In Vivo and In Vitro Activity of Neutrophil Alkaline Phosphatase In Acute Myelocytic Leukemia With 8;21 Translocation

By Nanao Kamada, Hiroo Dohy, Kosuke Okada, Nobuo Oguma, Atsushi Kuramoto, Kimio Tanaka, and Haruto Uchino

Cytogenetic studies were made on 160 patients with acute nonlymphocytic leukemia (ANLL) between 1963 and 1979, of whom 115 had acute myelocytic leukemia with 67 patients showing aneuploidy (58.3%). Among these, 24 patients were found to have similar chromosome alterations that appeared to involve specifically chromosomes 8 and 21. Banding studies on at least 7 of these patients confirmed the presence of a translocation between these two chromosomes. Of 160 ANLL patients, 142 were scored for neutrophil alkaline phosphate (neutrophil AP) at the time of diagnosis. Fifty-nine patients showed a low neutrophil AP score, 42 a normal value, and 41 a high value. All patients with 8:21 (or C/G) translocation had a low neutrophil AP score and leukemic cells with maturation (M4 of FAB classification) in the bone marrow. In vitro liquid culture for 2 wk of 8:21 translocated leukemic cells revealed no increase of neutrophil AP activity nor increase of mature granulocytes, whereas 9:22 translocated chronic myelocytic leukemia cells with a low neutrophil AP score did so. Neutrophil AP score at the time of diagnosis in acute myelocytic leukemia is very useful for detecting 8:21 translocation AML and for studying the pathophysiology and genetic alterations of the characteristic subgroup of AML with 8:21 translocation.

Since 1973 when Rowley first reported an 8:21 translocation in a patient with acute myelocytic leukemia (AML) using the quinacrine technique, a number of AML patients with this abnormality have been studied and such characteristic findings as missing sex chromosome, morphological abnormalities of the cells including Auer rods, nuclear blebs, peroxidase-negative neutrophils and maturation dysplasia, milder clinical course, and relatively good response to chemotherapy have been observed. In this article we will report on the neutrophil alkaline phosphate (neutrophil AP) activity of the peripheral blood in AML and also on the in vitro study of neutrophil AP in 8:21 translocation AML in comparison with those in diploid-M2-AML with low neutrophil AP score, 9:22 translocation chronic myelocytic leukemia (CML), and blastic crisis of CML with low neutrophil AP score. The study was performed to clarify the significance of low activity of neutrophil AP in 8:21 translocation AML.

MATERIALS AND METHODS

Cytogenetic Studies

Chromosome preparations were made using a modification of the direct bone marrow technique of Tjio and Whang. Peripheral blood cultures without PHA for 24 hr were performed in some patients. A modification of Seabright's method was used for Giemsa-banding and Caspersson's method for quinacrine-banding. Usually about 40 metaphases, but always at least 10, were scored from each marrow specimen and 5-10 karyotypes were prepared from each clone.

Histochmical Studies

Neutrophil AP activity was measured at the time of diagnosis by the method (naphthol AS-MX phosphate) described by Tomonaga et al. In our laboratory the normal range of the positive rate is 70%-90% and the score 181-320. Peroxidase stain was also employed.

In Vitro Culture Studies

Liquid suspension culture of marrow cells was performed by modification of a previously described technique. Briefly, bone marrow cells from patients with AML and CML were separated by Isopaque-Ficoll technique. Equal parts (2 ml) of bone marrow specimen and 0.9% NaCl were mixed, layered over with Isopaque-Ficoll, and then centrifuged at 800 g for 30 min. The cells recovered from the interface region were used for liquid culture. They were composed of approximately 90% immature leukemic cells (leukemic blasts, promyelocytes, myelocytes, less than 2% mature granulocytes, and a small percentage of lymphocytes and erythroblasts. They were almost devoid of polymorphs and erythrocytes. Aliquots of 5 x 10⁶ nucleated cells were placed in 30-ml plastic tissue culture flasks (Falcon Plastic) having 10 ml of RPMI 1640 medium containing 20% fetal calf serum. Cells were harvested by pipetting at periods of 7 days of culture. At each time, smears of culture cells were prepared by cytocentrifuge and stained for May-Grünwald-Giemsa, peroxidase, and neutrophil AP. In four patients, leukocyte differential and pulse height analysis (PHA) of peroxidase by means of Technicon Hemalog D were made prior to and 1 and 2 wk after culture.

Hematologic Studies

Morphological analysis of bone marrow smears stained by May-Grünwald-Giemsa and peroxidase included such items as presence or absence of Auer rods, granule anomalies, abnormal lobation (double nucleus), and nucleo-cytoplasmic maturation asynchrony. On the basis of these findings, acute nonlymphocytic leukemia

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(ANLL) patients were divided into 6 subgroups according to FAB classification. Differential count of cultured cells was made in the usual manner.

RESULTS
Histochemical, Cytogenetic, and Hematologic Studies

Of the 160 patients, 35 patients were AML without maturation (M1), 41 AML with maturation (M2), 9 acute promyelocytic leukemia (M3), 4 myelomonocytic leukemia (M4), 12 acute monocytic leukemia (M5), 20 erythroleukemia (M6), and 39 others (unusual types of AML such as hypoplastic leukemia and transitional cases from preleukemia). Neutrophil AP activity of M1 patients, M2 without 8;21 (or C/G) translocation, and others was equally distributed among high, normal, and low scores, whereas that of M2 patients with 8;21 (or C/G) translocation was extremely inclined toward a low score (Fig. 1). All patients with M6 of FAB classification showed normal or high neutrophil AP activity. According to neutrophil AP activity, ANLL patients were divided into three groups: low (score 0–180, 59 patients); normal (score 181–320, 42 patients); and high (score 321–500, 41 patients). Table 1 presents FAB classification versus neutrophil AP activity of the peripheral blood versus chromosome abnormality.

Cytogenetic studies of 160 ANLL revealed chromosome abnormalities in 98 patients (61.3%). Of the 35 FAB-M1 patients, 15 had abnormal karyotypes. Thirty-six of 41 FAB-M2 patients showed chromosome abnormalities, of whom 24 patients had consistent karyotype changes that were initially reported by us as C/G translocation. In Giemsa banding studies performed on 7 of the 24 patients, an 8;21 translocation was clearly identified. Of 24 patients with 8;21 (or C/G) translocation, 14 were found to have translocation alone, 8 the translocation plus –Y or –X, and 2 the translocation plus Cq–. Missing Y was found in 7 of 11 male patients (63.6%). The 8;21 (or C/G) translocation was found only in the group with low neutrophil AP activity of FAB-M2 patients (Table 1 and Fig. 1).

Table 1. FAB Classification, Neutrophil AP Activity of the Peripheral Blood, and Chromosome Aberrations in 160 ANLL Patients

<table>
<thead>
<tr>
<th>FAB Classification</th>
<th>No. of Patients</th>
<th>Neutrophil AP Activity and Chromosome Abnormalities (Normal, Abnormal)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>High</td>
</tr>
<tr>
<td>M1</td>
<td>35</td>
<td>12 (8, 4)§</td>
</tr>
<tr>
<td>M2</td>
<td>41</td>
<td>3 (1, 2)</td>
</tr>
<tr>
<td>M3</td>
<td>9</td>
<td>2 (0, 2)</td>
</tr>
<tr>
<td>M4</td>
<td>4</td>
<td>0</td>
</tr>
<tr>
<td>M5</td>
<td>12</td>
<td>4 (0, 4)</td>
</tr>
<tr>
<td>M6</td>
<td>20</td>
<td>7 (0, 7)</td>
</tr>
<tr>
<td>Others‡</td>
<td>39</td>
<td>13 (8, 5)</td>
</tr>
<tr>
<td>Total</td>
<td>160</td>
<td>41 (17, 24)</td>
</tr>
</tbody>
</table>

*Scoring of neutrophil AP was not done.
†Of these, 24 patients showed 8;21 (or C/G) translocation.
‡“Others” include patients with unusual type of AML such as hypoplastic leukemia and transitional cases from preleukemia.
§Number of patients with normal karyotype in left and with abnormal karyotype in right.
ACTIVITY OF NEUTROPHIL ALKALINE PHOSPHATASE

from patients with 8;21 (or C/G) translocation showed a cytologic picture that was typical of AML. Common morphological features of these samples included Auer rods (79.1%), nuclear abnormalities (79.1%) such as pseudo-Pelger anomaly and binucleated granulocytes, granule abnormalities (83.3%) as evidenced by eccentric localization of granules, decreased number of granules, and peroxidase-negative neutrophils, and the existence of some degree of maturation (100%) accompanied by nucleocytoplasmic maturation asynchrony.

Bone marrow cells from M2 patients (four with 8;21 translocation and four with normal diploid karyotype) and CML patients (six in chronic phase and five in blastic crisis) were cultured for 2 wk in a CO2 incubator. Of the four patients with 8;21 translocation, three had translocation alone and one the translocation plus −Y chromosome. Of the six CML patients in chronic phase, four had 9;22 translocation and the other two were Ph+ positive (nonbanded) cases. Karyotypes of the five patients with low neutrophil alkaline phosphatase activity in blastic phase were 46,XX,t(9;22)(q34;q11); 46,XX,t(9,22) (q34;q11) −22, +22q−; 47,XX,t(9;22) (q34; q11), +7; and 47,XY,t(9;22) (q34;q11), +9q+,−17,+i(17q).

Mean and range of neutrophil AP score in the peripheral blood and in vitro culture are shown in Table 2. No increase of neutrophil AP activity at 7 days was observed in cultured leukemic cells from patients with M2 and 8;21 translocation, and patients with M2 and diploid karyotype. In May-Grünewald-Giemsa stained smears, the maximum increase in days was observed in cultured leukemic cells from patients with 8;21 (or C/G) translocation and 47,XY,t(9;22) (q34;q11), +9q+,−17,+i(17q).

Mean and range of neutrophil AP score in the peripheral blood and in vitro culture are shown in Table 2. No increase of neutrophil AP activity at 7 days was observed in cultured leukemic cells from patients with M2 and 8;21 translocation, and patients with M2 and diploid karyotype. In May-Grünewald-Giemsa stained smears, the maximum increase in matured leukemic cells (band form plus segmented cells in Table 2) was observed in 14-day culture in 8;21 translocation AML and in 7-day culture in M2 and diploid AML. The degree of maturation in both groups seemed to be low and similar. In contrast, immature granulocytes from CML patients in the chronic phase showed a remarkable increase in neutrophil AP score and maturation toward segmented neutrophils on 7-day culture, though the percentage of neutrophil AP positive cells (positive rate) was small compared to the control culture from nonhematologic diseases. CML in blastic crisis of granulocytic conversion showed a moderately elevated neutrophil AP score and percentage of matured cells.

On pulse height analysis display of the peroxidase channel of Technicon Hemalog D, 8;21 translocated leukemic cells prior to culture showed a single peak with medium cell size and moderately positive peroxidase reactivity. After 7 days of culture, cultured cells showed two peaks with different cell size and decreased peroxidase reactivity (Fig. 2).

DISCUSSION

It is well known that CML cells with 9;22 translocation have differentiation ability in vivo and in vitro and low neutrophil AP activity. Based on a relatively small number of leukemic patients we have reported previously the presence of acute myelocytic leukemia with low neutrophil AP activity similar to that of CML, especially in patients with 8;21 translocation.7,14

The present study of 160 ANLL patients clearly demonstrated that: (1) about 40% of AML patients have low neutrophil AP activity (42.3%); (2) most AML patients with M2 type of FAB classification show low neutrophil AP score (81%); (3) patients with

<table>
<thead>
<tr>
<th>Type of Leukemia</th>
<th>No. of Patients</th>
<th>WBC (×10⁹/Liter)</th>
<th>M. + promyl. in BM (%)</th>
<th>Karyotype</th>
<th>N-AP Score in PB</th>
<th>N-AP Score 7 Days</th>
<th>N-AP Score 14 Days</th>
<th>Band + Seg. 7 Days</th>
<th>Band + Seg. 14 Days</th>
</tr>
</thead>
<tbody>
<tr>
<td>AML</td>
<td>4</td>
<td>46.8 15.8</td>
<td>63.6</td>
<td>45-46,t(8;21)*</td>
<td>42</td>
<td>11.5</td>
<td>14.1</td>
<td>29.8</td>
<td></td>
</tr>
<tr>
<td>(M2, 8; 21)</td>
<td>(12-67)</td>
<td>(5.1-29)</td>
<td>(43-82)</td>
<td>(18-85)</td>
<td>(6-17)</td>
<td>(7-18)</td>
<td>(12-48)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>AML</td>
<td>4</td>
<td>53.5 17.2</td>
<td>61.4</td>
<td>46</td>
<td>68.3</td>
<td>47.8</td>
<td>28.8</td>
<td>10.5</td>
<td></td>
</tr>
<tr>
<td>(M2, diploid)</td>
<td>(26-81)</td>
<td>(2.5-43)</td>
<td>(42-88)</td>
<td>(30-105)</td>
<td>(15-66)</td>
<td>(15-49)</td>
<td>(8-13)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CML chronic phase</td>
<td>6</td>
<td>52.3 103.8</td>
<td>7.7</td>
<td>46,t(9;22)</td>
<td>70.7</td>
<td>191.8</td>
<td>62</td>
<td>43.2</td>
<td></td>
</tr>
<tr>
<td>CML in blastic crisis</td>
<td>5</td>
<td>14.4 122.0</td>
<td>42.9</td>
<td>46-47,t(9;22)†</td>
<td>64.4</td>
<td>84.8</td>
<td>45.8</td>
<td>35.3</td>
<td></td>
</tr>
<tr>
<td>(granulocytic</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>conversion)</td>
</tr>
</tbody>
</table>

Cyto centrifuged immature leukemic cells at the time of diagnosis were cultured for 2 wk. WBC, M. + promyl. in BM, and N-AP score in PB indicate the data at diagnosis. Band + seg: Leukemic cells superficially resembled band or segmented neutrophils in culture, but they differed from normally matured neutrophils.

*Karyotype of these patients were 46, XX, t (8; 21) (q22; q22); 46, XX, t (8; 21) (q22; q22); 46, XY, t (8; 21) (q22; q22); 46, XY, t (8; 21) (q22; q22); and 45, X, −Y, t (8; 21) (q22; q22).
†Two patients had 46, XX, t (9; 22) (q34; q11) and the other three were 46, XY, t (9; 22) (q34; q11), −22, +22q−; 47, XX, t (9; 22) (q34; q11), +7; and 47, XY, t (9; 22) (q34; q11), +9q+,−17,+i(17q).
Fig. 2. Pulse height analysis (PHA upper) and the X-Y display (lower) of the peroxidase channel in 8;21 translocation AML. Upper curve of PHA shows frequency of cell size (Y axis: frequency; X axis: cell size) and lower curve frequency of peroxidase positive cells (Y axis: frequency; X axis: reactivity to peroxidase). In PHA, two small peaks appeared on 7-day culture with different cell size and weak peroxidase reactivity. X-Y display also showed left shift in the cell size and peroxidase reactivity. These findings as well as morphological studies seem to suggest an ability of leukemic cells with 8;21 translocation to undergo disorganized differentiation to some extent in culture.

8;21 translocation constitute 15% of ANLL and 58.5% of M₂; (4) 8;21 translocation patients show a low neutrophil AP activity (100%) without any exception; (5) 8;21 translocation AML patients have a high frequency of loss of the Y chromosome in their leukemic cells (63.6%); (6) bone marrow smears of 8;21 translocation AML show a high frequency of morphological abnormalities specific to these patients (about 80%) such as nuclear, granular abnormalities and nucleocytoplasmic maturation asynchrony.

A possible relation among FAB classification, neutrophil AP activity, and chromosome aberration of 8;21 translocation is schematically presented in Fig. 3. In ANLL, patients with low neutrophil AP activity are distributed mainly in M₂ and less in M₁ of FAB classification. Patients with 8;21 translocation belong only to M₂, with all showing low neutrophil AP activity. The specificity of the hematologic, histochemical, and cytogenetic changes found in these AML patients is remarkable and demands further investigation. Our findings showing a correlation between the translocation and M₂ morphology agree with the observations made at the Second Workshop. Our in vitro study revealed completely different features of neutrophil AP activity between leukemic cells with 8;21 and those with 9;22 translocation. CML cells have been demonstrated to have increased myeloid cell proliferation with normal maturation in culture and to recover neutrophil AP activity with maturation. The present study confirmed these earlier findings, showing a high neutrophil AP score ranging from 84 to 243 and a high percentage of matured cell population in culture (Table 2). CML cells in blastic crisis, especially of granulocytic conversion, have also shown the same tendency as in the chronic phase, but the activity seemed to be low in grade.

Studies on the recovery of neutrophil AP activity of AML cells in culture have not yet been reported. In AML patients with low neutrophil AP activity, the leukemic bone marrow cells were able to mature to some extent in culture (Table 2), but no increase of neutrophil AP activity could be observed. It has been shown that leukemic blasts taken from AML patients failed to develop into normally matured cells in culture and to show a variable amount of disorganized differentiation. Neither increase of neutrophil AP activity nor maturation tendency of leukemic cells with 8;21 was observed in our in vitro culture study. These findings may indicate the concept that neutrophil AP activity of leukemic cells mainly reflects maturity in culture, or alternatively, they may imply a different genetic control of neutrophil AP activity between CML and AML. The study of cell hybridization may shed some light to this question.

The present study clarified an unique situation of 8;21 (or C/G) translocation AML among ANLL, especially from the activity of alkaline phosphatase (in vivo and in vitro and from FAB classification and suggested a possible difference in introduction of enzyme activity in vitro between CML and AML culture. The data presented here are useful in the clinical diagnosis of acute leukemia and in understanding the unique situation of 8;21 translocation AML. Further study on leukemia with 8;21 translocation, which accounts for 15% of ANLL patients and has hematologic and histochemical specificity, should be made for a better elucidation of the mechanisms of leukemogenesis.

REFERENCES

ACTIVITY OF NEUTROPHIL ALKALINE PHOSPHATASE

In vivo and in vitro activity of neutrophil alkaline phosphatase in acute myelocytic leukemia with 8;21 translocation

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