Cytochemistry of Acute Promyelocytic Leukemia (M3): Leukemic Promyelocytes Exhibit Heterogeneous Patterns in Cellular Differentiation

By Masao Tomonaga, Yoshiru Yoshida, Masako Tagawa, Itsuro Jinnai, Kazutaka Kuriyama, Tatsuhiko Amenomori, Akira Yoshioka, Tatsuki Matsuo, Hiroaki Nonaka, and Michito Ichimaru

Cytochemical investigation of leukemic promyelocytes from 25 cases of acute promyelocytic leukemia (M3) disclosed two major cellular differentiation categories: (1) the pure neutrophilic (N) type (16 cases) with strong myeloperoxidase (MPO) and naphthol-ASD chloroacetate esterase (Es-chl), but lacking the monocytoenic enzyme NaF-sensitive alpha-naphthyl butyrate esterase (Es-b), and (2) the mixed monocytopoietic (M/N) type (seven cases) with strong Es-b as well as strong MPO, all cases exhibiting Es-dual (Es-b + Es-chl) positive cells. Two more cases with unusual phenotypes were noted: one with intense lycosome activity but without Es-b and the other with toluidine blue-methachromasia and negative MPO. Promyelocytes from the control group, consisting of nine cases of (8;21) M2 AML and ten cases with normal bone marrow, lacked such cytochemical heterogeneity. HL-60, an M3 cell line that can be induced to differentiate toward monocytic lineage in vitro, was almost negative for Es-b in the uninduced condition. Cytogenetically, eight cases of N type and five of N/M type had the t(15;17) abnormality. Thus at least two differentiation patterns were observed in M3 leukemia with fertility (N type) and infidelity (N/M type) for normal granulocytic differentiation. In this series, there was no statistically significant difference in clinical features (remission rate and survival) between the two types. Our study suggests that the development of M3 leukemia is not exclusively restricted to the neutrophilic pathway, but more heterogeneously related to myelomonocytic differentiation.

© 1985 by Grune & Stratton, Inc.

From the Department of Hematology, Atomic Disease Institute, Nagasaki University School of Medicine, Nagasaki, Japan.

Submitted Sept 17, 1984; accepted Feb 11, 1985.

Address reprint requests to Dr Masao Tomonaga, Department of Hematology, Atomic Disease Institute, Nagasaki University School of Medicine, Sakamoto-machi 12-4, Nagasaki 852, Japan.

© 1985 by Grune & Stratton, Inc.

0006-4971/85/6602-002/$03.00/0
**Heterogeneous Differentiation in APL**

**Cytogenetic analysis.** In 22 out of 25 cases, BM samples were processed directly. Metaphases were analyzed by standard and G-banding methods. In five cases no mitosis was noted, and in two cases metaphases were inadequate for analysis.

**Transmission electron microscopic (TEM) study.** Eight patients were studied by standard TEM method. On magnified photographs of M3 cells, the diameter of primary granules was measured in at least 25 cells for each case, and mean diameters and standard deviations were calculated.

**Chemotherapy protocols.** In this series, induction regimens consisted of three combinations of drugs, all including one anthracycline (daunorubicin [DR] or aclacinomycin [AR]) and cytosine arabinoside (ara-C): (1) DCMP regimen for 16 cases (DR 40 mg/m²/d for five to ten days, ara-C 120 mg/m²/d for seven to 14 days, 6-mercaptopurine 100 mg/d for seven to ten days, and prednisolone 30 mg/m²/d for seven to 14 days); (2) DCVP for six cases (DR and ara-C at the same doses for five and seven days, respectively, vincristine 2 mg on the second day, prednisolone 30 mg/d for seven days). Therapeutic outcomes were classified into complete remission (CR), partial remission, or early death and failure, according to Acute Leukemia Cooperative Group B’s criteria.

**RESULTS**

**Morphological Observations**

Except for three cases of M3V and one with metachromatic granules, most of the cases showed typical morphology, such as bilobed nuclei, hypergranularity, and faggots of Auer’s rod, as shown in Fig 1A. In two cases, faggots were absent, but single Auer’s rods were observed. Three cases of M3V showed low percentages of hypergranular cells—7%, 8%, and 10%, respectively (Fig 2A). Faggots were also noted in these cases. The case with basophil-like cells was a 25-year-old female. More than half of the leukemic cells had moderately coarse granules that stained metachromatic with May-Grünwald-Giemsa (Fig 3A). Bilobed nuclei, hypergranularity, and faggots were typical of M3. This patient showed a fulminating course with extensive DIC and relative resistance to DCVP chemotherapy.

**Cytochemistry of M3 Promyelocytes**

**MPO.** All but the case with basophil-like feature showed 100% 3+ activity (Fig 1B). In the latter, the positive rate was 47%; all positive cells showed 3+ activity, 53% of the leukemic cells were completely negative, and some MPO-negative Auer’s rods were noted (Fig 3B).

**Es-chi.** The positive rate ranged from 55% to 100%. Most of the leukemic cells showed 3+ activity, but in a few cases, including two of M3V, 1+ or negative cells were observed in more than half the leukemic cells.

**Es-b.** In seven cases, cells with strong activity were found (Fig 4C), the positive rate ranging from 9% to 95% with diffuse granular staining. One of the M3V cases was included in this group. The Es-b activity was almost completely inhibited by the addition of sodium fluoride (NaF) in all of these cases (Fig 4D). In the other seven cases, weak (1+) activity was observed in occasional cells, but this was resistant to NaF.

**Es-dual.** Of 22 cases examined, six cases that showed NaF-sensitive Es-b activity had single cells exhibiting a mixture of blue (Es-chi) and reddish brown (Es-b) tone, namely, purple; the positive rate ranged from 1% to 30% (Fig 4B). Cases with NaF-resistant weak Es-b activity did not have Es-dual cells.

**AP.** The activity varied considerably; 3+ to 2+ positive rate ranging from 0% to 86%. In one case of M3V, a complete absence of AP was noted.

**BG.** In contrast to AP, BG activity showed less variation; 3+ to 2+ positive rate ranged from 35% to 100%. The M3V cases with AP-negative cells showed the weakest activity.

**Lysosome.** Three out of eight cases examined exhibited definite lysis of bacilli, indicating positive enzyme activity; the positive rate ranged from 4% to 47%. One of three M3V cases showed a positive rate of 46%, and the activity of each cell was as strong as leukemic or normal monocytes (Fig 2B).

**PAS.** The positive rate ranged from 0% to 45%, and the reaction pattern was diffuse.

**Toluidine blue.** The unique case with metachromatic granules on May-Grünwald-Giemsa–stained smear, also showed a metachromasia with this dye in 42% of the leukemic cells (Fig 3C). In the other two cases, all leukemic cells were completely negative.

**Cytochemical Categorization of M3 Cases**

Based on the cytochemical heterogeneity observed, differentiation patterns of M3 cells in 25 cases were classified into two major categories: (1) pure neutrophilic (N) type (16 cases) and (2) mixed neutrophilic/monocytoid (N/M) type (seven cases). N type was defined by the presence of strong activities for two major neutrophilic enzymes, ie, MPO and Es-chi, but the absence of monocytic enzymes, ie, NaF-sensitive Es-b. N/M type was characterized by the simultaneous expression of neutrophilic and monocytic enzymes. The results of cytochemical staining are summarized in Table 1. The unique M3V case with strong lysozyme but lacking Es-b and the curious case with toluidine blue metachromasia were excluded from this categorization because of the scarcity of cases examined for these parameters in the present series.

**Comparative Studies Between N-Type and N/M-Type**

**Clinical and hematologic parameters.** Age, sex, hemoglobin concentration, WBC count, platelet count, hemostatic parameters (fibrinogen and fibrin degradation product), and degree of bleeding and organomegaly were compared, but no significant difference was noted between the two types (Table 2).

**Light-microscopic morphology.** The percentages of cells with hypergranularity and bilobed nuclei were not significantly different. One of three M3V cases belonged to N type and the other one to N/M type (Table 1).

**Electron microscopic morphology.** TEM morphology revealed a typical microgranular pattern in cells from two cases of M3V. The mean diameter of primary granules was 168 ± 49 and 221 ± 66 nm, respectively. Four cases of N type showed a mean diameter of 257 ± 65, 286 ± 71, 299 ± 83, and 329 ± 83 nm, respectively. In two cases of N/M type with high percentages of typical Es-dual positive cells, the...
A typical case of the pure neutrophilic (N) type showing confluent azurophilic coarse granules and nuclear lobulation, typical of M3 leukemia. This case carries t(15;17) abnormality. May-Grünwald-Giemsa (MG) stain (original magnification x 1,000; current magnification x 900). (B) MPO stain. Most intensive MPO activity is observed in all leukemic cells (original magnification x 1,000; current magnification x 900).

Fig 2. (A) A typical case of the M3 variant form shows absence of confluent azurophilic granulation but occasional cells carry faggots of Auer’s rod (MG stain. original magnification x 1,000; current magnification x 900). (B) Cytobacterial lysozyme stain. 46% of the leukemic cells show modest to strong lysis of Micrococcus lysodeikticus surrounding them, indicating positive lysozyme activity. Counterstained with MG (original magnification x 400; current magnification x 360).

Fig 3. (A) A case with basophil-like features. More than half the leukemic cells exhibit metachromasia of granules for MG stain (original magnification x 400; current magnification x 360). Hypergranulation and nuclear lobulation are similar to those of typical M3 cases. (B) MPO stain. Forty-seven percent of the cells are strongly MPO positive, but the remaining 53% are completely negative and even MPO-negative faggots of Auer’s body are observed (original magnification x 1,000; current magnification x 900). (C) Toluidine blue stain. Forty-three percent of the cells are stained metachromatic with this dye, suggesting basophil-like feature together with MPO negativity (original magnification x 1,000; current magnification x 900).

Fig 4. (A) A typical case of the mixed neutrophilic-monocytoid type shows similar azurophilic hypergranulation as in cases of N type but less prominent lobulation of the nucleus. This case also carries t(15;17). MG stain (original magnification x 1,000; current magnification x 900). (B) Dual staining for alpha-naphthyl butyrate esterase (Es-b) and naphthol ASD-chloroacetate esterase (Es-chl). Ten percent of the leukemic cells are Es-dual positive (purple staining) and remaining cells being solely Es-chl positive (blue staining) (original magnification x 1,000; current magnification x 900). (C) Es-b single stain. Eighty-five percent of the leukemic cells show modest to strong Es-b activity of diffuse cytoplasmic pattern (original magnification x 1,000; current magnification x 900). (D) Sodium fluoride inhibition test for Es-b activity. Almost complete inhibition of Es-b activity is demonstrated, indicating appearance of monocytic enzyme in these fundamentally neutrophilic cells (original magnification x 1,000; current magnification x 900).
HETEROGENEOUS DIFFERENTIATION IN APL

Table 1. Morphological and Cytochemical Findings of M3 Promyelocytes, Compared Between Two Cytochemical Subtypes

<table>
<thead>
<tr>
<th></th>
<th>Pure Neutrophilic Type (n = 16)</th>
<th>Mixed Neutrophilic-Monocytoid Type (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>May–Grünewald-Giemsa morphology</td>
<td>15.3 (2–51)</td>
<td>9.4 (1–19)</td>
</tr>
<tr>
<td>Bilobed nuclei</td>
<td>80.9 (8–100)</td>
<td>80.5 (10–100)</td>
</tr>
<tr>
<td>Hypergranular cells</td>
<td>3.8 (0–9)</td>
<td>10.0 (8.0–11.9)</td>
</tr>
<tr>
<td>Faggots of Auer’s rod (case)</td>
<td>11</td>
<td>2</td>
</tr>
<tr>
<td>2+</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>1+</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Cytochemistry</td>
<td>0.4 (0–1)</td>
<td>100.0 (10–100)</td>
</tr>
<tr>
<td>MPO</td>
<td>63.1 (10–93)</td>
<td>72.5 (20–100)</td>
</tr>
<tr>
<td>Es-chl (3–2+)</td>
<td>14.0 (1–32)*</td>
<td>32.3 (5–85)†</td>
</tr>
<tr>
<td>Es-b</td>
<td>0.0</td>
<td>18.0 (4–48)†</td>
</tr>
<tr>
<td>3–2+</td>
<td>9.4 (1–19)</td>
<td>80.5 (10–100)</td>
</tr>
<tr>
<td>1+</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>0</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Es-dual</td>
<td>2.9 (0–86)</td>
<td>9.2 (0–23)</td>
</tr>
<tr>
<td>GL (3–2+)</td>
<td>86.1 (35–100)</td>
<td>87.1 (61–99)</td>
</tr>
<tr>
<td>PAS</td>
<td>3.8 (0–9)</td>
<td>15.7 (1–45)</td>
</tr>
<tr>
<td>Lysozyme</td>
<td>0.4 (0–1)</td>
<td>25.5 (4–47)</td>
</tr>
</tbody>
</table>

Each value except for faggots of Auer’s rod is the mean percentage and the range (in parentheses) of positive cells. MPO, myeloperoxidase; Es-chl, naphthol ASD-chloracetate esterase; Es-b, alpha-naphthyl butyrate esterase; Es-dual, dual staining for Es-chl and Es-b; AP, acid phosphatase; GL, beta-glucuronidase; PAS, periodic-acid Schiff reaction.

*Es-b activity was resistant to sodium fluoride (NaF) inhibition.
†Es-b activity was markedly sensitive to NaF inhibition.

mean diameter was 260 ± 93 and 318 ± 80 nm, respectively. Except for the two M3V cases, there was no statistically significant difference in the diameter of primary granules between the two types. Other TEM findings, such as nuclear indentation and lobulation with increased fibril formation and dilated saccules of rough endoplasmic reticula, were not significantly different.

Outcomes of chemotherapy. As summarized in Table 3, CR was obtained in 11 (68.7%) out of 16 cases of N type and in five (71.4%) out of seven cases of N/M type. No statistically significant difference was noted in CR rates between the two types; early death and chemotherapy failure occurred equally. There was also no significant difference in CR duration and survival. Although all four patients surviving beyond two years belonged to N type, the difference of percentages of long-remitters among CR cases between the two types was not significant \( (\chi^2 = 1.2, \chi^2\) analysis) because of the small number of long-remitters in the latter type.

Table 2. Clinical and Hematologic Data of 23 Cases of Acute Promyelocytic (M3) Leukemia, Compared Between Two Cytochemical Subtypes

<table>
<thead>
<tr>
<th></th>
<th>Pure Neutrophilic Type (n = 16)</th>
<th>Mixed Neutrophilic-Monocytoid Type (n = 7)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>37.4 (15–64)</td>
<td>38.1 (17–56)</td>
</tr>
<tr>
<td>Sex (male/female)</td>
<td>8/8</td>
<td>4/3</td>
</tr>
<tr>
<td>Hemoglobin (g/dL)</td>
<td>10.2 (7.3–17.3)</td>
<td>10.0 (8.0–11.9)</td>
</tr>
<tr>
<td>WBC ( \times 10^9/L )</td>
<td>17.8 (1.6–86.1)</td>
<td>9.6 (1.2–30.2)</td>
</tr>
<tr>
<td>Platelet ( \times 10^9/L )</td>
<td>28.9 (1–127)</td>
<td>41.4 (3–92)</td>
</tr>
<tr>
<td>Leukemic cells (%)</td>
<td>62.4 (4–97)</td>
<td>56.0 (0–98)</td>
</tr>
<tr>
<td>in PB</td>
<td>88.4 (73–96)</td>
<td>41.4 (3–92)</td>
</tr>
<tr>
<td>in BM</td>
<td>191.8 (111–466)</td>
<td>139.7 (40–220)</td>
</tr>
<tr>
<td>Fibrinogen (mg/dL)</td>
<td>52.7 (12–100)</td>
<td>36.0 (10–80)</td>
</tr>
<tr>
<td>Fibrin degradation products (µg/mL)</td>
<td>62.4 (4–97)</td>
<td>56.0 (0–98)</td>
</tr>
<tr>
<td>Bleeding (case)</td>
<td>6</td>
<td>2</td>
</tr>
<tr>
<td>Severe</td>
<td>8</td>
<td>4</td>
</tr>
<tr>
<td>Moderate</td>
<td>2</td>
<td>0</td>
</tr>
<tr>
<td>Organomegaly (case)</td>
<td>4</td>
<td>2</td>
</tr>
<tr>
<td>Liver</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Spleen</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>

Normal range for fibrinogen is 175 to 342 mg/dL. Normal range for fibrin degradation products is 0 to 5 µg/mL.
Cytogenetic abnormality. Eight of 14 cases among N type and five of seven among N/M type showed t(15;17) abnormality (Table 3). One of N type had iso-17 abnormality. Unfortunately, cytogenetic data were not available for the case with toluidine blue-metachromasia. Two M3V cases exhibited t(15;17) also. However, no metaphase was obtained in the M3V case with marked lysozyme activity.

Cytochemistry of Promyelocytes in t(8;21) M2 AML

In all cases the leukemic promyelocytes showed 100% 3+ activity for MPO, strong activity for Es-chl, and no activity for Es-b (Table 4). Es-dual positive cells were also absent. A few cells showed slight lysozyme activity. MPO and Es-chl activities were 3+ to 2+ in most cells (Table 4).

DISCUSSION

It is widely accepted that APL comprises two major morphological categories, ie, M31 and M3V2; the former being predominant.23 Our present investigation on 25 M3 cases using cytochemical methods disclosed further heterogeneity, confirming and extending the observations by Liso et al7 and Gombot et al.12 The present study on MPO and Es-chl of M3 cells confirmed the view that fundamental differentiation of M3 cells proceeds by way of the neutrophilic pathway.18 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific esterase activity at the same time. Cytochemical activity of nonspecific esterase in normal blood cells is strongest in monocytes and stained diffusely in the cytoplasm.14 It is noteworthy, however, that seven (28.0%) of 25 cases showed monocyte-specific...
esterase (Es-A) activity, which constitutes 85% of the total Es-A activity of monocytes estimated biochemically. This band was not found among 11 bands from the granulocyte sample. Oertel and Kastner also demonstrated five different molecular weight isoenzymes of Es-A by two-dimensional IFGE and confirmed their specificity for monocytes. Both groups, however, did not analyze these bands using alpha-naphthyl butyrate as substrate. Therefore, we cannot conclude at present that the NaF-sensitive Es-b activity cytotoxicologically found in M3 cells is the specific isoenzyme(s) for the monocytic cell line. IFGE study on M3 cells will be necessary to draw a conclusion.

In the present study, we have found neither monocytic-equivalent strength of Es-b activity nor Es-dual-positive cells in normal promyelocytes. The leukemic promyelocytes from t(8;21) M2 AML also do not show Es-b activity or Es-dual cells. These findings suggest that the appearance of definite 3+ Es-b activity and Es-dual cells in fundamentally neutrophilic granulocytes of M3 leukemia reflects a kind of differentiation infidelity. This infidelity seems to be a constant phenotype of such leukemic clones because relapsing M3 cells again exhibited strong Es-b activity in one case of N/M type (data not shown). There are some reports concerning such an Es-duality in leukemia and related disorders. Tavasoli et al.28 and Gordon and Hubbar29 noted an increase of such cells in M4 (myelomonocytic) AML, and Scott et al.30 observed Es-dual-positive neutrophils in some cases of myelodysplastic syndrome. The latter group examined the Es-dual cells for their monocytic-specific surface marker but failed to demonstrate it.

From a diagnostic point of view, M3 leukemia of N/M type must be classified as APL primarily based on May-Grünwald-Giemsa morphology and cytogenetic abnormality of t(15;17), not on cytochemical findings. Positive results in single staining of NaF-sensitive Es-b do not necessarily mean monocytic leukemia.

To examine further the appearance of monocytic features in these aberrant M3 promyelocytes, we tried to demonstrate lysozyme activity, which is regarded as one of the enzymes rich in monocytes.30 This enzyme is used for the diagnosis of M4 and M5 AML with monocytic proliferation.31 In two cases with Es-dual cells, we observed a strong to modest degree of this enzyme activity using a cytochemical method. One of M3V cases also showed a marked activity equivalent to that of leukemic monocytes, although Es-b activity was almost negligible. Thus lysozyme activity can appear either combined with Es-b or alone in M3 cells. Because the test for lysozyme activity was done in a small group in our series, and weak to moderate activity can be also demonstrated in mature neutrophils, it must be delineated further whether the strong expression of this enzyme activity in promyelocytes is an abnormal expression of monocytic feature.

It is well known that HL-60 cells show monocytic differentiation when induced in vitro by phorbol ester. Both enzymatic and functional (immunophagocytosis32 and surface markers33) characteristics of monocytes can be easily induced. Our cytochemical observation on uninduced HL-60 cells disclosed an almost complete lack of Es-b activity. Gallagher et al.34 reported that there was no nonspecific esterase activity in fresh APL cells from which HL-60 was derived. They also demonstrated weak lysozyme activity in cell lysates and conditioned media of HL-60. According to Koeffler35 HL-60 can also be induced to differentiate toward monocytic lineage by vitamin D metabolites. These in vitro findings and our observations on fresh M3 cells suggest that the differentiation program may be more or less set for myelomonocytic lineage in APL.

Other than the appearance of monocytic enzymes, there were some minor abnormalities in the expression of other lysosomal enzymes. Weak Es-chl activity observed in four cases, including two of M3V, can be regarded as abnormal because no dissociation of enzyme activity between MPO and Es-chl was found in normal and M2 promyelocytes. AP activity varies considerably, lower activity being more frequent in N/M type. BG activity is less variable, but almost complete absence or weak activity of BG as well as AP was noted in two of three M3V cases. Therefore, all M3V cases in our series showed some cytochemical abnormalities. The pathophysiologic meaning of these abnormally low activities of lysosomal enzymes remains to be delineated.

The cytochemical findings in the case with basophil-like M3 cells may reflect another interesting aspect of APL. It is reasonable to suspect the presence of two subpopulations in this case: one with pure neutrophilic phenotypes and another with basophil-like phenotypes. We could not find intermediate cells showing a mixture of MPO-positive and MPO-negative granules in single cells. In contrast to our observation, Liso et al.36 demonstrated toluidine blue-positive granules in APL cells in one of five M3 cases, in which the leukemic cells with 100% MPO activity also were 100% positive for toluidine blue metachromasia. Although these observations of basophil-like features in M3 cells have potential significance in understanding the pathophysiology of APL, the data now available are so scarce that it is premature to draw any conclusion. More cases must be found and examined carefully for their true expression of basophil phenotype, eg, chemical contents of granules such as histamine and heparin-like substance and the presence of t(15;17).

The present comparative cytochemical investigation on M3 cells, M2 promyelocytes, and normal promyelocytes disclosed that enzymatic pattern in M3 N/M type and all t(8;21) M2 cases can be regarded as examples of fidelity, and that in M3 N/M type is an example of infidelity for normal enzymatic differentiation programs of neutrophil lineage. The complete fidelity observed in all M2 cells of our series is compatible with a recent report on 30 t(8;21) M2 cases by Swirsky et al.37 It is difficult to resolve the question of whether this cytochemical heterogeneity reflects some biological aspects of M3 leukemia. Age distribution, hematologic parameters, M3 cell morphology, and severity of bleeding and organomegaly are not related to this heterogeneity. It is notable that a moderate degree of gingival swelling, characteristic of monocytic leukemia, was observed in one case of N/M type. We also compared, retrospectively, the therapeutic outcomes between the two types. In the present series, there was no statistically significant difference in CR rate, CR duration, and survival time. McKenna et al.38

HETEROGENEOUS DIFFERENTIATION IN APL

355
reported a significantly shorter CR duration of M3V based on the comparative study between 31 M3 cases and eight M3V cases. Their finding is of special importance in the present situation of M3 chemotherapy. Increasingly higher CR rates are being obtained by current intensive regimens including DR and M3 leukemia has been recognized as a special entity with more long-duration remissions compared with other subtypes of AML.5,6,36

Concerning the infidelity of leukemia cell differentiation, Smith et al., using double fluorescent-staining methods with various monoclonal antibodies, recently demonstrated infidelities such as interlineage or intralineage biphenotypes in eight of 20 cases of acute leukemias. They found lymphoid markers on AML cells and also noted erythroid and/or megakaryocytic markers on leukemic granulocytes and monocytes. In their AML series, a significantly lower CR rate was obtained in the infidelity group. Mertelsman38 also compared the survival in AML cases with and without terminal transferase activity and found poorer prognoses in the former group. Our study of the cytochemical infidelity of M3 cells, however, could not support their conclusion. Therefore, it should be further delineated by systematic investigation whether the differentiation infidelity determines the biological feature of leukemias.

REFERENCES


Cytochemistry of acute promyelocytic leukemia (M3): leukemic promyelocytes exhibit heterogeneous patterns in cellular differentiation

M Tomonaga, Y Yoshida, M Tagawa, I Jinnai, K Kuriyama, T Amenomori, A Yoshioka, T Matsuo, H Nonaka and M Ichimaru