t(14;14)(q11;q32) in Biphenotypic Blastic Phase of Chronic Myeloid Leukemia

By Nicole Dastugue, Emilienne Kuhlein, Eliane Duchayne, Francis Roubinet, Georges Bourrouillou, Michel Attal, Jacques Pris, and Pierre Colombies

A blastic crisis of chronic myeloid leukemia without a detectable chronic phase is reported. At diagnosis, blast cells present t(9;22)(q34;q11), t(14;14)(q11;q32) translocations and early B cell phenotype (DR+, TdT+, B4+, BA1+, J5+). At relapse, the malignant clone evolves to a biphenotypic expression, the initial markers remain unchanged, and two myeloid antigens (My 7, My 9) appear. The wide overlap in percentages of blast cells displaying lymphoid and myeloid markers shows that a single clone bears antigens of both lineages. Simultaneous occurrence of a t(14;14)(q11;q32) translocation, usually found in T cell malignancies, and of a B cell phenotype raises the question of the relationship between chromosomal changes and surface marker expression. The malignant cell is assumed to be a progenitor cell, already committed to lymphoid lineage and retaining the potential to switch to myeloid lineage.

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MATERIALS AND METHODS

Cytogenetic studies. Peripheral blood was cultured with phytohemagglutinin (PHA) for 96 hours. Peripheral blood without stimulation and bone marrow were analyzed after 24 hours or after 72 hours (see Table 2). G banding was performed with the Searle bright technique. Before harvesting, the same culture medium was diluted (1:10 in RPMI), centrifuged on slides by cytopsin, and stained by May-Grünwald-Giemsa. This study helped us to identify the type of dividing cell.

Immunologic studies. The leukemic cells used were either fresh or thawed at the time of the study. Viability of the sample on thawing was 90% as judged from the dye exclusion test, stained with...
trypan blue. The three samples were also centrifuged by cytospin and showed a 95% blast population. An indirect immunofluorescent staining of cells in suspension was used. Briefly, 1 x 10^6 cells were treated with either 200 μL of a 1:160 dilution (for all antibodies, in a saturating dilution) of the specific monoclonal antibody to be tested, or 200 μL of a 1:160 dilution of an unreactive control antibody of an identical isotype. After incubation and repeated washing, they were stained with a fluorescein-conjugated goat antimouse IgG antiserum (GAM-FITC Coultronics). After repeated incubation at 4 °C for 30 minutes, the cells were washed three times and analyzed. Analysis was performed on an EPICS C flow cytometer (Coulter Electronics, Hialeah, Fla) equipped with an argon ion laser. For each sample, 10,000 cells were analyzed with a log amplifier. Windows on the display defining immunofluorescence-positive cells were set so that less than 2% of the cells stained with the unreactive control antibody were counted as positive. Monoclonal antibodies used are summarized in Table 1.

RESULTS

Cyto genetic analysis. Studies of peripheral blood cultured with PHA stimulation revealed a normal 46,XX karyotype. Karyotypes performed from bone marrow and blood without stimulation are listed in Table 2. Normal metaphases were never present. At diagnosis, a single clone with (t(9;22)(q34;q11) and (t(14;14)(q11;q32) translocations was identified. In remission, the (t(14;14) translocation disappeared. A clone with Ph positive alone was present. At the onset of relapse, the two former clones were simultaneously observed. In the terminal phase, a clonal evolution occurred. The only clone present was 46,XX,t(9;22) (q34;q11),t(14;14)(q11;q32),−5,+der(5) (5pter → q32:1qter → q23). A simultaneous study of the cytology and the cell karyotypes from the same culture medium showed that immature granulocytes bore only Ph, whereas blast cells bore both Ph and t(14;14).

Immunologic studies. The results of the immunologic studies are listed in Table 3. At diagnosis, blasts had an early B cell phenotype (TdT, DR, B4, BA1, J5). At relapse, TdT, DR, B4, BA1, and J5 were found in percentages similar to those found in the first sample, and two myeloid antigens (My 7, My 9) appeared. The percentages of cells expressing lymphoid and myeloid markers widely overlapped. These results suggest a biphenotypic expression.

DISCUSSION

Our case shows (t(9;22) (q34;q11),t(14;14)(q11;q32) in a biphenotypic (B lymphoid-myoeloid) blast phase of CML. Ph and t(14;14) have seldom been reported together. One instance was non-T-, non-B-AL and another was a lymphoid blast crisis of CML, the phenotype of which is unknown. Although our case was originally diagnosed as ALL, we can assume that it was actually a blast phase of CML because normal metaphase has never been observed. During the short remission period, Ph did not disappear, whereas the tandem translocation did. At this time, all hematopoietic cell lines were represented and only 5% of blast cells were present. Immature granulocytes (but not blast cells) are the probable bearers of this Ph+ (see Table 2). Such findings argue in favor of CML rather than ALL, since granulocytes are not thought to be involved in the Ph+ positive ALL. At the time of diagnosis, the blast population bearing both (t(9;22)(q34;q11) and t(14;14)(q11;q32) translocations was quite homogeneous. Ninety percent of the blast cells were present, all suggesting lymphoid lineage (morphology and cytochemistry). This hypothesis was confirmed by immunologic characterization: the association of DR, TdT, B4, BA1, and J5 markers, all present in high percentages, made the lymphoid nature unequivocal and was strongly suggestive of B cell lineage at an early stage of differentiation. This result is not surprising and is consistent with former findings reported in literature, that most BP-CML express the same phenotype as common ALL, and thus are usually of B cell lineage. Marker profiles observed in such malignancies represent the early stages of normal B cell maturation at or before the time of light chain rearrangement. The association of DR, B4, and J5 is the stage 3 of such a maturation (stage 1, DR alone; stage 2, DR, B4; stage 4, DR, B4, J5, B1). At relapse, two myeloid antigens (My 7, My 9) appeared beside the unchanged initial markers. These two markers are restricted to myelomonocytic lineage and usually allow differentiation between lymphoid and...
myeloid leukemias. The wide overlap in percentages of positive cells with myeloid and lymphoid markers suggests that blast cells simultaneously exhibit antigens of both lineages. The morphology of blast cells did not show any sign of myeloid differentiation during the phenotypic evolution. Later, in the terminal phase of relapse, a minor population of promonocytes appeared. Myeloperoxidase reaction and sodium fluoride-sensitive esterase reaction remained negative throughout the evolution. At the onset of relapse, a major clone with both t(9;22)(q34;q11) and t(14;14)(q11;q32) reappeared, followed in the terminal phase by an additional change involving chromosomes 1 and 5 in this clone. Thus, the presence of identical cytogenetic markers at diagnosis and relapse shows that the same malignant clone evolved from a classic early B precursor phenotype to a biphenotypic lymphoid-myeloid phenotype.

The translocation t(14;14)(q11;q32) has been observed in AT and is also closely related to T cell disorders. It has been found in T cell lymphoma,\(^2\) in the Sézary syndrome,\(^2\) and in T cell CLL.\(^2\) In the latter, as well as in the case reported by

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**Table 3. Expression of Differentiation Markers**

<table>
<thead>
<tr>
<th>Surface Marker</th>
<th>Bone Marrow Diagnosis</th>
<th>Bone Marrow Relapse 1</th>
<th>Bone Marrow Relapse 2</th>
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<th>Peripheral Blood</th>
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<tr>
<td></td>
<td>Positive Cells (%)</td>
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<tr>
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</table>

ND, not done; clg, cytoplasmic immunoglobulin.
McCaw et al., the malignant clone has emerged from a preexisting AT lymphocyte clone. This tandem translocation affects the 14q11 region of chromosome 14, in which the α-chain gene of the T cell receptor was mapped. In fact, a molecular analysis of translocations with a breakpoint in 14q11 has seldom been carried out. In a recent report concerning one case of T acute leukemia with a t(11;14)(p13;q11) translocation, the α-chain gene has been found to be split by the chromosomal breakpoint. Our case shows a simultaneous occurrence of B cell markers and a translocation usually found in T malignancies, thus raising the question of the relationship between chromosomal changes and surface marker expression. No history of AT was reported in the patient’s family. Acid phosphatase reaction was negative and the percentages of the two T cell markers studied are too low to indicate a T cell phenotype. Unfortunately, we could not study a more immature marker, such as a CD4. Considering the different definitions of chromosomal techniques (>5,000 kb) and of molecular analyses (<50 kb), we can interpret our case as an exception in which, despite a typical aspect of tandem translocation, the breakpoint occurs far from the α-chain gene locus. Alternatively, we can hypothesize that even if the breakpoint does occur within the α-chain gene, it may be an ineffective rearrangement, without affecting the T cell activation.

The significance of the biphenotypic expression remains unclear. It has been interpreted as an aberrant gene expression of malignant cells. Alternatively, the question arises of whether or not it may represent a clonal expansion of a normal counterpart. In our case, the third chromosomal change involving chromosomes 1 and 5 cannot account for the expression of myeloid antigens because this change occurred only in the terminal phase (relapse), 3 months after the phenotypic evolution. Because of the constant presence of Ph1, a reliable marker of stem cells, we can assume that this case represents an early precursor recently committed to B cell lineage but retaining the potential to switch to myeloid lineage. The expression of My 7 and My 9 antigens, usually found on granulocytic and monocytic lineages as early as the CFU-C stage, may reflect this potential switch. Furthermore, the emergence of promonocytes, only detectable by morphology, may also indicate a monocytic process of differentiation. Only a molecular approach, together with an immunophenotypic and a karyotypic study, will allow a better understanding of such puzzling cases and the relationship between gene rearrangement and gene function.

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