Case reports

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Reciprocal translocation between chromosomes 8 and 9 in atypical chronic myeloid leukaemia

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SUMMARY A balanced translocation t(8;9) (p11;q34) was present in the peripheral blood, bone marrow, and spleen cells of a patient with Ph negative chronic myeloid leukaemia. Subsequent transformation into acute leukaemia was associated with the emergence of trisomy 8 and der(8)(8qter→cen→8p11::9q34→9qter). This is the third reported case of t(8;9) (p11;q34) and raises the question of the role of c-abl in the pathogenesis of this myeloproliferative disorder.

Chronic myeloid leukaemia (CML) is a clonal disease with well defined clinical and haematological features. The initial chronic phase has a median duration of three years and is characterised by leucocytosis and splenomegaly when the leucocyte count reaches 50 to 100×10^9/l.\(^1\) The peripheral blood differential count shows a preponderance of myelocytes and neutrophils together with eosinophilia, basophilia, and relative monocytosis in the majority of cases.\(^1\) There is a subsequent inevitable transformation of the disease which may either begin insidiously or as an abrupt change into an acute leukaemia. The Philadelphia (Ph) chromosome which results from a balanced translocation t(9;22)(q34;q11) is found in over 90% of cases of CML.\(^2\) Other cases have either a complex or masked rearrangement.\(^3\) Very rarely, patients have presented with the clinical and haematological features of CML and have developed the Ph chromosome during the course of the illness.\(^5\) Clinical transformation of CML is often associated with karyotypic evolution.\(^2\)

It is important to distinguish typical Ph +ve CML from atypical Ph –ve cases.\(^1\) The latter are characterised clinically by an aggressive course with a median survival of one year. The typical leucocyte differential count is not seen and an absolute monocytosis and dysmyelopoietic features are sometimes present. Splenomegaly is commonly present at a lower total leucocyte count than in Ph +ve CML. The majority of these cases have a normal karyotype at presentation.\(^3\)

The c-abl oncogene has been located in or near band q34 on chromosome 9 and its activation and translocation to chromosome 22 have been implicated in the pathogenesis of Ph +ve CML.\(^6\)\(^7\) Recently, several cases of Ph –ve CML with chromosome abnormalities involving band 9q34 have been reported.\(^8\) These cases are of particular interest in evaluating the role of c-abl in diseases similar to but distinct from classical Ph +ve CML.\(^7\) For this reason, we describe in detail the third reported case of atypical CML with a translocation involving chromosomes 8 and 9 (p11;q34).

Case report

A 32 year old man presented in October 1982 with one month’s history of generalised weakness, weight loss, and night sweats. On examination, he was pale and the liver and spleen were palpable 4 cm and 10 cm below the costal margin respectively. There was no lymphadenopathy. Full blood count showed Hb 6.3 g/dl, WBC 60×10^9/l, (N15%, L3%, M8%, eosinophils 16%, basophils 16%, eosinophilic myelocytes 7%, basophilic myelocytes 2%, myelocytes 17%, promyelocytes 4%, blasts 12%) and platelets 195×10^9/l. A proportion of neutrophils were hypogranular and showed Pelger Huet abnormalities. A bone marrow aspirate and trephine showed 100% cellularity. Myelopoiesis was markedly hyperplastic, blast cells were increased in numbers, and eosinophilic and basophilic precursors were prominent. Serum B12 was >2000 ng/l (NR 150 to 900), ferritin 1000 ng/l (NR 15 to 400), and neutrophil alkaline phosphatase score 10 (NR 33 to 160). Plasma LDH was raised at 910 IU/l (NR 30 to
90). Initial treatment with hydroxyurea (1 to 2 g daily) and 6 thioguanine (80 mg daily) reduced the white cell count but he continued to require red cell transfusions and there was no reduction in the spleen size. In view of this, in February 1983 he underwent splenectomy. In April 1983 he developed a firm tender mass in the right parasternal region and a biopsy showed it to be a chloroma which was treated with local radiotherapy. In May 1983 the number of blast cells increased in the peripheral blood and bone marrow. The dysmyelopoietic features which were noted on presentation became more marked. He was treated with daunorubicin 90 mg on days 1, 3, and 5, cytarabine 180 mg bd for 10 days and 6 thioguanine 180 mg/d for 10 days. Three courses of this treatment failed to induce a remission. He subsequently transformed into acute myeloblastic leukaemia (FAB M4) in December 1983 and died a month later. Eosinophilia, basophilia, and dysplastic features persisted throughout the course of his illness.

Materials and methods

In vitro culture studies of the bone marrow were performed on three different occasions during the course of his illness. The samples were taken into Iscove’s modified Dulbecco’s medium and layered over Ficoll-sodium diatrizoate (density 1.077). Interface mononuclear cells were collected after centrifugation at 400 g for 25 to 30 minutes. The mononuclear cells were washed three times in Hanks’ balanced salt solution (Ca ++ and Mg ++ free) with 2% fetal calf serum (FCS). The cells were suspended in media containing methylcellulose (0-9% final concentration), 30% FCS, 5% phytohaemagglutinin leucocyte conditioned medium (PHA-LCM), 5 x 10^-5 2 mercaptoethanol (2ME), and Iscove’s medium to a final concentration of 2 x 10^8 cells/ml. Aliquots of 1 ml were cultured in 35 mm plastic dishes at 37°C in a 5% CO₂ environment with 100% humidity. The colonies were scored on days 7 and 14. For erythroid progenitor growth, erythropoietin 3 IU/plate was added to the culture. Individual colonies were picked from the dishes and stained with MGG and Alcian blue. Specimens of bone marrow, peripheral blood, and spleen cells were used for cytogenetic analyses. Direct preparations and short term cultures were made from bone marrow cells. Mitogen free and phytohaemagglutinin stimulated peripheral blood cells were incubated for 48 hours at 37°C. Spleen cells were cultured for seven days in the presence of lipopolysaccharide to a final concentration of 50 μg/ml. Cytogenetic analyses were carried out using the trypsin banding technique of Seabright.

Results

There was an increase in the number of granulocyte and macrophage colonies and clusters together with reversal of the colony/cluster ratio, and the absence of erythroid growth was observed on each occasion. There was a preponderance of pure basophilic colonies as demonstrated by MGG and Alcian blue.

<table>
<thead>
<tr>
<th>Date</th>
<th>Treatment received</th>
<th>Specimen</th>
<th>Karyotype</th>
<th>No of cells</th>
</tr>
</thead>
<tbody>
<tr>
<td>October 1982</td>
<td>Hydroxyurea</td>
<td>Bone marrow</td>
<td>46,XY.t(8;9)(p11;q34)</td>
<td>20</td>
</tr>
<tr>
<td>6 thioguanine</td>
<td></td>
<td></td>
<td>In all cells (see figure)</td>
<td></td>
</tr>
<tr>
<td>February 1983</td>
<td>Splenectomy</td>
<td>Bone marrow</td>
<td>46,XY.t(8;9)(p11;q34)</td>
<td>10</td>
</tr>
<tr>
<td>P blood (−PHA)</td>
<td></td>
<td></td>
<td>46,XY.t(8;9)(p11;q34)</td>
<td>30</td>
</tr>
<tr>
<td>P blood (+PHA)</td>
<td></td>
<td></td>
<td>46,XY</td>
<td>36</td>
</tr>
<tr>
<td>June 1983</td>
<td>After 2 courses of DAT*</td>
<td>Bone marrow</td>
<td>46,XY.t(8;9)(p11;q34)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>47,XY,+16</td>
<td>2</td>
</tr>
<tr>
<td>October 1983</td>
<td>6 thioguanine</td>
<td>Bone marrow</td>
<td>46,XY.t(8;9)(p11;q34)</td>
<td>8</td>
</tr>
<tr>
<td>December 1983</td>
<td>6 thioguanine</td>
<td>P blood (−PHA)</td>
<td>46,XY.t(8;9)(p11;q34)</td>
<td>4</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>48,XY,+8,+der(8)(8qter→cen→8p11::)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>9q34→9qter,t(8;9)(p11;q34)</td>
<td>2</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>46,XY</td>
<td>3</td>
</tr>
</tbody>
</table>

*Each course of DAT consisted of daunorubicin 90 mg x 3, cytarabine 180 mg bd for 10 days, 6 thioguanine 180 mg for 10 days.
stains. Attempts to karyotype the colonies proved unsuccessful because of sparse metaphases and poor banding. Results of the cytogenetic studies on peripheral blood, bone marrow, and spleen cells are summarised in tables 1 and 2. A constant finding was the presence of an apparently balanced translocation between 8p11 and 9q34 which was seen in peripheral blood, bone marrow, and spleen cells (figure). Transformation to an acute leukaemia was associated with the emergence of trisomy 8. Throughout the course of his disease no Ph chromosome was detected.

Discussion

This patient demonstrated the clinical and laboratory features of atypical CML. At presentation he had gross anaemia, splenomegaly, and leucocytosis. The differential count lacked the neutrophil and myelocyte peaks, but as in Ph +ve CML basophilia and eosinophilia were present throughout the course of his illness. The clinical course was aggressive and unresponsive to intensive chemotherapy and the patient died 14 months later in acute transformation. The Ph chromosome was not present but a reciprocal translocation t(8;9)(p11;q34) was consistently found in bone marrow, peripheral blood, and spleen cells. The in vitro colony growth pattern was not typical of chronic phase Ph +ve CML in which there is a huge increase in myeloid and erythroid colony growth.10 Pure and mixed basophilic colonies have previously been described in patients with CML and more recently in normal peripheral blood.11 Their presence in large numbers in this patient is unusual.

Two other patients with t(8;9)(p11;q34) have been described. Lewis et al8 reported a child with massive splenomegaly, a high leucocyte count with cells in all developmental stages in the peripheral blood, and, as in our patient, eosinophilia and basophilia were prominent. She responded initially to hydroxyurea and then underwent a bone marrow allograft. Friedhoff et al12 reported an 84 year old woman with no splenomegaly who presented with a leucocyte count of 107×109/L without the typical differential of Ph +ve CML, but no comment was made on the presence of eosinophilia, basophilia, or dysmyeloipoietic features. She responded initially to busulphan but died six weeks later of unrelated causes. A further three cases have been documented in which chromosomal analysis showed a normal pair of chromosomes 22, a break at band 9q34, and a reciprocal translocation between the distal segment of chromosome 9 and another chromosome. The
first such case was reported in a 10 year old girl by Warburton and Shah in 1976.13 Her karyotype was 46,XX,t(9;11)(q34;q12). She responded well to busulphan and was alive 29 months after diagnosis. The patient reported by Verhest and Lustman14 had a complex karyotype with t(3;9)(Y)(q35;q34;q12). A third case of Ph −ve CML with a break at 9q34 was listed in Sandberg’s review.15

c-abl has been shown by in situ hybridisation to be translocated from chromosome 9q34 to chromosome 22 in Ph +ve CML, although it appears that the break site on chromosome 9 is variable.2 Amplification of c-abl occurs in the Ph +ve k562 cell line and also in Ph +ve CML.7 In addition, Canaani et al16 have demonstrated the presence of an 8 kb RNA transcript in five out of six patients with Ph +ve CML. These data have led to the suggestion that c-abl may be of importance in the pathogenesis of CML. However, the rare cases of CML with variant translocations apparently not involving chromosome 9 and those cases who develop the Ph chromosome during the course of the disease would not lend support to this hypothesis.3 No translocation of c-abl was found in two cases of atypical CML with a normal karyotype.8 We are currently studying the cells of this patient using blot hybridisation with the human c-abl probe. The patient described in this report illustrates the differences in the clinical, haematological, and probably molecular behaviour of patients with Ph negative CML from those who possess the Ph chromosomal abnormality.

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References


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Partial 2p deletion in a girl with a complex chromosome rearrangement involving chromosomes 2, 6, 11, and 21

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SUMMARY We describe the clinical and cytogenetic findings of a 9½ month old girl with a complex chromosome rearrangement resulting in a probable deletion of band 2p14. She does not resemble other reported cases of del(2p). Deletions of the short arm of chromosome 2 are exceedingly rare, having been reported in only five

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