Hypergranular Promyelocytic Leukemia (APL): Cytogenetic and Ultrastructural Specificity

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Cytogenetic and ultrastructural findings were important diagnostic indicators of hypergranular promyelocytic leukemia (APL) in a patient whose bone marrow morphology appeared, by light microscopy, to be similar to that in acute myeloblastic leukemia (AML) with maturation. Peripheral blood smears and bone marrow specimens examined by light microscopy showed few cells with the numerous coarse, azurophilic granules typical of APL. Cytogenetic analyses, with several banding techniques, of cells from bone marrow and unstimulated peripheral blood revealed the 15;17 translocation, which has been observed only in APL. A reinterpretation of the reciprocal translocation, based on R banding, suggests that the breakpoints are distal to q24 in No. 15 and at or near the junction of q21 and q22 in No. 17. In addition, the patient had disseminated intravascular coagulation. The characteristic morphology of granules seen in APL was observed in this case only when transmission electron microscopy was used, since the granules were quite small. Since treatment for AML differs from that for APL, identification of the 15;17 translocation and ultrastructural evidence of granules represent valuable diagnostic aids for APL.

RECENTLY, Golomb et al. observed that myeloid cells of two patients with hypergranular promyelocytic leukemia (APL) had an abnormally small chromosome 17, which was identified by quinacrine (Q) banding. These two were the only patients with a deleted No. 17 and the only ones with APL in a series of 50 adults with acute nonlymphocytic leukemia (ANLL).2-3 The proposal that this chromosomal abnormality might be specific for APL was supported by the prior observation of Engel et al.4 of a deleted No. 17 or 18 in a 6-yr-old girl with this disease. Subsequently, we studied a third patient whose bone marrow (BM) chromosomes had more distinct banding, and we identified a rearrangement involving No. 15 as well as No. 17.5 The chromosomal rearrangement in two cases of APL recently studied with Giemsa (G) banding was identified as a reciprocal translocation [t(15;17)(q22;q21)].6-7

We now report a patient whose BM cells showed the 15;17 translocation but whose marrow morphology appeared, by light microscopy, to be similar to that seen in acute myeloblastic leukemia (AML) with maturation. The characteristic morphology of primary granules seen in patients with APL was ob-
served in this case only when transmission electron microscopy (TEM) was utilized. In addition, this patient had disseminated intravascular coagulation (DIC), which is often associated with APL.8

MATERIALS AND METHODS

Peripheral blood (PB) and BM aspirates were examined, and differential cell counts were done. The PB and BM specimens were studied with peroxidase, periodic acid Schiff, α-naphthyl acetate esterase (with and without sodium fluoride), α-naphthyl butyrate, naphthol AS-D-chloroacetate esterase, and acid phosphatase reactions.

We classify all cases of acute leukemia at The University of Chicago according to the conventional morphologic and cytochemical criteria of the French-American-British Co-operative Group.9 Spicules from BM aspirates were prepared for TEM by conventional methods. Preparation and analysis of chromosomes from BM and PB were as previously described.10 All samples were analyzed with Q banding, G banding, and reverse (R) banding were each used in the analysis of one sample to allow precise identification of the 15;17 rearrangement.

CASE REPORT

The patient, a 20-yr-old black male, was referred for evaluation of bleeding gums and fatigue of 1 mo duration. On physical examination, on 12/1/76, he was found to be pale, with active gingival bleeding. There were no ecchymoses or petechiae. His temperature was 39.0°C; neurologic findings were normal. The hemoglobin was 9.0 g/dl, with a hematocrit of 26.9%, WBC count of 47.0 x 10⁹/liter, platelet count of 38 x 10⁹/liter, prothrombin time of 16.0 sec (control 12.0 sec), partial thromboplastin time of 25.3 sec (control 30.3 sec), plasma fibrinogen of 1.5 g/liter, and fibrin degradation products of >1000 µg/ml (normal <20 µg/ml). Examination of the PB smear showed 60% blasts, as well as 31% cells (counted as promyelocytes) with fine, dustlike azurophilic granules. Folded, indented, and dumbbell-shaped nuclei were present in many immature cells. The bone core biopsy specimen was 100% cellular, with marked diminution in the numbers of erythroid precursors and megakaryocytes. A BM specimen revealed 71% blasts, 20% promyelocytes with fine granules, 2% myelocytes, 5% erythroid precursors, and 1% lymphoid cells. Some cells in both the PB and BM had Auer rods; multiple Auer rods were rarely seen (<1/500 cells). The diagnosis, based on the BM findings, was AML with maturation. Lumbar puncture yielded leukemic cells.

The patient was treated initially with broad-spectrum antibiotics and heparin; cytosine arabinoside (ara-C), 6-thioguanine (6TG), and intrathecal methotrexate were begun 48 hr after admission. On the seventh hospital day, the patient became lethargic and confused. A computerized tomographic brain scan was normal, but a lumbar puncture yielded xanthochromic fluid without any blast cells. The patient's condition improved after the administration of mannitol, platelets, and decadron. Three weeks after his admission, the gingival bleeding had eased, and all coagulation tests were normal.

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One month after relapse, the patient was readmitted with a fever of 39.5°C. The hemoglobin now was 8.9 g/dl, with a hematocrit of 26.3%, WBC count of 50.3 x 10⁹/liter, platelet count of 29 x 10⁹/liter, prothrombin time of 16.6 sec, partial thromboplastin time of 25 sec, plasma...
fibrinogen of 2.0 g/liter, and fibrin degradation products > 2000 µg/ml. Although no bleeding was observed, broad-spectrum antibiotics and heparin were administered. Chemotherapy with ara-C and daunorubicin was initiated 48 hr later. Four days after this admission the patient developed a left hemiparesis that was rapidly followed by a comatose state. Computerized brain tomography revealed massive bilateral intracerebral hemorrhages. The platelet count decreased to 13 x 10^9/liter. Despite the administration of platelets, heparin, decadron, and mannitol, the patient died on the seventh hospital day, 8 mo from the date of diagnosis.

An autopsy showed leukemic infiltrates in the BM and spleen. Fibrin thrombi were present in the gastrointestinal tract and lungs. Massive intracerebral hemorrhage and brain stem herniation were the immediate causes of death.

RESULTS

Light microscopy of Wright-Giemsa-stained PB smears and BM specimens showed few cells with the numerous coarse, azurophilic granules typical of APL. Instead, the cytoplasm of the leukemic cells was either agranular or showed only a few fine granules (Fig. 1). The nuclear irregularity of many of the immature cells, however, was similar to that described by others as being characteristic of APL. Cytochemical analysis showed that 40% of the leukemic cells had slight to moderate peroxidase activity, with intense activity in only a small number. A similar proportion of cells (38%) showed naphthol AS-D-chloroacetate esterase positivity, varying from plus-minus to moderately intense activity. Acid phosphatase was present in 79% of the leukemic cells; most showed only a weak reaction, but in some, intensely positive granules were seen either scattered diffusely or accumulated in one area of the cell cytoplasm.

Fig. 1. Two representative fields of the initial BM aspirate as seen with light microscopy. Although azurophilic granules are present in a few cells, most have agranular cytoplasm. Some cells show irregularly-shaped nuclei. Wright's stain. × 1000.
Fig. 2. (A) Low-magnification transmission electron micrograph (TEM) of a BM spicule from a previously reported case of typical APL, as determined by light microscopy. Note the large, reniform nuclei as well as the distribution of dark primary granules throughout the cytoplasm. ×4400. (B) Low-magnification TEM of a BM spicule from the present case at the time of first admission. Large, reniform nuclei are readily seen. Although the dark granules are smaller than those in Fig. 2A, their distribution is similar in both cases. ×4400. (C) High-magnification view of a portion of the cytoplasm from a cell of the patient in Fig. 2A. Granules are usually larger than mitochondria. ×40,300. (D) High-magnification view of a portion of cytoplasm from a cell of the present patient. Dark granules are numerous but usually much smaller than mitochondria. ×39,700.
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Fig. 3. Partial karyotypes of chromosome pairs 15 and 17 from three G-banded metaphases obtained from unstimulated PB at the time of first admission. Long arm (q) of the abnormal 17 is small (17q-) due to a reciprocal translocation with one No. 15. Additional material on 15q+ appears to correspond to the distal end of q in the normal 17, including most of the lightly stained band 17q21.

The nonspecific esterase reactions (α-naphthyl acetate esterase with and without sodium fluoride and α-naphthyl butyrate) were positive in less than 5% of the leukemic cells; this finding appeared to rule out significant monocytic differentiation. Examination of BM elements by TEM revealed granules of irregular size and shape (Figs. 2B, 2D). The size and distribution of granules in this case were compared with those in a case easily diagnosed as APL by light microscopy5 (Figs. 2A, 2C). The distribution of granules was similar in the two cases, but the primary granules were usually much smaller than mitochondria in our present case, whereas in the easily diagnosed case the granules were usually larger than mitochondria.

Cytogenetic analysis showed that the rearrangement involving chromosomes 15 and 17 was present at the time of the patient's first admission, prior to treatment. Normal metaphases in the initial sample were indicative of the patient's constitutional karyotype. It was evident from the G-banding study that the rearrangement was a reciprocal translocation, with a possible designation of t(15;17)(q22;q21) (Fig. 3). The results of the cytogenetic analyses are summarized in Table I. A 48-hr culture of PB from 5/24/77, when the patient was in relapse, showed 100% abnormal metaphases. All metaphases examined had the 15;17 translocation, and all showed additional abnormalities. The modal karyotype was 48,XY,+8,+13,t(15;17). R banding indicated that more accurate locations of the breakpoints were in No. 15 distal to band q24 and in No. 17 at or near the junction of q21 and q22 (Fig. 4). All subsequent samples had abnormal cells, nearly all of which showed extra chromosomes in addition to the t(15;17) (Table I).

DISCUSSION

The 15;17 translocation appears to be highly specific for APL. The four patients with a t(15;17) seen at the University of Chicago had APL and were the only ones to show this karyotypic abnormality among 83 patients with ANLL who were observed over a 7-yr period.14 The only other reported cases of APL in which chromosomes were banded also showed this rearrangement.6,7
Table 1. Summary of Cytogenetic Findings

<table>
<thead>
<tr>
<th>Sample Date</th>
<th>Sample Source</th>
<th>Clinical Status</th>
<th>Blasts/ Promyel (%)</th>
<th>No. of Metaphases</th>
<th>No. of Chromosomes</th>
<th>Percent</th>
<th>With t(15,17)</th>
<th>Comments</th>
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<tr>
<td>12/01/76</td>
<td>BM, PB</td>
<td>AD, H, DIC</td>
<td>71/20</td>
<td>44</td>
<td>33(14)</td>
<td>79</td>
<td>Only t(15,17) in the abnormal metaphases</td>
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<tr>
<td>12/16/76</td>
<td>BM</td>
<td>AD</td>
<td>48/17</td>
<td>44</td>
<td>10(4)</td>
<td>0</td>
<td>Normal</td>
<td></td>
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<tr>
<td>01/10/77</td>
<td>BM</td>
<td>CR§</td>
<td>7/1.5</td>
<td>44</td>
<td>24(10)</td>
<td>0</td>
<td>Normal**</td>
<td></td>
</tr>
<tr>
<td>02/17/77</td>
<td>BM</td>
<td>CR§</td>
<td>6/2.5</td>
<td>44</td>
<td>41(17)</td>
<td>0</td>
<td>Normal</td>
<td></td>
</tr>
<tr>
<td>03/23/77</td>
<td>BM</td>
<td>CR§</td>
<td>6/2</td>
<td>44</td>
<td>40(12)</td>
<td>0</td>
<td>Normal</td>
<td></td>
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<tr>
<td>05/05/77</td>
<td>BM</td>
<td>CR§</td>
<td>8/1</td>
<td>44</td>
<td>25(9)</td>
<td>11</td>
<td>+8, +13,t(15,17)† †</td>
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<tr>
<td>05/24/77</td>
<td>PB</td>
<td>RL, DIC</td>
<td>89/1</td>
<td>44</td>
<td>49(39)</td>
<td>100(15%)</td>
<td>+8,t(15,17)</td>
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<tr>
<td>06/13/77</td>
<td>BM</td>
<td>RL, DIC</td>
<td>50/1</td>
<td>44</td>
<td>28(12)</td>
<td>8</td>
<td>+8, +13,t(15,17)§††</td>
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<td></td>
<td></td>
<td>+4C,+D,-G(no banding)††</td>
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<tr>
<td>06/30/77</td>
<td>BM</td>
<td>RL, DIC</td>
<td>87/6</td>
<td>44</td>
<td>88(13%)</td>
<td>88(13%)</td>
<td>Only t(15,17)††</td>
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<tr>
<td>07/06/77‡‡</td>
<td>PB</td>
<td>RL, DIC</td>
<td>Not Done</td>
<td>44</td>
<td>73(23)</td>
<td>100(4%)</td>
<td>Only t(15,17)††</td>
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*BM, bone marrow; PB, peripheral blood (without PHA).
†AD, active disease; DIC, disseminated intravascular coagulation; H, hemorrhaging; CR, complete remission; RL, relapse.
‡Blasts/promyelocytes expressed as percentage of total nucleated cells (400 cells counted) for each BM.
§Number in parentheses indicates number of metaphases examined in detail with fluorescence.
|| Calculated as percentage of metaphases examined with fluorescence.
*One abnormal metaphase had an extra No. 18.
**One abnormal metaphase had a pericentric inversion of a chromosome 12.
††Only one metaphase in the sample had this karyotype.
‡‡Patient died 1 wk after this date.
§§A review of the BM at a later date for this study showed that the patient was only in partial remission, since the percentage of blasts was greater than 5%.
|| These metaphases showed random loss of chromosomes; however, the t(15,17) and extra chromosomes present were consistent with those listed in the comments column.
Fig. 4. Karyotype of an R-banded metaphase cell obtained from unstimulated PB during relapse. Cell has t(15;17) and extra chromosomes 8 and 13 [48,XY,+8,+13,t(15;17)]. Inset, partial karyotypes of pairs 15 and 17 from this cell (top row) and another cell. Densely stained band q24 is present both in the normal 15 and in the 15q+, suggesting a translocation breakpoint distal to q24. Two narrow bands in 17q21 are present both in the normal 17 and in the 17q-, suggesting a breakpoint at or near the junction of q21 and q22. Probable breakpoints are identified with arrows, and the number above each arrow identifies the band proximal to the breakpoint [t(15;17)(q25;q22)].

There are several observations of cytogenetic interest. For example, the translocation was present in most BM cells on 12/1/76, before the patient had had any therapy. No evidence of the translocation was found in the next four samples, obtained soon after the initiation of therapy or when the patient was in clinical complete remission (Table I). The disappearance of the abnormal clone (or clones) during a complete remission is typically seen in ANLL. On 5/24/77, when the patient was in overt clinical relapse, all cells showed t(15;17) and further evolution of the karyotype. The additional No. 8 probably arose first, since the sample of 5/24/77 included six metaphases (15%) with only an extra No. 8, while none had only an extra No. 13. Therefore it appears that clonal karyotypic evolution progressed as follows during the course of the disease: 46,XY→46,XY,t(15;17)→47,XY,+8,t(15;17)→48,XY,+8,+13,t(15;17). Besides the major line of evolution of the abnormal clone, some cells had additional aberrations superimposed. An example is the extra No. 9 that was present in 8% of the cells on 5/24/77 and in 26% in the last sample on 7/6/77.

This patient is the first reported case of APL in whom karyotypic evolution was seen. Rowley and Potter observed a patient (their case 30) with AML who also acquired an extra No. 8 and an extra No. 13, which were superimposed on a clone carrying a 6;9 translocation. While an additional No. 13 is rare, an extra No. 8 is the most common abnormality seen in ANLL, both initially
and during the further clonal evolution in these patients. In each case, the evolution affected the initial abnormal clone.\(^2\)

G banding, both by us and by others,\(^6,7\) suggested that the breakpoints in the t(15;17) were at q22 in No. 15 and at the proximal end of q21 in No. 17. R banding indicated, however, that the breakpoints were distal to q24 in No. 15 and at or near the junction of q21 and q22 in No. 17. Multiple banding techniques afford greater precision in the interpretation of rearrangements. Since bands 15q24 and 17q21 stain distinctively with the R-banding technique (Fig. 4), this method was particularly valuable in the reinterpretation of the t(15;17) breakpoints.

Unlike the three previous cases of APL examined by us, this patient did not show the typical coarse, azurophilic granules of APL by light microscopy. The three earlier cases had variations in granule size, two having classic large granules and the third having smaller granules. Nevertheless, in each case more than 50% of the BM cells were classified as promyelocytes by light microscopy. From our TEM study, it appears that the granules in the present case were much smaller than mitochondria and just beyond the limit of resolution of the light microscope. Myeloblasts from acute granulocytic leukemia have been shown to have essentially no primary granules.\(^6\)

Sultan et al.\(^8\) examined 140 consecutive cases of what they termed AML, which was essentially ANLL, to assess the relationship between blast cell morphology and the occurrence of DIC. Of 18 patients with abnormal coagulation tests and DIC, 17 had the morphologic features of APL. It was the finding of DIC in association with a t(15;17) that led us to examine closely the ultrastructure of the malignant cells in this case.

Previously, treatment of APL with classic chemotherapy had been unsuccessful, and death was usually due to bleeding. In 1973, however, Bernard et al.\(^17\) reported remarkable results in the treatment of APL. More than 50% of the patients who received daunorubicin achieved complete remissions, with a median survival of 26 mo, compared to only 13% complete remissions of those treated with other kinds of chemotherapy. Therefore if a remission is obtained in APL it appears that it is longer than that in other acute leukemias. Our patient was not treated with daunorubicin until his last admission because he had responded fairly well to ara-C and 6TG up to that time and because BM morphology by light microscopy was more consistent with a diagnosis of AML than of APL. Whether or not this patient would have achieved a longer remission if he had been given daunorubicin therapy earlier in his disease course is impossible to know.

A recent editorial\(^18\) emphasized that more patients with malignancies should be studied cytogenetically in order "...to determine whether, as in the case of chronic myeloid leukaemia, cytogenetic criteria can improve the accuracy of diagnosis and provide a basis for subclassification of cases, whereby therapy can be planned with greater precision." The patient reported here is an example of the improved accuracy of diagnosis that can be achieved with the aid of both cytogenetics and TEM. Since the correct diagnosis of APL can have important therapeutic consequences,\(^17\) DIC, ultrastructural evidence of granules, and the t(15;17) are all important indicators of APL.
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REFERENCES

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