Abnormal Karyotype Findings in Bone Marrow and Lymph Node Aspirates of a Patient with Malignant Lymphoma

By J. H. Tjio, John C. Marsh, Jacqueline Whang and Emil Frei III

RECENT ADVANCES in cytogenetics have permitted accurate chromosome analysis of some human neoplastic cells, particularly in patients with leukemia.16 A characteristic abnormal chromosome (Ph$^+$) occurs in the leukemic cells of the majority of patients with chronic myelocytic leukemia.7-12 The neoplastic cells of patients with other forms of leukemia and cancer have not shown consistent qualitative changes.5,6

The present report concerns a patient with lymphosarcoma and a leukemoid reaction who had consistent pseudodiploid chromosomal abnormalities in the cells of the bone marrow and lymph node aspirates.

The chromosome studies of the bone marrow and lymph node aspirates were performed without prior in vitro growth by the direct air-drying technic.12 Peripheral blood cultures and skin cultures were used for chromosome studies of leukocytes and fibroblasts.

CASE REPORT

H. B. (CC 03-49-18) is a 35-year-old white mother of five who was in good health until 2 days following the delivery of her fifth child in January, 1961, when she developed tender, diffuse adenopathy. She also noted dysphagia due to enlarged tonsils, easy fatigability, early morning sweats, and a weight loss of 20 pounds. On March 1 she was admitted to another hospital where an axillary lymph-node biopsy was thought to be consistent with a lymphoma, while the bone marrow and peripheral blood picture were suggestive of chronic myelocytic leukemia. There was no history of exposure to radiation or toxic chemicals. She was referred to the National Cancer Institute and admitted to the Clinical Center, National Institutes of Health, on April 4, 1961.

Positive physical findings included massive enlargement of the tonsils, anterior cervical, and submandibular lymph nodes, moderate enlargement of the posterior cervical, supravclicular, axillary, epitrochlear, and inguinal lymph nodes, a diffuse excoriated macular rash on all extremities, a small hemorrhage in the right nasal optic fundus, a grade 1 apical systolic murmur, splenomegaly (9 cm.), hepatomegaly (2 cm.), and 2 plus edema of the right leg and ankle. The blood pressure was 120/70, pulse 108, respirations 20, and temperature 37.4 C.

On admission the hemoglobin was 12.1 Gm. per cent, the white blood cell count was 27,800 with 53 per cent mature neutrophils, 8 per cent band forms, 4 per cent metamyelocytes, 4 per cent myelocytes, 1 per cent promyelocytes, 22 per cent eosinophils, 5 per cent lymphocytes, and 3 per cent monocytes. The platelet count was 375,000 (direct method). The blood urea nitrogen was 7 mg. per cent, blood glucose 98 mg. per cent, serum glutamic oxaloacetic transaminase 10 units, serum alkaline phosphatase 8 King-Armstrong units, total serum bilirubin 0.5 mg. per cent, thymol turbidity 3 units, and serum uric acid 1.2 mg. per cent. The total protein was 7.4 Gm. per cent with 3.37 Gm. per cent albumin, 0.39 Gm. per cent alpha-1 globulin, 0.88 Gm. per cent alpha-2 globulin, 0.94 Gm. per cent beta

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Table 1.—Admission Bone Marrow Differential*
(April 5, 1961)

<table>
<thead>
<tr>
<th>Myeloid Series</th>
<th>%</th>
<th>Erythroid Series</th>
<th>%</th>
</tr>
</thead>
<tbody>
<tr>
<td>Myeloblasts</td>
<td>2.7</td>
<td>Pronormoblasts</td>
<td>0.7</td>
</tr>
<tr>
<td>Promyelocytes</td>
<td>17.0</td>
<td>Basophilic normoblasts</td>
<td>4.0</td>
</tr>
<tr>
<td>Myelocytes</td>
<td></td>
<td>Polychromatophilic normoblasts</td>
<td>7.7</td>
</tr>
<tr>
<td>Neutrophilic</td>
<td>9.0</td>
<td>Acidophilic normoblasts</td>
<td>8.3</td>
</tr>
<tr>
<td>Eosinophilic</td>
<td>5.3</td>
<td>Total</td>
<td>20.7</td>
</tr>
<tr>
<td>Metamyelocytes</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophilic</td>
<td>10.0</td>
<td>Other</td>
<td>%</td>
</tr>
<tr>
<td>Eosinophilic</td>
<td>2.3</td>
<td>Lymphocytes</td>
<td>3.3</td>
</tr>
<tr>
<td>Band forms</td>
<td></td>
<td>Monocytes</td>
<td>0.7</td>
</tr>
<tr>
<td>Neutrophilic</td>
<td>10.0</td>
<td>Plasma cells</td>
<td>0.3</td>
</tr>
<tr>
<td>Eosinophilic</td>
<td>2.7</td>
<td>Total</td>
<td>4.3</td>
</tr>
<tr>
<td>Polymorphonuclear</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Neutrophilic</td>
<td>11.7</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Eosinophilic</td>
<td>4.3</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>75.0</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*Three hundred nucleated cells counted.

globulin, and 1.82 Gm. per cent gamma globulin. A urinalysis showed moderate numbers of white blood cells, a stool specimen was negative for occult blood, and the VDRL test was negative. A bone marrow examination showed granulocytic hyperplasia with eosinophilia (table 1). Two other bone marrow aspirates prior to therapy were similar, including the first one used for chromosome analysis (4/13/61). A classification of mitoses based on cytoplasmic characteristics was made on Giemsa-stained smears. The differential was 30 per cent erythroid forms, 39 per cent myeloid forms, and 31 per cent uncertain (100 mitoses counted).

Two subsequent bone marrow examinations following the initiation of chemotherapy, when the leukocyte count was in the range of 4,000-5,000, showed relative erythroid hyperplasia. One of these used for chromosome analysis (7/20/61) had a mitotic differential of 60 per cent erythroid forms, 16 per cent myeloid forms and 24 per cent uncertain (50 mitoses counted). No invasion of the marrow by lymphoma cells was seen at any time.

An axillary lymph node biopsy on 4/6/61 revealed replacement of the normal architecture by a diffuse infiltrate composed primarily of medium-sized lymphocytes. Many reticulum cells and eosinophils but no Reed-Sternberg cells were present. The histologic appearance was thought to be consistent with a lymphoblastic type of lymphosarcoma.

An inguinal lymph node aspirate on 4/13/61 examined with phase microscopy and Giemsa stain showed predominantly small lymphocytes with about 15 per cent medium to large mononuclear cells in which were found numerous mitoses. A few eosinophils and a very rare normoblast were seen. There was no infiltration with cells of the myelocytic series.

Laboratory values relevant to the question of the diagnosis of chronic myelocytic leukemia included leukocyte alkaline phosphatase values of 17 units (μmoles of p-nitrophenol liberated per hour per 10⁹ cells) in April, 1961, when the leukocyte count was 36,700 with 50 per cent cells in the neutrophilic series, and 98 units in September 1961, when the leukocyte count was 24,800 with 67 per cent cells in the neutrophilic series. (Normal range for this method is 25–173 units.14 A serum B₁₂ level in April, 1962 was 162 μg./ml.*}

*Courtesy of Dr. Charles Rath, Georgetown University School of Medicine. The normal range for his laboratory is 100–300 μg./ml.
The patient was treated with x-irradiation and a number of chemotherapeutic agents as outlined in Table 2. The enlarged lymph nodes responded initially to dichloromethotrexate but did not respond well to this or other chemotherapeutic agents subsequently. Major difficulties included gastrointestinal and hematologic toxicity, and frequent infection including bacterial tracheobronchitis, sinusitis, and Trichomonas vaginitis. The tumor tissue was strikingly radiosensitive. When last seen on August 16, 1962, the patient had persistent hepatosplenomegaly, moderate axillary lymphadenopathy and a leukocytosis of 90,000.

**CHROMOSOME STUDIES**

Table 3 shows the chromosome distribution in cells from the bone marrow, lymph node aspirate, cultured leukocytes and skin fibroblasts. The first
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Table 3.—Chromosome Distribution in Cells from Bone Marrow and Lymph Node Aspirates Without Prior In Vitro Growth and in Cultured Leukocytes and Skin Fibroblast Cells

<table>
<thead>
<tr>
<th>Date</th>
<th>Specimen</th>
<th>Treatment</th>
<th>Cumulative and Current Treatment*</th>
<th>Chromosome Number</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td>45</td>
<td>46</td>
</tr>
<tr>
<td>4/13/61</td>
<td>bone marrow</td>
<td>none</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7/20/61</td>
<td>bone marrow</td>
<td>UM, (DCM)</td>
<td>53</td>
<td>53</td>
</tr>
<tr>
<td>9/19/61</td>
<td>bone marrow</td>
<td>UM, DCM</td>
<td>7</td>
<td>7</td>
</tr>
<tr>
<td>10/6/61</td>
<td>bone marrow</td>
<td>UM, DCM (GAG)</td>
<td>55</td>
<td>55</td>
</tr>
<tr>
<td>10/16/61</td>
<td>bone marrow</td>
<td>UM, DCM, GAG</td>
<td>105</td>
<td>1</td>
</tr>
<tr>
<td>11/24/61</td>
<td>bone marrow</td>
<td>UM, DCM, GAG (GAG)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1/13/62</td>
<td>peripheral</td>
<td>none</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>lymph node</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>4/21/62</td>
<td>peripheral</td>
<td>none</td>
<td>150</td>
<td></td>
</tr>
<tr>
<td></td>
<td>blood</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>6/25/62</td>
<td>skin culture</td>
<td>UM, DCM, GAG, IsoTIC, x-ray, (x-ray)</td>
<td>150</td>
<td></td>
</tr>
</tbody>
</table>

*Abbreviations for treatment: UM = uracil mustard; DCM = dichloroacetyl methane; GAG = methylbis-glyoxal-guanylhydrazone; IsoTIC = isoterephthalanilide. Current therapy is indicated by parentheses.

marrow specimen, peripheral blood and lymph node aspirate were taken prior to therapy. The other specimens were taken at different times after or during chemotherapy or irradiation. The modality of all the samples was 46. Only the last two bone marrow samples and the lymph node aspirate had cells with deviating numbers. Analyses of the metaphases from the peripheral blood and skin fibroblast cultures showed a normal female karyotype (figs. 1 and 2). The bone marrow and lymph node specimens revealed an abnormal karyotype in nearly all metaphases examined (80-90 per cent). There were consistently two chromosomes missing, one each in groups 6-12 and 13-15 respectively. Instead, two new chromosome types were present: one was very similar to pair 3 and the other was acrocentric and slightly longer than pair 21 (figs. 3-5). The cells with 47 and 48 chromosomes in the lymph node aspirate had one and two small, apparently acentric, fragments respectively, in addition to the normal karyotype. This was also the case with the cells from the marrow which had 47 chromosomes.

The same chromosome abnormality was found in all marrow specimens—i.e., before and after treatment. No changes in distribution or morphology were found. The fact that the skin fibroblast and peripheral leukocyte cultures have a normal female karyotype indicates that the somatic karyotype of the patient was not congenitally abnormal.

The new chromosome type in the marrow and lymph node aspirates may have arisen during the progression of the disease as a result of interchromosomal translocations after breaks in certain regions, presumably involving chromosomes in groups 6-12 and 13-15. Therapy did not alter the abnormal karyotype although the patient did improve and was in partial remission when the second bone marrow specimen was obtained. The only significant change was a drop in the mitotic index in the third marrow specimen. In the subsequent sample the mitotic index was again increased.

DISCUSSION

While it is clear that this patient had lymphosarcoma the nature of the myelocytic leukocytosis deserves comment. The diagnosis of chronic myelo-
Fig. 1.—Metaphase from peripheral blood grown in vitro with normal female karyotype (X2600).

cyctic leukemia was considered but the normal leukocyte alkaline phosphatase, the absence of the Ph1 chromosome, and the normal serum $B_{12}$ value make this diagnosis unlikely. The diagnosis of leukemia, however, cannot be excluded since a minority of patients with chronic myelocytic leukemia will have normal leukocyte alkaline phosphatases and karyotypes. Only one of 69 patients with chronic myelocytic leukemia had a serum $B_{12}$ value within the normal range. Normal or elevated serum $B_{12}$ values have been reported in leukemoid reactions. Leukemoid reactions have been
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Fig. 2.—Metaphase from a skin fibroblast culture with normal female karyotype (X2400).

described frequently in Hodgkin's disease\textsuperscript{21-24} but they are much less common in lymphosarcoma.\textsuperscript{25} We have tentatively concluded that this patient has a leukemoid reaction secondary to her lymphosarcoma.

No consistent chromosome abnormalities have been found in the few
Fig. 3.—Pseudodiploid metaphase from lymph node aspirate, taken on 4/13/61, before treatment. Two chromosomes are missing, one each in groups 6–12 and 13–15 respectively. There are two new chromosome types: one is very similar to pair 3 and the other is acrocentric and slightly larger than pair 21 (X2600).
Fig. 4.—Pseudodiploid metaphase from bone marrow without prior in vitro culture, from a sample taken on 4/13/61, before treatment. Two chromosomes are missing, one each in groups 6–12 and 13–15 respectively. There are two new chromosome types: one is very similar to pair 3 and the other is acrocentric and slightly larger than pair 21 (X2400).
Fig. 5.—Pseudodiploid metaphase from bone marrow without prior in vitro culture, from a sample taken on 11/24/61, after the patient had been treated for about 5½ months. Two chromosomes are missing, one each in group 6–12 and 13–15 respectively. There are two new chromosome types: one is very similar to pair 3 and the other is acrocentric and slightly larger than pair 21 (X1600).
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...cytogenetic studies of human lymphoma cells thus far reported. In view of the findings in this patient, further chromosome studies of the lymph nodes of patients with lymphoma are under way. In the closely related lymphatic leukemias, aneuploidy has been frequent and qualitative chromosomal abnormalities may occur, but less commonly and they are not consistent.

The presence of the abnormal karyotype in the majority of the cells of the lymph node and bone marrow and its persistence in the marrow throughout a major course of the disease suggests that it is intrinsically related to the pathologic process. The presence of the identical karyotype abnormality in the morphologically normal myeloid cells of the marrow as well as in the tumor cells of the lymph node is unique and unexplained. The fact that 80–90 per cent of the mitoses examined were abnormal and that at least 30–60 per cent of the marrow mitoses were erythroid is strong presumptive evidence of involvement of the red cell series as well.

It has been suggested that the lymphocyte may be pluripotential and serve as the progenitor of the hemopoietic cells. It could be hypothesized that the patient’s chromosome abnormality occurred in a lymphocyte which served as a precursor both for the malignant lymphoblasts and and quantitatively abnormal marrow cells. It must be admitted however that there is considerable evidence against the theory that the lymphocyte serves as a hematopoietic precursor. Recent observations that the Ph chromosome of chronic myelocytic leukemia is present in the erythroid as well as the myeloid cells of the marrow, but not in the peripheral blood in remission, is additional evidence against this hypothesis. An alternative possibility might be that the neoplastic process, whatever its nature, affected separate cell types of the lymphocytic, myelocytic, and perhaps erythrocytic series simultaneously.

The abnormal karyotype is presumably limited to the lymphosarcoma and marrow cells since it was not demonstrated in a skin fibroblast culture. In addition, the chromosome analysis of the single blood culture taken did not reveal the abnormal karyotype. Its failure to be demonstrated in the peripheral blood culture may be due to the fact that the neoplastic cells were at a comparative disadvantage with normal cells in vitro. The frequency of observed abnormal karyotypes in acute leukemia has been shown to be less when blood or marrow is cultured in vitro than when direct preparations are made.

SUMMARY

A patient with lymphoblastic lymphosarcoma, leukocytosis, eosinophilia, and granulocytic hyperplasia of the bone marrow was treated with chemotherapy and radiation with many changes in her clinical state but maintained a consistent chromosome abnormality in the bone marrow before and during treatment. A lymph node aspirate obtained before treatment showed the same abnormality. There were consistently two chromosomes missing, one each in group 6–12 and 13–15 respectively. These were replaced by two new chromosomes: one was very similar to pair 3 and the other was acrocentric and slightly longer than pair 21. The skin and peripheral blood leukocyte...
culture had a normal female karyotype. The leukemoid blood picture was
differentiated from chronic myelocytic leukemia by the absence of the Ph1
chromosome, normal serum B12, and normal leukocyte alkaline phosphatase
activity.

**SUMMARIO IN INTERLINGUA**

Un patiellte feminin con lymphosarcoma lymphoblastic, leucocytosis, eosino-
ophilia, e hyperplasia granulocytic del medulla ossee esseva tractate con
chimotherapia e radiation. Isto resultava in numeros alteraciones del stato
clinic del patiellte, sed illa manteneva un persistente anormalitate del chromo-
smas in le medulla ossee que esseva notate ante e durante le tractamento.
Un aspirato de nodo lymphatic obtenite ante le tractamento monstrava le
mesme anormalitate. Uniformemente duo chromosomas esseva absente, un in
gruppo 6–12 e un in gruppo 13–15. Istos esseva reimpliciate per duo nove
chromosomas: le un esseva simile al par 3, e le altere esseva acrocentric e
evermente plus longe que par 21. Le pelle e le circulation peripheric resultava
in culturas de sanguine con leucocytos de normal karyotypo feminin. Le
tableau de sanguine leucemoide esseva differentiate ab chronic leucemia
myelocytic per le absentia del chromosome Ph1, normal vitamina B12 in le
sero, e normal activitate de phosphatase alcalin del leucocytos.

**ADDENDUM**

After the preparation of this manuscript, it was learned that the patient died at home on
September 14, 1982. No autopsy was performed.

**ACKNOWLEDGMENT**

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helpful advice and criticism.

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