Emergence of a B-Cell Lymphoblastic Lymphoma in a Patient With B-Cell Chronic Lymphocytic Leukemia: Evidence for the Single-Cell Origin of the Two Tumors

By Vito Pistoia, Silvio Roncella, Paola Franca Di Celle, Mario Sessarego, Giovanna Cutrøna, Giannamaria Cerruti, Gian Piero Boccaccio, Carlo Enrico Grossi, Robin Foà, and Manlio Ferrarini

A patient is described who presented with a chronic lymphocytic leukemia (CLL) and later developed a lymphoblastic lymphoma. The cells from the CLL were typical mature B lymphocytes as could be assessed by morphologic, cytochemical, and surface marker analyses. The cells from the lymphoblastic lymphoma were immature B cells that expressed CD10, CD20, and HLA-DR markers, but not surface IgM or cytoplasmic IgM chains, and were negative for terminal deoxynucleotidyl transferase (TdT). The cells of two continuous cell lines, obtained from the bone marrow and the peripheral blood of the patient, had the same phenotype as the lymphoblastic lymphoma cells, did not contain the Epstein-Barr virus genome, and displayed malignant features in vitro, including the capacity to form colonies in agar. The two cell lines also shared identical chromosomal abnormalities, a finding which suggests that they derived from the same malignant cell already present in vivo. Such chromosomal abnormalities were not seen in the karyotype of the peripheral blood cells at the onset of the disease. Analysis of the Ig heavy chain genes using a DJ-specific probe showed the very same monoclonal rearrangement in the cells from the B-CLL, the lymphoblastic lymphoma and the two cell lines, thus demonstrating their common clonal origin. By contrast, a monoclonal rearrangement of the λ chain gene locus was found in the B-CLL cells only, a finding consistent with their exclusive capacity to express surface IgM λ. This patient represents a rare case in whom a chronic lymphoproliferative disorder with mature malignant cells transforms into a lymphoblastic lymphoma characterized by cells frozen at a very early maturational stage. The possible mechanisms leading to such transformation within the same cell clone are discussed.

© 1991 by The American Society of Hematology.

LYMPHOPROLIFERATIVE disorders usually originate from the clonal expansion of a single malignant cell frozen at a certain maturational stage. Polyclonal or oligoclonal lymphoproliferative disorders are rare and occur mostly in immunodepressed patients, in whom the infection of B cells by Epstein-Barr virus (EBV) may play a pathogenic role. Occasionally patients with two distinct malignant lymphoid clones have been described. In some instances, these clones were found to be totally unrelated, thus suggesting their likely independent origin. In other cases, the two malignant lymphoid clones shared a number of markers, a finding that indicated their common origin. The studies of cases of the latter type have provided clues on the process of maturation of lymphoid cells and have offered support to the hypothesis of the multistep genesis of neoplasias.

Here, we describe a patient with a B-cell chronic lymphocytic leukemia (B-CLL) who subsequently developed a lymphoblastic lymphoma characterized by an accumulation of immature B cells. Cell lines comprised of immature malignant B cells were also obtained from the peripheral blood and the bone marrow of the patient. Marker studies indicated that the cells from the two neoplasias, as well as those from the cell lines, had a common clonal origin, but were frozen at different stages of maturation. Models proposing different steps of malignant transformation can be devised to explain the observation reported.

CASE HISTORY

A 71-year-old man was referred to the Department of Internal Medicine, University of Genoa, Italy, because of the recent onset of fatigue, malaise, sweating, nausea, mild fever, and occasional gingival bleeding. On admission, the patient had an elevated white blood cell (WBC) count (32,000/mm³) and thrombocytopenia (50,000/mm³); hemoglobin (Hb) was 12.2g% and hematocrit (Ht) 35%. The WBC differential showed 65% lymphocytes (Fig 1A), 30% neutrophils, and 15% monocytes. A bone marrow aspirate showed a predominance of lymphocytes with a characteristic CLL pattern (Fig 1B). Surface marker analysis of peripheral blood mononuclear cells (MNC) showed the presence of a monoclonal B-cell proliferation with surface IgM λ. The patient was diagnosed as suffering from B-CLL and enrolled in a protocol of treatment with recombinant interferon (IFN)α2 (Intron A; Schering-Plough, Milan, Italy) at the dosage of 3 megaunits daily i.m. A temporary remission was achieved, with a decrease of the WBC (4,000/mm³) and a normalization of the differential count after 15 to 20 days of treatment. Two months after starting IFN treatment, IFNα2 was discontinued and the patient was hospitalized. WBC, 2,500/mm³ with 10% lymphoid blasts, red blood cells (RBC) with anisopoikilocytosis, and occasional macrocytes and promyelocytes were observed in the peripheral blood. Platelets were 5,000/mm³, Hb 7.6 g% and Ht 22.5%. A bone marrow biopsy showed an infiltration of lymphoid blast cells (70% to 80% of the total cellularity) with large indented nuclei and nucleoli (Fig 1C). Cytologic analysis of a needle aspirate from the scalp mass showed a homogeneous infiltration of the same lymphoid cells. The mass was subsequently excised and examined. Morphologic, phenotypic, and molecular analyses (see below) were consistent with a diagnosis of B-cell lymphoblastic lymphoma.

The patient was treated with intensive chemotherapy. The American Society of Hematology.
less, he appeared confused and disoriented, with serious memory disturbances, diffuse tremor and nistagmus. A CAT scan of the brain was normal. CSF examination showed the presence of WBC (1,800/mm³) almost exclusively comprised of lymphoid blast cells with numerous mitoses. These cells were analyzed for surface markers and found to express a phenotype consistent with that of immature B cells (see below). The patient was treated with TCT for a total dose of 1,000 rad followed by intrathecal administration of methotrexate, 6-methylprednisolone, and cytarabine. The treatment induced a complete clinical remission and the patient was released from the hospital. He died at home, 1 month later, of myocardial infarction apparently unrelated to his recent clinical history. Autopsy could not be performed.

 MATERIALS AND METHODS

Cell separation and culture. Peripheral blood and bone marrow MNC were separated on a Ficoll-Hypaque (FH) density gradient. Adherent cells were removed by adherence to plastic. MNC were also isolated from a CSF sample and from the surgically removed scalp mass after gentle mincing. In an attempt to generate continuous cell lines, peripheral blood and bone marrow MNC were cultured at the concentration of 1 × 10⁶/mL in RPMI 1640 medium (GIBCO, Paisley, Scotland) supplemented with 10% fetal calf serum (FCS; GIBCO), sodium pyruvate, L-glutamine, and nonessential amino acids. After 2 weeks of culture, clusters of proliferating cells were observed. An increase in rapidly dividing cells was subsequently observed and two cell lines were generated, one from the peripheral blood MNC and the other from the bone marrow MNC.

In vitro colony formation. Colony formation in agar was assessed by culturing the cell lines (1 × 10³ cells/well) in 0.5 mL of a mixture containing 0.3% agar (Bacto Agar; Difco, Detroit, MI), α-medium (GIBCO), 10% FCS, L-glutamine, sodium pyruvate and penicillin-streptomycin. Colonies were counted after 5 days in culture.

Immunophenotypic analysis. Mouse monoclonal antibodies (MoAbs) to human MNC were obtained from different sources, as listed in Table 1. Fluorescein-conjugated goat polyclonal antibodies specific for human Ig heavy and light chains (Southern Biotechnology Associates, Birmingham, AL) were used in direct immunofluorescence. Mouse MoAbs were detected by indirect immunofluorescence with a fluorescein-conjugated goat antimouse Ig serum (polyvalent) (New England Nuclear, Florence, Italy). Cell preparations were analyzed by flow cytometry (Facstar; Becton-Dickinson) or with a Leitz Orthoplan fluorescence microscope (Leitz, Wetzlar, Germany). Nuclear staining for terminal deoxynu-
B-CLL ORIGINATES A B-CELL LYMPHOBLASTIC LYMPHOMA

DNA was isolated, digested with the appropriate restriction enzymes, size-fractionated by electrophoresis in 0.7% to 1% agarose gel, and blotted to nitrocellulose membrane filters. Filters were subsequently hybridized with 32P-labeled probes, washed at high stringency, and autoradiographed with intensifying filters. Filters were subsequently hybridized with "P-labeled probes, were stained with hematoxylin and eosin (H-E). Peripheral blood and bone marrow aspirates were stained with May-Grünwald-Giemsa. Cytochemical staining was performed on cytoplasmic staining for Ig chains was performed by indirect immunofluorescence. Flow cytometry.

Morphology and cytochemistry. Portions of the biopsy tissue obtained after appropriate informed consent were fixed in phosphate-buffered formalin and embedded in paraffin, and sections were stained with hematoxylin and eosin (H-E). Peripheral blood smears and bone marrow aspirates were stained with May-Grünwald-Giemsa. Cytochemical staining was performed on cytogenetic preparations. The tartrate-resistant acid phosphatase (TRAP) activity was determined by the addition of Na-tartrate to the incubation mixture.

Cytopathic studies. Chromosome analysis was performed using routine methods for culture harvest, slide preparation, and G and Q banding. DNA analysis. DNA was isolated, digested with the appropriate restriction enzymes, size-fractionated by electrophoresis in 0.7% to 1% agarose gel, and blotted to nitrocellulose membrane filters. Filters were subsequently hybridized with 32P-labeled probes, washed at high stringency, and autoradiographed with intensifying screens. The following probes were used: the 3-kb BamHI W (internal repeat) fragment of EBV, kindly donated by Dr A. Pflizer (Tumor Virology Laboratory, Dana Farber Cancer Institute, Boston, MA); a 3.3-kb EcoRI-HindIII fragment of the joining region of the Ig heavy chain gene (JH); a 2.7-kb EcoRI fragment carrying the constant portion of the Ig κ light chain gene (Cκ); a 1.2-kb BamHI fragment carrying the constant Cκ 2 region that encodes the Kern−Oz−isotype of the Ig λ light chain gene.

RESULTS

Phenotypic characterization of the neoplastic cells. At presentation, peripheral blood leukocytes comprised approximately 65% lymphocytes. These were small cells with a high nuclear to cytoplasmic ratio (Fig 1A) and were negative for TRAP. Cells with identical morphologic features were predominant also in a bone marrow aspirate obtained on the same occasion (Fig 1B). Surface marker analysis of the blood lymphocytes showed that more than 90% had a B-cell phenotype (CD19+, CD20+, HLA-DR+); 40% of them expressed monoclonal surface IgM (Table 2) with a dim pattern of staining. CD5 was expressed on 45% of the lymphocytes, which were negative for CD10 and TdT (Table 2) or for CD25, H2C, and CD11c (not shown).

Histology of the surgically excised parietal mass showed a morphologically homogenous population of lymphoblasts with large indented nuclei and prominent nucleoli. When analyzed in suspension with a battery of MoAbs, blast cells displayed a phenotype consistent with that of immature B cells (CD19+, CD20+, HLA-DR+, CD10+). They did not express surface Ig or cytoplasmic μ chains and were TdT-negative (Table 2). Cells with identical morphology and phenotype were first detected in the peripheral blood concomitantly with the appearance of the scalp mass. However, the proportion of these circulating cells never exceeded 10% to 15%.

An identical population of lymphoblasts was also predominant in a bone marrow biopsy obtained at the time when the scalp mass appeared (Fig 1C). Lymphoid blast cells with large, indented nuclei and an intensely basophilic cytoplasmic histochemistry were seen in the bone marrow biopsy and in the parietal scalp mass. A similar population of lymphoblasts was also found in the CSF and in the bone marrow derived from the iliac crest biopsy. However, the proportion of these circulating cells never exceeded 10% to 15%.

**Table 1. MoAbs Used in This Study**

<table>
<thead>
<tr>
<th>MoAbs</th>
<th>Specificity</th>
<th>Source</th>
</tr>
</thead>
<tbody>
<tr>
<td>OKT11-CD2</td>
<td>T cells, NK cells</td>
<td>Ortho*</td>
</tr>
<tr>
<td>OKT3-CD3</td>
<td>T cells</td>
<td>Ortho</td>
</tr>
<tr>
<td>OKT4-CD4</td>
<td>Helper T cells</td>
<td>Ortho</td>
</tr>
<tr>
<td>OKT8-CD8</td>
<td>Suppressor/cytotoxic T cells</td>
<td>Ortho</td>
</tr>
<tr>
<td>Leu9-CD7</td>
<td>T cells</td>
<td>Becton-Dickinson</td>
</tr>
<tr>
<td>OKM1-CD11b</td>
<td>Monocytes, granulocytes, NK cells, T-cell subset</td>
<td>Ortho</td>
</tr>
<tr>
<td>Leu11b-CD16</td>
<td>Granulocytes, NK cells</td>
<td>Becton-Dickinson</td>
</tr>
<tr>
<td>Leu7-CD57</td>
<td>NK cell and T-cell subsets</td>
<td>Becton-Dickinson</td>
</tr>
<tr>
<td>NKH1-CD56</td>
<td>NK cells</td>
<td>Coulter</td>
</tr>
<tr>
<td>OKIa1</td>
<td>HLA-DR antigens</td>
<td>Ortho</td>
</tr>
<tr>
<td>LeuM5-CD11c</td>
<td>Macrophages, granulocytes</td>
<td>Becton-Dickinson</td>
</tr>
<tr>
<td>IL2-CD25</td>
<td>Activated cells</td>
<td>Becton-Dickinson</td>
</tr>
<tr>
<td>B4-CD19</td>
<td>B cells</td>
<td>Coulter</td>
</tr>
<tr>
<td>B1-CD20</td>
<td>B cells</td>
<td>Coulter</td>
</tr>
<tr>
<td>B2-CD21</td>
<td>B cells</td>
<td>Coulter</td>
</tr>
<tr>
<td>H2C</td>
<td>Activated B cells</td>
<td>Dr D. Posnett‡§</td>
</tr>
<tr>
<td>Leu1-CD5</td>
<td>T cells, B-cell subset</td>
<td>Becton-Dickinson</td>
</tr>
<tr>
<td>My9-CD33</td>
<td>Pan-myeloid</td>
<td>Coulter</td>
</tr>
<tr>
<td>My4-CD14</td>
<td>Monocytes</td>
<td>Coulter</td>
</tr>
<tr>
<td>My7-CD13</td>
<td>Granulocytes, monocytes</td>
<td>Coulter</td>
</tr>
<tr>
<td>Fcε-CD23</td>
<td>Activated B cells</td>
<td>Dr N. Chiorazzi^</td>
</tr>
<tr>
<td>Ki1-CD30</td>
<td>Activated cells</td>
<td>Dako®</td>
</tr>
<tr>
<td>OKT7-CD38</td>
<td>Activated cells</td>
<td>Ortho</td>
</tr>
<tr>
<td>HPCA1-CD34</td>
<td>Early hematopoietic progenitors</td>
<td>Becton-Dickinson</td>
</tr>
</tbody>
</table>

**Table 2. Phenotypic Markers of Peripheral Blood Lymphocytes (PBL) of Cells Isolated From the Scalp Mass and CSF, and of a Spontaneous Cell Line Derived From the Bone Marrow**

<table>
<thead>
<tr>
<th>Cell Marker</th>
<th>PBL*</th>
<th>Scalp Mass*</th>
<th>CSF†</th>
<th>Ga1 Line*§</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD19</td>
<td>95</td>
<td>70</td>
<td>75</td>
<td>70</td>
</tr>
<tr>
<td>CD20</td>
<td>95</td>
<td>75</td>
<td>68</td>
<td>60</td>
</tr>
<tr>
<td>CD10</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>90</td>
</tr>
<tr>
<td>HLA-DR</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
<tr>
<td>CD5</td>
<td>48</td>
<td>&lt;1</td>
<td>ND</td>
<td>&lt;1</td>
</tr>
<tr>
<td>sigκ3</td>
<td>40</td>
<td>&lt;1</td>
<td>ND</td>
<td>&lt;1</td>
</tr>
<tr>
<td>sigκ5</td>
<td>40</td>
<td>&lt;1</td>
<td>ND</td>
<td>&lt;1</td>
</tr>
<tr>
<td>sigλ3</td>
<td>40</td>
<td>&lt;1</td>
<td>ND</td>
<td>&lt;1</td>
</tr>
<tr>
<td>sigλ5</td>
<td>40</td>
<td>&lt;1</td>
<td>ND</td>
<td>&lt;1</td>
</tr>
<tr>
<td>CD3</td>
<td>5</td>
<td>&lt;1</td>
<td>&lt;1</td>
<td>&lt;1</td>
</tr>
</tbody>
</table>

*Cells were stained with the appropriate MoAbs and enumerated by flow cytometry.

†Owing to the low number of cells obtained, they were stained as described above and counted with a fluorescence microscope.

‡Surface markers of the Ga2 cell line were superimposable to those reported for Ga1 cells.

§, surface.

Fig 1A: CD38, CD19, and CD10 expression in a representative B-cell lymphoblastic lymphoma (B-CLL). Figure 1B: CD5 expression in a representative B-CLL. Figure 1C: CD20 expression in a representative B-CLL.
plasm were also detected in the CSF. Their phenotypes are reported in Table 2.

Characterization of lymphoid cell lines established from the peripheral blood and the bone marrow. Two spontaneously growing cell lines were raised from the peripheral blood and from the bone marrow cells in two different occasions. The cell lines, termed Ga1 and Ga2, respectively, originated when peripheral blood analyses suggested that the patient was in apparent remission (ie, before the appearance of the scalp mass). These cell lines do not need the addition of exogenous growth factors and proliferate as single cell carpets with a doubling time of approximately 30 hours.

Cells from both lines are large lymphoid blasts with indented nuclei, multiple nucleoli, and a deeply basophilic cytoplasm (Fig 1D). The surface phenotype of both cell lines is identical. Blasts are positive for CD10, CD19, CD20, and HLA-DR surface markers. They do not express surface Ig, nuclear TdT, or cytoplasmic \( \mu \) chains (Table 2). The surface phenotype of Ga1 and Ga2 cells, studied at monthly intervals, remained stable. Both Ga1 and Ga2 cells are not infected by EBV, as shown by the failure to detect the EBV genome by Southern blot analysis (not shown). Both cell lines form colonies in agar with a cloning efficiency of 40% to 50%, which is similar to that observed for other malignant cell lines.22,33

Ig gene rearrangements. DNA was extracted from the peripheral blood MNC at presentation, digested, and probed with a JH-specific cDNA. The results of these experiments are shown in Fig 2. A monoclonal rearrangement of the Ig heavy (H) chain gene locus was detected in the DNA fragments cut with both HindIII (Fig 2, JH, lane 1) and EcoRI (not shown) restriction enzymes. Hybridization of the same DNA sample with a \( \kappa \) light chain gene cDNA probe showed that the \( \kappa \) genes were in the germ line configuration (Fig 2, Ck, lane 1). Hybridization with a \( \lambda \) light chain gene cDNA probe showed a major germ line band together with a minor rearranged band in the prepara-

![Fig 2](http://www.bloodjournal.org)
tions cut with both HindIII and EcoRI (Fig 2, Cα, lanes 1). This result is consistent with the finding that a proportion of the leukemic cells expressed surface IgM λ.

When the DNA extracted from the cells of the scalp mass was probed with the same JH cDNA used above, a pattern of rearrangement identical to that of the peripheral blood cells was observed (Fig 2, JH, lane 2). By contrast, the minor rearranged band detected in the peripheral blood with the cDNA λ probe was no longer found and all of the DNA appeared in the germ line configuration (Fig 2, Cα, lanes 2). A very faint germ line band was detected with a κ chain-specific cDNA, a finding that possibly suggests a deletion of the κ chain genes in the majority of the cells (Fig 2, Cκ, lane 2).

DNA extracted from Ga1 and Ga2 cell lines showed the same JH rearrangement seen in the peripheral blood MNC and the scalp mass DNA (Fig 2, JH, lanes 3 and 4). In the DNA from both lines, the λ chain gene locus was in germ line configuration (Fig 2, Cα, lanes 3 and 4) while the κ chain genes were deleted (Fig 2, Cκ, lanes 3 and 4). The latter finding supports further the aforementioned indication that there was a deletion of the κ chain genes in the malignant cells from the scalp mass.

**Karyotypic studies.** Karyotypic analyses were first performed on peripheral blood cells at the onset of disease. Cells were incubated in culture with or without stimuli and analyzed after 24 hours. Stimulation with TPA and ionomycin failed, however, to induce cell proliferation as compared with the unstimulated cell suspensions. Thus, the results reported refer to the analysis of the mitoses detected in the unstimulated cultures.

A total of 18 metaphases could be observed. None of these had a normal diploid karyotype, whereas a loss of chromosome Y was detected in the remaining seven (not shown). Karyotypic analysis of the cells from the scalp mass was unsuccessful owing to the poor viability of the recovered cells in vitro.

Ga1 and Ga2 cell lines were studied at 3-month intervals over a 1-year period. The karyotype of both lines was consistently the following: 48, X, −Y, +1, −19, +21, −22, del(13)(q13q22), +m1, +m2, +m3 (Fig 3). The karyotype reported in Fig 3A was obtained by a Q banding technique; to investigate further whether or not the extra chromosome 21 was in fact a chromosome 22, the karyotype was determined using a G banding technique. As shown in Fig 3B, these studies indicated that extra 21 chromosome was not a chromosome 22. The finding that the two cell lines, which were raised from two different cell samples (bone marrow and peripheral blood) and on two different occasions, had identical karyotypes indicates that they originated from the same malignant cells already present in vivo and that no chromosomal changes were acquired subsequently in vitro.

**DISCUSSION**

This report describes a patient with a chronic lymphoproliferative disorder that evolved into an acute lymphoid malignancy. Problems related to this patient’s case concern:

1. the definition of the chronic disorder;
2. the nature of the acute malignancy;
3. the relationship between the cells of the two clonal expansions; and
4. the possible steps that may have caused the evolution of one disorder into the other.

Most of the findings at presentation were consistent with a chronic lymphoproliferative disorder, most likely a B-CLL, as indicated by the morphologic and phenotypic features of the neoplastic cells.

The second malignancy that manifested at a later stage of the disease fulfilled the criteria of lymphoblastic lymphoma. The cells were lymphoblasts that expressed the B-cell markers CD19, CD20, CD10, but did not have surface Ig or cytoplasmic μ chains. In addition, the cells displayed a monoclonally rearranged H chain gene locus in the absence of detectable rearrangements of the light (L) chain gene loci. Somewhat surprisingly, the cells were negative for TdT.

Immature B-cell markers were also found on the Ga1 and Ga2 cell lines. Both of them did not contain the EBV genome and had malignant features, such as the ability to form colonies in agar with a high cloning efficiency and to grow in culture at a low cell density as a single cell carpet. Cells from the two lines had the same karyotype and an identical Ig H chain gene rearrangement; the latter was superimposable to that of the tumor cells present in vivo. Furthermore, both Ga1 and Ga2 cells displayed a germ line configuration of the λ chain gene locus as the cells from the lymphoblastic scalp mass. These findings suggest that the characteristics of the cell lines reflected those of the original neoplastic cells present in vivo. Because the origin of the lines preceded the clinical manifestation of the lymphoblastic lymphoma, it can be inferred that a number of immature neoplastic cells were already present when the patient was in temporary remission during IFN therapy.

The hypothesis that the two malignancies from the patient were closely related is clearly demonstrated by the identical Ig H chain gene rearrangement found in the peripheral blood leukemic cells at the onset of the disease as well as in the cells from the lymphoblastic lymphoma. The absence of the Y chromosome noted at presentation in some of the mitoses and confirmed later in the cell lines could also provide some evidence that the two malignant clones also shared a common chromosomal marker. However, a note of caution may be suggested by the relatively small proportion of mitoses examined in the peripheral blood and by the notion that the loss of the Y chromosome can be detected not only in some hematologic malignancies but also in normal cells from aging males.

The feature of this clinical case that deserves consideration is the mechanism underlying the origin of the two lymphoid clones that were frozen at two distinct developmental stages. Previously reported cases have generally described the transformation of a chronic B-cell lymphoproliferative disorder into a more aggressive disease with cells that retained some properties of the well-differentiated B cells, including expression of surface Ig. A typical example is the Richter syndrome, characterized by the transformation of a B-CLL into a large B-cell lymphoma. Only
exceptionally an acute lymphoblastic transformation of B-CLL has been reported, but, in these studies, the clonal origins of the two lymphoproliferative disorders could not be investigated in detail.

There are two possible interpretations for our findings. The first postulates that the two malignancies derived from a common progenitor that was subsequently responsible for the generation of the two subclones. One of these was capable of maturation into B cells with surface IgM λ molecules, while the other remained frozen at an earlier B-cell stage. According to this hypothesis, the common progenitor cells were characterized by the presence of the same H chain gene rearrangement observed in the two malignant clones and perhaps by the loss of the Y chromosome. This scheme fits well with some analogous models proposed as an explanation for the simultaneous presence, in certain myeloma patients, of mature plasma cells together with pre-B cells both sharing the same H chain idiotypic marker as the serum M component. A similar explanation has also been offered for the appearance, in the blast crisis of chronic myelogenous leukemia, of pre-B cells that express the same monoclonal markers as the malignant myeloid cells.

The major limitation of this and analogous models resides in their incapacity to fully account for the monoclonal L chain gene rearrangement observed in the cells from the more mature clone. If an early progenitor cell, albeit neoplastic, can differentiate up to the mature stages of the B-cell lineage, then it should be conceivably able to originate a full set of mature B cells each with differently rearranged L chain loci. In this connection, it is perhaps worth recalling that, in acute myeloid leukemias...
and myelodysplastic syndromes, the B cells that show the same monoclonal glucose-6-phosphate dehydrogenase (G6PD) marker as the malignant cells can express either κ or λ chains when infected with EBV in vitro. In addition, in murine pre-B cell lines transformed by the Abelson-murine leukemia virus, there is a continuing L chain gene rearrangement. Therefore, for the common progenitor model to hold true, one has to postulate that a specially selected L chain gene rearrangement took place in the development of the more mature B-cell clone or else that there was a clonal selection following a random process of L chain gene rearrangement. The latter mechanism has been actually postulated to explain the origin of monoclonal follicular lymphomas in patients presenting oligoclonal expansions of pre-B cells.

The second model proposes that the more immature cell clone originated from the mature one through a multistep process of "dedifferentiation" consisting of a progressive accumulation of mutations. For example, the loss of chromosome 22, where Ig λ chain genes map, may have been instrumental in determining the failure of the resulting cell population to synthesize complete Ig molecules. This hypothesis would help explain a rather unusual feature observed at the disease onset consisting in the presence of circulating B cells that were CD10-negative, slg-negative (see Table 2) and most likely did not have a monoclonal rearrangement of the λ chain gene locus (Fig 2). These cells could have represented an intermediate stage of transformation from the chronic into the acute lymphoproliferative disorder. The above hypothesis would also offer an explanation for the TdT negativity of the immature B-cell blasts. Unfortunately, the differences encountered in analyzing the malignant cell karyotype at the onset of the disease make it difficult to ascertain the changes that may have occurred in the cells during the hypothetical "dedifferentiation" process.

ACKNOWLEDGMENT

We thank Drs D. Posnett and N. Chiaraippi for the kind gift of monoclonal antibodies; Dr A. Plizer for the generous supply of the EBV cDNA probe; Drs Giuseppe Saglio and Giovanni Pizzolo for helpful discussion; and Teresa Tavilla for precious secretarial assistance.

REFERENCES


Emergence of a B-cell lymphoblastic lymphoma in a patient with B-cell chronic lymphocytic leukemia: evidence for the single-cell origin of the two tumors

V Pistoia, S Roncella, PF Di Celle, M Sessarego, G Cutrona, G Cerruti, GP Boccaccio, CE Grossi, R Foa and M Ferrarini