Hematologic and Cytologic Characterization of 8/21 Translocation Acute Granulocytic Leukemia

By José M. Trujillo, Ann Cork, Michael J. Ahearn, Ehsan L. Youness, and Kenneth B. McCredie

Cytogenetic studies were performed in 546 patients with acute leukemia between 1968 and 1975. Two hundred thirty-four patients were aneuploid (42.9%), and 312 patients were diploid (57.1%). Among these, 32 patients were found to exhibit similar chromosomal alterations that appeared to involve specifically chromosomes 8 and 21. Banding studies in at least 15 of these patients confirmed the presence of a translocation between these two chromosomes. The cytogenetic findings were correlated with the hematologic and clinical data. It was found that each of these individuals had a typical picture of acute granulocytic leukemia with Auer rod-positive and peroxidase-positive cells. Ultrastructurally, the patients in this group also consistently demonstrated the presence of a nuclear bleb that has been positively associated with aneuploidy in acute leukemia. Clinically, they seemed to respond better to therapy than other adult patients with acute granulocytic leukemia. It is proposed that the 8/21 translocation acute leukemia represents a definite subgroup within the general category of acute granulocytic leukemia, with an incidence of approximately 7.3%.

APPLICATION of banding techniques to the study of chromosomal abnormalities found in patients with acute leukemia has revealed the presence of karyotypic alterations that involve the same chromosomes. The specificity of some of these structural changes has been emphasized by several recent publications that have documented similar observations in a number of patients with acute leukemia.1-9 In this publication we will report on a series of 32 patients with acute granulocytic leukemia (AGL), all of whom present a specific type of aneuploidy that we initially categorized as the "complex" profile but that now has been definitely identified as an 8/21 translocation.

MATERIALS AND METHODS

Cytogenetic Studies

Chromosome studies using standard cytogenetic techniques were performed on hematopoietic (bone marrow and peripheral blood) samples obtained from the 32 patients. Briefly, these techniques included setting up 24-hr bone marrow cultures, 24-, 48-, and 72-hr unstimulated peripheral blood cultures, and 72-hr peripheral blood cultures stimulated with PHA. Whenever possible, hematopoietic samples were obtained during the initial period of the disease before therapy at periodical intervals (every 3 mo) during remission and relapse. In those patients initially referred to this institution during remission, samples also were collected during relapse.

Giemsa-banding studies were performed on the samples obtained from 15 of the 32 individuals. The methodology employed in our laboratory for Giemsa-banding is a modification of Seabright's method.10 Briefly, this included the following basic steps: Routinely prepared air-dry slides were
exposed to a 0.25% trypsin solution diluted 5:45 with saline for a period of 45–90 sec or more, as required by the specimen, washed in two changes of saline, and stained with Gurr's Giemsa stain at pH 6.8. At least two well-banded metaphases were photographed, and the chromosomes were arranged in a karyotype according to the Paris Conference.12

Hematologic Studies

The hematologic studies included detailed morphologic analysis of bone marrow smears stained by the Wright-Giemsa method. In all cases, peroxidase stains were also performed.

Ultrastructural Studies

The bone marrow specimens for ultrastructural examination were concentrated using Wintrobe tubes. Initial fixation was carried out in 2.5% glutaraldehyde in Sörensen's phosphate buffer (300 mOsm) for 1 hr at 25°C. The specimens were washed in phosphate buffer and incubated in 3,3'-diaminobenzidine tetrahydrochloride reagent (K&K Laboratories) for detailing endogenous peroxidase at the electron microscopic level. The specimens were postfixed in 1% osmium tetroxide at 4°C, dehydrated in graded acetones, and embedded in Epon 812. Sections were cut using a diamond knife mounted on a Porter Blum ultramicrotome and subsequently stained with uranyl acetate for 1 hr at room temperature, followed by Reynold's lead citrate for 2 min. The specimens were examined using a Siemens Elmiskop IA at 80 kV.
Fig. 1. Cytogenetic and hematologic observations in an 8/21 translocation AGL patient. A: Giemsa-banded karyotype showing the 8/21 translocation and the loss of an X chromosome. B: Myeloblasts with Auer rods. C: Dysplastic erythropoiesis.
Clinical Data

The chemotherapeutic regimens used were combinations of cytosine arabinoside (ara-C), initially with Cytoxan, vincristine, and prednisone, and subsequently with anthracycline antibiotics (either daunomycin, Adriamycin, or rubidazone), vincristine, and prednisone.

The chemotherapy schedules were chosen and modified on an individual basis according to patient response and degree of drug toxicity, without any regard to the cytogenetic alterations. Supportive therapy in these patients was in the form of antibiotics and platelet, granulocyte, and packed red cell transfusions. Patients were treated in the protected environment when needed.

The length of survival was determined from the time of diagnosis, and the survival curves were constructed using nonparametric procedures.13

RESULTS

Cytogenetic Findings

Cytogenetic studies during the acute phase of the disease in all 32 patients revealed consistent karyotype changes that were initially reported by us as the “complex” profile.14 After Giemsa-banding studies were performed in 15 of the 32 patients, an 8/21 translocation was clearly identified. In 8 of our 19 male patients (approximately 42%) the Y chromosome was found to be missing, whereas in 2 of 13 female patients (approximately 15%) 1 of the 2 X chromosomes was absent (Fig. 1A).

Chromosomal studies during complete remission, done whenever feasible (16 patients), revealed absence of the 8/21 translocation clone and the presence of diploid cells. However, studies of hematopoietic samples obtained during relapse always resulted in reappearance of the same 8/21 translocation clone. Cytogenetic studies during the acute phase showed the presence of cells with the 8/21 translocation and additional chromosomal alterations (secondary clones) in 8 patients (25%). In 4 of these 8 patients the additional changes could not be adequately characterized because banding techniques were not available at that time. Of the remaining 4 patients studied with G-banding techniques, 2 patients exhibited an extra 8 chromosome, 1 patient had an extra 21 chromosome, and 1 patient had an additional translocation involving the 19 and Y chromosomes. Since the 8/21 translocation appeared to be present in most of these secondary clones, the findings could be interpreted as evidence of clonal evolution.

Hematologic Picture

Routine hematologic studies of the bone marrow smears and clot sections obtained during the acute phase of the disease showed a cytologic picture that was typical of AGL. Common morphologic features of all of these samples included Auer rods (100%), peroxidase-positive granules (100%), and the existence of some degree of differentiation, as evidenced by the presence of maturing elements from both the granulocytic and erythroid lines (Fig. 1B and 1C). In 20 of 22 patients who were studied during the initial phase of the disease, the percentage of leukemic blasts in the bone marrow ranged from 20.0% to 97.4%. The other 2 patients had 11.4% and 12.0% blasts in their first bone marrow specimens. All of these samples also exhibited an increase in cellularity ranging from 20%–30% to 100%. The granulocytic cells exhibited maturation dysplasia, nucleocytoplasmic maturation asynchrony, and abnormal lobation. The erythroid elements also exhibited dysplastic features, such as maturation asynchrony and, less frequently, binucleation and megaloblastiform changes. The megakaryocytic elements were decreased
and exhibited little evidence of platelet formation. The few lymphocytic elements (3%-12%) observed in the marrow samples of these patients were unremarkable. During the remission periods the bone marrow showed a normal picture, with total disappearance of leukemic cells and reestablishment of the normal ratio of maturation. This return to normalcy of the hematopoietic tissue was reflected in the peripheral blood, with normal WBC and platelet counts and an increase in the hemoglobin levels.

Ultrastructural Studies

Ultrastructurally, the nucleocytoplasmic asynchrony noted in the leukemic cells of these patients consistently demonstrated a more differentiated cytoplasm than nucleus. The chromatin was characteristically euchromatic in blastic conformation.

Fig. 2. This leukemic progranulocyte demonstrates asynchronous differentiation (maturation dysplasia), a nuclear bleb, and the presence of both peroxidase-positive azurophilic granules (small arrows) and peroxidase-negative specific granules (large arrows) in the cytoplasm (×9500).
with a very distinct nucleolus. A high frequency of nuclear blebs was present in the immature myeloid cells of all these patients. Auer rods were found as membrane-bound structures with crystalline cores. In addition to the previously mentioned malignant features, bundles of cytoplasmic fibrils were regularly noted in the juxtanuclear area. In contrast with the series of acute myelogenous leukemias studied by Bainton et al.,\textsuperscript{15} the differentiated cytoplasm contained peroxidase-positive azurophilic and peroxidase-negative specific granules (Fig. 2). During remission, all of the cells with the abnormal features described disappeared from the bone marrow.

\textit{Clinical Features}

Of the 32 patients studied, 19 were male and 13 were female. The age distribution, dates of diagnosis, and survival times are shown in Table 1. Complete clinical and hematologic remission was observed in 27 patients (84.4\%). In 11

<table>
<thead>
<tr>
<th>Patient</th>
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<th>Date of Diagnosis</th>
<th>Survival Time (wk)</th>
</tr>
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<td>R.V.</td>
<td>13</td>
<td>01-30-73</td>
<td>149</td>
</tr>
<tr>
<td>J.D.</td>
<td>14</td>
<td>12-15-75</td>
<td>Still Alive*</td>
</tr>
<tr>
<td>P.H.</td>
<td>16</td>
<td>06-08-72</td>
<td>253</td>
</tr>
<tr>
<td>K.H.\textsuperscript{t}</td>
<td>17</td>
<td>03-06-71</td>
<td>3</td>
</tr>
<tr>
<td>T.M.</td>
<td>19</td>
<td>05-10-75</td>
<td>27</td>
</tr>
<tr>
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<td>20</td>
<td>06-07-70</td>
<td>83</td>
</tr>
<tr>
<td>R.G.\textsuperscript{t}</td>
<td>23</td>
<td>08-30-70</td>
<td>81</td>
</tr>
<tr>
<td>L.T.\textsuperscript{t}</td>
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<tr>
<td>O.F.</td>
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<td>D.W.\textsuperscript{t}</td>
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<td>L.C.</td>
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<tr>
<td>C.M.</td>
<td>33</td>
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<td>R.P.</td>
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<td>C.C.\textsuperscript{t}</td>
<td>49</td>
<td>01-09-68</td>
<td>114</td>
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<td>H.W.\textsuperscript{t}</td>
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<td>A.H.</td>
<td>63</td>
<td>03-27-75</td>
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\textsuperscript{*At cutoff date (1975).}
\textsuperscript{tPreviously reported in Cancer (1974).}
patients a second remission was achieved (34.4%). Two patients also had a third remission (6.3%). Three patients had an initial remission period that lasted between 4 and 6 wk. However, the majority of the remaining patients experienced longer periods of remission that lasted between 24 and 54 wk (approximately 6 to 13 mo). In 7 patients the initial remission period extended from 89 to 215 wk (approximately 2 yr 6 mo to 4 yr 2 mo). The second periods of remission were, in general, shorter and ranged between 2 and 59 wk. The median survival time (MST) in these 32 patients was 81 wk (Fig. 3). Eleven patients between 13 and 29 yr of age showed a median survival time of 90 wk, and 16 patients between 30 and 49 yr of age showed a median survival time of 81 wk. These survival figures compare favorably with those in 5 patients over 50 yr of age who had a median survival time of 8.5 wk. The longest survival (215 wk) was observed in 1 female patient who was 39 yr of age at time of diagnosis. This patient was still alive at the time this report was written (367 wk or 7 yr 3 wk).

CASE REPORT

A typical case history is that of D.L., a 39-yr-old Latin American female who was admitted to the hospital on July 23, 1968, with a clinical diagnosis of papillary cystadenoma of the ovary, stage I-C. She received cobalt and betatron therapy from August 15, 1968, to October 11, 1968, and had a total hysterectomy on July 31, 1969. On July 2, 1971, she returned to the hospital with anemia, possible leukemia. A bone marrow aspiration on that day showed 38.8% blasts (Auer rod-positive). The peripheral blood picture included a white blood cell count of 8400/cu mm with 40% blasts (Auer rod-positive) and 34,000 platelets. Cytogenetic preparations of this initial bone marrow specimen revealed only 11 metaphases. Chromosomal analysis of all of these metaphases showed pseudodiploidy, with alterations that were characterized as 46,XX,−C,+D,+E,−G. At that time no chromosomal banding studies were available. Chemotherapy with daunomycin, vincristine, ara-C, and prednisone (DOAP) was initiated on July 2, 1971, and by September 1, 1971, after three courses, she was in complete remission (2.8% blasts in the bone marrow). A chromosomal study done on the bone marrow aspirate on September 14, 1971, revealed 100% diploid (46,XX) metaphases. Since September 1, 1971, she has remained in complete hematologic and clinical remission and is still alive. Maintenance therapy since December, 1972, has consisted of immunotherapy with BCG. Chromosomal studies of multiple samples during this long remission period have shown no abnormalities.
DISCUSSION

The specificity of the chromosomal changes found in these patients is remarkable and worthy of further investigation. As previously mentioned, before the banding techniques were available, the chromosomal alterations seemed to involve preferentially the C, D, E, and G groups, and for that reason it was proposed that it be called the “complex” profile, to be requested generically by the formula $-C,+D, +E,-G$. In our first discussion of these cases, however, we suggested that one possible explanation for these changes could be the presence of a translocation between a C-group chromosome and a G-group chromosome, resulting in two new elements morphologically similar to those of the D and E groups \[t(Cq_-,Gq_+).\]

In 1973 Rowley first reported on a patient with AGL having similar karyotypic changes in whom the use of banding techniques permitted clear identification of a translocation between the 8 and 21 chromosomes. According to Rowley, the sites of chromosomal breakage involved specific bands of both chromosomes, as illustrated in the formula \(t(8;21)(q22;q22)\). Soon afterward, Sakurai and associates, also with the use of banding techniques, confirmed the presence of a similar translocation in a patient with AGL. He called this cytogenetic aberration the “prototypic” karyotype, suggesting that this translocation could be a basic and initial change found in a group of patients with AGL. A larger series of patients (15 patients) with similar karyotypic alterations was recently reported by Kamada et al. In 15 of the 32 patients reported here, application of the Giemsa-banding techniques has definitely confirmed the presence of the 8/21 translocation. Unfortunately, this improved methodology was not available at the time the other 17 patients were studied. However, they do exhibit the karyotypic changes initially described as the “complex” profile, and for that reason they are included in this series. Since the end of 1975, the time selected as the cutoff date for the preparation of this report, 16 new patients with the 8/21 translocation (4 of whom are under 12 yr of age), all confirmed by banding techniques, have been studied in our laboratory. However, data from these patients have not been included here.

An interesting finding in our series is the high incidence of sex chromosome involvement seen in these patients. As previously mentioned, 8 of 19 male patients (42%) were missing the Y chromosome, and in 2 of 13 female patients (15%) the X chromosome was absent (Table 2). Rowley and Sakurai have made similar observations in their cases. Pierre and Hoagland, in their initial studies of this phenomenon in normal human hematopoietic tissue, noted that loss of the Y chromosome was directly related to age and occurred more frequently in older people. Other investigators confirmed this apparent physiologic loss of the Y chromosome with increasing age. Loss of the Y chromosome unrelated to age also has been frequently observed in myeloproliferative disorders, as well as in other neoplasias. Sakurai and Sandberg have observed that in Ph$^+$-positive chronic granulocytic leukemia (CGL) the 45,X,Ph$^+$ cell lines are less prone to the development of additional karyotypic abnormalities than are the 46,XY,Ph$^+$ cells; according to these authors, this reflects the relative resistance of these cells to blastic transformation and may, therefore, explain the long survival noted in some CGL patients with missing Y chromosomes. In the 8/21 translocation cases reported in the literature and in those studied in our laboratory, no direct
relationship between loss of the Y chromosome and age could be found. In a recent review of this problem, DiLeo et al. suggested that loss of the Y chromosome confers selective advantage to the neoplastic cells. Since the Y chromosome carries the histocompatibility gene responsible for the H-Y antigen, it has been theorized that loss of the Y chromosome changes the antigenic property of the tumor cells, thus contributing to their uncontrolled proliferation. The mitotic error leading to loss of the Y chromosome is not well understood. It is obviously not exclusively related to neoplasia, since it is also observed in older normal males. However, the fact that this phenomenon is frequently observed in myeloproliferative disorders and in 8/21 translocation leukemia is of interest and is suggestive of some sort of relationship between these clinical conditions.

Experimental work done by Mitelman and Levan has shown that malignant transformation induced by chemical carcinogens can result in specific chromosomal changes. The specificity of these karyotypic aberrations was demonstrated by the fact that in histologically identical tumors induced by different oncogenic agents the occurrence of a particular chromosomal alteration was always related to the same carcinogen. Results obtained by these investigators with experimental tumors clearly showed that the type of carcinogenic agent could be of decisive importance in determining the aneuploid alterations found in these neoplasias. Later on, Rowley also postulated that specific chromosomal changes in human neoplasias could be related to certain previously unidentified oncogenic agents. As in the case of the Philadelphia chromosome (9/22 translocation), the specificity of the changes observed in 8/21 translocation leukemia suggests that these individuals may have been exposed to a heretofore unknown common oncogenic agent. Thus it may be of importance to carefully investigate the social, environmental, and geographic backgrounds of these patients. A preliminary study of the clinical histories of our patients does not indicate any familial relationship, nor does it
indicate any common ethnic or geographic features. As previously mentioned, one of these patients (D.L.) had been treated with radiotherapy for papillary cystadenoma of the ovary 3 yr before the appearance of the disease. However, in none of the other patients in this series could evidence of x-ray exposure be identified.

As illustrated in Table 2, all patients showed a typical hematologic picture of AGL with varying degrees of differentiation still present in the hematopoietic tissue. In most cases the maturing elements exhibited a great deal of dysplasia. It is possible that the good response to therapy observed in these patients is related to the persistence of bone marrow maturation. The percentage of leukemic blasts in the hematopoietic tissue varied widely and exhibited no direct relationship to clinical behavior. Sakurai and Sandberg have postulated that in patients with AGL those who have some normal (diploid) cells in the marrow (AN patients) experience a longer survival than those who have only aneuploid cells (AA patients). So far, our data on the 8/21 translocation are not supportive of this conclusion, since no significant difference has been found between survivals of these two groups of patients. In fact, the patient with longest survival had 100% aneuploidy in her initial study (see the Case Report). A constant feature (100%) was the presence of Auer rods and peroxidase-positive granules, which, of course, is characteristic of AGL. It is interesting to point out that in his series of patients Kamada consistently found low alkaline phosphate levels. Unfortunately, this feature was not studied in our patients.

The good therapy response noted in these patients was emphasized as a clinical characteristic of the “complex” profile in a previous publication. As summarized in Table 2, the 8/21 translocation patients had a median survival time (81 wk) that compared favorably with the median survival time of other patients with AGL. It is possible that this good clinical response to chemotherapy is related to age, since most of these individuals were under 50 yr of age. It is of importance to mention that the cytogenetic and ultrastructural alterations (8/21 translocation and nuclear blebs) are good morphologic markers to monitor the presence or absence of leukemic cells, since the cells carrying these abnormal features disappear totally during remission and return during relapse.

In the span of the 7 yr covered by this investigation, our laboratory has successfully studied hematopoietic samples obtained from 546 patients with acute leukemia either before therapy or during the relapse stage of their disease. As illustrated in Table 3, 234 of these patients were aneuploid and 312 were diploid.

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**Table 3. Incidence of 8/21 Translocation Group in Acute Leukemia (1968–1975)**

<table>
<thead>
<tr>
<th></th>
<th>Number of Patients</th>
<th>Diploid Patients</th>
<th>Aneuploid Patients</th>
<th>Patients With 8/21 Translocation</th>
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</thead>
<tbody>
<tr>
<td>Acute granulocytic leukemia</td>
<td>438 (100%)</td>
<td>245 (55.9%)</td>
<td>193 (44.1%)</td>
<td>32 (7.3% of total AGLs)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(16.6% of aneuploid AGLs)</td>
</tr>
<tr>
<td>Other acute leukemias</td>
<td>108 (100%)</td>
<td>67 (62.0%)</td>
<td>41 (38.0%)</td>
<td>0</td>
</tr>
<tr>
<td>All acute leukemias</td>
<td>546 (100%)</td>
<td>312 (57.1%)</td>
<td>234 (42.9%)</td>
<td>32 (5.9% of total acute leukemias)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>(13.7% of aneuploid acute leukemias)</td>
</tr>
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</table>
which gives an incidence of aneuploidy of 42.9%. When the 8/21 translocation group is related to all of the acute leukemia patients studied in our laboratory before 1975, this subgroup constitutes 5.9% of all the acute leukemias and 13.7% of all aneuploid acute leukemias. If only AGL is considered, the 8/21 translocation patients represent 7.3% of all AGLs and 16.6% of all aneuploid AGLs (Table 3). These figures indicate that 8/21 translocation leukemia constitutes a small but significant fraction to the total number of AGL patients studied. However, the fact that all of these patients present common cytologic features and, in general, behave clinically in a more or less similar manner supports categorization of this entity as a definite subgroup within the general category of AGL.

ACKNOWLEDGMENT

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