Chromosome 3q21 Abnormalities Associated With Hyperactive Thrombopoiesis in Acute Blastic Transformation of Chronic Myeloid Leukemia

By R. Bernstein, A. Bagg, M. Pinto, D. Lewis, and B. Mendelow

Two patients with acute blastic transformation of chronic myeloid leukemia (CML) associated with strikingly elevated platelet counts showed abnormalities of chromosome 3q in addition to the standard Philadelphia (Ph1) chromosome translocation. The first patient had an inversion of chromosome 3 (q21q26) cytologically identical to an inversion previously reported in de novo acute megakaryoblastic leukemia, and the second patient showed a translocation between chromosome 3q and the chromosome 9 homologue not involved in the Ph1 translocation, [t(3;9)(q21;q34)]. Previous studies had incriminated either 3q21 or 3q26 as the locus for a regulatory thrombopoietic gene, but the current study suggests that 3q21 is the relevant site.

The specific association of chromosome 3q homologous translocation1-7 or paracentric inversion of 3q3.5-6 with acute nonlymphocytic leukemia (ANLL) characterized by abnormal thrombopoiesis suggests that these 3q abnormalities identify a particular subtype of ANLL with features of acute megakaryoblastic leukemia (M7).8 The French-American-British (FAB) classification of acute leukemia has recently been amended to include an M7 category.9

The breakpoints common to these 3q abnormalities were 3q21 and 3q26 (or 3q25). An elevated serum thrombopoietin level in one of the aforementioned patients with acute megakaryoblastic leukemia and inv(3)(q21q26)8 led to the suggestion that either 3q21 or 3q26 contains the locus of a gene regulating thrombopoiesis.

We here report on two patients with Philadelphia (Ph1) chromosome-positive chronic myeloid leukemia (CML) in whom acute blast transformation associated with markedly elevated platelet counts developed. Abnormalities of 3q were again found; inv(3)(q21q26) was noted in one patient and a t(3;9)(q21;q34) in the other. The common breakpoint on chromosome 3 is 3q21.

CASE REPORTS

Patient M.M. A 59-year-old white female presented in Sept 1982 with the clinical and hematologic features of chronic-phase CML. Details of the patient's subsequent course and therapy are presented in Figs 1 and 2. The chronic phase was unremarkable except for a course punctuated by an extremely high platelet count that was difficult to control. In July 1984 a platelet count of 2,950 x 10^9/L necessitated treatment with 5 mCi ^31P (Fig 1).

In Feb 1985 she showed acceleration of her disease, and in Aug 1985, 35 months after initial presentation, acute blast transformation was observed. The platelet count at the time was >1,000 x 10^9/L (Fig 2). Marrow aspirate tests revealed 38% blasts that had an undifferentiated morphology (Fig 3A) and were negative for myeloperoxidase, Sudan black, and periodic acid-Schiff (PAS). They did not express common acute lymphoblastic leukemia antigen (CALLA) or myeloid antigens, as defined by the monoclonal antibodies J5 and MY 906, respectively, and were negative for terminal deoxynucleotidyl transferase (TdT), but 68% of mononuclear cells expressed the Ia antigen. Unfortunately, neither platelet peroxidase nor platelet monoclonal antibodies were used in this study.

An induction course of DOAP was followed by an initial pancytopenia, but the WBC count rose again within a week to 123 x 10^9/L with 30% blasts, and the platelet count reached a level of >2,000 x 10^9/L. A marrow aspirate contained 50% blasts at this stage. A marrow biopsy specimen consisted almost entirely of solid sheets of abnormal megakaryocytes, including numerous micromegakaryocytes (Fig 3B). There was a marked increase in background fibrosis. The patient was not given any further chemotherapy and died of renal failure and septicemia in Sept 1985, 5 weeks after transformation to the acute phase.

Patient D.T. A 32-year-old black manual worker presented at a peripheral hospital in Oct 1982 with a 5-month history of left upper abdominal pain. There was no known occupational or therapeutic exposure to carcinogens. A diagnosis of CML in the chronic phase was made on the basis of his clinical presentation and hematologic findings. He received busulphan therapy for 2 months but was subsequently lost to follow-up for 6 months.

He again presented in June 1983 when he was acutely ill. On examination, he had a lobar pneumonia, numerous subcutaneous nodules (possibly infiltrative), generalized lymphadenopathy, a 10-cm splenomegaly, and a 4-cm hepatomegaly. Blood cultures revealed a staphylococcal septicaemia. Peripheral blood indexes were indicative of acute blast transformation of CML. The hemoglobin concentration was 5.5 g/dL, and the WBC count was 550 x 10^9/L with a differential count of 69% blasts, 3% promyelocytes, 2% myelocytes, 3% metamyelocytes, 15% neutrophils, 5% basophils, 1% eosinophils, 2% lymphocytes, and five normoblasts per 100 white cells. Auer's rods were not observed. The platelet count was 1,028 x 10^9/L. Bone marrow aspiration yielded a dry tap. Bone marrow trephine biopsy results showed a marked, diffuse increase in cellularity, predominantly because of a pronounced proliferation of blasts and micromegakaryocytes that were clonally distributed; micromegakaryocytes were prominent (Fig 4). Focal residual areas of granulopoiesis with maturation were present. Erythropoiesis was markedly depressed. The patient received packed red cells, antibiotics, and a single dose of chemotherapy consisting of prednisone, hydroxyurea, Adriamycin, and vincristine, but died of septicemia five days after admission.

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CHROMOSOME 3q21 ABNORMALITIES IN CML

MATERIALS AND METHODS

Metaphases were obtained from the bone marrow aspirate by direct processing and a 24-hour unstimulated culture. Unstimulated and control phytohemagglutinin (PHA)-stimulated 48- and 72-hour cultures were established from peripheral blood. Chromosome analyses were performed on Giemsa- (GTG) and quinacrine (QFQ)-banded metaphases. An abnormal clone was defined as at least two cells with the same structural abnormality or the same extra chromosome and at least three cells with the same missing chromosome. Random loss of chromosomes was not specified in the results.

RESULTS

Patient M.M. Initial chromosome studies performed when the patient first presented in Sept 1982 revealed a Ph'-positive karyotype of 46,XX,t(9;22)(q34;q11). No other abnormalities were noted, but the chromosomes were short, and their banded morphology was poor. Cytogenetic studies were not repeated until July 1985 when the patient was in the accelerated phase of her disease. At this stage the karyotype appeared to be unchanged, but chromosome preparations were again of poor quality. The final analysis was performed in Sept 1985 when acute blastic transformation had already occurred. All 29 cells analyzed had the Ph' translocation, but in addition, 25 of these cells showed an inversion of 3q, karyotype 46,XX,t(9;22),inv(3)(q21q26). (Table 1 and Fig 5). No polyploid metaphases were detected in over 100 cells systematically screened. Metaphases from previous chromosome analyses were retrospectively reviewed, and in two of 13 cells analyzed during the accelerated phase, the inv(3q) was detected.

Patient D.T. Initial chromosome studies at presentation were unsuccessful. Chromosome analysis during the acute phase of his illness, 8 months later, revealed 13 cells with a standard Ph' translocation, t(9;22), and they all showed another translocation of chromosome 3q to the chromosome 9 homologue not involved in the Ph' translocation, t(3;9)(q21;q34). In seven of these cells clonal evolution was noted, with duplication of the chromosome 9 derivative of the t(3;9), disomy Y (confirmed by the quinacrine fluorescence method), and trisomies of chromosomes 8, 19, and 21 (Table 1 and Fig 6). Three normal cells with a 46,XY karyotype were found among PHA-stimulated metaphases. No polyploid metaphases were detected.

DISCUSSION

The association of megakaryocytic and thrombopoietic abnormalities observed in patient M.M. together with a cytologically identical inv(3)(q21q26) to that found in previously described cases of de novo acute leukemia with abnormal thrombopoiesis, is most unlikely to be coincidental. This inversion therefore points to a gene situated either at 3q21 or 3q26 that is intimately involved in the regulation of normal thrombopoiesis. Bitter et al suggest that whether the abnormality of 3q is an inv(3q), a t(3q;3q), or an ins(5;3q) the important event in determining abnormal
thrombopoiesis is the aberrant juxtaposition of genes on 3q21 and 3q26. The t(3;9) found in patient D.T. affects only the q21 breakpoint on chromosome 3, localizing region 3q21 as the more likely site for the locus.

The translocation detected in patient D.T. is of great interest because the translocated segment 3q21–>3qter was attached to the same 9q34 region cytogenetically as the Ph1 translocation, but in this instance, affected the 9 homologue not involved in the t(9;22). The recent molecular identification of a new fusion gene on the Ph1 chromosome by the transposition of the oncogene c-abl from 9q34 to the ber of 22q11 clearly incriminates both the 9q34 and 22q11 breakpoints in the pathogenesis of CML and affords a rational explanation for a series of cytogenetic observations that had been made previously. In this case, transposition of a thrombopoietic gene from 3q21 to 9q34 could have been an important mechanism in dictating the type of evolution in patient D.T. instead of the usual juxtaposition of 3q21 and 3q26 as suggested by Bitter et al. Furthermore, the duplication of this 9 derivative of t(3;9) may be analogous to the duplication of the Ph1 chromosome frequently observed in the progression of CML, even when present in a masked form.

A review of karyotypic findings in previously documented cases of CML with suspected or confirmed megakaryoblastic transformation was informative in only two instances. Carbonell et al27 reported a patient with Ph1-positive CML in whom an ins(3;3)(q26;q21q26) was identified during blast crisis; this patient’s course was characterized by hyperplasia of morphologically abnormal megakaryocytes and thrombocytopenia, but decreased megakaryocytes and thrombocytopenia were observed when blast crisis supervened. Pintado et al7 described a patient with blastic transformation of Ph1-positive CML and thrombopoietic abnormalities associated with an inv(3)(q21q26). These reports and our own suggest a possible utility for the cytogenetic identification of megakaryoblastic transformation.

The other cases were not contributory in assessing whether chromosome 3q abnormalities were present or not. All cases, with one exception11 were Ph1-positive, but the only published karyotype was unbanded.22 Abnormalities such as a paracentric inversion of 3q would not be detected in unbanded or poorly banded chromosomes (for instance, the earlier studies performed on patient M.M.).

On the other hand, a review of previously described chromosome 3q abnormalities in blastic CML were not definitive in establishing an association with abnormal thrombopoiesis. Mecucci et al5 described three patients with Ph1-positive CML in blast crises who had a t(3q–;3q+) abnormality and a further four patients in blastic CML with
translocations involving chromosome 3q, but no details concerning thrombopoietic activity are specified. Barlogie et al also recorded a case of Ph-positive CML with a t(3;3) during blastic crisis, but here again the characteristics of the transformation are unknown.

In summary, the cytogenetic findings in two cases of CML with transformation, which is very suggestive of a megakaryoblastic acute phase, and previous findings in both de novo acute leukemia or preleukemia with abnormal thrombopoiesis and a few cases of blastic CML point to a locus on chromosome 3, which is the site of a thrombopoietic regulatory gene. Too few cases have been reported to categorically state that 3q21 is a more likely site than 3q26 and that abnormalities at one or both of these sites are specific.

Table 1. Chromosome Results

<table>
<thead>
<tr>
<th>Analysis</th>
<th>Karyotype</th>
<th>Number of Cells Analyzed</th>
<th>Total</th>
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<td>10</td>
</tr>
<tr>
<td></td>
<td>46,XX,t(9;22),inv(3)(q21q26)</td>
<td>2†</td>
<td>2</td>
</tr>
<tr>
<td>9/12/85</td>
<td>46,XX,t(9;22),inv(3)(q21q26)</td>
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<td></td>
<td>46,XX,t(9;22),inv(3)(q21q26)</td>
<td>18</td>
<td>7</td>
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<tr>
<td>Patient D.T.</td>
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<tr>
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<tr>
<td></td>
<td>51,XY,t(9;22),t(3;9),+der(9)t(3;9),+8,+19,+21</td>
<td>4</td>
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</table>

Abbreviations: BM, bone marrow aspirate culture; PB, peripheral blood cultures.

*Chromosome morphology was too poor to determine retrospectively whether chromosome 3 had an inversion or not.
†Retrospective review of chromosome 3 banding pattern.
Fig 5. (A) GTG-banded karyotype of patient M.M., 46,XX,t(9;22)(q34;q11),inv(3)(q21q28). (B) Partial GTG-banded karyotypes of three metaphases from patient M.M. showing the inverted 3 (large arrow) and the Ph' translocation, t(9;22) (small arrows). (There is a suspicion in the third partial karyotype that both homologues of 3q may be inverted, but the homologue on the left is technically more elongated than the inverted 3q, thus making the diagnosis of an inv(3q) in this homologue uncertain.)

Fig 6. (A) GTG-banded karyotype of patient D.T., 51,XY,Y,t(9;22) (q34;q11),t(3;9)(q21;q34),der(9),-t(3;9),+8,+19,+21. (B) Partial GTG-banded karyotype of patient D.T. showing the 3q– and the duplicated chromosome 9q + derivatives of the t(3;9) and the Ph' translocation, t(9;22) (arrowed).
cally associated with megakaryoblastic leukemia, but a nonrandom association has been established. This gene could be activated by juxtaposition of 3q21 and 3q26 as suggested by Bitter et al., or alternatively, based on our findings in patient D.T. by the juxtaposition of 3q21 and 3q34. There is a need in future studies to correlate cytogenetic studies with other morphologic, cytochemical, ultrastructural, and immunologic parameters of the blast cell in the acute blastic phase of CML.

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REFERENCES


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