Acquired XO/XY Clones in Bone Marrow of a Patient With Paroxysmal Nocturnal Hemoglobinuria (PNH)

By Jacqueline Whang-Peng, Turid Knutsen, Elaine C. Lee, and Brigid Leventhal

Cytogenetic studies showed both 45XO and 46XY clones in the bone marrow of a 76-yr-old male with a 17-yr history of paroxysmal nocturnal hemoglobinuria (PNH). \(^{55}\)Fe incorporation studies demonstrated that both clones involved the hematopoietic stem cells. The loss of the Y chromosome may reflect an aging phenomenon, rather than be related to the PNH.

The loss of the Y chromosome in the bone marrow has been reported in several males with Ph'-positive chronic myelogenous leukemia. One case of Ph'-negative CML has been described with a 45XO karyotype and there have been several reports of a missing fluorescent Y body in malignant tumors. Here we report the first case of a patient with a 17-yr history of paroxysmal nocturnal hemoglobinuria and a missing Y chromosome in the majority of his bone marrow cells.

MATERIALS AND METHODS

Case History

The patient was born in 1899. He enjoyed good health until 1958 when he was found to be anemic and had hemoglobinuria. For the first 5 yr of his disease he was treated with infusions of dextran and whole blood transfusions. In 1963, folic acid and methyltestosterone were added to his management regimen. He was first admitted to the NIH in 1965. At that time he was a well-developed, well-nourished, white male in no distress. His blood pressure was 160/70, and pulse 88 and regular. He was not icteric, and there was no hepatosplenomegaly. The remainder of the physical examination was unremarkable. His hemoglobin was 8.4 g/100 ml, platelet count 193,000/cu mm, white count 2600/cu mm with 12% polymorphonuclears, 86% lymphocytes, and 2% eosinophils. Reticulocyte count was 7.2%. Urinalysis showed 1+ protein, no sugar, and 1+ to 4+ hemoglobin. His leukocyte alkaline phosphatase score value was 9 (normal 37-98), and the Ham test was positive. The plasma hemoglobin ranged from 20 to 30 mg/100 ml. The diagnosis of paroxysmal nocturnal hemoglobinuria was later definitely established by studies demonstrating increased lysis of his red cells by complement in an immune lysis assay. During the intervening years, his course was complicated by benign prostatic hypertrophy which required a suprapubic prostatectomy in 1968; repair of a right inguinal hernia in 1969; episodes of loss of consciousness associated with incontinence without demonstrable EEG abnormality or cardiac arrhythmia in 1966 and 1967, for which he was given Dilantin therapy for about 4 yr; angina pectoris and two myocardial infarctions in 1971 and 1972 which have resulted in persistent ST elevations in V1-V3 on EKG, consistent with a ventricular aneurysm; and adult onset diabetes which is controlled by diet alone. Persistent mild abnormalities in liver function tests are at present unexplained, and representative recent values include: total bilirubin 1.6 mg/100 ml (0.20 mg/100 ml direct), alanine aminotransferase/GPT 91 U/liter (normal 2-45 U/liter), aspartate aminotransferase/GOT 131 U/liter (normal 6-52 U/liter), total protein 9.1 g/100 ml (normal 6.0-8.5 g/100 ml) with albumin 4.6 g/100 ml (normal 3.1-5.4 g/100 ml). Neither Australia antigen (HBsAg) nor antibody to HBsAg are present in the peripheral blood. His hemoglobin was maintained with...
transfusions, approximately 2 units of washed red cells every 3 wk until about 1970 when his transfusion requirement began to decrease. Since 1971 he has ceased to require transfusions. Therapeutic trials were carried out with vitamin K, halotestin R, and salicylates, none of which appeared to have a beneficial effect on his disease. In 1974 the bone marrow was moderately hypocellular with decreased stainable iron, slight lymphocytosis and plasmacytosis, and a decreased number of megakaryocytes. At that time the hemoglobin was 15.7 g/100 ml, platelets 67,000/cu mm, reticulocytes 2.1%, white count 5100/cu mm with 19% neutrophils, 72% lymphocytes, 8% monocytes, and 1% eosinophils. Although much improved, the patient continues to have hemoglobin in his urine intermittently and still has a component of cells showing sensitivity to complement, consistent with PNH. The present improvement in erythropoiesis appears to be spontaneous.

Chromosome Studies

Serial chromosome studies in this patient were performed on bone marrow using the direct air-dry technique of Tjio and Whang and on peripheral blood (Moorhead et al.). Peripheral blood cytogenetic studies were also done on his only son.

The methylcellulose technique for in vitro culture of bone marrow cells was used for long-term growth of these cells and has been previously described. Cytogenetic analysis of pooled colonies from these cultures was done according to the procedure described by Duttena et al.

Positive identification of erythropoietic cells in chromosomal preparations of bone marrow cells was made using a modification of $^{55}$Fe labeling techniques as described by Rastrick. Four drops of bone marrow aspirate were placed in a medium consisting of 4 ml McCoy's 5A (Gibco), 2 ml fetal calf serum, 20 units of heparin/cc, and 100 μCi $^{55}$FeCl$_3$ (specific activity 10 mCi/mg Fe$^{3+}$, New England Nuclear). This suspension was incubated for up to 24 hr, including a 2-hr treatment with colchicine. Chromosome preparations were made using the air-dry technique.

Autoradiographs were prepared by covering slides of the chromosome preparations with AR-10 stripping film (Eastman Kodak). These were exposed for 3-4 wk, developed, and stained with Giemsa stain. All the cells with 15 or more grains were considered to be labeled red cell precursors. These were scored and photographed, the film removed, and the slide restained in order to analyze the chromosome complement of these cells.

Previously, Giemsa-stained chromosome preparations from bone marrow and peripheral blood, including autoradiographs from which the film had been removed, were destained in acid alcohol. The slides were then stained with quinacrine mustard according to the method of Caspersson et al. in order to verify the presence of a brightly fluorescing Y chromosome.

RESULTS

Table 1 presents the results of cytogenetic studies on peripheral blood and bone marrow from this patient. Repeated chromosome analyses of the peripheral blood revealed a normal male karyotype, except on one occasion in 1972, when 2% of the cells were missing a Y chromosome (45XO) and 8% of the cells showed a random loss of one or more chromosomes. The 45XO cells could also have represented random chromosome loss.

The metaphases in the bone marrow had both 46XY and 45XO karyotypes (Fig. 1). In February 1965, 84% of the cells had a 45XO karyotype and in January 1974, 92% were missing the Y chromosome. In December 1971, both direct and in vitro studies were done of the bone marrow cells. In the 11-day methylcellulose culture, 20% of the metaphases were missing the Y chromosome and 80% had a 46XY karyotype. This ratio was the reverse of that found in the direct bone marrow preparation. $^{55}$Fe-labeling studies showed that one-third to two-thirds of the metaphases incorporated $^{55}$Fe. The majority of the labeled and unlabeled bone marrow cells had a 45XO karyotype (Fig. 2), with less than 30% of the labeled cells and fewer than 45% of the unlabeled cells
Table 1. Serial Cytogenetic Studies in Peripheral Blood (PB) and Bone Marrow (BM)

<table>
<thead>
<tr>
<th>Date</th>
<th>Specimen</th>
<th>No. of Cells</th>
<th>Chromosome Distribution</th>
<th>45 (%)</th>
<th>45 XO (%)</th>
<th>46 XY (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>2-17-65</td>
<td>BM direct</td>
<td>50</td>
<td>2</td>
<td>84</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>PB 3-day culture</td>
<td>16</td>
<td>0</td>
<td>0</td>
<td>100</td>
<td></td>
</tr>
<tr>
<td>4-09-68</td>
<td>BM direct</td>
<td>30</td>
<td>3</td>
<td>87</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td>12-16-71</td>
<td>BM direct</td>
<td>98</td>
<td>0</td>
<td>86</td>
<td>14</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BM $^{55}$Fe (24-hr culture)</td>
<td></td>
<td>Labeled 37%</td>
<td>0</td>
<td>85</td>
<td>15</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>Unlabeled 63%</td>
<td>0</td>
<td>56</td>
<td>44</td>
</tr>
<tr>
<td></td>
<td>BM agar culture, 11 days</td>
<td>5</td>
<td>0</td>
<td>20</td>
<td>80</td>
<td></td>
</tr>
<tr>
<td>11-15-72</td>
<td>BM direct</td>
<td>59</td>
<td>7</td>
<td>83</td>
<td>10</td>
<td></td>
</tr>
<tr>
<td></td>
<td>BM $^{55}$Fe (24-hr culture)</td>
<td></td>
<td>Labeled 69%</td>
<td>0</td>
<td>71</td>
<td>29</td>
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<tr>
<td></td>
<td></td>
<td></td>
<td>Unlabeled 31%</td>
<td>0</td>
<td>75</td>
<td>25</td>
</tr>
<tr>
<td></td>
<td>PB 3-day culture</td>
<td>40</td>
<td>8</td>
<td>2</td>
<td>90</td>
<td></td>
</tr>
<tr>
<td>1-21-74</td>
<td>BM direct</td>
<td>12</td>
<td>0</td>
<td>92</td>
<td>8</td>
<td></td>
</tr>
</tbody>
</table>

having the normal male 46XY karyotype (Fig. 3). No Y-fluorescence was seen in cells with 45 chromosomes.

Cytogenetic studies of the peripheral blood from the only son of the patient revealed a normal male karyotype.

**DISCUSSION**

In this patient, we report the coexistence of two abnormalities in the bone marrow, namely paroxysmal nocturnal hemoglobinuria and an XY/XO

![Fig. 1. Karyotype of a bone marrow cell with 45 XO.](image-url)
Fig. 2. (A) An $^{55}$Fe-labeled 45XO metaphase, the karyotype of which is shown below. The arrows indicate the G group chromosomes. (B) The same metaphase as in A; the stripping film has been removed and the slide restained with quinacrine mustard. No brightly fluorescent Y can be seen.

chromosome pattern. The clinical picture of PNH has been well described. The red cells from patients with PNH consist of at least two populations, one very sensitive to the lytic action of complement (C), the other less sensitive, but still somewhat more sensitive than normal. The cause of PNH remains unknown; Dacie has suggested that it might be caused by a somatic mutation resulting in overgrowth of normal marrow by an abnormal clone of cells. Indeed, in a study by Oni et al., a patient with PNH who was heterozygous for G6PD, it appeared that the PNH was produced by the overgrowth of a single clone of cells as defined by G6PD typing.

A number of investigators have studied the chromosomes in patients with PNH in an attempt to define further the clonal nature of the defect. The medi-
Fig. 3. (A) An $^{55}$Fe-labeled 46XY metaphase, the karyotype of which is shown below. The arrows indicate the G group chromosomes and the Y chromosome. (B) The same metaphase as in A; the stripping film has been removed and the slide restained with quinacrine mustard. The arrow indicates the brightly fluorescent Y chromosome.

cal literature contains 18 cases of PNH with cytogenetic studies on bone marrow and/or peripheral blood (Table 2). In ten cases only the peripheral blood was studied, and no cytogenetic abnormalities were found. It should be noted, however, that cytogenetic studies of the peripheral blood represent an analysis of the chromosomes of the lymphocytic cells in the majority of individuals rather than of the myeloproliferative cells. Undetected marrow abnormalities may have existed in these patients and, in fact, the peripheral blood studies in our patient showed a much lower percentage of abnormal metaphases than did the bone marrow. Two other patients revealed no lymphocyte abnormalities in the face of marked abnormalities in marrow. Eight cases had chromosome studies performed on bone marrow. In three of the cases, no abnormalities were observed. Aneuploidy was seen in five cases, but the type
Table 2. Cytogenetic Studies in Patients With PNH

<table>
<thead>
<tr>
<th>Case</th>
<th>Age/Sex</th>
<th>Bone Marrow Cytogenetic Status</th>
<th>Peripheral Blood Cytogenetic Status</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>76/M</td>
<td>45XO (84%–92%) + N</td>
<td>46XO (0%–3%) + N</td>
<td>Present case</td>
</tr>
<tr>
<td>2</td>
<td>24/F</td>
<td>Aneuploidy (40%) + N</td>
<td>Aneuploidy (40%) + N</td>
<td>17</td>
</tr>
<tr>
<td>3</td>
<td>38/M</td>
<td>Aneuploidy (35%) + N</td>
<td>Aneuploidy (?) + N</td>
<td>17</td>
</tr>
<tr>
<td>4</td>
<td>52/M</td>
<td>−C and/or −Gp− (74.4%–90%) + N</td>
<td>N</td>
<td>18</td>
</tr>
<tr>
<td>5</td>
<td>43/M</td>
<td>Bq− (95%) + N</td>
<td>N</td>
<td>19</td>
</tr>
<tr>
<td>6–8</td>
<td>?/?</td>
<td>N</td>
<td>N</td>
<td>20</td>
</tr>
<tr>
<td>9</td>
<td>45/M</td>
<td>45X−C (95%) + N</td>
<td>N</td>
<td>21</td>
</tr>
<tr>
<td>10</td>
<td>?/?</td>
<td>−</td>
<td>N</td>
<td>20</td>
</tr>
<tr>
<td>11–12</td>
<td>?/?</td>
<td>−</td>
<td>N</td>
<td>22</td>
</tr>
<tr>
<td>13</td>
<td>7.5/F</td>
<td>−</td>
<td>N</td>
<td>23</td>
</tr>
<tr>
<td>14–19</td>
<td>?/?</td>
<td>−</td>
<td>N</td>
<td>24</td>
</tr>
</tbody>
</table>

of abnormality varied from patient to patient. In two patients there was no identifiable clone of abnormal cells. In addition to the present case, definite clonal abnormalities were seen in three patients; one patient was missing a chromosome in either the C or G group in some of his cells and, in addition, had a small G chromosome in the majority of cells; the second had 95% of the marrow cells with a Bq− chromosome (i.e., a portion of the long arm in one of the B group chromosomes was deleted); and the third was missing a C group chromosome in 50% of his cells. Our patient was similar to these last three patients in that he had a definite clonal abnormality with deletion of the Y chromosome in addition to a normal population of cells.

Pierre et al.25 published direct bone marrow chromosome analyses in 88 male patients with no common disease; of the 52 patients who were between the ages of 60 and 89, six had a 45XO cell line which ranged from 48% to 100% of the total cells. Three of these six patients had a question of preleukemia, one had pernicious anemia, one had agnogenic myeloid metaplasia, and one had pancytopenia following x-ray therapy to the parotid gland following lymphoblastic lymphoma. None of the remaining 36 males, all of whom were less than 60 years of age, were found to have a 45XO cell line. The authors suggested that the loss of the Y chromosome in the bone marrow may be a normal process associated with aging in the male.

In an earlier study O’Riordan et al.26 reported analyses of marrow chromosomes in 32 male subjects who had no primary hematologic disease. Three of these cases had a high percentage of marrow cells with 45XO chromosomes; in a fourth patient, the Y chromosome was hard to identify, and the authors could only state that the missing chromosome was in the G or Y group. The incidence of aneuploidy in this study was 9.9% in the patients under 65 and 13.1% in those over 65. Walker27 studied 23 hematologically normal males aged 48–73 and found that the two oldest (both 73 yr of age) had a significant loss of G group chromosomes in their aneuploid cells.

Sandberg and Sakurai28 reported that 9 of 62 males (15%) over the age of 60 without leukemia or other malignancy in the marrow were missing the Y chromosome from some of their bone marrow cells, whereas only 1 out of 38
such males under 60 had such cells. They were impressed that in over 200 males
of unstated age with acute leukemia none had cells missing the Y chromosome
from an otherwise normal karyotype, although there were several cases with a
missing Y chromosome; these patients were all in stable condition and had not
developed blast crisis. These authors felt that the absence of a Y chromosome
might protect patients from the development of the acute phase of a marrow
malignancy. We have reported a patient with Ph1 positive CML, coexistent
CLL, and a missing Y chromosome; this patient is also in stable condition
3 yr after diagnosis. Shifman5 has reviewed the association of deletion of the
Y chromosome with CML and discussed 19 Ph1-positive patients. He felt that
there was not a significant increase in survival of these patients when compared
to the overall group of patients with CML. One 69-yr-old patient with Ph1-
negative CML has also been reported to have 45X0 cells in the bone marrow.6

The association of the Y chromosome with solid tumors, the majority of
which were carcinomas, has been investigated by Vass and Sellyei.7 They found
that 23/136 or 17% of such tumors studied were negative for Y chromosome
fluorescence compared to 96 (71%) of tumors that were positive; the remainder
were scored as Y-positive, negative. There was no significant difference in the
mean age of these two groups, 65.5 yr for Y-positive tumors, 64.5 yr for Y-nega-
tive tumors. Bone marrow studies were not done on these patients.

It appears then, that the missing Y chromosome as it occurs in elderly males
is either a phenomenon associated with aging or in association with a variety
of hematologic disorders. The incidence in acute leukemia or in the blastic
phase of chronic myelogenous leukemia may be lower than that expected when
compared to the incidence in other disorders. It appears, however, that the
incidence of Y deletion in solid tumors in elderly males is similar to that seen
in the bone marrow of patients of comparable age.

We attempted to study the chromosomes of the erythropoietic tissue in this
patient to demonstrate if the diploid clone or the clone with Y deletion was
producing the PNH cells. One patient with a clonal abnormality in the marrow
has been reported in whom it was felt that the red cell hypoplasia represented
the poor erythropoietic capacity of the abnormal clone of cells.9 However, in
our patient, it was clear that stem cells with both 46XY and 45X0 karyotypes
were incorporating iron and presumably making red cells. Complement treat-
ment of marrow in an attempt to allow only the resistant cells to survive was
unsuccessful in that no growth of either cell line occurred after such treatment.
We were unable to prove, therefore, that either of the clones in the bone mar-
row were in fact, producing the PNH red cells. The marked clinical improve-
ment in this patient despite the persistent high percentage of XO cells in the
marrow, indicated that the PNH cells were produced either from both the XO
and XY cells or by the XY cells exclusively. After growth for 11 days in methyl-
cellulose, one cell (20%) of the metaphases was missing the Y chromosome and
four cells (80%) had a normal karyotype. Since culture in methylcellulose selec-
tively allows growth of myeloid colonies,\textsuperscript{29} it seems that both clones of cells are indeed stem cells, giving rise to both erythroid and myeloid elements. However, in comparison with the original bone marrow sample, the percentage of 45XO cells was much lower in the methylcellulose culture system. This finding may represent inability of the 45XO cells to adapt as easily as 46XY cells to the in vitro environment.

PNH has also been considered to be a "generalized growth abnormality" of the bone marrow similar to other myeloproliferative disorders.\textsuperscript{30} This view is prompted by the fact that at least two patients have been shown to have PNH in the presence of myelofibrosis,\textsuperscript{31} six patients have developed acute leukemia,\textsuperscript{32, 37} and two other patients have developed chronic myelogenous leukemia\textsuperscript{38, 39} in the course of PNH. Furthermore, both PNH and active leukemia often occur in the setting of marrow hypoplasia, whether idiopathic, congenital,\textsuperscript{15} or drug induced.\textsuperscript{40} We feel that the coexistence of a Y chromosome deletion and PNH in our patient tends to support the view that PNH is likely to be associated with a variety of myeloproliferative disorders. Indeed, where bone marrow studies have been done, five of eight patients with PNH have shown some abnormality in chromosome complement of their marrow cells.

REFERENCES

Acquired XO/XY clones in bone marrow of a patient with paroxysmal nocturnal hemoglobinuria (PNH)

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