“Microgranular” Acute Promyelocytic Leukemia: A Distinct Clinical, Ultrastructural, and Cyto genetic Entity

By Harvey M. Golomb, Janet D. Rowley, James W. Vardiman, Joseph R. Testa, and Ann Butler

Three patients with acute leukemia, disseminated intravascular coagulation, and a specific acquired chromosome abnormality [t (15;17)] were found by transmission electron microscopy to have the typical distribution of granules seen in promyelocytes. However, the average granule sizes were 120, 170, and 180 nm, respectively, for the three patients, significantly less than the 250-nm resolution of light microscopy. We regard the leukemia in these three patients as comprising a distinct clinical, ultrastructural, and cytogenetic entity that we have chosen to call “microgranular” acute promyelocytic leukemia.

According to the criteria of the French-American-British (FAB) cooperative study group, the diagnosis of acute promyelocytic leukemia (APL) should be made only when the “great majority” of cells are abnormal promyelocytes whose cytoplasm is packed with coarse granules and which have prominent Auer-like bodies. Clinically, APL is a form of acute leukemia characterized by episodes of hemorrhage and by disseminated intravascular coagulation (DIC).² Cyogenetically, a specific chromosome abnormality [t (15;17)] has been identified in patients with APL;³ this translocation has not been reported in any of the other acute leukemias of the FAB classification.⁴

In 1978, we reported on a patient whose bone marrow appeared, by light microscopy, to be similar morphologically to that in acute myeloblastic leukemia with maturation. In addition to DIC, the patient was found on cytogenetic analysis to have the 15;17 translocation. The characteristic distribution and morphology of granules seen in APL were observed only when transmission electron microscopy (TEM) was used.³ More recently, in reviewing our experience from 1973 to 1978,⁴ we briefly alluded to a similar case. The present report was prompted by a third identical patient who was first examined in early 1979. We now describe in detail three patients with DIC, a t(15;17), and a typical distribution of granules in promyelocytes, which, in most cells, could be observed only with TEM.

MATERIALS AND METHODS

Wright-stained peripheral blood (PB) and bone marrow (BM) aspirates obtained from the 3 patients were examined, and 500-cell differential counts were done. The PB and BM specimens were studies with peroxidase, periodic acid-Schiff, α-naphthyl acetate esterase (ANAE) with and without sodium fluoride, α-naphthyl butyrate, naphthol AS-D-chloroacetate esterase, and acid phosphatase reactions.

Spicules from BM aspirates were prepared for TEM by conventional methods. Preparation and analysis of chromosomes from BM and PB were as previously described.⁵ All samples were analyzed with Q banding,⁶ G banding⁷ and reverse (R) banding⁸ were each used in the analysis of one sample from patient 1 to allow precise identification of the 15;17 rearrangement.

CASE REPORTS

Case 1

A detailed case report was presented previously.⁷

Case 2

The patient, a 27-yr-old black woman, was admitted to the Obstetrics Service in June 1978, at approximately 15 wk into her first pregnancy, because of bleeding gums and hematuria. One week prior to admission, the patient experienced dull lower abdominal pain that caused no distress. At the same time, she had bleeding gums. She saw her dentist, who cleaned her teeth and started her on penicillin tablets. Her gums continued to be painful, and 1 day prior to admission she noticed blood-tinged urine. On the day of admission, gross hematuria had developed. The patient also had bruised easily for several days, but she denied having rectal or vaginal bleeding. No fever or cough was present. The past medical history included a previous admission 5 yr earlier for repair of an umbilical hernia.

Physical examination revealed a blood pressure of 120/60 mm Hg, a regular pulse of 90/min, and regular respirations of 16/min; the patient was afebrile. The skin contained multiple ecchymoses. The ocular fundi showed no evidence of hemorrhage or exudate. The gums were swollen and bleeding and were covered with petechiae. The chest was clear, and the abdomen was protuberant without a palpable liver or spleen. The findings in the remainder of the examination were unremarkable; no localized neurologic signs were discovered.

The initial laboratory examination revealed a white blood cell (WBC) count of $10^9$ cells/liter with 15% bands, 5% lymphocytes, 4% monocytes, and 90% myeloblasts. The hematocrit (H) was 29.3% and the platelet count was $10^9$ cells/liter. The patient was immediately transferred to the Hematology Service. Routine chemistry laboratory values on the day of admission were within normal limits. However, the plasma fibrinogen was 0.07 g/liter (normal, 0.1-0.4 g/liter). The prothrombin time (PT) was 20.3 sec, with a control of 12.4 sec, and the partial thromboplastin time (PTT) was

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38.5 sec with a control of 26.2 sec. Fibrin degradation products (FDP) were reported between 40 and 80 μg/ml (normal, less than 20 μg/ml).

The patient was diagnosed as having acute myelogenous or myelomonocytic leukemia at the time of admission, underwent a therapeutic abortion, and was begun on chemotherapy 1 day later. Chemotherapy consisted of our TAD regime, which included 6-thioguanine (100 mg/sqm every 12 hr for 5 days), cytosine arabinoside (200 mg/sqm with continuous 24-hr infusion for 5 days), and daunorubicin (60 mg/sqm i.v. push daily for 3 days). A BM biopsy done approximately 1 mo after the initiation of therapy showed a cellularity of 15% with clusters of blasts. A second cycle of chemotherapy was started 5 wk after the initial one; at the start the hematocrit was 27.9%, the WBC count 3.7 x 10^9/liter, and the platelet count 200 x 10^9/liter. A BM specimen obtained 1 mo later showed residual acute leukemia; at that time, the Hct was 31.9%, WBC count 22.3 x 10^9/liter, and the platelet count 367 x 10^9/liter. The patient now refused any further inpatient therapy and was referred to a school physician, who noted a WBC count of 4.35 x 10^9/liter, petechiae on her thighs and lower legs. About 2 days prior to admission when she noted multiple ecchymoses as well as melena, hematemesis, or hematuria, but complained of persistent oozing from the BM aspiration site in the sternum. During physical examination, her temperature was 37.8°C, the pulse 100/min and regular, respirations 14/min and regular, and the blood pressure 130/80 mm Hg. The patient was slightly pale, with multiple ecchymoses on her extremities and on her soft palate. There was oozing from the sternal marrow site. The remainder of the findings in the examination were unremarkable; no adenopathy, hepatosplenomegaly, or abnormal neurologic findings were observed. Admission laboratory values included a WBC count of 2.1 x 10^9/liter, with 18% neutrophils, 76% small lymphocytes, 4% monocytes, 1% blasts, and 1% metamyelocytes. The platelet count was 5 x 10^9/liter and Hct 18%. Electrolyte levels and other blood chemistry tests at the time of admission were normal. Fibrinogen was 0.1 g/liter (normal, 0.1–0.4 g/liter). The initial PT was 18.5 sec (control, 11.9 sec), and the PTT was 23.8 sec (control, 25.1 sec). An admission cardiodiogram showed a sinus tachycardia with a rate of 120/min and borderline left ventricular hypertrophy. Nonspecific T-wave inversion was also seen. A chest x-ray showed no abnormality.

A BM aspirate obtained at the time of admission revealed a hypercellular marrow, with numerous blasts replacing the normal marrow elements. Many blasts had convoluted nuclei and very fine cytoplasmic granules. A bone core biopsy done at the same time showed an overall cellularity of 99%; no megakaryocytes and few erythroid precursors were detected. The abnormal cells had folded,
irregular nuclei with large nucleoli. Approximately 60% of the cells contained fine cytoplasmic granules; large, dark azurophilic granules were seen in only 10% of the cells. One day after admission, FDP were between 1280 and 2560 µg/ml (normal, less than 20 µg/ml). Within 24 hr of admission and upon return of the FDP results, the patient was begun on combination chemotherapy for her acute leukemia. The treatment consisted of TAD, as for case 2. In addition, continuous-infusion heparin was begun at 2000 U intravenous push, to be followed by 600 U every hour continuously.

A repeat BM aspirate obtained 13 days after the start of treatment, when the hemocrit was 35% (posttransfusion), WBC count 0.4 x 10⁹/liter, and the platelet count 69 x 10⁹/liter, showed 5% cellularity, with the majority of cells consisting of promyelocytes and blasts. At this time, the second course of TAD was begun at the same dosages and completed 5 days later. A BM specimen obtained approximately 3 wk after the start of the second cycle of TAD showed an overall cellularity of less than 1%, with very few hematopoietic elements. No clusters of immature myeloid cells were noted. A repeat BM aspirate 2 wk later, at a time when the WBC count was 0.1 x 10⁹/liter, showed little cellularity. Two weeks later, or approximately 2 mo after the initial admission, the patient had a WBC count of 2.4 x 10⁹/liter with 52% neutrophils, 40% lymphocytes, 7% monocytes, and no blasts. The platelet count was 44 x 10⁹/liter and the hematocrit 32%. Evaluation 11 wk after initiation of TAD demonstrated normal blood counts, however, a BM aspirate showed residual leukemia.

RESULTS

Clinical

The clinical characteristics of all patients are summarized briefly in Table 1. All three patients presented with bleeding complaints; the first two had bleeding gums, the third had multiple ecchymoses as well as a prolonged menstrual cycle. All three had laboratory evidence of DIC with increased FDP.

Light and Electron Microscopy

Light-microscopic evaluation showed all three patients to have less than 50% hypergranular promyelocytes in their BM, but to have a significant percentage of promyelocytes with fine, dust-like granules, often concentrated in one area of the cytoplasm (Table 2). In each case, more than 50% of the cells had irregular, folded, and bilobed nuclei that resembled monocytic nuclei (Fig. 1). Cytochemically, cells from
patients 1 and 2 were positive for peroxidase and naphthol AS-D-chloroacetate esterase, and fewer than 5% of the cells showed any positivity with the nonspecific esterase reaction. For patient 3, more than 95% of the abnormal cells had an intense peroxidase and naphthol AS-D-chloroacetate esterase reaction. However, 40% of the cells showed a moderate reaction with alpha-naphthyl acetate, and this esterase activity was inhibited with sodium fluoride.

Ultrastructural examination demonstrated a dilated endoplasmic reticulum in all three cases (Fig. 2). The average granule sizes were 120, 170, and 180 nm for patients 1, 2, and 3 respectively. (The limit of resolution of light microscopy is approximately 250 nm.) All granules were dense; those in case 3 were not typical of monocytic differentiation despite the cytochemical reactions observed. A histogram showing the distribution of granules, according to size, of each of the three patients is given in Fig. 3. Granules larger than 250 nm comprised only 2%, 15% and 9% of the total population of granules in patients 1, 2, and 3 respectively. In addition to the data from these three patients, the distribution of granule size is included for two previously reported cases of classical hypergranular promyelocytic leukemia from our institution (Fig. 3, A and B). One of these patients1 had granules of an average size of 640 nm, and all were larger than 250 nm; the second patient2 had granules of an average size of 270 nm, approximately 50% being larger than 250 nm.

Cytogenetic

In each of the three patients, a 15;17 rearrangement was present in a high percentage (79%–100%) of the metaphases from the initial sample (Table 3). The structural rearrangement seemed to be identical in all of the abnormal metaphases from patients 1 and 2 and in one of the metaphases from patient 3 [t (15;17) (q25?; q22?)]. All of the other abnormal metaphase cells in patient 3, however, had another rearrangement; whereas the translocation between nos. 15 and 17 appeared to involve the breakpoints mentioned above, the 17q− chromosome had additional unidentified bands on the short arm (Fig. 4). The 17p+ q− chromosome was submetacentric with conventional stain and did not appear to be an isochromosome of the long arm of the deleted no. 17 when it was examined with quinacrine fluorescence.

All three patients had one or more cytogenetic analyses during the period of response, and each had a normal karyotype during this time. Patient 1 showed clonal evolution of the karyotype to further complexity during relapse.7 Patients 2 and 3 continued to have normal karyotypes as of May 1979.

DISCUSSION

It seems clear that these three patients belong to a subgroup of ANLL that has only recently been identified.7,8,14,15 There is disagreement as to the correct classification of these patients.8,15 It is our contention that the clinical syndrome of DIC, the ultrastructural determination of numerous primary granules, and the
Table 3. Summary of Cytogenetic Findings

<table>
<thead>
<tr>
<th>Patient</th>
<th>Sample Date</th>
<th>Sample Source</th>
<th>No. of Metaphases</th>
<th>No. of Chromosomes</th>
<th>Percent With t(15;17)t</th>
<th>Karyotype</th>
</tr>
</thead>
<tbody>
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<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1</td>
<td>12-01-76</td>
<td>BM, PB</td>
<td>5</td>
<td>44</td>
<td>31(14)</td>
<td>46,XY/46.XY,t(15;17)(q25?;q22?)f</td>
</tr>
<tr>
<td>2</td>
<td>6-12-78</td>
<td>BC</td>
<td>3</td>
<td>44</td>
<td>22(14)</td>
<td>46.XX$/$46.XX,t(15;17)(q25?;q22?)</td>
</tr>
<tr>
<td>3</td>
<td>2-6-79</td>
<td>BM</td>
<td>1</td>
<td>45</td>
<td>35(21)</td>
<td>46.XX,t(15;17)(q25?;q22?) $|$</td>
</tr>
</tbody>
</table>

*BC, bone core biopsy; BM, bone marrow aspirate; PB, peripheral blood (without PHAI).
†Number in parentheses indicates number of informative metaphases examined with fluorescence.
‡Calculated as percent informative metaphases examined with fluorescence.
§Only one metaphase seen in this sample showed a normal karyotype.
||Subsequent samples at the time of relapse showed clonal evolution to a more complex karyotype.
\[In 20 of 21 banded metaphases, the 17q- had additional unidentified bands on the short arm.

Cytogenetic findings of the t(15;17) combine to identify this subgroup as a variant of acute promyelocytic leukemia, which we have chosen to call the “microgranular” type.

All 3 patients had DIC, which is consistent with the observation of Sultan et al.16 that, of 140 consecutive patients who had ANLL, 17 of 18 patients with abnormal coagulation tests had APL.

Although our three patients had less than 10% hypergranular cells, with a larger percentage of cells containing only dust-like granules by light microscopy, TEM showed that the majority of cells in these patients contained many granules that were less than 250 nm in size and thus smaller than the limit of resolution of light microscopy. By light microscopy, however, similarities were observed in the nuclear configuration in the cells of these three patients and in four of our patients with the more classic variety of

**Fig. 4.** Karyotype of a Q-banded metaphase cell obtained from a BM sample from patient 3. The metaphase has a translocation between the long arms of one no. 15 and one no. 17 [t(15;17)(q25?;q22?)]. The abnormal 17 also has additional unidentified bands on the short arm. Inset: partial karyotype of pairs 15 and 17 from another metaphase from the same BM sample; there is a t(15;17)(q25?;q22?), but without any alteration of the short arm of the abnormal 17.
APL; reniform, folded, and bilobed monocyte-like nuclei were numerous in each. Cells from all three patients contained extremely dilated rough endoplasmic reticulum (RER), although one patient also had cells with nondilated RER. Dilated RER in the promyelocytes of APL patients was recently reported by Parkin and Brunning.17

The specificity of the 15;17 translocation for APL has been suggested by our group in a study of 90 ANLL patients, 4 of whom (including one from the present study) had the t(15;17); all 4 had APL.18 Recently Van Den Berghe et al.5 reported a t(15;17) (q26;q22) in 11 of 16 APL patients. A 15;17 translocation has also been reported for APL patients seen by several other investigators.19-21 This rearrangement was observed in 9 of 17 APL patients reviewed at the First International Workshop on Chromosomes in Leukemia;6 in contrast, it was not seen in any of the 224 patients who had other forms of ANLL.

The 15;17 structural rearrangement in patients 1 and 2 was identical to that seen in four of our previously reported patients with classic hypergranular promyelocytic leukemia.4 The breakpoints in the long arm of nos. 15 and 17 in patient 3 also appeared to be identical to those in these other patients; however, in patient 3 the 17q chromosome had additional unidentified material on the short arm in all but one of the abnormal cells. The metaphase with the usual t(15;17) in patient 3 probably represents the original abnormal clone, whereas the metaphases with the 17p+q represent a subsequent structural rearrangement in this clone.

Although the diagnosis of “microgranular” APL seems warranted from the data presented, the positive ANAE reaction in case 3 remains to be explained. Analysis of case 3 by TEM showed only primary granules; there was no evidence of monocytic granules. Although the positive ANAE reaction is more in keeping with a monocytic origin of some of the cells, a similar pattern has been described previously for the cytochemistry of APL.22,23 Thus, the positive ANAE reaction in case 3 would suggest a FAB classification of M2, yet TEM evaluation would demand an M3 designation. Cases 1 and 2 would have been classified as an M2 by light microscopy and cytochemistry, but TEM analysis would require an M4 designation.

Recently, Berger et al.14,15 reported on three patients with a t(15;17) who showed a type of ANLL similar to that of the patients in this report. They classified their patients as AML, although they acknowledged that they were atypical. Their cases were characterized by a high WBC count, monocytic-like myeloblasts, DIC, and a t(15;17) in the majority of leukemic cell mitoses. Light microscopy showed reniform folded nuclei, as described in our three cases; cytochemistry yielded positive peroxidase, but essentially negative napthal AS-D-chloracetate esterase and ANAE. Transmission electron micrographs were not labeled by cases; most cells had reniform nuclei and sparse primary granules; there was one example of a cell with multiple Auer rods.

We recently used TEM to analyze the cell ultrastructure of 25 patients with ANLL (Butler A, et al., unpublished observations). One patient, a 37-year-old white woman, had no DIC, had a normal karyotype, and was classified by the FAB system as M2 (i.e., acute myelogenous leukemia with some maturation). By TEM analysis, the majority of cells were found to contain numerous granules that averaged 190 nm in size and were similar in distribution to those in our 3 patients with microgranular APL (Fig. 5).

It seems to us, therefore, that there are patients with ANLL that form a distinct subgroup, although there is some variability in the morphology and cytochemistry of the leukemic cells. This group is characterized by a hematologic history and laboratory evidence of DIC; by cytochemical findings that are predominantly myelocytic, but with an occasional case with monocytic staining characteristics; by ultrastructural findings of few to many cytoplasmic primary granules that are usually smaller than the resolution of light microscopy; and by the presence of the t(15;17). In view of the number of features that are typical of classical APL, we prefer to classify these patients as APL, “microgranular” variant. It is apparent, however, that there is a spectrum of morphological forms from myeloblastic through promyelocytic to monocytic; the precise position within this continuum of the patients presented in this report must await the careful cytogenetic, cytochemical, and TEM analysis of additional patients.

It is important clinically to make the correct diagnosis of APL as early as possible in order to initiate appropriate therapy. Bernard et al.24 showed that

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**Fig. 5.** Histogram represents the distribution of primary granule size in the cells of a patient with acute myelogenous leukemia with maturation (M2). The distribution is similar to that seen in the cases of microgranular acute promyelocytic leukemia.
daunorubicin can induce remission in about 50% of APL patients with a median survival of 26 mo. Drapkin et al.\textsuperscript{25} suggested that prophylactic heparin along with chemotherapy may allow a better chance of induction remission. Thus, it is important that possible cases of APL be studied cytochemically, ultrastructurally, and cytogenetically; the determination of the diagnosis of APL has therapeutic importance.

REFERENCES


ADDENDUM

Since acceptance of this manuscript, the French-American-British (FAB) Group have suggested recognition of a variant form of hypergranular promyelocytic leukemia (M3) in which the cells are characterized by bilobed, multilobed, or reniform nuclei, and cytoplasm with minimal or no granulation. A few cells with the features of typical M3 will be present in such instances. The cases in this report correspond to this variant group. [Bennet JM, Catovsky D, Daniel MT, Flandrin G, Galton DAG, Gralnick HR, Sultan C: A variant form of hypergranular promyelocytic leukemia (M3). Br J Haem (in press)].
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