Association of a Chromosomal 3;21 Translocation With the Blast Phase of Chronic Myelogenous Leukemia


An identical reciprocal translocation between the long arms of chromosomes 3 and 21 with breakpoints in bands 3q26 and 21q22, t(3;21)(q26;q22), was found in three male patients with the blast phase of chronic myelogenous leukemia (CML). The abnormality was clonal in all three patients and was always accompanied by either a standard or variant 9;22 translocation resulting in a Philadelphia chromosome (Ph1). In two cases, the t(3;21) was the only abnormality other than a t(9;22) in the primary clone. Serial studies of one patient demonstrated that the t(3;21) occurred as a result of clonal evolution near the time of development of the blast phase. We have not observed the t(3;21) in >500 patients with CML in the chronic phase. Thus, the t(3;21) is a new recurring cytogenetic abnormality associated with the blast phase of CML.

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THE LEUKEMIA CELLS of virtually all patients with chronic myelogenous leukemia (CML) are characterized by the Ph1 or Philadelphia chromosome, a product of a reciprocal 9;22 translocation, t(9;22)(q34;q11), or of a variant of this translocation. The t(9;22) is present at the time of diagnosis of CML and throughout the course of the disease. Evidence is accumulating that the molecular consequence of this rearrangement, namely, fusion of sequences of the ABL and bcr genes, plays a critical role in the pathogenesis of CML.

At diagnosis and during the chronic phase of CML, the t(9;22) is usually the sole cytogenetic abnormality; additional abnormalities are found in 9% to 30% of cases. Subsequently, karyotypic evolution occurs in 80% of individuals before or during the blast phase. Numerous secondary chromosomal abnormalities have been observed; however, only a few recurring abnormalities have been identified. The most common of these are +8, +Ph1, and i(17q); at least one of these is found in 80% of individuals with evolution. Rarely, specific abnormalities first identified in acute non-lymphocytic leukemia (ANLL) are noted. These include the t(15;17) of acute promyelocytic leukemia and inv(3) and t(3;3) [also interpreted in some reports as an ins(3;3)] of ANLL with abnormal megakaryopoiesis; in cases of CML with these abnormalities, the acute phase has the characteristic features of the corresponding ANLL.

We identified a new recurring cytogenetic abnormality in association with the blast phase of CML in three patients. This finding will provide a focus for molecular studies aimed at understanding the conversion of CML from chronic to blast phase.

MATERIALS AND METHODS

The patients we studied had been referred to the University of Chicago or Michael Reese Hospitals; specimens of bone marrow and/or blood were analyzed in the Hematology/Oncology Cytogenetics Laboratory. Each patient presented with clinical and morphological features typical of CML in the chronic phase. The diagnosis of CML was confirmed in all cases by identification of the Philadelphia chromosome in hematopoietic tissue. Morphological characterization of the blast phase was based on examination of peripheral blood smears and of bone marrow biopsy specimens and aspirates obtained before institution of acute phase therapy.

Cytogenetic analyses using trypsin-Giemsa, quinacrine fluorescence, and chromomycin-A3-methyl green reverse fluorescence banding techniques were performed on bone marrow aspirates or peripheral blood samples. All three patients were studied during the blast phase; patient 1 was also studied during the chronic and accelerated phases. Metaphase cells from short-term (24-hour) unstimulated cultures and methylxanthate-synchronized cells cultured for 48 hours in phytohemagglutinin (PHA)-stimulated leukocyte-conditioned medium were analyzed. The criteria first proposed by Rowley and Potter and accepted at the First International Workshop on Chromosomes in Leukemia were used for identification of abnormal clones. Chromosomal abnormalities were described according to the ISCN (1985).

Southern blot analysis of DNA from patient 1 was performed as previously described; we used DNA probes specific for the human transferrin receptor (TFRC) and Hu-ets-2 (ETS2) genes. The pTR4 probe contains ~1.6 kilobases (kb) of the 3' untranslated region of a human TFRC cDNA clone; the pTR1/7 probe contains 2.5 kb from the TFRC cDNA, including the entire coding region; and the pH33 probe contains 1.0 kb of human genomic sequences from the ETS2 locus.

RESULTS

Clinical and hematologic features. The clinical and hematologic features of the three patients who had CML and a t(3;21) are summarized in Table 1. All of the patients were men. Each had a relatively long chronic phase during which hydroxyurea and/or busulfan was administered. All three patients had splenomegaly, and patient 2 had lymphadenop-
Abbreviations: PB, peripheral blood; BM, bone marrow aspirate; 24 h, cells cultured for 24 hours; 48 h MTX, cells cultured for 48 hours in phytohemagglutinin-stimulated leukocyte-conditioned medium and synchronized with methotrexate.

<table>
<thead>
<tr>
<th>Course/Feature</th>
<th>Patient 1</th>
<th>Patient 2</th>
<th>Patient 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age/sex</td>
<td>41/M</td>
<td>66/M</td>
<td>30/M</td>
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<tr>
<td>Chronic phase: treatment</td>
<td>Hydroxyurea, Busulfan</td>
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<td>Busulfan</td>
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<tr>
<td>Duration (mo)</td>
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<td>36</td>
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<tr>
<td>Accelerated phase</td>
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<tr>
<td>Manifestations</td>
<td>Weight loss, splenomegaly</td>
<td>—</td>
<td>Fever, pancytopenia</td>
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<tr>
<td>Treatment</td>
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<td>—</td>
<td>Fluoxymesterone</td>
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<tr>
<td>Duration (mo)</td>
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<td>2</td>
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<tr>
<td>Blast phase</td>
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<tr>
<td>Hemoglobin (g/dL)</td>
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<td>9.0</td>
<td>8.7</td>
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<tr>
<td>Platelets (× 10^9/L)</td>
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<td>50</td>
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<tr>
<td>WBC (× 10^9/L)</td>
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<td>23</td>
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<tr>
<td>Blasts (%)</td>
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<tr>
<td>Blasts (%)</td>
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<td>80</td>
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<tr>
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<td>Nonlymphoid*</td>
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<tr>
<td>TdT</td>
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<td>Negative</td>
<td>Negative</td>
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<tr>
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<tr>
<td>Number</td>
<td>Abnormal</td>
<td>Normal*</td>
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<tr>
<td>Morphology</td>
<td>Decreased</td>
<td>Normal*</td>
<td>Normal*</td>
</tr>
<tr>
<td>Treatment and response</td>
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<td>VP → NR, DAT → NR</td>
<td>VP → NR, DAT → NR</td>
</tr>
<tr>
<td>Survival (mo)</td>
<td>46</td>
<td>37</td>
<td>64</td>
</tr>
<tr>
<td>Overall survival (mo)</td>
<td>46</td>
<td>37</td>
<td>64</td>
</tr>
</tbody>
</table>

*Bone marrow aspirates could not be obtained because of fibrosis; blast cell features were determined from peripheral blood samples, and megakaryocyte morphology could not be assessed fully.

Cytogenetic analyses. The results of cytogenetic analyses are summarized in Table 2 and are illustrated in Fig 1. Patient 1 was studied at three times during the course of his disease. The first study was performed during the chronic phase, 19 months following diagnosis. All 19 bone marrow cells examined had only a typical t(9;22)(q34;q11). The patient subsequently entered an accelerated phase characterized by weight loss and increasing splenomegaly, which was
clones were found in patient 3; one was pseudodiploid and the remaining 7 contained a t(3;21)(q26;q22) in addition to the t(9;22).

Peripheral blood from patients 2 and 3 was studied in our laboratory at the time of blast phase. In patient 2, the predominant clone, comprising 21 of 22 metaphase cells studied, had 48 chromosomes. A three-way translocation, t(X;9;22)(p22;q34;q11), which is a variant of the standard t(9;22), was present. In addition, these cells had extra chromosomes 8 and 11, an isochromosome of the long arm of one chromosome 8 homolog, and a t(3;21)(q26;q22). The remaining cell had 49 chromosomes and contained these abnormalities as well as a second Ph'. Three related subclones were found in patient 3; one was pseudodiploid and the other two were hyperdiploid. The simplest of these subclones had 46 chromosomes and had a standard t(9;22) and a t(3;21)(q26;q22) as the only abnormalities. The two other subclones had undergone further evolution, with one containing an extra chromosome 15 and a second Ph', and the other containing these abnormalities plus a deletion of a part of the long arm of one chromosome No. 7 [del(7)(q32q36)].

DNA analysis. At the cytogenetic level of resolution, the locations of the breakpoints of the t(3;21) at bands 3q26 and 21q22 correspond to the mapped positions of the TFRC gene and of the human homolog 2 of the ets sequence of the transforming avian erythroblastosis virus E26 (ETS2), respectively. To determine whether the TFRC or ETS2 gene loci were rearranged as a consequence of the translocation, we performed Southern blot analysis of DNA from peripheral blood cells of patient 1; placental DNA was used as a control. DNA, which had been digested with EcoRI, HindIII, or PvuII, was hybridized sequentially to two TFRC probes and an ETS2 probe. The sample had been obtained at blast phase, concurrently with the sample used for cytogenetic analysis. The WBC count at that time was 86.4 x 10^9/L; of these cells, 41% were blasts and 36% were micromegakaryocytes. Only germline restriction fragments were detected with the probes. Thus, in this patient, there was no evidence for a translocation breakpoint within the TFRC or ETS2 genes.

DISCUSSION

We identified an identical t(3;21)(q26;q22) in three male patients with Philadelphia chromosome-positive CML during blast phase. In one patient, whom we studied serially, we showed that the translocation has occurred in association with the development of the blast phase as the result of clonal karyotypic evolution. We have not observed a t(3;21) in >500 other patients with CML in the chronic phase (M. M. Le Beau and J. D. Rowley, unpublished observations). A review of the literature and of Mitelman's Catalogue of Chromosome Aberrations in Cancer failed to reveal any other cases with a t(3;21)(q26;q22). One patient has been reported as having a t(3;21)(q12;q22) in the blast phase of CML; however, the breakpoint on chromosome 3 differs from that in our cases. Thus, we have identified a new recurring abnormality associated with the blast phase of CML.

The t(3;21) is not entirely specific for CML, however; the same rearrangement has been observed as the only abnormality in bone marrow from two other patients studied in our laboratory, both of whom developed a myelodysplastic syndrome following cytotoxic chemotherapy for a prior malignancy (M. M. Le Beau and J. D. Rowley, unpublished observations). Because the t(3;21) is likely to be therapy induced in these two patients, the same abnormality found in the patients with CML may also be a result of exposure to mutagenic agents rather than a spontaneous evolutionary event. All three patients with CML and a t(3;21) had received chemotherapy during the chronic phase; two of these received the alkylating agent busulfan.

The breakpoints of the t(3;21) are of interest with respect to previously identified cytogenetic abnormalities in leukemia. The breakpoint in 3q26 corresponds to one of the breakpoints in the t(3;3)(q21;q26) and the inv(3)(q21q26), both of which are associated with ANLL and abnormal megakaryocytopenia. Patient 1 had a high platelet count and numerous micromegakaryocytes in his bone marrow in the blast phase, reminiscent of the findings in ANLL patients with t(3;3) or inv(3). In contrast, patients 2 and 3...
had low platelet counts and a marked reduction of morphologically identifiable megakaryocytes in the marrow at blast phase, suggesting that factors other than a breakpoint at 3q26 are important in determining the presence of megakaryocyte abnormalities. A breakpoint in 3q21, rather than 3q26, in the blast phase of CML has been proposed to be associated with a high platelet count and abnormal megakaryocytes. Bernstein et al reported two patients who had secondary cytogenetic abnormalities involving 3q21 at the time of blast phase of CML. One had an inv(3)(q21q26) like that seen in ANLL, and the other had a t(3;9)(q21;q34); both had elevated platelet counts and numerous micromegakaryocytes at the time of blast phase. The common breakpoint in these two cases is 3q21. In the present report, the findings in patient 1 suggest that a breakpoint at band 3q26 without a 3q21 abnormality may also influence the megakaryocytes in certain cases; however, additional examples are needed for confirmation of this possibility.

The breakpoint in the t(3;21) at band 21q22 also corresponds to one breakpoint in another specific abnormality found in ANLL, namely, the t(8;21)(q22;q22). The t(8;21) has not been identified in any reported cases of CML. Alimena et al, however, tabulated 256 breakpoints observed during blast phase of CML and found a cluster of eight occurring at 21q22, suggesting that this may be an important site with respect to progression of CML.

By analogy to other recurring chromosomal rearrangements in human leukemia that have been studied at the molecular level, the consistency of the breakpoints in the t(3;21) implies that specific genes in bands 3q26 and 21q22 participate in this rearrangement. Furthermore, the t(3;21) may play an important role in disease progression. The latter is suggested by the absence of the t(3;21) during the chronic phase in patient 1 and by the presence of the t(3;21) at blast phase as the only chromosomal rearrangement other than the t(9;22) in the primary clone in patients 1 and 3. Therefore, an investigation of this abnormality at the DNA level might be helpful for an understanding of the basis for the conversion of CML from chronic to blast phase. Our initial approach has been to determine by standard Southern blot analysis whether TFRC or ETS2, which have been localized near bands 3q26 and 21q22, respectively, was rearranged in DNA from patient 1. By using this method, we did not find abnormalities of either gene; however, new techniques such as pulsed-field gel electrophoresis, by which one can examine very large regions of DNA, may allow detection of rearrangements located farther away from these genes.

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