Unusual Karyotypic Changes and B Cell Involvement in a Case of Lymph Node Blast Crisis of Chronic Myelogenous Leukemia

By Donna E. Hogge, Shinichi Misawa, Joseph R. Testa, Richard D. Leavitt, Avrom Pollak, and Charles A. Schiffer

A patient with Philadelphia chromosome (Ph') positive chronic myelogenous leukemia (CML) entered a blast crisis localized to lymph nodes. On light microscopy, by morphology and histochemical staining, the blasts were undifferentiated. In spite of terminal deoxynucleotidyl transferase positivity, some of the lymph node cells expressed a myeloid differentiation antigen, OKM1, and were peroxidase positive by transmission electron microscopy (TEM). However, the majority of cells were peroxidase negative on TEM and expressed OKT-10, a marker found on both primitive myeloid and lymphoid cells. Cultures of lymph node cells stimulated with Epstein-Barr virus or lipopolysaccharide (LPS) revealed the Ph', indicating B cell involvement in the CML. T cells from cultures stimulated with L4-phytohemagglutinin and T cell growth factor were negative for the Ph'. In unstimulated lymph node cells, the uncomplicated Ph' could not be demonstrated; instead, a unique complex karyotype involving a masked Ph' was identified in these and the LPS cultures. This karyotype was not found in bone marrow (BM) metaphase cells. Instead, BM cells showed either the simple Ph' or the Ph' with a rearrangement involving chromosomes 13 and 20. The patient had transient responses to three chemotherapy regimens, two of which were designed to treat acute lymphocytic leukemia, but he died 8 months after disease acceleration without BM blast crisis. These findings are compatible with an extramedullary blast crisis originating in a primitive cell with both myeloid and lymphoid characteristics.

The clonal origin of malignancy in chronic myelogenous leukemia (CML) has been demonstrated using cytogenetic analysis and glucose-6-phosphate dehydrogenase (G6PD) isoenzyme studies.1-5 These techniques have also shown that the cell of origin is an early hematopoietic progenitor capable of giving rise to myeloid and erythroid cells, megakaryocytes, monocytes, and some populations of lymphocytes.1-6 A characteristic cytogenetic abnormality, the Philadelphia chromosome (Ph'), has been identified in 90% of patients with CML.7 When disease acceleration occurs in the clinical course of these patients, approximately 80% develop additional karyotypic changes in malignant blasts.8 In about 30% of cases, the blast transformation occurs in lymphoid cells,9 and in a small proportion, the blast transformation may be extramedullary in sites such as the lymph nodes, spleen, skin, or meninges.10-14

We present a patient with Ph'-positive CML who developed extramedullary blast crisis in lymph nodes with unusual cytogenetic abnormalities, including a masked Ph' in the blasts. We studied the morphological and histochemical characteristics of these blasts on light and electron microscopy and performed a series of cell lineage markers by immunofluorescence. We were also able to demonstrate the uncomplicated Ph' in some of the patient's lymph node cells stimulated with Epstein-Barr virus (EBV) and lipopolysaccharide (LPS).

CASE HISTORY

The patient, a white male, was 24 years old when the diagnosis of CML was made on routine blood counts in August 1979. The white blood cell count (WBC) was 55,000/μL (38% neutrophils, 22% bands, 11% metamyelocytes, 7% myelocytes, 3% monocytes, 4% eosinophils, 15% lymphocytes), the hematocrit (Hct) 37%, and the platelets 271,000/μL. The leukocyte alkaline phosphatase score was 0 (normal 15–100), and bone marrow cytogenetics showed a Ph'. The patient was treated with intermittent oral busulfan and allopurinol for three years. In November 1982, he developed malaise, fever, and progressive bilateral swelling of the neck and axillae secondary to massive cervical and axillary adenopathy. The liver and spleen were palpable 2 and 4 cm below the right and left costal margins, respectively. The Hct was 41%, platelets 42,000/μL, WBC 10,100/μL (72% neutrophils, 4% bands, 4% metamyelocytes, 15% lymphs, 5% monocytes). A bone marrow aspirate was consistent with chronic phase CML with a reduced number of megakaryocytes. Three percent of the nucleated cells were blasts. A lymph node excised from the left side of the neck showed replacement by primitive cells. There was congestive hepatosplenomegaly, but no evidence of leukemic involvement in these organs or elsewhere. Subsequent therapy with vincristine and prednisone, and later a combination of methotrexate, vincristine, l-asparaginase and deca-drone, led to a partial response in the enlarged nodes but persistent pancytopenia of increasing severity. The patient succumbed to bacterial and fungal sepsis in July 1983. At autopsy, the bone marrow was found to be severely hypocellular, with no evidence of leukemic infiltration. Lymph nodes, although enlarged, were also depleted of cells. There was congestive hepatosplenomegaly, but no evidence of leukemic involvement in these organs or elsewhere. While receiving the various forms of chemotherapy outlined above,
the patient had multiple bone marrow examinations, which consistently showed hypopcellularity with absent megaloblasts and small numbers of mature erythroid and myeloid precursors. At no time did blasts represent more than 8% of the nucleated cells in the bone marrow specimen.

MATERIALS AND METHODS

Lymph nodes and bone marrow from the patient were examined histologically using standard sections stained with hematoxylin and eosin. Lymph node sections were also stained with Wright's stain for cell morphology and differential, and for terminal deoxynucleotidyl transferase (TdT) by immunofluorescence (Bethesda Research Laboratory, Inc; Gaithersburg, Md). For transmission electron microscopy (TEM), portions of the lymph node were fixed in a phosphate-buffered formaldehyde-glutaraldehyde mixture and further processed for TEM in a routine fashion. For the ultrastructural localization of myeloperoxidase, the tissue was first fixed in a tannic acid-aldehyde formaldehyde-glutaraldehyde mixture, followed by incubation in 3,3'-diaminobenzidine (DAB) medium.

Cells were isolated from the sectioned nodes and fractionated on a Ficoll-Hypaque gradient. A cytotoxic preparation was made from the separated lymph node cells, stained with Wright's stain, and examined by light microscopy and by immunofluorescence for TdT. The cells were also analyzed for cell markers, cytogenetic abnormalities, and studied in long-term culture systems.

Bone marrow aspiration specimens from the patient were examined histologically after staining with Wright-Giemsa. A portion of two specimens was used for cytogenetic studies, one from the chronic phase (2/82) and one taken at the outset of the acute phase of the disease (11/82). Bone marrow was studied twice for the presence of TdT during the accelerated phase (2/83 and 5/83).

Cell Culture Techniques

Heparinized bone marrow aspiration specimens were suspended in complete medium (RPMI with 15% fetal bovine serum, 100 U/mL penicillin, 100 μg/mL streptomycin, and 0.3 mg/mL L-glutamine) at a concentration of 10⁶ cells/mL. Half of the cultures were synchronized with methotrexate (MTX) for 17 hours, followed by incubation with thymidine for a further six hours prior to harvest for cytogenetic analysis. The remainder of the bone marrow cultures were harvested routinely after incubating for 24 hours.

Lymph node cells were suspended in complete medium at a concentration of 10⁶ cells/mL for unstimulated cultures. A further series of cultures was initiated to preferentially stimulate the growth and division of subgroups of lymph node cells:

1. B cells were stimulated with 75 μg/mL lipopolysaccharide (LPS) from Escherichia coli 055:B5 (No. 3120-25 Difco Laboratories, Detroit), added to lymph node cells at 10⁴ cells/mL. Cells were harvested for cytogenetics on day 6 of culture.

2. EBV-containing supernatant from an EBV-producing marmoset cell line, B958 (Dr. George Miller, New Haven, Conn), was used to establish continuous B cell growth of patient lymph node cells according to standard technique. Cultured cells were harvested on days 16 and 113 and were examined by immunofluorescence for the presence of the Epstein-Barr virus nuclear antigen (EBNA) after 8 weeks in culture.

3. T cell growth was established by suspending lymph node cells (10⁶/mL) with L4-phytohemagglutinin (L4-PHA) at 3 μg/mL. After 3-day incubation, the L4-PHA-stimulated cells were resuspended at 0.5 × 10⁶ cells/mL in complete medium containing 20% T cell growth factor (TCGF) (TCGF-Delectinated; tenfold concentrated; Cellular Products, Buffalo). Every three days, one-half of the medium was replaced with fresh complete medium containing 20% TCGF. Cells were harvested on day 16.

All cultures were incubated at 37°C in a humidified atmosphere containing 5% CO₂. At the time of harvest of EBV and TCGF-stimulated lymph node cultures, cells were studied morphologically using a Wright's stained cytocentrifuge preparation and were analyzed for cell surface markers and cytogenetics.

Preparations for Cytogenetic Analysis

All cultures from bone marrow and lymph nodes were harvested according to our usual procedures. Slide preparations were stained conventionally with Giemsa, destained, and restained with quinacrine mustard to obtain Q-bands when examined under ultraviolet light. Chromosomes were identified according to the ISCN (1978), and karyotypes were expressed as recommended by this system.

Cell Surface Marker Studies

The number of lymph node cells with membrane antigens recognized by monoclonal antibodies was measured by indirect immunofluorescence using standard techniques. The percent fluorescent cells in at least 100 cells was counted using a Zeiss fluorescence microscope with epifluorescence. The monoclonal antibodies used, their source, and their distribution in normal human blood cells is shown in Table 1. Surface immunoglobulin was determined by direct immunofluorescence with affinity-purified goat anti-human immunoglobulin, monospecific for heavy chains (IgM, IgG, IgA), kappa light chain, or lambda light chain.

Cytoplasmic immunoglobulin was determined on ethanol-fixed cytocentrifuge preparations. At least 100 cells were scored by phase and fluorescent microscopy.

RESULTS

Cytopathology

Bone marrow aspiration and biopsy specimens from September 1979, February 1982, and November 1982 showed a hyperplastic bone marrow with marked increase in myeloid elements at all stages of maturation. Less than 5% of the nucleated marrow cells were blasts. TdT performed on two marrow specimens obtained subsequent to the development of lymphadenopathy showed less than 1% of the cells to be positive.

Lymph node pathology from November 1982 showed a diffuse infiltration of the node with primitive undifferentiated blasts. A Leder stain for chloroaceta esterase was negative. Lymph node touch preparations showed that 83% of the cells present were undifferentiated, with large primitive nuclei, prominent nucleoli, and plentiful agranular blue cytoplasm. The remainder of the cells were small mature lymphocytes. Less than 1% of the cells present were mature granulocytes. The blasts did not stain with Sudan black, chloroaceta esterase or alpha-naphthyl butyrate esterase or PAS.

Electron Microscopy

Figure 1A is representative of the majority of malignant cells infiltrating the lymph node. The cells appear...
Table 1. Cell Lineage Markers on Lymph Node Cells

<table>
<thead>
<tr>
<th>Antigen</th>
<th>Normal Cell Distribution</th>
<th>Unstimulated Lymph Node</th>
<th>EBV Cultures Day 16</th>
<th>EBV Cultures Day 113</th>
<th>PHA/TCGF Cultures Day 16</th>
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<tr>
<td>TdT*</td>
<td>Thymocyte</td>
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<td>Early thymocyte</td>
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</table>

*Bethesda Research Labs, Bethesda, Md.  
†Becton Dickinson, Sunnyvale, Calif.  
‡Ortho Diagnostics, Raritan, N.J.  
§Dr. Jerry Ritz, Boston.  
‖Dr. Jun Minowada, Buffalo.  
‖‖Coulter, Inc., Hialeah, Fla.  
***Kallestad Laboratories, Chaska, Mich.  
††UPC 120 mouse IgG myeloma protein.

to be immature, and there is little evidence of differentiation. Approximately 30% of the cells had small peroxidase-positive granules (Fig 1B). Most often, these granules were observed in cells possessing folded or clefted nuclei.

Cell Lineage Markers

Table 1 presents cell lineage marker data. TdT was present in approximately 40% of freshly isolated lymph node cells. However, common ALL antigen (CALLA) was absent. Further study of cell surface markers did not determine an immunologic phenotype that unambiguously established that the malignant cells represented a single cell lineage. OKT10, which was present on most of the patient’s node cells, is normally present on normal thymocytes, but is also widely distributed on other immature lymphoid and hematopoietic cells and may occur in nonlymphocytic leukemia. Only 21% of the cells expressed the T-6 antigen of common thymocytes and T-ALL. Small populations of T lymphocytes (T-3 positive) and B lymphocytes (B-1 or surface immunoglobulin positive) were present. The myeloid antigen OKM1 (44%) was present, but not the antigens MCS-1 and MCS-2, which are present on normal myeloid precursors and many nonlymphocytic leukemias.

Wright’s stained cytocentrifuge preparations from EBV or TCGF-stimulated lymph node cultures demonstrated greater than 99% of the cells to be mature.
lymphocytes or lymphoblasts. Marker studies on cells from stimulated cultures showed that more than 90% of those infected with EBV were B cells (positive for SmIg). More than 50% of these cells were also positive for EBNA. Those cells that grew in the presence of L4-PHA followed by TCGF were 90% T cells (positive for Leu-1 or OKT-3).

Cytogenetics

Cytogenetic results are summarized in Table 2.

**Bone marrow.** Two specimens were analyzed the first 9 months before the onset of lymphadenopathy (2/82), when the patient was in stable chronic phase, and the second at the onset of node enlargement (11/82). From the first specimen, 15 metaphase cells were analyzed with Q-banding and all contained the Ph' resulting from the usual 9;22 translocation; the karyotype was 46,XY,t(9;22)(q34;q11). No other karyotypic abnormality was identified at that time.

From the second bone marrow specimen, all 25 banded metaphases contained the typical Ph' translocation. In 18 cells, this was the only abnormality. The remaining 7 cells had a new submetacentric chromosome, resulting from a translocation between one No. 20 and an extra No. 13; the karyotype was 46,XY,—20,+ der(20)t(13;20)(q14;q13.3),t(9;22)(q34;q11).

Unstimulated lymph node cells. Twenty-five metaphase cells were analyzed with banding. Three cells had a normal karyotype. Twenty-two cells were abnormal with a complex karyotype. Although a 9q+ was identified in all abnormal cells, the typical Ph' was not present. Instead, the 22q was translocated to the short arm of one chromosome No. 12, resulting in a masked Ph'. In addition, a second translocation between the short arms of chromosomes No. 7 and the other No. 12 was observed. The karyotype of these cells was: 45,XY,t(7;12)(p15;p13),t(9;22;12)(9pter---9q34::22q11---22qter;9qter---9q34::22q11---22p11?::12p11---12qter) (Fig 2).

[Fig 2. Karyotype of Q-banded bone marrow metaphase cell: 46,XY,—20,+ der(20)t(13;20)(q14;q13.3),t(9;22)(q34;q11).]
Fig 3. Karyotype of Q-banded, unstimulated lymph node metaphase cell with masked Ph': 45,XY.t(7;12)(p15;p13).t(9;22;12)(9pter→9q34:: 22q11→22qter;9qter→9q34::22q11→22p11:: 12p11→12qter).

Stimulated lymph node cells. Cells from the lymph node biopsy specimen were studied after stimulation with three different agents.

1. LPS: Thirty-five metaphases were analyzed with banding. Two cells contained the typical Ph' as the only abnormality. The remaining 20 metaphases had the complex karyotype identified in the unstimulated lymph node cells.

2. EBV: Thirty-nine metaphases were analyzed with banding on day 16 of culture. Ten (26%) had the typical Ph' translocation. The remainder had a normal karyotype. After 113 days in culture, a further 40 metaphases were analyzed, of which 4 (10%) had the typical Ph' abnormality. The remaining 36 cells were normal. In none of the cells analyzed after either 16 or 113 days in culture was the complex abnormality seen in unstimulated lymph node cells identified.

3. L4-PHA/TCGF: Twenty-seven metaphases were analyzed with banding, and a further 66 cells were studied after conventional Giemsa staining only to determine the presence of 46 chromosomes with four normal G-group chromosomes. There was no clonal abnormality detected in 93 metaphases examined.

Discussion

Our patient followed the usual clinical course of CML by initially responding well to therapy with busulfan, but after several years evolving into a more difficult, less responsive phase. The presentation of blast crisis was quite atypical, with generalized lymphadenopathy appearing when the bone marrow continued to show chronic phase CML. Massive lymphadenopathy in CML is uncommon, but has been well described, usually in association with disease acceleration. Often, the bone marrow shows a predominance of blasts simultaneously or within several months of node involvement. However, there are several reported cases similar to our patient, in whom bone marrow blast crisis did not develop despite lymphadenopathy with infiltration of the nodes by immature malignant cells that were either lymphoid or undifferentiated.

The presence of the Ph' in malignant cells of CML is evidence for the clonal origin of this disease in a primitive stem cell capable of differentiation. Evidence for B cell involvement in the chronic phase of this disease comes from a study by Fialkow et al, in which B lymphocytes from a G6PD heterozygote with CML, grown in the presence of EBV, gave rise to a series of lymphoid cell lines of which a majority expressed the B isoenzyme and some the Ph' as well. Our patient was a white male, and G6PD isoenzyme studies were not applicable. However, we were able to establish continuous B cell growth in culture by infecting lymph node cells with EBV. A significant proportion of these B cells contained a Ph' after up to 4 months in culture. At the time cytogenetics was performed, no myeloid cells could be identified in the cultures by morphology. Cell surface markers similarly failed to identify a significant nonlymphoid population that could have been expressing the abnormal chromosome.

The lymph node culture, stimulated with a polyclonal activator of B cells, LPS, showed the typical Ph' in 2 and the masked Ph' in 20 of 35 metaphase cells. The presence of both the simple Ph' and the
complex karyotype identified in unstimulated malignant lymph node cells in this culture suggests a B cell origin for this patient's blasts. Unfortunately, this result is inconclusive, because cell surface markers were not done on the LPS culture at the time of harvest for cytogenetic analysis. Therefore, we cannot exclude the possibility that undifferentiated or nonlymphoid blasts persisted in culture long enough to be harvested on day 6 for karyotypic analysis.

Thus far, it has not been possible to conclusively demonstrate mature T cell involvement in chronic phase CML. As previous investigators have found, we were unable to identify the Ph in T cells cultured from our patient. There are several possible reasons for this. First, T cells may not be involved in the malignant process at all. Second, T cells have a much longer lifespan in the circulation than myeloid cells, and many of the cells studied may have antedated the onset of the CML. In addition, the growth characteristics of T cells both in vitro and in vivo are different than those of other blood cells. It is possible that the Ph and other subsequent chromosomal abnormalities in CML confer a proliferative advantage to the malignant myeloid cells, but are disadvantageous to T cells or their precursors.

The involvement of stem cells capable of lymphoid differentiation in the accelerated phase of CML is suggested by the finding that 30%–50% of these patients express TdT and lymphoid markers such as CALLA on their blasts. Recently, such blasts have been shown in many cases to have rearrangement of immunoglobulin genes coding for heavy chain production, a feature of early B cell differentiation, and a single case of blast crisis expressing T cell surface antigens has been described. A significant proportion of the malignant lymph node cells from our patient expressed TdT. However, the CALLA antigen was absent. TdT positivity has been previously associated with CALLA-negative leukemia in blast crisis of CML. In our case, histochemical staining and a series of cell surface markers for a number of myeloid and lymphoid antigens failed to distinguish a pattern characteristic of the common forms of acute leukemia or lymphoma.

OKM1, which is a myeloid differentiation antigen, and peroxidase positivity on TEM were found in a substantial minority of the cells examined for these features. This suggests that at least some of the blasts had a nonlymphocytic origin. However, the majority of the lymph node cells were negative for OKM1 and other myeloid cell surface markers and were undifferentiated by histochemical staining and on TEM. The only antigen expressed by most of the lymph node cells (OKT10) is found on many primitive cells of both lymphoid and myeloid origin. Perhaps the most logical explanation for the somewhat contradictory laboratory and clinical information in this case is that the blast transformation in this patient had taken place in a very primitive stem cell capable of differentiation along both lymphoid and myeloid pathways. Such an outcome appears plausible, as we have cytogenetic evidence for the involvement of such a stem cell with the Ph demonstrated in both bone marrow cells and B lymphocytes from the lymph node. Morphological and cytochemical evidence for mixed lymphoid and myelomonocytic populations of blasts has been presented for both blast crisis of CML and acute leukemia arising de novo or following therapy for Hodgkin's disease.

The karyotype of the malignant cells in CML has been shown to evolve from the presence of only the Ph to the subsequent acquisition of additional abnormalities in 80% of cases as the disease enters its more aggressive phase. In our patient, the second bone marrow specimen showed a small clone [46,XY,–20,+der(20)(t(13;20),t(9;22))] with abnormalities in addition to the Ph. However, the morphology of the marrow continued to show only chronic phase disease. It has been previously noted that such complex karyotypic abnormalities may precede overt marrow blast crisis by several months or years. In the malignant lymph node cells, the simple Ph abnormality was confined to B cells in the EBV or LPS-stimulated cultures. The 13;20 chromosomal rearrangement identified in the bone marrow was not seen in any lymph node cells. Instead, a completely different karyotype appears to have evolved from Ph-positive lymph node cells. Mitelman et al have described extramedullary karyotypic evolution in CML in the spleen, whereas the bone marrow retained the Ph only. Karyotypic evolution with development of a new malignant clone with a Ph and additional changes unique to the lymph nodes, as illustrated by our patient, has been described by Stoll et al in three other cases. The late abnormalities in our case include a masked Ph with the original Ph (22q–) translocated to the short arm of chromosome No. 12. The normal short arm of this chromosome No. 12 appears to have been lost.

A masked Ph is extremely rare, having been reported in 0.6% of all Ph-positive cases of CML. Chromosome No. 12 has been involved in at least one other case of masked Ph, but the abnormality involved the long arm of this chromosome. Variant Ph translocations may involve almost any chromosome in the genome, but No. 12, especially its short arm, is particularly frequently involved. Five such cases appear in the literature, and all involved band
A masked Ph\(^1\) may be identified during the chronic phase of CML, but can evolve from a typical 9;22 translocation in association with the accelerated phase of the disease, as in our case and another described by Oshimura et al.\(^3\) However, our case is unique in that the masked Ph\(^1\) was found only in malignant lymph node cells during disease acceleration. In our patient, the clone with the complex karyotype and masked Ph\(^1\) appeared only to be missing a G-group chromosome when examined with conventional stain. It was not until Q-banding was analyzed that the complex changes were recognized. Before the advent of chromosome banding techniques, cases of CML with masked Ph\(^1\) would probably have been classified as Ph\(^1\)-negative, and it is therefore possible that the true incidence of “Ph\(^1\)-negative” CML is lower than was reported in the older literature.

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