debated, it appears that the patient described by us (case No. 3) is the first AML in which the Ph' was shown to originate from a translocation of the distal part of the long arm of chromosome 22 to chromosome 9, i.e., t(9;22)(q34;q11 or 12), a situation identical to that in CML. In our case and in most of the Ph'-positive AML
cases reported, the Ph$^+$ was present in only a portion of the marrow cells; characteristically, in CML nearly all the cells have had a Ph$^+$ in the well-established disease. It could be debated that the case presented represents an extreme variation of the blastic phase of CML and, hence, finding the Ph$^+$ would then not be unusual, as well as some of the other chromosomal changes. However, the aplastic phase and hypocellularity of the marrow and its persistence during the disease, the lack of basophilia and organ enlargement, the presence of a substantial percentage of normal diploid cells in the marrow, the normalization of the karyotypes following therapy, and several other facets of the case point to this as being one of AML and to be associated with a Ph$^+$, a chromosome identical in its origin to that seen in CML. Incidentally, the presence of trisomy 8 and a missing Y is not unusual in AML.11

REFERENCES

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