Clonal Evolution in Atypical Chronic Granulocytic Leukemia: A Non-Philadelphia Translocation

By Grover C. Bagby, Jr., Barbara Kaiser-McCaw, Frederick Hecht, Robert D. Koler, and James W. Linman

Hemopoietic cells in chronic granulocytic leukemia (CGL) frequently contain a chromosome translocation involving chromosome 22 and another autosome, usually number 9. The translocated chromosome 22 is known as the Philadelphia (Ph) chromosome. The appearance of a second Ph chromosome is the most common cytogenetic abnormality in CGL signaling the blastic phase. For 6 yr we serially studied a man with atypical CGL whose marrow cells were marked by a translocation from chromosome 18 to chromosome 11 [46XY, t(11;18)(q23;q12)]. Three months prior to blast transformation there appeared an extra copy of the marker chromosome 18: 47XY,t(11;18)(q23;q12),+(18p11→18q12). This man presents a new cytogenetic pattern of clonal evolution in CGL. The pattern is analogous to that of the Ph chromosome and is characterized by a balanced chromosomal rearrangement and the subsequent acquisition of an extra copy of the small translocation chromosome immediately prior to blast transformation.

Approximately 90% of patients with chronic granulocytic leukemia (CGL) have the Philadelphia (Ph) chromosome in their hemopoietic cells. The Ph chromosome is a number 22 chromosome with material from the long arm translocated usually onto the long arm of chromosome 9, or, less frequently, onto other sites in the genome.

Blast transformation in CGL is generally heralded by the evolution of additional chromosome abnormalities in cells with the original chromosome aberration. Three aberrations account for 90% of those seen in the clonal evolution of CGL: (1) a second Ph chromosome (most often), (2) an extra chromosome 8, or (3) an isochromosome 17. Although the reasons for the prevalence of...
these three aberrations in clonal evolution are not understood, their temporal association with acute leukemia suggests that they play a role in the evolution of blastic transformation.

We report herein serial cytogenetic studies in a man with atypical CGL whose marrow cells exhibited a non-Philadelphia chromosomal rearrangement. A new clone of cells evolved prior to blast transformation. The new clone of cells was marked not only by the original balanced translocation but also by an extra copy of one of the chromosomes involved in the original translocation.

METHODS

Patient

A 35-yr-old male was well until 1968, when a routine blood cell count documented mild neutrophilic leukocytosis. He was referred to the University of Oregon Health Sciences Center in August 1970, where splenomegaly was first noted. At that time the WBC was 54.4 x 10^9/liter (88% segmented neutrophils, 7% bands, 2% lymphocytes, 1% eosinophils, 1% monocytes, 1% metamyelocytes), platelet count was 243 x 10^9/liter, and PCV was 0.46. Leukocyte alkaline phosphatase score was 148 (30-70 normal) in 1970 and 360 in 1975. Serum vitamin B12 level was 4000 pg/ml in 1975. No teardrop-shaped or nucleated red blood cells were seen on peripheral blood smears. Bone marrow samples showed marked granulocytic hyperplasia but no fibrosis or basophilia. Morphologic features of bone marrow aspirates obtained serially from 1970 to 1976 are summarized in Table 1. In 1970 a direct marrow cytogenetic study yielded 32 cells with a normal 46XY karyotype.

The clinical course is summarized in Fig. 1. Between 1970 and 1975 the patient was asymptomatic and did not receive cytotoxic therapy until January 1976. In September 1975 the spleen had enlarged to 14 cm below the left costal margin. Over the next 5 mo there ensued a progressively accelerated course characterized by bone pain, fever, weight loss, progressive anemia, leukocytosis, and thrombocytopenia and extensive extramedullary disease involving retina, skin, and spinal epidural space. He received spinal irradiation in January 1976. Subsequently, busulfan therapy was initiated but was followed by worsening thrombocytopenia. Splenectomy was performed in June 1976.

Double-layer agar cultures (CFU-C) of both peripheral blood and marrow cells were performed prior to splenectomy according to methods previously described.6-8 The cloning efficiency of peripheral blood was markedly increased (155 ± 8 colonies/2 x 10^5 cells) and equaled the cloning efficiency of marrow cells (147 ± 4).

In July 1976 bone marrow myeloblasts had increased to 37% and hydroxyurea and 6-thiogua-

Table 1. Bone Marrow Morphology

<table>
<thead>
<tr>
<th>Date</th>
<th>Blasts</th>
<th>Progranulocytes</th>
<th>Myelocytes</th>
<th>Metamyelocytes</th>
<th>Neutrophils</th>
<th>Megakaryocytes*</th>
<th>Erythroblastosis*</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aug. 1970</td>
<td>1</td>
<td>4</td>
<td>12</td>
<td>20</td>
<td>36</td>
<td>27</td>
<td>Increased</td>
</tr>
<tr>
<td>Sept. 1975</td>
<td>2</td>
<td>4</td>
<td>17</td>
<td>16</td>
<td>25</td>
<td>36</td>
<td>Normal</td>
</tr>
<tr>
<td>Mar. 1976</td>
<td>3</td>
<td>10</td>
<td>27</td>
<td>18</td>
<td>19</td>
<td>23</td>
<td>Normal</td>
</tr>
<tr>
<td>May 1976</td>
<td>7</td>
<td>19</td>
<td>30</td>
<td>11</td>
<td>17</td>
<td>16</td>
<td>Moderate decrease</td>
</tr>
<tr>
<td>June 1976</td>
<td>4</td>
<td>15</td>
<td>30</td>
<td>17</td>
<td>11</td>
<td>23</td>
<td>Moderate decrease</td>
</tr>
<tr>
<td>July 1976</td>
<td>12</td>
<td>9</td>
<td>12</td>
<td>12</td>
<td>20</td>
<td>35</td>
<td>Moderate decrease</td>
</tr>
<tr>
<td>Oct. 1976</td>
<td>49</td>
<td>16</td>
<td>9</td>
<td>8</td>
<td>8</td>
<td>10</td>
<td>Marked decrease</td>
</tr>
</tbody>
</table>

*Estimated cellularity from bone marrow particle preparation.
nine were administered. A bone marrow examination in October showed 49%, myeloblasts. The patient died of massive intrapleural hemorrhage in November 1976.

Histologic sections of the 1432-g spleen and Wright-stained spleen-cell suspensions showed active granulopoiesis (30%, myeloblasts), scattered Gaucher-like cells, and no megakaryocytes; less than 1% of the nucleated cells were erythroid precursors. Postmortem examination confirmed the diagnosis of acute nonlymphocytic leukemia with nodular myeloblastic infiltration of epidural space, skin, periaortic nodes, liver, and multiple areas of cortical bone.

Cytogenetic techniques. Aspirated marrow cells were placed in heparinized McCoys 5A medium for 45-60 min. Cells were accumulated in metaphase by adding colchicine (0.1 μg/ml) to the culture 1 hr before harvest. The cells were treated twice with hypotonic KCl (0.075 M), fixed in methanol:acetic acid (3:1), placed on glass slides, and air dried. Slides were sequentially stained with Giemsa, metaphases were located, and then, after destaining, slides were stained with quinacrine for Q banding.9 Cells were photographed with a Zeiss

Table 2. Progression of Clonal Evolution

<table>
<thead>
<tr>
<th>Date</th>
<th>Clinical Status</th>
<th>46 Normal</th>
<th>46, Translocation</th>
<th>47, Translocation</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 1970</td>
<td>Stable</td>
<td>100%</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Sept. 1975</td>
<td>Stable</td>
<td>15%</td>
<td>85%</td>
<td>0</td>
</tr>
<tr>
<td>Mar. 1976</td>
<td>Accelerated disease</td>
<td>0</td>
<td>25%</td>
<td>75%</td>
</tr>
<tr>
<td>May 1976</td>
<td>Accelerated disease</td>
<td>0</td>
<td>20%</td>
<td>80%</td>
</tr>
<tr>
<td>Oct. 1976</td>
<td>Blastic phase</td>
<td>0</td>
<td>0</td>
<td>100%</td>
</tr>
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Fig. 2. See opposite page.
photoscope (model 1) equipped for light and fluorescent microscopy with an HB mercury light source. Photographic enlargements (2400×) were scored for chromosome number, breaks, and morphology. Karyotypes were arranged according to the Paris Conference. Five separate direct marrow studies were done, and banding studies were performed on the last four samples.

RESULTS

The first marrow study was done in 1970 and showed a normal 46XY karyotype in all cells analyzed. The progression of chromosomal evolution is shown in Table 2. Prior to the accelerated course of his leukemia, the majority of the patient’s marrow cells exhibited a translocation (t) of material from the long arm (q) of chromosome 18 to 11q, which can be coded 46XY,t(11q;18q) (Figs. 2 and 3). During the accelerated course, but before the development of blastic transformation, the cells with the translocation had acquired an extra small chromosome 18, which is coded +(18p11→18q12) (Fig. 3). Blast crisis rapidly ensued. At the time of death the new clone with 47 chromosomes made up 100% of the marrow cells examined (Table 2).

DISCUSSION

We were reluctant to categorize this man’s myeloproliferative disorder until a postmortem examination had been performed because of the absence of a Philadelphia chromosome, persistently elevated leukocyte alkaline phosphatase, and lack of basophilia. The diagnosis of CGL was made on the basis of the following features of his case: 6–8 yr of stable granulocytic hyperplasia and splenomegaly in a nonanemic, nonthrombocytopenic middle-aged male, characteristic spleen history (including Gaucher-like cells but little erythropoiesis), elevated serum vitamin B12 level, increased peripheral blood cloning efficiency (CFU-C), extramedullary granulocytic tumors developing during an accelerated clinical phase, clonal evolution followed by death in blastic crisis, and absence of myelofibrosis and myeloid metaplasia at postmortem examination.

Agar cloning efficiency of peripheral blood cells in our patient was consistently and markedly increased, a phenomenon known to be a characteristic feature of CGL of both Ph-positive and -negative varieties. While such marked increases may also be seen in other chronic myeloproliferative disorders, these were ruled out in our case. Thus we feel that the peripheral blood cloning efficiency observed in our patient supports the diagnosis of CGL.

Early in the course of this patient’s disease, chromosomes in marrow cells appeared normal. This suggests either that a visible abnormality in chromosomal structure was not essential to the initial clinical signs of disease or that the translocation may have been present in some marrow cells that we did not sample. The translocation was present before he received irradiation or cytotoxic chemotherapy and thus cannot be attributed to these agents.

Although visible abnormalities of chromosome structure are not required

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**Fig. 2.** Representative karyotypes from direct bone marrow cell preparations. Chromosomes stained with quinacrine. (A) Initial translocation; symbol “t” with the arrow indicates the balanced translocation between long arms of chromosomes 18 and 11. (B) Karyotype showing both original translocation and supernumerary small 18. This abnormality was found immediately prior to blast transformation.
for the neoplastic state, chromosome abnormalities are common in many established neoplastic cells. An orderly progression of karyotype rearrangements often occurs in neoplastic cells of many types, a process known as clonal evolution. Clonal evolution occurs in myeloproliferative disorders other than CGL. However, the conversion of a balanced translocation to a hyperdiploid rearrangement by the specific duplication of the shortened chromosome (e.g., the Ph chromosome is the "shortened" chromosome in CGL) seems to occur with regularity only in CGL.

Thus the pattern of clonal evolution in our patient is remarkably similar to the major pattern of clonal evolution in the Ph-positive form of CGL. First, a clone of cells emerged marked by a balanced chromosomal rearrangement \([46XY, t(11;18)(q23;q12)]\). This rearrangement is analogous to the most common one in Ph-positive CGL \([46XY, t(9;22)(q34;q11)]\). In both chromosome rearrangements the translocation process involves unequal exchange of material so that there is an abbreviated chromosome, no. 18 in our case and no. 22 in the usual form of CGL. In Ph-positive CGL the acquisition of a supernumerary Ph chromosome is the most common second step in chromosome
evolution and almost always heralds blast transformation. Similarly, in our patient the gain of an extra copy of the small 18 was noted 3 mo prior to the onset of blast transformation, while the balanced translocation (analogous to Ph) was found long in advance of blast crisis.

The precise mechanism(s) involved in the production of the extra chromosome is unknown. The initial translocation may predispose to subsequent mitotic errors that result in the acquisition of a copy of the “donor” translocation chromosome. Possible mechanisms include nondisjunction, anaphase lag, and selective endoreduplication. Regardless of the mechanisms involved, neoplastic cells with an extra chromosome commonly have a selective advantage over cells with the original translocation.

Adult CGL is cytogenetically heterogeneous and not strictly limited to those patients whose bone marrow cells show the Ph chromosome. Bone marrow cells from about 10% of patients with CGL will lack a Ph chromosome. The duration of survival in the Ph-negative group is shorter than in the Ph-positive group. Although it is clear that this patient showed some atypical clinical features, the time course of his disease was more compatible with that of patients with Ph-positive CGL. Nonetheless, had we not repeated the marrow chromosome study our patient would have been considered Ph negative. Therefore we suggest that all patients with CGL, including those in whom the initial chromosome study is normal, warrant serial chromosome studies. Discovery of similar chromosome changes should permit comparative analysis of the relationship between chromosomal evolution and blastic leukemia.

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